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ABOUT COVER

Editorial Board Member of *World Journal of Hepatology*, Igor Skrypnyk, MD, MDS, PhD, Professor, Internal Medicine #1, Poltava State Medical University, Poltava 36011, Ukraine. inskrypnyk@gmail.com

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The primary aim of *World Journal of Hepatology* (*WJH*, *World J Hepatol*) is to provide scholars and readers from various fields of hepatology with a platform to publish high-quality basic and clinical research articles and communicate their research findings online.

WJH mainly publishes articles reporting research results and findings obtained in the field of hepatology and covering a wide range of topics including chronic cholestatic liver diseases, cirrhosis and its complications, clinical alcoholic liver disease, drug induced liver disease autoimmune, fatty liver disease, genetic and pediatric liver diseases, hepatocellular carcinoma, hepatic stellate cells and fibrosis, liver immunology, liver regeneration, hepatic surgery, liver transplantation, biliary tract pathophysiology, non-invasive markers of liver fibrosis, viral hepatitis.

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FRONTIER

Role of endoscopic ultrasound in the field of hepatology: Recent advances and future trends

Jahnvi Dhar, Jayanta Samanta

ORCID number: Jahnvi Dhar 0000-0002-6929-4276; Jayanta Samanta 0000-0002-9277-5086.

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Jahnvi Dhar, Jayanta Samanta, Department of Gastroenterology, Post Graduate Institute of Medical Education and Research, Chandigarh 160012, India

Corresponding author: Jayanta Samanta, MBBS, MD, DM, Assistant Professor, Doctor, Department of Gastroenterology, Post Graduate Institute of Medical Education and Research, Sector 12, Chandigarh 160012, India. dj_samanta@yahoo.co.in

Abstract

The role of endoscopic ultrasound (EUS) as a diagnostic and therapeutic modality for the management of various gastrointestinal diseases has been expanding. The imaging or intervention for various liver diseases has primarily been the domain of radiologists. With the advances in EUS, the domain of endosonologists is rapidly expanding in the field of hepatology. The ability to combine endoscopy and sonography in one hybrid device is a unique property of EUS, together with the ability to bring its probe/transducer near the liver, the area of interest. Its excellent spatial resolution and ability to provide real-time images coupled with several enhancement techniques, such as contrast-enhanced (CE) EUS, have facilitated the growth of EUS. The concept of "Endo-hepatology" encompasses the wide range of diagnostic and therapeutic procedures that are now gradually becoming feasible for managing various liver diseases. Diagnostic advancements can enable a wide array of techniques from elastography and liver biopsy for liver parenchymal diseases, to CE-EUS for focal liver lesions to portal pressure measurements for managing various liver conditions. Similarly, therapeutic advancements range from EUS-guided eradication of varices, drainage of bilomas and abscesses to various EUS-guided modalities of liver tumor management. We provide a comprehensive review of all the different diagnostic and therapeutic EUS modalities available for the management of various liver diseases. A synopsis of all the technical details involving each procedure and the available data has been tabulated, and the future trends in this area have been highlighted.

Key Words: Endoscopic ultrasound; Liver disease; Elastography; Varices; Liver tumor; Liver biopsy

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Core Tip: The advancements in the field of endoscopic ultrasound (EUS) have enabled endosonologists to rapidly expand their wings in the field of hepatology. "Endo-hepatology" encompasses the wide range of diagnostic and therapeutic endoscopic procedures that can be used for the management of various liver diseases. Diagnostic advancements range from elastography for liver parenchymal diseases, contrast-enhanced EUS for a focal liver lesion to portal pressure measurements. Therapeutic advancements range from EUS-guided eradication of varices to drainage of abscesses to liver tumor ablation. In this comprehensive review, all the various diagnostic and therapeutic EUS modalities available for the management of liver diseases have been detailed.

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INTRODUCTION

The armamentarium of endoscopic ultrasound (EUS) has grown considerably in recent years, both as an investigative and a therapeutic modality. The established diagnostic tools for the study of liver diseases include trans-abdominal ultrasound (USG), computed tomography (CT) scan and magnetic resonance imaging (MRI). While in the past, interventions in liver disease have predominantly been performed by the percutaneous or vascular route, EUS is now more and more being used for both diagnostic and therapeutic purposes. The ability to combine endoscopy and sonography in one hybrid device is a unique property of EUS, together with the ability to bring its probe/transducer in close proximity to the liver, the area of interest. In addition, its excellent spatial resolution and ability to provide real-time images, along with additional techniques, such as contrast-enhanced (CE) EUS, have facilitated the growth of EUS.

Furthermore, EUS guided intervention is also used as a rescue modality when the percutaneous approach is not favorable. EUS has opened doors to a variety of other procedures which are being explored, such as portal vein (PV) sampling for cancer cells, delivery of chemotherapy in the PV, measurement of portosystemic pressure gradient, and EUS guided transjugular intrahepatic portosystemic shunt (TIPS) creation. Harnessing its use in various liver-related interventions paves the way for a new zone of specialty, "Endo-hepatology." Herein we provide a comprehensive review on the use of EUS in the field of hepatology, both diagnostic and therapeutic, discussing the various recent advances and future trends (Figure 1).

LITERATURE SEARCH

A search was performed in PubMed and Embase and the search strategy is outlined in Supplementary Doc 1. All studies such as case reports, series, clinical studies, animal models and reviews regarding EUS applications in liver disorders, including portal hypertension (PHTN), were reviewed. Non-English language literature was not included in the review. EUS applications for extrahepatic bile duct obstruction, gallbladder, *etc.*, including their interventions, are beyond the scope of this review and have been excluded.

EUS FOR LIVER PARENCHYMA ASSOCIATED DISEASES

EUS can be used for the diagnosis, assessment and therapeutic management of ascites, liver parenchymal pathologies, space-occupying lesions (SOLs), liver biopsy, drainage of liver abscesses, bilomas and the management of hepatic tumors.

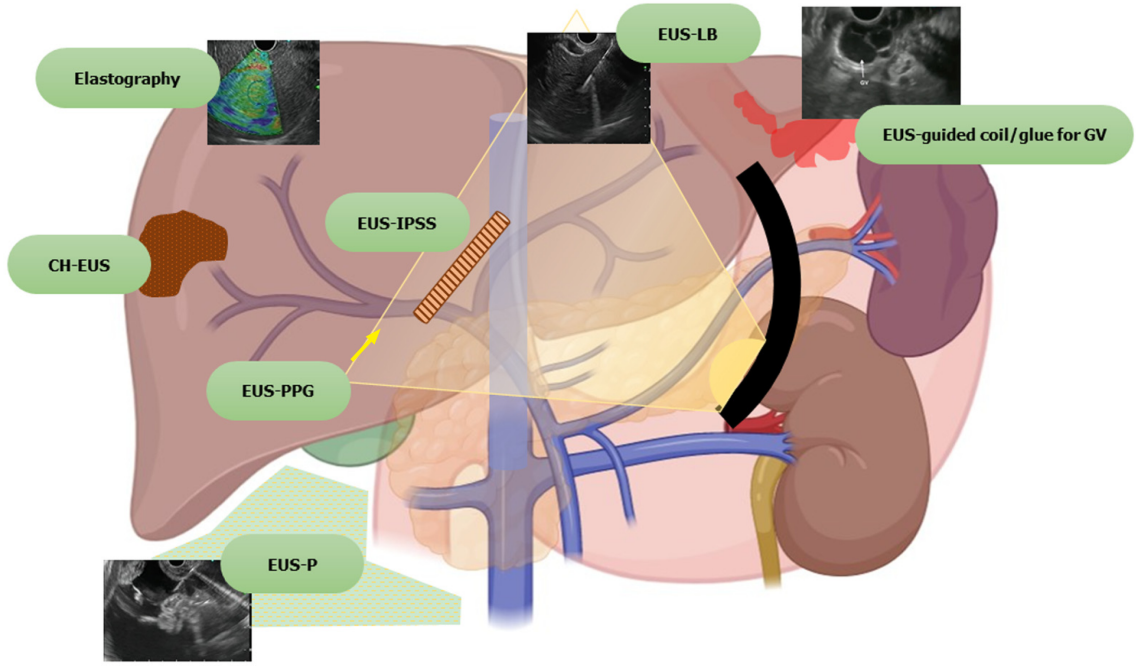

Figure 1 Spectrum of endoscopic ultrasound in hepatology. EUS: Endoscopic ultrasound; CH-EUS: Contrast harmonic endoscopic ultrasound; EUS-IPSS: Endoscopic ultrasound guided intrahepatic portosystemic shunt; EUS-LB: Endoscopic ultrasound guided liver biopsy; EUS-PPG: Endoscopic ultrasound guided portal pressure gradient; EUS-P: Endoscopic ultrasound guided paracentesis; GV: Gastric varices.

Ascites: Assessment and paracentesis

Ascites can be due to benign or malignant diseases. Although the differential diagnosis is broad, around 80%-90% of cases are attributed to underlying cirrhosis and PHTN [1]. Traditionally, routine paracentesis is performed bedside and sometimes with abdominal ultrasound guidance. However, abdominal paracentesis may become difficult in the presence of multiple abdominal scars, previous puncture marks, obesity, dilated bowel loops, dilated/tortuous veins, or the presence of omental or peritoneal nodules[1-3]. EUS guided paracentesis (EUS-P) is more sensitive than CT in detecting ascites[2,4]. The presence of ascites not visualized on imaging (CT/USG) as well as compartmentalization of fluid (such as benign etiologies like tuberculosis or tumor implants in peritoneal carcinomatosis) makes EUS-P a very promising tool in these areas[4,5]. With EUS-P, even small amounts of fluid (as little as 2.7 mL) can be aspirated and provide valuable diagnostic information[6]. In addition, EUS-P can be used as a rescue procedure in the case of previously failed percutaneous paracentesis or part of diagnostic workup during diagnostic EUS (Figure 2).

Additionally, EUS guided fine needle aspiration (EUS-FNA) of suspicious nodules in the omentum/peritoneum can be performed simultaneously while performing paracentesis for targeted cytological diagnosis[7]. Contrast-enhanced EUS (CE-EUS) has also been evaluated to identify enhancement patterns of peritoneal nodules or omental caking and differentiate benign or malignant causes of undiagnosed ascites [8].

The technique of EUS-P: The technique of EUS-P is detailed in Table 1.

Future trends: Since the first report of EUS-FNA of ascites and pleural fluid performed in 1995, various reports of EUS-P with/out FNA of peritoneal deposits have been published subsequently with excellent diagnostic capability and correlation with intraoperative findings[12]. Some cases of development of infectious complications (attributed to traversing the contaminated gastrointestinal wall) such as self-limited fever (3.3%) and bacterial peritonitis (4%) have been reported[5,10]. Recent developments include the deployment of double plastic stents in loculated ascites (benign/malignant), leading to internal drainage causing significant improvement in quality of life[13,14] (Figure 3). A clinical trial is also recruiting patients for EUS guided placement of a plastic prosthesis for refractory malignant ascites[15]. The various studies on EUS-P are summarized in Table 2.

Thus, EUS-P is an excellent tool (sensitivity 94%, specificity 100%) to detect a small quantity of ascites[10] and therapeutic drainage where the percutaneous approach is

Table 1 Technique of endoscopic ultrasound guided paracentesis[1-3,9-11]

Pre-procedure requirements
(1) No recommendations exist for EUS-P, although most studies have been performed under the cover of pre/peri-procedural antibiotics; and (2) Patient is usually fasted for 4-6 h before the procedure
Technical aspects
(1) EUS-P is usually performed using a 22 G/25 G FNA needle. A specialized spring-loaded 22 G FNA needle can also be used for the same; (2) The approach can be transgastric or transduodenal. The tip of the needle is visualized under EUS guidance in the ascites; (3) At the time of puncture, care is taken to avoid a trajectory involving any tumor/vessels to avoid peritoneal seeding or bleeding; (4) For therapeutic paracentesis, a suction tube attached to a vacuum canister can be used; (5) Repositioning of the needle is carried out in case it gets blocked by the tumor or omentum; (6) Two and fro motion is usually not needed; (7) CE-EUS followed by FNA of the peritoneal/omental nodules can also be done for added diagnostic value; and (8) The sample aspirated is sent for routine cytological assessment and for any additional tests that might be needed
Post procedure
The administration of albumin post 5 L of paracentesis and post procedure observation are carried out as per standard recommendations (EASL, AASLD)

EUS: Endoscopic ultrasound; EUS-FNA: Endoscopic ultrasound guided fine needle aspiration; EUS-P: Endoscopic ultrasound guided paracentesis; G: Gauge; CE-EUS: Contrast enhanced endoscopic ultrasound; EASL: European Association for the Study of Liver; AASLD: American Association for the Study of Liver Diseases.

not amenable. Furthermore, FNA of peritoneal/omental nodules is an added advantage that can increase the diagnostic yield.

Assessment of liver parenchyma/SOLs: Anatomy of the liver, its segments and surrounding structures

The requirement for three-dimensional conceptualization of the liver parenchyma makes EUS assessment of the liver and surrounding structures different from the conventional methods of USG/CT/MRI. Depending on the position of the EUS scope, either in the stomach or duodenum, various structures can be identified (Table 3 and Figure 4) such as[17]: (1) From the gastric end: Segments I (caudate lobe), left lobe segments (II, III, IV), right lobe (V, VIII), umbilical part of the left PV and ligamentum teres, ligamentum venosum, inferior vena cava, and hilum; and (2) From the duodenal bulb: Segments VI, VII; the hepatoduodenal ligament structures and PV and hepatic artery branches, the liver hilum and the segmental divisions of the right PV and hepatic artery.

Although transabdominal USG or CT scan is the first-line approach for evaluation of liver parenchyma or focal lesions, EUS has additional features which can add to its diagnostic/therapeutic potential[18,19]: (1) Transducer proximity enables better identification of the structures; (2) Combination of real-time images with elastography enables semi-quantitative measurements of liver parenchymal stiffness; (3) Newer generation EUS machines with color, power and pulsed Doppler systems helps easy assessment of the vasculature; (4) CE-EUS or harmonic EUS increases the diagnostic performance of focal liver lesions; and (5) Simultaneous assessment and interventions such as management of varices and liver biopsy can be performed in a single setting.

Techniques of assessment: Elastography and contrast enhancement techniques

Real-time elastography (RTE) has been developed for the assessment and quantification of liver tissue stiffness. Qualitative RTE uses the degree of deformation by the compression of structures as an indicator of tissue stiffness and is depicted using a color map wherein hard tissue is blue, intermediate stiffness is green and soft tissue is red. Quantitative RTE, on the other hand, uses hue histogram and strain ratio. While the former is a graphical representation of the color distribution in a selected image field, the strain ratio is calculated as the ratio of the target area (A) by reference area (B) (Figure 5)[20].

CE-EUS is a more valuable technique to improve the diagnostic performance of focal liver lesions. It is of 2 types: CE-EUS with the Doppler method (CE-EUS-D) and CE-EUS with harmonic imaging (CE-EUS-H). The former helps distinguish vascular-rich and hypovascular areas of a liver SOL, whereas the latter helps provide a detailed roadmap of the vasculature of the same. Of the contrast agents available, Sonovue and Sonazoid are more commonly used[21].

The concept of CE-EUS depends on the dual blood supply of the liver and has 3 phases: arterial phase (20-45 s), portal venous phase (lasting up to 120 s), and the late phase (contrast agent clearance, around 6 min)[21].

Table 2 Studies on endoscopic ultrasound guided paracentesis

Ref.	Study design	Patient population	Imaging	Age (yr)	Gender (M/F)	Needle	Route (TG/TD)	Amount of fluid aspirated	Diagnosis on EUS	Actual diagnosis	Complications
Chang *et al*[12], 1995	Case report	2 cases	CT (pleural effusion and ascites)	-	-	-	-	-	-	Malignant effusion and ascites	-
Romero-Castro *et al*[14], 2017	Case series	3 cases	DLBCL (1 case), HCC (2 cases)	60/74/55	3/-	19 G FNA (all cases)	TG (3 cases)	Double Pigtail placement (3 cases)	-	Malignant ascites (3 cases)	None
Wardeh *et al*[16], 2011	Retrospective study	101	Ascites not detected in 6/9 cases on CT	68.3	54/47	19 G FNA	NA	10 mL (max) in 90 cases, 2 smears in 11 cases	74 negative	84 malignant	None
Suzuki *et al*[11], 2014	Retrospective study	11 cases	CT (no ascites in 4)	66.4	7/4	22 G (automatedspring-loaded)	NA	14.1 mL (range 0.5-38 mL)	Benign 5; malignant 6	NA	None
Kaushik *et al*[10], 2006	Retrospective study	25	NA	66-70	16/9	22/25 G FNA	Both	6.8 mL (range, 1-20 mL)	64% malignant (benign 9; malignant 16)	Benign 8; malignant 17	1 cases (4%) (bacterial peritonitis)
Lee *et al*[4], 2005	Retrospective study	250 cases	CT in all	60.3	160/90	NA	NA	NA	37% ascites, 28% peritoneal metastasis	All malignant	None
Dewitt *et al*[5], 2007	Retrospective study	60	CT/MRI/USG in all (ascites 31 cases (51%)	67	33/27	22 G	55 (TG), 5 (TD)	8.9 (1-40) mL	Benign 42; malignant/atypical 18	Benign 15; malignant 45	2 cases fever
Köck *et al*[13], 2018	Case report	2 cases	Rectal cancer, ovarian cancer	36, 56	-/2	19 G	Both TG	Pigtail (plastic) placed	-	-	None
Nguyen and Chang[2], 2001	Retrospective study	31 cases (of 85)	CT had ascites in 14/79 (18%)	NA	NA	NA	NA	7.9 (1-40 mL)	Malignant 5; benign 26	NA	None
Varadarajulu and Drelichman[3], 2008	Case report	1	SCC anus	31	-/1	19 G	TG (1)	10 mL (diagnostic); 5 L (therapeutic)	Malignant ascites	NA	None

DLBCL: Diffuse large B cell lymphoma; TG: Transgastric; TD: Transduodenal; M: Male; F: Female; G: Gauge; EUS: Endoscopic ultrasound; CT: Computed tomography; FNA: Fine needle aspiration; SCC: Squamous cell carcinoma; USG: Ultrasound; MRI: Magnetic resonance imaging.

The advantages of CE-EUS over CT and MRI are that: (1) It provides real-time imaging; (2) Contrast is not excreted by the kidneys, and thus can be used in cases with renal insufficiency; (3) Contrast is confined to the vascular space only and so has prolonged enhancement of vascular system; (4) Higher resolution helps in targeted biopsies; and (5) Can characterize lesions less than 1 cm.

EUS imaging in chronic liver diseases

Certain tests such as transient elastography (TE), Fibroscan, and RTE can aid in the diagnosis of the degree of liver fibrosis. However, these tests are fraught with

Table 3 Structures visualized with endoscopic ultrasound in the liver

Structure	Features	Doppler
Portal vein branches	Thick and hyperechoic walls	Positive signal
Hepatic vein branches	Thin, non-reflective walls, straight course	Positive signal
Biliary radical	Hyperechoic walls, irregular course	Negative signal
Ligaments (teres and venosum)	Thick, hyperechoic (no lumen) (between vessels and Glisson's capsule)	Negative signal
Gallbladder	Cystic structure, hyperechoic walls, anechoic content	Negative signal
Falciform ligament	Thick, hyperechoic (no lumen); on the left anterior to segment III, on the right anterior to segment IVa and IVb	Negative signal
Hepatic artery	Thick with reflective walls	Positive signal

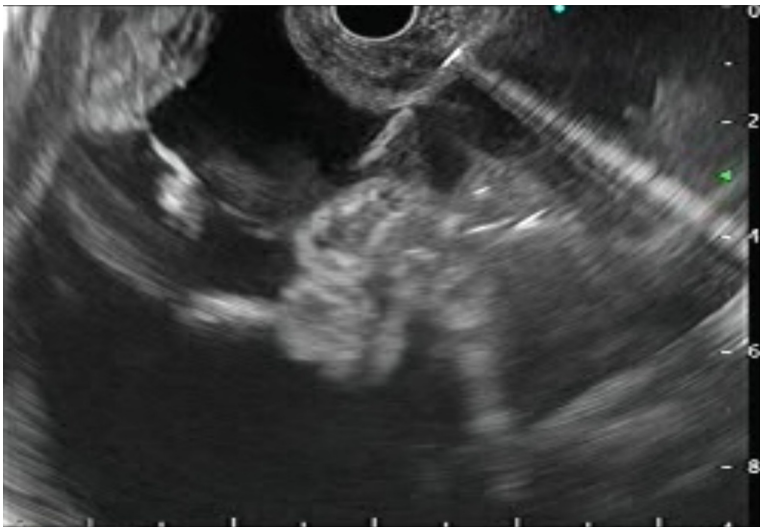

Figure 2 Endoscopic ultrasound guided paracentesis. Needle is visualized in the ascitic fluid.

limitations in people with obesity and ascites. EUS can be used similarly with probably better diagnostic sensitivity for the same. Schulman *et al*[22] reported that liver fibrosis index (LFI) correlated with abdominal imaging (LFI in normal, fatty liver and cirrhosis patients were 0.8, 1.4 and 3.2, respectively). Similar findings were replicated in liver fibrosis assessment for chronic hepatitis C cases (LFI of 2.38 had an area under the receiver operating characteristic curve of 0.73) compared with the gold standard of liver biopsy. Histogram acquisition was successful in 82% of patients[23]. A recent study by Tu *et al*[24] in early-stage cirrhosis showed that the accuracy of a combination of EUS, EUS-RTE, acoustic radiation force impulse (AFRI) and aspartate aminotransferase-to-platelet ratio (APRI) had the highest diagnostic rate (sensitivity 87%). Thus, EUS can provide a one-stop diagnostic modality to screen and rule out a host of conditions in patients with liver disease, from the screening of varices, pancreaticobiliary pathology to hepatic parenchymal/SOL assessment.

EUS imaging in focal liver lesions

The diagnostic accuracy of EUS in detecting focal liver lesions, mostly less than 1 cm, exceeds that of USG, CT, and MRI[25,26]. Singh *et al*[27] addressed the diagnostic yield of EUS *vs* CT for hepatic metastasis (98% *vs* 92%), wherein EUS identified a significantly greater number of metastatic lesions (40 *vs* 19). Diagnostic criteria proposed by Fujii-Lau *et al*[28] can be used to differentiate between benign and malignant metastatic hepatic lesions based on EUS findings with a positive predictive value of 82%. Lesion shape, borders, echogenicity, homogeneity, and size are used to delineate malignant lesions. It is said to be neoplastic if it meets at least three criteria: (1) Lack of isoechoic/slightly hyperechoic center; (2) Post-acoustic enhancement; (3) Adjacent structures distortion; (4) Hypoechogenicity (slightly or distinctly); and (5) At least 10 mm in size.

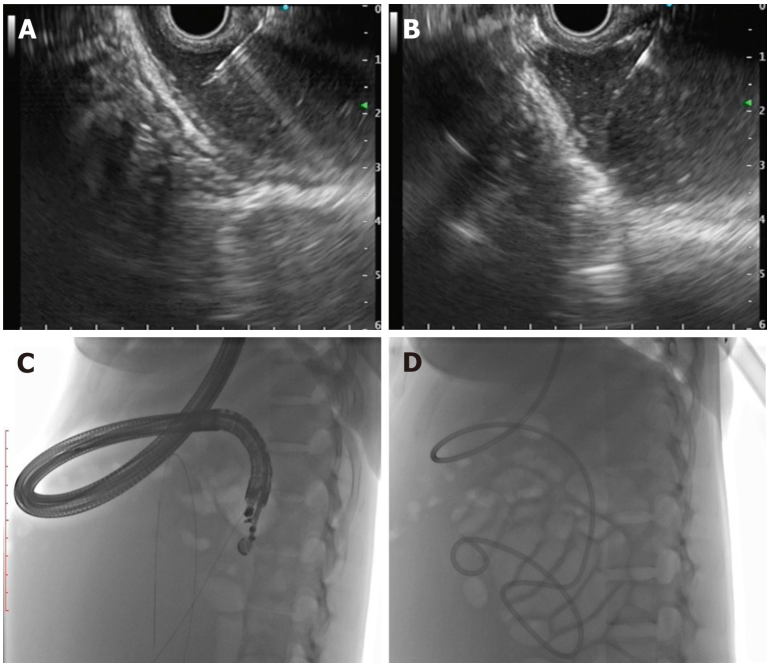

Figure 3 Endoscopic ultrasound-guided internal drainage of loculated ascites. A: Puncture of the loculated ascites with 19-G aspiration needle; B: Guidewire negotiated across as visualized on endoscopic ultrasound; C: Fluoroscopic view of guidewire coiled inside the loculated ascites; D: Naso-cystic drain placed inside the loculated ascites.

With the advent of EUS-RTE, the characterization of liver SOLs and their biopsies have become better (Figure 6). A study reported a hue histogram cutoff of 170 to discriminate between benign and malignant tumors (sensitivity 92.5%, accuracy 88.6%) [29]. In addition, the use of contrast agents in CE-EUS helps in differentiating primary tumors and metastasis[30]. CE-EUS has also been utilized for the assessment of treatment response in hepatocellular carcinoma (HCC) post-trans-arterial catheter embolization[31]. Hence, EUS with RTE, CE-EUS and CE-EUS-H might be a promising tool for diagnosing focal liver lesions and targeted intervention.

EUS-FNA of focal liver lesions

Several studies exist on the use of EUS-FNA/FNB (fine needle biopsy) for solid liver lesions with a complication rate of 0%-6% (Table 4). A recent systematic review by Ichim *et al*[42] showed the diagnostic yield of EUS-FNA to be 80%-100%.

Future trends

Studies have reported additional assessment of KRAS mutation in inconclusive cytological samples, which has resulted in an improved diagnostic yield from 89.3% to 96.4%[43]. Similarly, an animal study has evaluated the art of *in vivo* cytological observation using a high-resolution micro-endoscopy (HRME) system under EUS guidance[44] to decrease the number of needle-passes and subsequent adverse events. Recently, Minaga *et al*[45] have reported the additive role of CE-EUS-H in the detection of left lobe liver metastasis from pancreatic ductal adenocarcinoma. The diagnostic accuracy of CH-EUS was 98.4% compared to 90.6% with CECT.

EUS guided liver biopsy

Despite the advances in various non-invasive testing available to determine the degree of fibrosis, liver biopsy remains the gold standard method for accurate assessment in diagnosis and staging. As first described in 1883 by Dr. Paul Ehlrich, percutaneous liver biopsy (PC-LB) has evolved from a mere percussion method to an "image-guided" technique in the last ten years using ultrasound/CT imaging to accomplish it. However, despite image guidance, the risk of bleeding persists, occurring in up to 0.6% of cases, including other adverse events like pneumothorax and gallbladder puncture and even death in a few cases[46]. The transjugular technique of liver biopsy, introduced in 1973, can help reduce this risk, especially in patients with underlying coagulopathy. However, this method also carried added risks of local site hematoma, intraperitoneal bleeding, arrhythmia and carotid puncture[47].

Table 4 Studies on endoscopic ultrasound guided fine needle aspiration/fine needle biopsy of focal liver lesions

Ref.	Design	Patients	Diagnostic yield (%)	Needle passes (median)	Complications
EUS-FNA					
Nguyen *et al*[32]	Prospective	14	100	2	0
TenBerge *et al*[33]	Retrospective	26	88.6	-	3.8% (fever)
DeWitt *et al*[34]	Retrospective	77	91	3.4 (mean)	0
Hollerbach *et al*[35]	Prospective	33	94	1.4 ± 0.6	6.1% (self-limited bleeding)
McGrath *et al*[36]	Prospective	7	100	2	0
Singh *et al*[26]	Prospective	9	88.9	2	0
Singh *et al*[27]	Prospective	26	96	2.1	0
Crowe *et al*[37]	Retrospective	16	75	3 (minimum)	0
Prachayakul *et al*[38]	Retrospective	14	100		0
Oh *et al*[39]	Prospective	47	90.5	3	0
Ichim *et al*[25]	Prospective	48	98	2	0
EUS-FNB					
Lee *et al*[40]	Prospective	21	90.5	2	0
Chon *et al*[41]	Retrospective	58	89.7	2	1.7% (bleed)

EUS: Endoscopic ultrasound; FNA: Fine needle aspiration; FNB: Fine needle biopsy.

EUS guided liver biopsy (EUS-LB) initiated as early as 2007 is currently emerging as a cost-effective, safe and well-tolerated procedure and helps in more representative sampling. The American Association for the Study of Liver Diseases recommends a tissue length of at least 2-3 cm with ≥ 11 or more complete portal tracts (CPTs) for determining the adequacy of liver biopsy samples[48]. The mean tissue length and CPTs for EUS-LB, PC-LB and TJLB, as shown in various studies is 36.9, 9 and 17.7 mm, and 7.7, 13.5 and 6.8 mm, respectively[49,50]. This can be achieved with a regular 19 G EUS-FNA needle (71). Similarly, a meta-analysis on EUS-LB revealed that pooled successful histological diagnosis was achieved in 93.9% of cases. Adverse event rates with EUS-LB, PC-LC and TJLB were 2.3%, 0.09%-3.1% and 0.56%-6.5%, respectively [48,51,52]. A recent meta-analysis between the three techniques revealed that EUS-LB was comparable to PC-LB in terms of CPT, but tissue length was better with the former with no complication rates[53].

EUS-LB has been used in the setting where patients undergo other endoscopic procedures such as screening of the biliary tree, assessment of surrounding structures and lymph nodes and variceal screening in those not affected with ascites and obesity [50], thereby saving time and resources. Furthermore, EUS-LB is theoretically less painful as it does not require skin puncture, eliminates the need for breath-hold and allows visualization and avoidance of blood vessels even 1 mm in size and is suitable for anxious patients by using adequate sedation (Figure 7). Moreover, bilobar biopsy can be achieved, reducing sampling error and helping in better assessment of disease activity and fibrosis[54].

Technique: The technique of EUS-LB is described in Table 5.

Future trends: In attempts to acquire better quality and quantity of specimens, various studies have been published on different needles and methods of executing a EUS-LB procedure. A recent RCT comparing a 19 G FNB needle (fork-tip) *vs* 19 G standard FNA needle yielded better results with the former (pre-processing length 2.09 cm *vs* 1.47 cm and more CPTs)[55]. In contrast, a recent meta-analysis showed the superiority of FNA needles over core biopsy needles in terms of better tissue acquisition[51]. Thus, 19 G FNA needle may be used for EUS-LB procedures except for the cases where immunohistochemistry and architecture characterization are warranted, in whom core biopsy needle may be used.

Mok *et al*[56] showed that the "wet heparin" suction technique had greater tissue yield compared to "dry suction" (aggregate specimen length 49.2 mm *vs* 23.9 mm;

Table 5 Technique of endoscopic ultrasound guided liver biopsy[50,51]

Pre-biopsy: The following workup is needed in all cases of liver biopsy

(1) Coagulation work up including platelet count, PT/INR and BT/CT; (2) Prior to the biopsy, the medications should be stopped as follows: anti-platelet medications 7 d, warfarin 5 d, heparin and related products discontinued 12-24 h prior to biopsy; and (3) Use of conscious sedation such as midazolam and nalbuphine or propofol as per operator's preference or patient comfort

Procedural details of EUS-LB

(1) A linear array echoendoscope (Olympus GF-UCT180, Center Valley, United States) is generally used for the procedure; (2) Prior to the procedure, Doppler imaging is done to ensure that no vascular structures are present along the expected trajectory of the needle; (3) The EUS-LB can be performed using a 19 G EUS-FNA/FNB needle; (4) The left lobe is identified first, as that liver parenchyma which is a few centimeters below the gastro-esophageal junction with the scope torqued clockwise. The right lobe if needed to be biopsied, is accessed from the duodenal bulb. Two site biopsy can be undertaken at the discretion of the endosonographer; (5) A preferably long vessel free trajectory allowing free passage of the needle to a depth of at least 3 cm or more is usually selected; (6) For wet heparin suction, the stylet is removed and the needle is primed with a heparin flush and the suction syringe is reattached to the needle hub; (7) The needle is then introduced into the echoendoscope channel; (8) Once liver parenchymal penetration is achieved with the needle (around 1-2 cm), full suction is applied with the 20 mL vacuum syringe with fluid column; (9) One pass consists of a total of 4-5 to-and-fro needle motions using the fanning technique under direct EUS guided visualization of the tip of the needle. Two such passes are usually taken (maximum 10 actuations); and (10) The specimen is pushed from the needle directly into the formalin solution using the stylet or saline flush

Post-liver biopsy: The following instructions are to be followed in all cases post liver biopsy

(1) The patient post biopsy, irrespective of the type of procedure, is transferred to the post procedure recovery room and monitored as per the AASLD protocol[69]; (2) The minimum observation period is 2-4 h; (3) Post-procedure pain and need for analgesics to be noted and provided; and (4) Patient is asked to report adverse events at specific time intervals (as per institute policy)

EUS: Endoscopic ultrasound; PT Prothrombin time; INR International normalized ratio; BT: Bleeding time; CT: Clotting time; EUS-LB: Endoscopic ultrasound guided liver biopsy; FNA: Fine needle aspiration; FNB: Fine needle biopsy; AASLD: American Association for the Study of Liver Diseases.

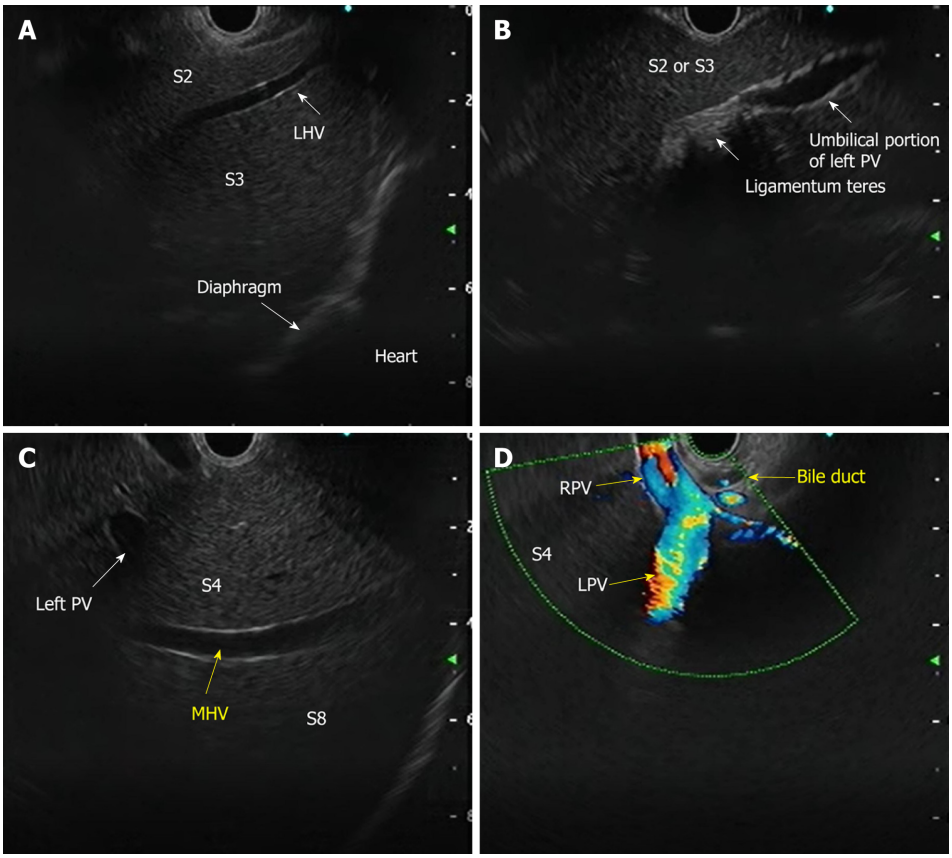

Figure 4 Endoscopic ultrasound anatomy of liver segments. A: Anatomy of the left lobe with S2 and S3 segments; B: Ligamentum teres with umbilical portion of the left portal vein; C: Middle hepatic vein with segments of the liver; D: Anatomy of the bifurcation of portal vein from the duodenal bulb. PV: Portal vein; MHV: Middle hepatic vein; LHV: Left hepatic vein; RPV: Right portal vein; LPV: Left portal vein.

mean CPT count 7 *vs* 4). Thus, the combination of wet-heparinized suction and a 19-G second-generation (FNA/FNB) needle might help achieve better specimens with minimal fragmentation.

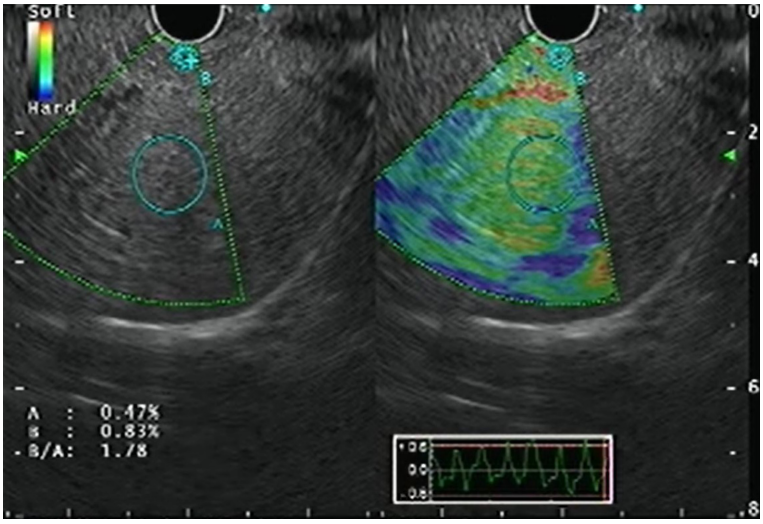

Figure 5 Endoscopic ultrasound elastography of the liver parenchyma.

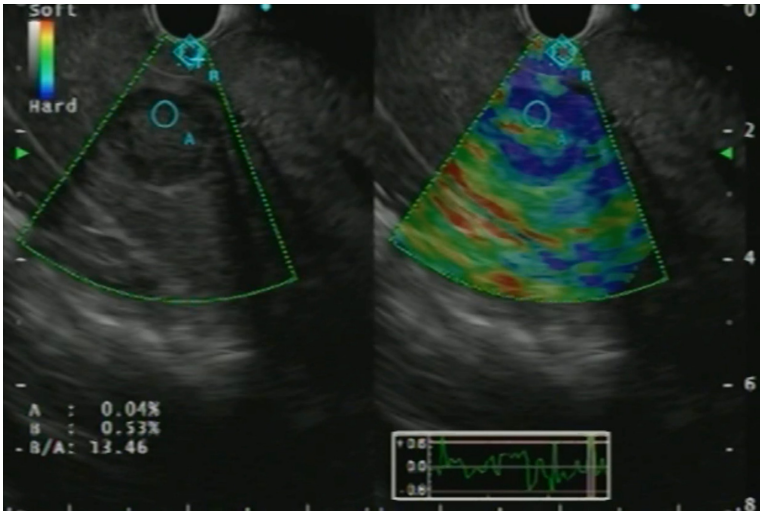

Figure 6 Endoscopic ultrasound elastography of a focal liver lesion with strain ratio calculation.

The various studies using EUS-LB (FNA/FNB) in patients with chronic liver disease are highlighted in Table 6. The average technical success and diagnostic yield for EUS-FNA and EUS-FNB-guided liver biopsy are 100% and 89.8%, respectively, with a complication rate of 3.3%, consisting entirely of minor events[70]. In addition, studies reporting the use of EUS-LB in patients with NAFLD (overall technical success rate 100%, yield 96.8% with 7.7% complication rate) are reported in Supplementary Table 1.

EUS guided therapeutic management of liver cysts, liver abscess and biloma

Symptomatic liver cysts, abscesses and bilomas may require drainage. Traditionally, these were approached through surgical or interventional radiology using percutaneous catheter drainage (PCD). Recently, EUS guidance has been used to drain simple intrahepatic cysts of varied etiologies, liver abscesses and bilomas. EUS guided drainage may be superior to PCD as it enables a one-step approach, leading to internal drainage and thus avoiding the complications of catheter dislodgement, pericatheter leak, multiple interventions and movement restrictions.

EUS guided treatment of hepatic cysts: The most frequent liver cysts encountered for drainage *via* EUS include simple hepatic cysts and intrahepatic pancreatic pseudocysts. Those located in the left lobe of the liver or the caudate lobe can be drained *via* EUS guidance. PCD would be preferred for right lobe cysts as it is difficult to access the right lobe in the duodenal bulb with an unstable scope position. Therapies offered by EUS include fine-needle aspiration, ethanol lavage and

Table 6 Studies on endoscopic ultrasound guided fine needle aspiration guided and endoscopic ultrasound guided fine needle biopsy guided liver biopsy in patients with chronic liver disease

Ref.	Design of the study	Patients	Technical success (%)	Diagnostic yield (%)	Specimen length (median, range) (mm)	CPT (median, range)	Needle used for EUS-LB	Needle passes (median)	Complications, *n* (%)
EUS-FNA guided liver biopsy									
Pineda *et al* [57]	Retrospective	110	100	98	38 (24-81)	14 (9-27)	19 G	-	0
Shuja *et al*[58]	Retrospective	69	100	100	45.8 (mean)	10.84 (mean)	19 G	3	0
Stavropoulos *et al*[50]	Prospective case series	22	100	91	36.9 (2-184.6)	9 (1-73)	19 G	2 (1-3)	0
Diehl *et al*[59]	Prospective non randomized	110	100	98	38 (0-203)	14 (0-68)	19 G	1.5 (1-2)	1 (0.9) (mild bleeding)
Gor *et al*[60]	Retrospective case series	10	100	100	13 (6-23)	8 (6-15)	19 G	-	0
EUS-FNB guided liver biopsy									
Shah *et al*[61]	Retrospective	24	100	96	65.6 (17-167.4)	32.5 (5-85)	19 G (SharkCore)	2 (1-3)	2 (8.3)
Nieto *et al*[62]	Retrospective	165	100	100	60 (43-80)	18 (13-24)	19 G (SharkCore)	1	3 (1.8)
Mathew[63]	Case report	2	100	100	-	-	19 G (QuickCore)	-	0
Ching *et al* [55]	Prospective (RCT)	20; 20	100; 100	100; 100	114 (mean); 153.2 (mean)	16.5 (6-38); 38 (0-81)	19 G (FNA); 19 G (Acquire)	--	8 (40); 7 (35)
Mok *et al*[56]	Prospective (RCT)	40; 40	100; 100	88; 68	-; -	-; -	19 G (FNA); 22 G (SharkCore)	-; -	0; 1 (2.5)
Patel *et al*[64]	Retrospective	30; 50; 28; 27	100; 100; 100; 100	66.7; 46; 82.1; 81.5	1.8 (mean); 4.7 (mean); 1.9 (mean); 8.4 (mean)	6.9 (mean); 3 (mean); 7.3 (mean); 16.9 (mean)	Acquire 22 G; QuickCore 19 G; ProCore 19 G; Expect 19 G	-; -; -; -	-; -; -; -
Gleeson *et al* [65]	Retrospective	9	100	100	13 (8-28)	7 (5-8)	19 G (QuickCore)	2 (1-3)	0
DeWitt *et al* [66]	Prospective case series	21	100	90.5	9 (1-23)	2 (0-10)	19 G (QuickCore)	3 (1-4)	0
Nakai *et al* [67]	Case report	1	100	100	15	8	ProCore 19 G		0
Sey *et al*[68]	Prospective cross sectional study	45; 30	100; 100	73.3; 96.7	9 (0-25); 20 (5-60)	2 (0-15); 5 (0-24)	QuickCore 19 G; ProCore 19 G	3; 2	2 (4.4); 0
Hasan *et al* [69]	Prospective (RCT)	40	100	100	55 (44.5-68)	42 (28.5-53)	Acquire 22 G	-	6 (15)

CPT: Complete portal triad; EUS-LB: Endoscopic ultrasound guided liver biopsy; FNA: Fine needle aspiration; FNB: Fine needle biopsy; RCT: Randomized controlled trial; G: Gauge.

transmural stent placement.

In a retrospective study by Lee *et al*[71], 19 cases of hepatic cysts were treated by PCD and EUS guided ethanol lavage and reported a 97.5% reduction in cyst volume at 11.5 mo of follow-up in the PCD group and a 100% reduction at 15 mo in the EUS arm. The studies on EUS guided treatment of hepatic cysts are outlined in Supplementary Table 2.

EUS guided drainage of liver abscess: Traditionally, pyogenic and amoebic liver abscesses have been drained by PCD with a high technical success rate. However, EUS guided drainage of liver abscesses is a promising new approach, especially for difficult-to-reach locations. Additionally, the advantage of internal drainage with a

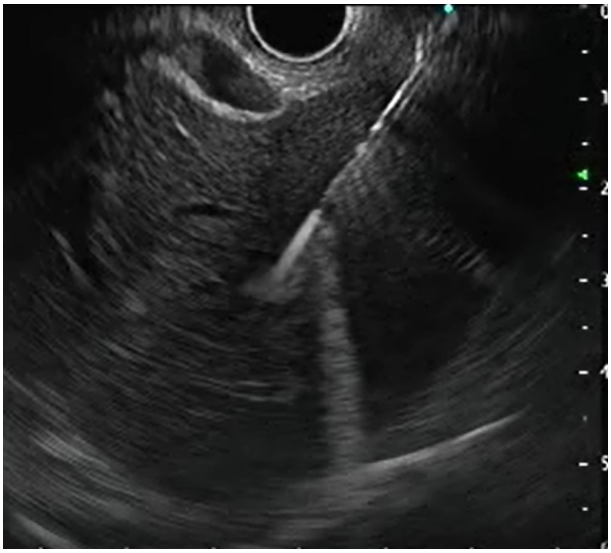

Figure 7 Endoscopic ultrasound-guided liver biopsy.

single-step procedure and easy access from the stomach makes transmural drainage of left and caudate lobe abscess convenient.

The technique was first described by Seewald *et al*[72], who reported complete resolution 4 weeks post-procedure. Literature on EUS guided drainage is limited to retrospective case series only in which the majority have been drained with double pigtail plastic stents[73-75]. Recently, data are emerging on the use of fully covered self-expandable metal stents (SEMS)[76] for the same. Ogura *et al*[77] reported retrospective comparative data on EUS *vs* PCD guided abscess drainage wherein EUS guided abscess drainage (EUS-AD) cases showed greater clinical success (100% *vs* 89%) with shorter hospital stay (21 d *vs* 41 d). Studies on EUS-AD are listed in Supplementary Table 3.

EUS guided drainage of biloma: Biloma is defined as a well-demarcated collection of bile outside the biliary tree, which can be extrahepatic or intrahepatic, encapsulated or without a capsule[78]. It is most frequently caused by iatrogenic biliary tree injury during cholecystectomy. It has been traditionally managed with PCD or surgery. However, large bilomas in opposition to the gastric wall can be taken up for transmural drainage (Figure 8). Similar to EUS-AD, earlier plastic stents were utilized for the same, but now SEMS has been in vogue for biloma drainage with excellent results. Post drainage, such patients should be evaluated to determine the need for endoscopic retrograde cholangiopancreatography, or sphincterotomy with/out biliary stenting or surgery[79]. Studies on EUS guided drainage of bilomas are described in Supplementary Table 4.

Despite it being a point of contention, EUS guided drainage of intrahepatic lesions (cysts, abscesses and bilomas) is an upcoming promising technique and may be considered in conditions where PCD is not amenable or has failed.

EUS guided treatment of liver tumors

A thrilling offshoot of EUS guided therapeutic interventions has been EUS guided local treatment of tumor lesions (both pancreatic and hepatic tumors)[80]. EUS-guided tumor management is a new experimental application that has shown promise in reaching difficult lesions (left lobe, caudate lobe), provided a rescue option in refractory cases, and has potential to improve quality of life by minimizing systemic side effects[81,82]. This procedure has been extensively studied in cases of pancreatic neoplasm, but its role in hepatic tumors (primary or metastatic) is still in its infancy.

Various techniques of EUS guided liver tumor management have been described.

Fine needle injection therapy: Ethanol ablation

Percutaneous injection of ablative injections is most commonly used worldwide to manage HCC, although EUS guided fine needle injection can be performed using acetic acid or ethanol (pure alcohol 95%-99%)[83]. Its advantage is that it enables real-time imaging during delivery of ethanol to the tumorous lesion and thus can help

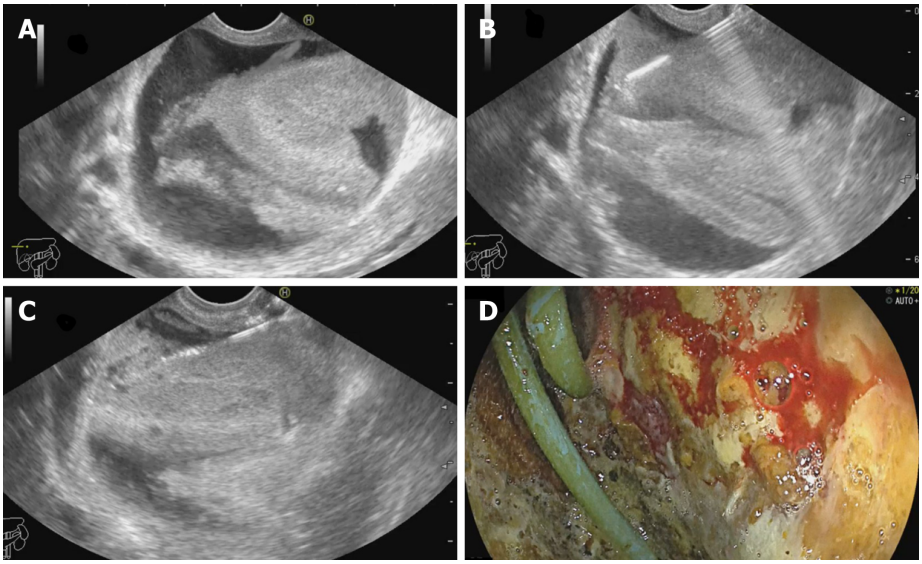

Figure 8 Endoscopic ultrasound-guided drainage of biloma. A: Post-operative biloma noted on endoscopic ultrasound (EUS) with internal echoes; B: EUS-guided puncture of the biloma; C: Guidewire negotiated into the collection followed by placement of naso-cystic drain; D: Endoscopic view of the cavity entered with catheter noted *in situ*.

avoid collateral damage.

Initial case reports using 22 G and 25 G FNA needles have been reported with excellent technical success and complete resolution of HCC[84-87]. For example, Nakaji *et al*[87] reported a high-resolution rate at 31 mo in 12 cases of caudate lobe HCC, whereas Jiang *et al*[88] only showed 30% complete resolution at 12 mo. This technique has also been evaluated for the treatment of hepatic metastasis from pancreatic adenocarcinoma[89].

Thermal ablative therapy

Radiofrequency ablation: Radiofrequency ablation (RFA) uses a high-frequency alternating current (375 kHz to 500 kHz) and is minimally invasive with good tolerability[90]. It can be delivered percutaneously, intraoperatively, *via* an endoluminal approach or endosonographic (transmural) route. Emerging data on the latter have resulted in its application in cases wherein the percutaneous approach fails. Obesity, tumor nodules in the left lobe or caudate lobe, deep-seated and sub-capsular/sub-diaphragmatic lesions that carry an inherent risk of hemothorax or pleural effusion are some of the conditions where it has been applied[81,90]. A specifically designed needle tip electrode for performing EUS-RFA (EUSRA RFA Electrode, STARmed, Koyang, Korea) with a designed internally cooled needle electrode is the most extensively studied. Only a few case reports exist on EUS-RFA using EUSRA in HCC[91-93]. Also, hybrid models combining EUS-RFA with cryoablation in the bovine liver have demonstrated better efficacy of the combination treatment[94].

Laser ablation by neodymium:yttrium-aluminum-garnet: Neodymium:yttrium-aluminum-garnet (Nd-YAG) is a type of LITT (laser interstitial thermotherapy) wherein laser waves are introduced through the EUS needle directly into the tumor tissue leading to cell apoptosis and eventual necrosis. Only two human studies have been published so far for the treatment of HCC. Di Matteo *et al*[95] reported complete HCC resolution in 2 mo in a case of previously failed caudate lobe HCC. Similarly, Jiang *et al*[96] reported resolution at 3 mo with an encouraging safety profile.

Cryotherapy ablation: Cryotherapy ablation (CYA) destroys tissue through multiple freezing-thawing cycles leading to osmotic dehydration and injury to the intracellular structures and cell death[90]. No human study exists for its use in liver lesions. However, a single animal study showed the efficacy of a hybrid EUS-RFA and cryoderm device in a porcine model[97].

High-intensity focused ultrasound: This is a non-invasive technique that causes tissue necrosis *via* heat generation and acoustic cavitation by the formation and collapse of bubbles produced by intense USG waves[90]. Its use in EUS has only been tested in

animal models[98,99], showing complete necrosis of the lesions with no immediate side effects.

Brachytherapy

This treatment modality has been used for various cancers with the advantage of less toxicity to surrounding tissues over external beam radiotherapy[81,90]. For example, EUS guided brachytherapy with permanent seed placement of Iodine (I125) or palladium (Pd103) has been performed for head-neck, esophageal, and pancreatic cancer[100-102]. In addition, Jiang *et al*[88] have used EUS guided I125 seed implantation for liver tumors with high efficacy and safety.

Studies on EUS guided liver tumor treatments are outlined in Table 7.

EUS GUIDED VASCULAR INTERVENTIONS

The presence of real-time, high-resolution sonographic imaging with Doppler, along with the relative proximity of the gastrointestinal tract to the major blood vessels in the abdomen and the mediastinum, has led to a growing interest to explore the role of EUS in the field of vascular interventions. EUS may be preferred over the percutaneous route, especially in obesity, ascites and overlying distended bowel[104].

Esophageal and gastric varices: diagnosis and management

EUS guided vascular intervention in patients with PHTN has been well established in managing varices (esophageal, gastric, duodenal, and ectopic).

Management of esophageal varices: Endoscopic variceal band ligation (EVL) has been the standard treatment of esophageal varices (EV) (both primary and secondary prophylaxis). However, re-bleeding rates of 15%-65% have been reported due to the failure to obliterate perforating veins and collaterals feeding the varices[105]. Lahoti *et al*[106] described the first report of EUS guided sclerotherapy in 5 cases, wherein sclerosant (sodium morrhuate) was injected under EUS guidance (2-4 mL per injection site) directed at the perforating vessels as determined by color Doppler with complete eradication of the varices. An RCT comparing EUS *vs* direct sclerotherapy revealed no difference in both arms[107]. Thus, although EUS carries a theoretical advantage for identifying the feeders, more studies are needed to assess its practical clinical benefit.

Management of gastric varices: In patients with PHTN, gastric varices (GV) are present in up to 20% with a 50%-65% re-bleeding rate[108]. Endoscopic injection of CYA glue for GVs has been the treatment of choice since its first description in 1986 but is still prone to a re-bleeding rate of 40%[109]. In the current era of EUS guided vascular interventions, management of GVs by EUS has many conceptual advantages, both diagnostic and therapeutic such as[110,111]: (1) A higher detection rate (6 times) over conventional endoscopy; (2) Greater success in differentiating varices from thick gastric folds; (3) Confirmation of the cessation of blood flow post-treatment; (4) Real-time varix visualization and hence accurate delivery of hemostatic agent to the varix; and (5) Targeted treatment for feeder vessels.

The first description of EUS guided CYA injection in GVs was given by Romero-Castro *et al*[111] and Lee *et al*[112]. To reduce the chances of embolization with CYA, stainless steel coils alone or in combination with CYA glue have been introduced. The advantage is three-pronged: additive hemostasis and varix obliteration, reducing the volume of glue needed and acting as a scaffold to retain the glue within the varix, thereby decreasing embolization. Various studies, including RCTs, have favored coil over glue. Bhat *et al*[113] reported a complete obliteration in 93% with only 3% re-bleeding rates using coils and glue combination. Similarly, two RCTs and a meta-analysis have reported the combination therapy of coil with glue to be superior to either agent alone[114-116]. Newer treatments of utilizing coils with gelatin sponge and sclerotherapy or isolated thrombin injection have been reported in various case series and have shown good results[117-119].

The technical steps of the EUS guided coil and glue placement for the obliteration of GV are outlined in Table 8 and Figure 9.

Use of EUS in the prediction of re-bleeding from EV/GV: EUS with Doppler has a higher sensitivity for detecting esophageal and GV than upper GI endoscopy and can also be used to predict re-bleeding. Certain parameters can help guide us in this direction[120,121]: (1) EUS can help in demonstrating collaterals or feeders, a strong

Table 7 Studies in humans demonstrating the role of endoscopic ultrasound guided therapies for liver lesions

EUS guided treatment	Study design	Patients	Location of the lesion	Technical success (%)	Response to therapy	Complications
Ethanol ablation in HCC						
Nakaji *et al*[84]	Case report	1	Segment 8	100	Complete	0
Lisotti *et al*[85]	Case report	1	Segment 2	100	Complete	0
Nakaji *et al*[86]	Case report	1	Segment 3	100	Complete	0
Nakaji *et al*[87]	Retrospective	12	Caudate lobe	100	Complete	2 (16.7%)
Jiang *et al*[88]	RCT	10	Left lobe	92	Partial (30%)	0
Alcohol ablation in liver metastasis						
Barclay *et al*[89]	Case report	1	Left lobe	100	Complete	Self-limited sub-capsular hematoma
Hu *et al*[103]	Case report	1	Left lobe	100	Complete	Low grade fever
RFA (radiofrequency ablation) in HCC						
Armellini *et al*[91]	Case report	1	Left lobe	100	Complete	None
Attili *et al*[92]	Case report	1	Segment 3	100	Complete	None
de Nucci *et al*[93]	Case report	1	Segment 2-3-4b	100	70% reduction	None
Ablation by Nd-YAG						
Di Matteo *et al*[95]	Case report	1	Caudate lobe	100	Complete	0
Jiang *et al*[96]	Prospective	10	Left lobe	100	Complete	0
Brachytherapy (Iodine-125)						
Jiang *et al*[88]	RCT	13	Left lobe	92	Near complete	0

EUS: Endoscopic ultrasound; HCC: Hepatocellular carcinoma; RCT: Randomized controlled trial; Nd-YAG: Neodymium:yttium-aluminum-garnet; RFA: Radiofrequency ablation.

Table 8 Steps of endoscopic ultrasound guided coil and glue placement for gastric varices obliteration

Pre-procedure requirements

(1) All procedures are done under the cover of pre/peri-procedural antibiotics; (2) Patient is usually fasted for 4-6 h before the procedure; and (3) Adequate resuscitation of the patient, in case of active bleeding is ensured, prior to the procedure

Technical aspects

(1) The echoendoscope is usually positioned either in the distal esophagus or the gastric fundus; (2) Water is filled intra-luminally in the fundus. This enables a good acoustic coupling for better visualization of the gastric varices. Adequate examination of the fundus, the intramural varices and the feeder vessels is carried out; (3) The approach can be trans-esophageal or transgastric, wherein the trans-esophageal route is given preference; (4) EUS-guided coil and glue embolization is usually performed using a 22 G/19 G (gauge) FNA needle. The size of the coil is determined by the short axis of the diameter of the varix; (5) After puncture of the varix, blood is aspirated to confirm the location. This is followed by flushing of the needle with saline; (6) The coils are then deployed into the varix using the stylet as a pusher. Once the coils are deployed, flushing of the needle is done with normal saline; (7) After coil deployment, 1-2 mL of cyanoacrylate glue is injected over 30-45 s followed by rapid flushing with saline; and (8) Once, the varix is obliterated, visualized by absence of flow on color Doppler, the sheath of the needle is advanced beyond the endoscope tip for 2-3 cm before withdrawing the scope. This avoids contact of glue with the endoscope tip. The sample aspirated is sent for routine cytological assessment as well as for any additional tests that might be needed

Post procedure

(1) The patients are kept under observation for 12 h; (2) Repeat EUS can be done after 2 d to look for residual varices; and (3) Follow-up EUS can be performed at 1- and 3-mo intervals

EUS: Endoscopic ultrasound; FNA: Fine needle aspiration; G: Gauge.

indicator to a future occurrence of a re-bleed; (2) Hematocystic spots on EVs identified as saccular aneurysms on EUS is associated with a high risk of variceal rupture; (3) Digital image analysis on EUS can help to determine the cross-sectional area of EVs in the distal esophagus and a cutoff of 0.45 cm^2 has a sensitivity of 83% for future re-

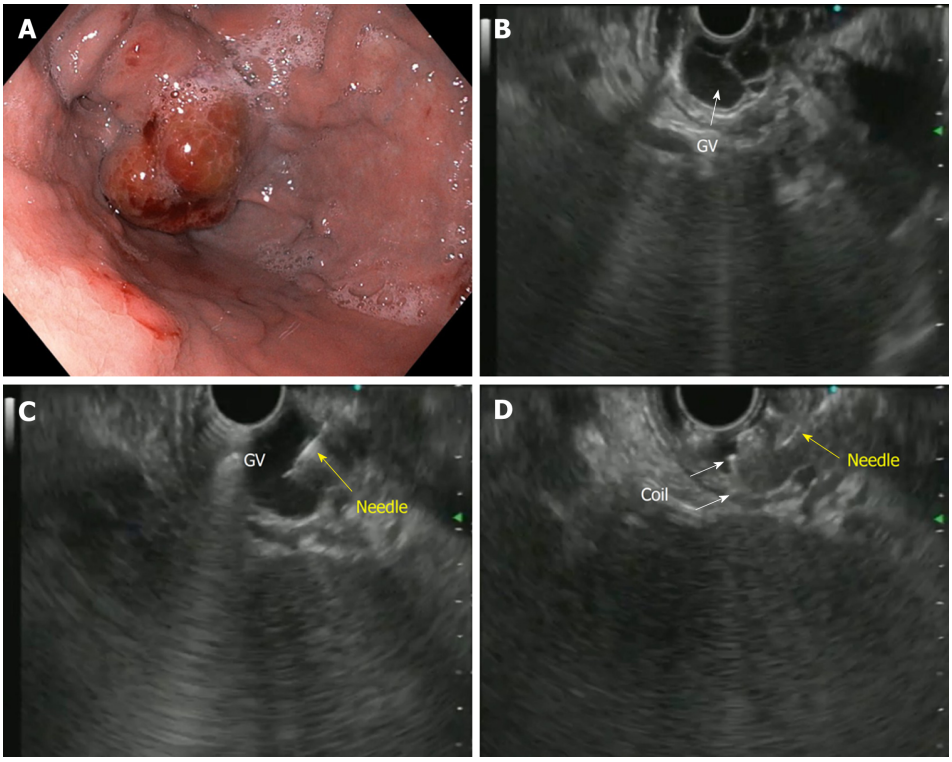

Figure 9 Endoscopic ultrasound-guided coil embolization of fundal varix. A: Endoscopic view of the fundal varix; B: Endoscopic ultrasound (EUS) view of the fundal varix; C: EUS guided puncture of the varix with a 22-G needle; D: Coil deployment inside the varix. GV: Gastric varices.

bleeding; and (4) Para-esophageal diameter after EVL is a better recurrence predictor (cutoff 4 mm has a 70.6% sensitivity).

Thus, there is a huge prospect for using EUS in PHTN, namely in the evaluation of vascular changes of the digestive wall, hemodynamic assessment by measurement of PV pressure gradient, management of variceal bleeding and re-bleeding prediction and currently liquid biopsy *via* PV sampling. Nonetheless, despite the diversity of possible uses, more data on efficacy and safety are warranted.

EUS guided PV access

The PV can be accessed from both the stomach and duodenum and is in very close juxtaposition with the tip of the echoendoscope. The most frequent location to target is the intrahepatic PV through the hepatic parenchyma. The other less commonly used technique is the extrahepatic PV *via* the duodenum[122,123].

Technique of the procedure: After confirming the vascular structure with color Doppler and pulse-wave verification, PV puncture is done *via* the EUS-FNA needle. Studies have shown that 25 G needle causes the least trauma. The trans-gastric, trans-hepatic approach is safer than the trans-duodenal approach. CO2 is better than using iodine as a contrast (allows better PV visualization and easier intravascular administration through the small-caliber FNA needle). After PV puncture, on withdrawal of the needle, the track is monitored with color Doppler to check for bleeding. In cases of blood flow being identified, the needle is kept in place until the flow has stopped[122,123].

Animal studies: The first case of PV access was reported in 2004 by Lai *et al*[124], wherein a EUS guided trans-duodenal access to extrahepatic PV was adopted with a 22 G FNA needle in 21 swine models, proving the technical feasibility of the procedure. Thereafter, PV angiography was reported for the first time in 2007 by Magno *et al*[125], wherein autopsies revealed no injuries with a 25 G needle and a hematoma with 19 G needle. Subsequently, Giday *et al*[123,126] reported trans-hepatic access to the PV with a 25 G needle.

EUS guided portal pressure gradient measurement

Measurement of PHTN is useful in determining the stage, progression, prognosis and complications of cirrhosis. Currently, the standard practice of measuring the portal

pressure gradient (PPG) is the percutaneous route. However, both direct PV access and hepatic venous pressure gradient (HVPG) measurement are invasive procedures and have high complication rates. Moreover, HVPG correlated poorly in presinusoidal PHTN cases. Therefore, EUS guided PPG can be performed to overcome these difficulties. Moreover, additional analyses such as assessment of varices and liver biopsy can be carried out in the same sitting. The technique of PPG measurement and the studies (human and animal models) on the same are shown in Supplementary Tables 5 and 6.

EUS guided TIPS

TIPS has an established role in managing PHTN-related complications like variceal bleeding (pre-emptive or rescue) and refractory ascites. EUS-guided TIPS creation in a live porcine model (8 cases) was first described by Buscaglia *et al*[127], wherein the hepatic vein (HV) and PV were sequentially punctured, and a metal stent was inserted with the distal end in the PV and proximal end in the HV. In addition, Binmoeller and Shah[128], and Schulman *et al*[129] have both reported using a lumen apposing metal stent (LAMS) in porcine models for the same purpose.

EUS guided PV sampling

"Liquid biopsy" for hepatobiliary malignancies is gaining momentum in view of the PV harboring circulating tumor cells (CTCs) from the primary tumor. These CTCs are the forerunners of future metastasis of solid organ cancers and help predict the development of liver metastasis[130]. They have been inconsistently found in the peripheral blood due to hepatic sequestration. They reflect tumor signature, help in prognostic stratification, and potentially form organoids for future tumor study.

Catenacci *et al*[131] reported the first human study of PV sampling wherein a 19 G FNA needle was used to sample the PV as four 7.5 mL aliquots of blood. CTCs were detected in 100% cases from the PV *vs* 4 (22.2%) cases from peripheral blood. Liu *et al*[132] reported similar findings in cases of advanced pancreatic cancer (100% detection of CTCs in PV *vs* 54% in peripheral blood). Besides these, further studies are needed to establish the clinical utility of EUS guided liquid biopsies.

EUS guided FNA of PV thrombosis

The presence of malignant PV thrombosis (PVT) usually portends a poor prognosis. Therefore, differentiating bland and malignant thrombus needs FNA confirmation. Various case reports have suggested the use of EUS guided FNA of the PVT by overcoming the complications encountered *via* the percutaneous route[133-135] with excellent results.

EUS guided PV injection chemotherapy

Both systemic palliative chemotherapy and transarterial microbead injection into the hepatic artery for diffuse liver metastasis are fraught with complications. However, Faigel *et al*[136] reported the feasibility of EUS guided PV injection chemotherapy in 24 porcine models using drug-eluting microbeads and nanoparticles. In comparison with systemic injection, systemic levels were halved, but the hepatic concentration of drugs was doubled. Human studies are warranted for the same.

EUS guided PV embolization

Preoperative PV embolization before liver resection in hepatobiliary malignancies induces affected lobe atrophy and ultimately hypertrophy in the functional liver[137]. However, preliminary studies in the animal model by Matthes *et al*[138] and Park *et al*[139] using EUS guided PV embolization using ethylene-vinyl alcohol copolymer and coil with CYA glue embolization, respectively, reported high success rates.

EUS guided PV stent placement

EUS directed PV access has opened up avenues for stent placement *via* this route in PV occlusion or thrombosis. Park *et al*[140] reported 100% technical success (all uncovered stents) in 6 swine models.

FUTURE ADVANCES

Photodynamic therapy

Photodynamic therapy (PDT) is a commonly used modality for treating malignant

biliary obstruction, requiring pretreatment with a photosensitizer followed by exposure to selective tissue wavelength of light-generating singlet oxygen species (tissue necrosis from 6-40 mm depth)[141]. Preliminary animal studies exist on the use of EUS guided PDT on the porcine pancreas[141,142] and pancreaticobiliary malignancies (with lesions in the caudate lobe)[143].

EUS guided fiducial marker placement

Stereotactic body radiation therapy demands high targeting accuracy to minimize toxicity to surrounding organs. Placement of fiducial markers can help localize and track the target and can be placed *via* a percutaneous or surgical approach. EUS guided fiducial marker placement has come into the forefront for targeting even deeper abdominal lesions not amenable *via* standard means[144,145]. However, no studies exist on its use in liver malignancies.

Artificial intelligence

Artificial intelligence (AI) is a prediction technique using mathematical algorithms to create automated learning and recognize patterns in the fed data. Artificial neural network (ANN) and deep learning (DL) are powerful machine-learning-based tools used to provide high yield predictions and are being used more and more in the medical field to aid in diagnosis. Just like its widespread use in the field of endoscopic diagnosis of polyps and other lesions, AI has also found its place in the arena of diagnostic EUS. Studies have used ANN for the interpretation of EUS-elastography and CE-EUS[146]. However, to date, only two studies have used DL for EUS image analysis. With the availability of additional studies, AI can add to the diagnostic armamentarium of EUS and lead to much better accuracy.

CONCLUSION

Hepatologists have always turned to radiologists for imaging and intervention of various liver-related conditions. However, with the expansion of this intersection of endoscopy in EUS and hepatology, the field of "Endo-hepatology" may soon evolve into a sub-specialty with hepatologists trained in interventional EUS. Starting from EUS-guided liver biopsy to PV interventions, the merger of EUS and hepatology seems to show invigorating scope in the future. However, more studies are needed to establish the safety and efficacy of these newer modalities in regular mainstream practice.

REFERENCES

1 **European Association for the Study of the Liver**. EASL Clinical Practice Guidelines for the management of patients with decompensated cirrhosis. *J Hepatol* 2018; **69**: 406-460 [PMID: 29653741 DOI: 10.1016/j.jhep.2018.03.024]

2 **Nguyen PT**, Chang KJ. EUS in the detection of ascites and EUS-guided paracentesis. *Gastrointest Endosc* 2001; **54**: 336-339 [PMID: 11522974 DOI: 10.1067/mge.2001.117544]

3 **Varadarajulu S**, Drelichman ER. EUS-guided therapeutic paracentesis. *Gastrointest Endosc* 2008; **67**: 758-759 [PMID: 18178210 DOI: 10.1016/j.gie.2007.08.025]

4 **Lee YT**, Ng EK, Hung LC, Chung SC, Ching JY, Chan WY, Chu WC, Sung JJ. Accuracy of endoscopic ultrasonography in diagnosing ascites and predicting peritoneal metastases in gastric cancer patients. *Gut* 2005; **54**: 1541-1545 [PMID: 15955787 DOI: 10.1136/gut.2004.055772]

5 **DeWitt J**, LeBlanc J, McHenry L, McGreevy K, Sherman S. Endoscopic ultrasound-guided fine-needle aspiration of ascites. *Clin Gastroenterol Hepatol* 2007; **5**: 609-615 [PMID: 17336593 DOI: 10.1016/j.cgh.2006.11.021]

6 **Chin MA**. EUS-guided paracentesis and ascitic fluid analysis. *Endosc Ultrasound* 2018; **7**: 223-227 [PMID: 30117483 DOI: 10.4103/eus.eus_31_18]

7 **Levy MJ**, Abu Dayyeh BK, Fujii LL, Clayton AC, Reynolds JP, Lopes TL, Rao AS, Clain JE, Gleeson FC, Iyer PG, Kendrick ML, Rajan E, Topazian MD, Wang KK, Wiersema MJ, Chari ST. Detection of peritoneal carcinomatosis by EUS fine-needle aspiration: impact on staging and resectability (with videos). *Gastrointest Endosc* 2015; **81**: 1215-1224 [PMID: 25660979 DOI: 10.1016/j.gie.2014.10.028]

8 **Que Y**, Wang X, Tao C, Zhang Y, Wan W, Chen B. Peritoneal metastases: evaluation with contrast-enhanced ultrasound. *Abdom Imaging* 2011; **36**: 327-332 [PMID: 20959976 DOI: 10.1007/s00261-010-9651-3]

9 **ASGE Standards of Practice Committee**, Khashab MA, Chithadi KV, Acosta RD, Bruining DH,

Chandrasekhara V, Eloubeidi MA, Fanelli RD, Faulx AL, Fonkalsrud L, Lightdale JR, Muthusamy VR, Pasha SF, Saltzman JR, Shaukat A, Wang A, Cash BD. Antibiotic prophylaxis for GI endoscopy. *Gastrointest Endosc* 2015; **81**: 81-89 [PMID: 25442089 DOI: 10.1016/j.gie.2014.08.008]

10 **Kaushik N**, Khalid A, Brody D, McGrath K. EUS-guided paracentesis for the diagnosis of malignant ascites. *Gastrointest Endosc* 2006; **64**: 908-913 [PMID: 17140897 DOI: 10.1016/j.gie.2005.11.058]

11 **Suzuki R**, Irisawa A, Bhutani MS, Hikichi T, Takagi T, Shibukawa G, Sato A, Sato M, Ikeda T, Watanabe K, Nakamura J, Annangi S, Tasaki K, Obara K, Ohira H. An automated spring-loaded needle for endoscopic ultrasound-guided abdominal paracentesis in cancer patients. *World J Gastrointest Endosc* 2014; **6**: 55-59 [PMID: 24567793 DOI: 10.4253/wjge.v6.i2.55]

12 **Chang KJ**, Albers CG, Nguyen P. Endoscopic ultrasound-guided fine needle aspiration of pleural and ascitic fluid. *Am J Gastroenterol* 1995; **90**: 148-150 [PMID: 7801920]

13 **Köck R**, Jürgensen C, Fischer-Lampsatis R. Endosonography-guided drainage of loculated malignant ascites using double-pigtail plastic stents. *Endosc Int Open* 2018; **6**: E902-E906 [PMID: 29978013 DOI: 10.1055/a-0605-3587]

14 **Romero-Castro R**, Jimenez-Garcia VA, Boceta-Osuna J, Castilla-Guerra L, Pellicer-Bautista F, Caunedo-Alvarez A, Herrerias-Gutierrez JM, Romero-Gómez M, Giovannini M. Endoscopic ultrasound-guided placement of plastic pigtail stents for the drainage of refractory malignant ascites. *Endosc Int Open* 2017; **5**: E1096-E1099 [PMID: 29250586 DOI: 10.1055/s-0043-118746]

15 **Romero-Castro R**. EUS-Guided Drainage of Refractory Malignant Ascites. [accessed 2021 Feb 15]. In: ClinicalTrials.gov [Internet]. Bethesda (MD): U.S. National Library of Medicine. Available from: https://clinicaltrials.gov/ct2/show/NCT03550560 ClinicalTrials.gov Identifier: NCT03550560

16 **Wardeh R**, Lee JG, Gu M. Endoscopic ultrasound-guided paracentesis of ascitic fluid: a morphologic study with ultrasonographic correlation. *Cancer Cytopathol* 2011; **119**: 27-36 [PMID: 21072835 DOI: 10.1002/cncy.20123]

17 **Bhatia V**, Hijioka S, Hara K, Mizuno N, Imaoka H, Yamao K. Endoscopic ultrasound description of liver segmentation and anatomy. *Dig Endosc* 2014; **26**: 482-490 [PMID: 24355092 DOI: 10.1111/den.12216]

18 **Friedberg SR**, Lachter J. Endoscopic ultrasound: Current roles and future directions. *World J Gastrointest Endosc* 2017; **9**: 499-505 [PMID: 29085560 DOI: 10.4253/wjge.v9.i10.499]

19 **Campos S**, Poley JW, van Driel L, Bruno MJ. The role of EUS in diagnosis and treatment of liver disorders. *Endosc Int Open* 2019; **7**: E1262-E1275 [PMID: 31579708 DOI: 10.1055/a-0958-2183]

20 **Iglesias-García J**, Lariño-Noia J, Domínguez-Muñoz JE. New Imaging Techniques: Endoscopic Ultrasound-Guided Elastography. *Gastrointest Endosc Clin N Am* 2017; **27**: 551-567 [PMID: 28918798 DOI: 10.1016/j.giec.2017.06.001]

21 **Alvarez-Sánchez MV**, Napoléon B. Contrast-enhanced harmonic endoscopic ultrasound imaging: basic principles, present situation and future perspectives. *World J Gastroenterol* 2014; **20**: 15549-15563 [PMID: 25400439 DOI: 10.3748/wjg.v20.i42.15549]

22 **Schulman AR**, Lin MV, Rutherford A, Chan WW, Ryou M. A Prospective Blinded Study of Endoscopic Ultrasound Elastography in Liver Disease: Towards a Virtual Biopsy. *Clin Endosc* 2018; **51**: 181-185 [PMID: 29566479 DOI: 10.5946/ce.2017.095]

23 **Marques S**, Carmo J, Túlio MA, Bispo M, Matos L, Chagas C. Diagnostic Performance of Real-Time Elastography in the Assessment of Advanced Fibrosis in Chronic Hepatitis C. *GE Port J Gastroenterol* 2016; **23**: 13-18 [PMID: 28868425 DOI: 10.1016/j.jpge.2015.10.008]

24 **Tu CH**, Li J, Wang CY, Zhou L, Ma Y, Gao M, Wang J, Zeng QM, Lu W. [Diagnostic value of endoscopic ultrasonography, fibroscan, acoustic radiation pulse imaging, serological index, and their combination for early stage liver cirrhosis]. *Zhonghua Gan Zang Bing Zazhi* 2019; **27**: 615-620 [PMID: 31594079 DOI: 10.3760/cma.j.issn.1007-3418.2019.08.006]

25 **Ichim VA**, Chira RI, Mircea PA, Nagy GA, Crisan D, Socaciu MA. Accuracy of endoscopic ultrasound-guided biopsy of focal liver lesions. *Med Ultrason* 2020; **22**: 20-25 [PMID: 32096783 DOI: 10.11152/mu-2078]

26 **Singh P**, Erickson RA, Mukhopadhyay P, Gopal S, Kiss A, Khan A, Ulf Westblom T. EUS for detection of the hepatocellular carcinoma: results of a prospective study. *Gastrointest Endosc* 2007; **66**: 265-273 [PMID: 17543307 DOI: 10.1016/j.gie.2006.10.053]

27 **Singh P**, Mukhopadhyay P, Bhatt B, Patel T, Kiss A, Gupta R, Bhat S, Erickson RA. Endoscopic ultrasound *vs* CT scan for detection of the metastases to the liver: results of a prospective comparative study. *J Clin Gastroenterol* 2009; **43**: 367-373 [PMID: 18981929 DOI: 10.1097/MCG.0b013e318167b8cc]

28 **Fujii-Lau LL**, Abu Dayyeh BK, Bruno MJ, Chang KJ, DeWitt JM, Fockens P, Forcione D, Napoleon B, Palazzo L, Topazian MD, Wiersema MJ, Chak A, Clain JE, Faigel DO, Gleeson FC, Hawes R, Iyer PG, Rajan E, Stevens T, Wallace MB, Wang KK, Levy MJ. EUS-derived criteria for distinguishing benign from malignant metastatic solid hepatic masses. *Gastrointest Endosc* 2015; **81**: 1188-96.e1 [PMID: 25660980 DOI: 10.1016/j.gie.2014.10.035]

29 **Sandulescu L**, Padureanu V, Dumitrescu C, Braia N, Streba CT, Gheonea DI, Cazacu S, Ciurea T, Rogoveanu I, Saftoiu A. A pilot study of real time elastography in the differentiation of focal liver lesions. *Curr Health Sci J* 2012; **38**: 32-35 [PMID: 24778839]

30 **Kitano M**, Kamata K. Contrast-enhanced harmonic endoscopic ultrasound: Future perspectives. *Endosc Ultrasound* 2016; **5**: 351-354 [PMID: 28000625 DOI: 10.4103/2303-9027.195852]

31 **Liu M**, Lin MX, Lu MD, Xu ZF, Zheng KG, Wang W, Kuang M, Zhuang WQ, Xie XY. Comparison of contrast-enhanced ultrasound and contrast-enhanced computed tomography in evaluating the treatment response to transcatheter arterial chemoembolization of hepatocellular carcinoma using modified RECIST. *Eur Radiol* 2015; **25**: 2502-2511 [PMID: 25702094 DOI: 10.1007/s00330-015-3611-9]

32 **Nguyen P**, Feng JC, Chang KJ. Endoscopic ultrasound (EUS) and EUS-guided fine-needle aspiration (FNA) of liver lesions. *Gastrointest Endosc* 1999; **50**: 357-361 [PMID: 10462656 DOI: 10.1053/ge.1999.v50.97208]

33 **TenBerge J**, Hoffman BJ, Hawes RH, Van Enckevort C, Giovannini M, Erickson RA, Catalano MF, Fogel R, Mallery S, Faigel DO, Ferrari AP, Waxman I, Palazzo L, Ben-Menachem T, Jowell PS, McGrath KM, Kowalski TE, Nguyen CC, Wassef WY, Yamao K, Chak A, Greenwald BD, Woodward TA, Vilmann P, Sabbagh L, Wallace MB. EUS-guided fine needle aspiration of the liver: indications, yield, and safety based on an international survey of 167 cases. *Gastrointest Endosc* 2002; **55**: 859-862 [PMID: 12024141 DOI: 10.1067/mge.2002.124557]

34 **DeWitt J**, LeBlanc J, McHenry L, Ciaccia D, Imperiale T, Chappo J, Cramer H, McGreevy K, Chriswell M, Sherman S. Endoscopic ultrasound-guided fine needle aspiration cytology of solid liver lesions: a large single-center experience. *Am J Gastroenterol* 2003; **98**: 1976-1981 [PMID: 14499774 DOI: 10.1111/j.1572-0241.2003.07638.x]

35 **Hollerbach S**, Willert J, Topalidis T, Reiser M, Schmiegel W. Endoscopic ultrasound-guided fine-needle aspiration biopsy of liver lesions: histological and cytological assessment. *Endoscopy* 2003; **35**: 743-749 [PMID: 12929021 DOI: 10.1055/s-2003-41593]

36 **McGrath K**, Brody D, Luketich J, Khalid A. Detection of unsuspected left hepatic lobe metastases during EUS staging of cancer of the esophagus and cardia. *Am J Gastroenterol* 2006; **101**: 1742-1746 [PMID: 16790035 DOI: 10.1111/j.1572-0241.2006.00665.x]

37 **Crowe DR**, Eloubeidi MA, Chhieng DC, Jhala NC, Jhala D, Eltoum IA. Fine-needle aspiration biopsy of hepatic lesions: computerized tomographic-guided *vs* endoscopic ultrasound-guided FNA. *Cancer* 2006; **108**: 180-185 [PMID: 16634071 DOI: 10.1002/cncr.21912]

38 **Prachayakul V**, Aswakul P, Kachintorn U. EUS guided fine needle aspiration cytology of liver nodules suspicious for malignancy: yields, complications and impact on management. *J Med Assoc Thai* 2012; **95** Suppl 2: S56-S60 [PMID: 22574530]

39 **Oh D**, Seo DW, Hong SM, Jun JH, Song TJ, Park DH, Son BK, Lee SS, Lee SK, Kim MH. The usefulness of contrast-enhanced harmonic EUS-guided fine-needle aspiration for evaluation of hepatic lesions (with video). *Gastrointest Endosc* 2018; **88**: 495-501 [PMID: 29859228 DOI: 10.1016/j.gie.2018.05.019]

40 **Lee YN**, Moon JH, Kim HK, Choi HJ, Choi MH, Kim DC, Lee TH, Cha SW, Kim SG, Kim YS. Usefulness of endoscopic ultrasound-guided sampling using core biopsy needle as a percutaneous biopsy rescue for diagnosis of solid liver mass: Combined histological-cytological analysis. *J Gastroenterol Hepatol* 2015; **30**: 1161-1166 [PMID: 25684303 DOI: 10.1111/jgh.12922]

41 **Chon HK**, Yang HC, Choi KH, Kim TH. Endoscopic Ultrasound-Guided Liver Biopsy Using a Core Needle for Hepatic Solid Mass. *Clin Endosc* 2019; **52**: 340-346 [PMID: 31302987 DOI: 10.5946/ce.2018.175]

42 **Ichim VA**, Chira RI, Mircea PA. Diagnostic yield of endoscopic ultrasound-guided biopsy of focal liver lesions. *Med Pharm Rep* 2019; **92**: 15-20 [PMID: 30957081 DOI: 10.15386/cjmed-1066]

43 **Choi HJ**, Moon JH, Kim HK, Lee YN, Lee TH, Cha SW, Cho YD, Park SH. KRAS mutation analysis by next-generation sequencing in endoscopic ultrasound-guided sampling for solid liver masses. *J Gastroenterol Hepatol* 2017; **32**: 154-162 [PMID: 27118240 DOI: 10.1111/jgh.13423]

44 **Suzuki R**, Shin D, Richards-Kortum R, Coghlan L, Bhutani MS. In vivo cytological observation of liver and spleen by using high-resolution microendoscopy system under endoscopic ultrasound guidance: A preliminary study using a swine model. *Endosc Ultrasound* 2016; **5**: 239-242 [PMID: 27503155 DOI: 10.4103/2303-9027.187867]

45 **Minaga K**, Kitano M, Nakai A, Omoto S, Kamata K, Yamao K, Takenaka M, Tsurusaki M, Chikugo T, Matsumoto I, Chiba Y, Watanabe T, Kudo M. Improved detection of liver metastasis using Kupffer-phase imaging in contrast-enhanced harmonic EUS in patients with pancreatic cancer (with video). *Gastrointest Endosc* 2021; **93**: 433-441 [PMID: 32592778 DOI: 10.1016/j.gie.2020.06.051]

46 **Tapper EB**, Lok AS. Use of Liver Imaging and Biopsy in Clinical Practice. *N Engl J Med* 2017; **377**: 756-768 [PMID: 28834467 DOI: 10.1056/NEJMra1610570]

47 **Rösch J**, Hanafee WN, Snow H. Transjugular portal venography and radiologic portacaval shunt: an experimental study. *Radiology* 1969; **92**: 1112-1114 [PMID: 5771827 DOI: 10.1148/92.5.1112]

48 **Rockey DC**, Caldwell SH, Goodman ZD, Nelson RC, Smith AD; American Association for the Study of Liver Diseases. Liver biopsy. *Hepatology* 2009; **49**: 1017-1044 [PMID: 19243014 DOI: 10.1002/hep.22742]

49 **Cholongitas E**, Senzolo M, Standish R, Marelli L, Quaglia A, Patch D, Dhillon AP, Burroughs AK. A systematic review of the quality of liver biopsy specimens. *Am J Clin Pathol* 2006; **125**: 710-721 [PMID: 16707372 DOI: 10.1309/W3XC-NT4H-KFBN-2G0B]

50 **Stavropoulos SN**, Im GY, Jlayer Z, Harris MD, Pitea TC, Turi GK, Malet PF, Friedel DM, Grendell JH. High yield of same-session EUS-guided liver biopsy by 19-gauge FNA needle in patients undergoing EUS to exclude biliary obstruction. *Gastrointest Endosc* 2012; **75**: 310-318 [PMID: 22248599 DOI: 10.1016/j.gie.2011.09.043]

51 **Mohan BP**, Shakhatreh M, Garg R, Ponnada S, Adler DG. Efficacy and safety of EUS-guided liver biopsy: a systematic review and meta-analysis. *Gastrointest Endosc* 2019; **89**: 238-246.e3 [PMID: 30389469 DOI: 10.1016/j.gie.2018.10.018]

52 **Kalambokis G**, Manousou P, Vibhakorn S, Marelli L, Cholongitas E, Senzolo M, Patch D, Burroughs AK. Transjugular liver biopsy--indications, adequacy, quality of specimens, and complications--a systematic review. *J Hepatol* 2007; **47**: 284-294 [PMID: 17561303 DOI: 10.1016/j.jhep.2007.05.001]

53 **Mony S**, Shah I, Vyas N, Sofi A, Das A. Su1375 Meta-analysis of EUS guided liver biopsy in comparison with percutaneous and transjugular liver biopsy for the diagnosis of parenchymal liver disease. *Gastrointest Endosc* 2018; **87**: AB328-AB329

54 **Khurana S**, Butt W, Khara HS, Johal AS, West SF, Chen ZE, Berger AL, Diehl DL. Bi-lobar liver biopsy *via* EUS enhances the assessment of disease severity in patients with non-alcoholic steatohepatitis. *Hepatol Int* 2019; **13**: 323-329 [PMID: 30993598 DOI: 10.1007/s12072-019-09945-4]

55 **Ching-Companioni RA**, Diehl DL, Johal AS, Confer BD, Khara HS. 19 G aspiration needle *vs* 19 G core biopsy needle for endoscopic ultrasound-guided liver biopsy: a prospective randomized trial. *Endoscopy* 2019; **51**: 1059-1065 [PMID: 31342474 DOI: 10.1055/a-0956-6922]

56 **Mok SRS**, Diehl DL, Johal AS, Khara HS, Confer BD, Mudireddy PR, Kirchner HL, Chen ZE. A prospective pilot comparison of wet and dry heparinized suction for EUS-guided liver biopsy (with videos). *Gastrointest Endosc* 2018; **88**: 919-925 [PMID: 30120956 DOI: 10.1016/j.gie.2018.07.036]

57 **Pineda JJ**, Diehl DL, Miao CL, Johal AS, Khara HS, Bhanushali A, Chen EZ. EUS-guided liver biopsy provides diagnostic samples comparable with those *via* the percutaneous or transjugular route. *Gastrointest Endosc* 2016; **83**: 360-365 [PMID: 26301407 DOI: 10.1016/j.gie.2015.08.025]

58 **Shuja A**, Alkhasawneh A, Fialho A, Shukri A, Harris C, Smotherman C, Malespin M, de Melo SW Jr. Comparison of EUS-guided *vs* percutaneous and transjugular approaches for the performance of liver biopsies. *Dig Liver Dis* 2019; **51**: 826-830 [PMID: 30755347 DOI: 10.1016/j.dld.2019.01.006]

59 **Diehl DL**, Johal AS, Khara HS, Stavropoulos SN, Al-Haddad M, Ramesh J, Varadarajulu S, Aslanian H, Gordon SR, Shieh FK, Pineda-Bonilla JJ, Dunkelberger T, Gondim DD, Chen EZ. Endoscopic ultrasound-guided liver biopsy: a multicenter experience. *Endosc Int Open* 2015; **3**: E210-E215 [PMID: 26171433 DOI: 10.1055/s-0034-1391412]

60 **Gor N**, Salem SB, Jakate S, Patel R, Shah N, Patil A. Histological adequacy of EUS-guided liver biopsy when using a 19-gauge non-Tru-Cut FNA needle. *Gastrointest Endosc* 2014; **79**: 170-172 [PMID: 23916397 DOI: 10.1016/j.gie.2013.06.031]

61 **Shah ND**, Sasatomi E, Baron TH. Endoscopic Ultrasound-guided Parenchymal Liver Biopsy: Single Center Experience of a New Dedicated Core Needle. *Clin Gastroenterol Hepatol* 2017; **15**: 784-786 [PMID: 28126424 DOI: 10.1016/j.cgh.2017.01.011]

62 **Nieto J**, Khaleel H, Challita Y, Jimenez M, Baron TH, Walters L, Hathaway K, Patel K, Lankarani A, Herman M, Holloman D, Saab S. EUS-guided fine-needle core liver biopsy sampling using a novel 19-gauge needle with modified 1-pass, 1 actuation wet suction technique. *Gastrointest Endosc* 2018; **87**: 469-475 [PMID: 28551024 DOI: 10.1016/j.gie.2017.05.013]

63 **Mathew A**. EUS-guided routine liver biopsy in selected patients. *Am J Gastroenterol* 2007; **102**: 2354-2355 [PMID: 17897349 DOI: 10.1111/j.1572-0241.2007.01353_7.x]

64 **Patel HK**, Saxena R, Rush N, Patel SK, Dasari CS, Mneimneh W, Quickery A, Rahal MA, Temnykh L, DeWitt J, Al-Haddad M. A Comparative Study of 22G *vs* 19G Needles for EUS-Guided Biopsies for Parenchymal Liver Disease: Are Thinner Needles Better? *Dig Dis Sci* 2021; **66**: 238-246 [PMID: 32128647 DOI: 10.1007/s10620-020-06165-x]

65 **Gleeson FC**, Clayton AC, Zhang L, Clain JE, Gores GJ, Rajan E, Smyrk TC, Topazian MD, Wang KK, Wiersema MJ, Levy MJ. Adequacy of endoscopic ultrasound core needle biopsy specimen of nonmalignant hepatic parenchymal disease. *Clin Gastroenterol Hepatol* 2008; **6**: 1437-1440 [PMID: 19081532 DOI: 10.1016/j.cgh.2008.07.015]

66 **Dewitt J**, McGreevy K, Cummings O, Sherman S, Leblanc JK, McHenry L, Al-Haddad M, Chalasani N. Initial experience with EUS-guided Tru-cut biopsy of benign liver disease. *Gastrointest Endosc* 2009; **69**: 535-542 [PMID: 19231495 DOI: 10.1016/j.gie.2008.09.056]

67 **Nakai Y**, Samarasena JB, Iwashita T, Park DH, Lee JG, Hu KQ, Chang KJ. Autoimmune hepatitis diagnosed by endoscopic ultrasound-guided liver biopsy using a new 19-gauge histology needle. *Endoscopy* 2012; **44** Suppl 2 UCTN: E67-E68 [PMID: 22396285 DOI: 10.1055/s-0031-1291567]

68 **Sey MS**, Al-Haddad M, Imperiale TF, McGreevy K, Lin J, DeWitt JM. EUS-guided liver biopsy for parenchymal disease: a comparison of diagnostic yield between two core biopsy needles. *Gastrointest Endosc* 2016; **83**: 347-352 [PMID: 26278654 DOI: 10.1016/j.gie.2015.08.012]

69 **Hasan MK**, Kadkhodayan K, Idrisov E, Ali S, Rafiq E, Ben-Ami Shor D, Abdel-Jalil A, Navaneethan U, Bang J, Varadarajulu S, Hawes R, Pernicone P. Endoscopic ultrasound-guided liver biopsy using a 22-G fine needle biopsy needle: a prospective study. *Endoscopy* 2019; **51**: 818-824 [PMID: 31365947 DOI: 10.1055/a-0967-3640]

70 **Sbeit W**, Kadah A, Mahamid M, Pellicano R, Mari A, Khoury T. A State-of-the-Art Review on the Evolving Utility of Endoscopic Ultrasound in Liver Diseases Diagnosis. *Diagnostics (Basel)* 2020; **10** [PMID: 32717886 DOI: 10.3390/diagnostics10080512]

71 **Lee S**, Seo DW, Paik WH, Park DH, Lee SS, Lee SK, Kim MH. Ethanol lavage of huge hepatic cysts by using EUS guidance and a percutaneous approach. *Gastrointest Endosc* 2014; **80**: 1014-1021 [PMID: 24890421 DOI: 10.1016/j.gie.2014.03.037]

72 **Seewald S**, Imazu H, Omar S, Groth S, Seitz U, Brand B, Zhong Y, Sikka S, Thonke F, Soehendra N. EUS-guided drainage of hepatic abscess. *Gastrointest Endosc* 2005; **61**: 495-498 [PMID: 15758937 DOI: 10.1016/s0016-5107(04)02848-2]

73 **Noh SH**, Park DH, Kim YR, Chun Y, Lee HC, Lee SO, Lee SS, Seo DW, Lee SK, Kim MH. EUS-guided drainage of hepatic abscesses not accessible to percutaneous drainage (with videos). *Gastrointest Endosc* 2010; **71**: 1314-1319 [PMID: 20400078 DOI: 10.1016/j.gie.2009.12.045]

74 **Itoi T**, Ang TL, Seewald S, Tsuji S, Kurihara T, Tanaka R, Itokawa F. Endoscopic ultrasonography-guided drainage for tuberculous liver abscess drainage. *Dig Endosc* 2011; **23** Suppl 1: 158-161 [PMID: 21535224 DOI: 10.1111/j.1443-1661.2011.01115.x]

75 **Medrado BF**, Carneiro FO, Vilaça TG, Gouveia TS, Frazão MS, de Moura EG, Sakai P, Otoch JP, Artifon EL. Endoscopic ultrasound-guided drainage of giant liver abscess associated with transgastric migration of a self-expandable metallic stent. *Endoscopy* 2013; **45** Suppl 2: E331-E332 [PMID: 24150733 DOI: 10.1055/s-0033-1344128]

76 **Alcaide N**, Vargas-Garcia AL, de la Serna-Higuera C, Sancho Del Val L, Ruiz-Zorrilla R, Perez-Miranda M. EUS-guided drainage of liver abscess by using a lumen-apposing metal stent (with video). *Gastrointest Endosc* 2013; **78**: 941-942 [PMID: 24016354 DOI: 10.1016/j.gie.2013.07.034]

77 **Ogura T**, Masuda D, Saori O, Wataru T, Sano T, Okuda A, Miyano A, Kitano M, Abdel-Aal UM, Takeuchi T, Fukunishi S, Higuchi K. Clinical Outcome of Endoscopic Ultrasound-Guided Liver Abscess Drainage Using Self-Expandable Covered Metallic Stent (with Video). *Dig Dis Sci* 2016; **61**: 303-308 [PMID: 26254774 DOI: 10.1007/s10620-015-3841-3]

78 **Della Valle V**, Eshja E, Bassi EM. Spontaneous biloma: a case report. *J Ultrasound* 2015; **18**: 293-296 [PMID: 26261461 DOI: 10.1007/s40477-013-0053-6]

79 **Sandha GS**, Bourke MJ, Haber GB, Kortan PP. Endoscopic therapy for bile leak based on a new classification: results in 207 patients. *Gastrointest Endosc* 2004; **60**: 567-574 [PMID: 15472680 DOI: 10.1016/s0016-5107(04)01892-9]

80 **Sbeit W**, Kadah A, Mari A, Mahamid M, Khoury T. A Comprehensive Narrative Review on the Evolving Role of Endoscopic Ultrasound in Focal Solid Liver Lesions Diagnosis and Management. *Diagnostics (Basel)* 2020; **10** [PMID: 32932960 DOI: 10.3390/diagnostics10090688]

81 **Lakhtakia S**, Seo DW. Endoscopic ultrasonography-guided tumor ablation. *Dig Endosc* 2017; **29**: 486-494 [PMID: 28171697 DOI: 10.1111/den.12833]

82 **Chua T**, Faigel DO. Endoscopic Ultrasound-Guided Ablation of Liver Tumors. *Gastrointest Endosc Clin N Am* 2019; **29**: 369-379 [PMID: 30846159 DOI: 10.1016/j.giec.2018.11.007]

83 **Schoppmeyer K**, Weis S, Mössner J, Fleig WE. Percutaneous ethanol injection or percutaneous acetic acid injection for early hepatocellular carcinoma. *Cochrane Database Syst Rev* 2009; CD006745 [PMID: 19588401 DOI: 10.1002/14651858.CD006745.pub2]

84 **Nakaji S**, Hirata N, Iwaki K, Shiratori T, Kobayashi M, Inase M. Endoscopic ultrasound (EUS)-guided ethanol injection for hepatocellular carcinoma difficult to treat with percutaneous local treatment. *Endoscopy* 2012; **44** Suppl 2 UCTN: E380 [PMID: 23139031 DOI: 10.1055/s-0032-1309918]

85 **Lisotti A**, Piscaglia F, Fusaroli P. Contrast-enhanced harmonic endoscopic ultrasound-guided ethanol injection for a small hepatocellular carcinoma. *Endoscopy* 2019; **51**: E317-E318 [PMID: 31163490 DOI: 10.1055/a-0915-1385]

86 **Nakaji S**, Hirata N, Kobayashi M, Shiratori T, Sanagawa M. Endoscopic ultrasonography-guided ethanol injection as a treatment for ruptured hepatocellular carcinoma in the left hepatic lobe. *Endoscopy* 2015; **47** Suppl 1: E558-E560 [PMID: 26610083 DOI: 10.1055/s-0034-1393394]

87 **Nakaji S**, Hirata N, Mikata R, Kobayashi M, Shiratori T, Ogasawara S, Ooka Y, Tsuyuguchi T, Yamaguchi T, Yokosuka O. Clinical outcomes of endoscopic ultrasound-guided ethanol injection for hepatocellular carcinoma in the caudate lobe. *Endosc Int Open* 2016; **4**: E1111-E1115 [PMID: 27747288 DOI: 10.1055/s-0042-116146]

88 **Jiang TA**, Deng Z, Tian G, Zhao QY, Wang WL. Efficacy and safety of endoscopic ultrasonography-guided interventional treatment for refractory malignant left-sided liver tumors: a case series of 26 patients. *Sci Rep* 2016; **6**: 36098 [PMID: 27958384 DOI: 10.1038/srep36098]

89 **Barclay RL**, Perez-Miranda M, Giovannini M. EUS-guided treatment of a solid hepatic metastasis. *Gastrointest Endosc* 2002; **55**: 266-270 [PMID: 11818938 DOI: 10.1067/mge.2002.120784]

90 **Nault JC**, Sutter O, Nahon P, Ganne-Carrié N, Séror O. Percutaneous treatment of hepatocellular carcinoma: State of the art and innovations. *J Hepatol* 2018; **68**: 783-797 [PMID: 29031662 DOI: 10.1016/j.jhep.2017.10.004]

91 **Armellini E**, Leutner M, Stradella D, Ballarè M, Occhipinti P. EUS-guided radiofrequency ablation: an option for the extrapancreatic region. *Endosc Ultrasound* 2018; **7**: 282-283 [PMID: 29536950 DOI: 10.4103/eus.eus_103_17]

92 **Attili F**, Boškoski I, Bove V, Familiari P, Costamagna G. EUS-guided radiofrequency ablation of a hepatocellular carcinoma of the liver. *VideoGIE* 2018; **3**: 149-150 [PMID: 29916463 DOI: 10.1016/j.vgie.2018.02.006]

93 **de Nucci G**, Della Corte C, Reati R, Imperatore N, Arena I, Larghi A, Manes G. Endoscopic ultrasound-guided radiofrequency ablation for hepatocellular carcinoma in cirrhosis: a case report test for efficacy and future perspectives. *Endosc Int Open* 2020; **8**: E1713-E1716 [PMID: 33140029 DOI: 10.1055/a-1236-3105]

94 **Hines-Peralta A**, Hollander CY, Solazzo S, Horkan C, Liu ZJ, Goldberg SN. Hybrid radiofrequency and cryoablation device: preliminary results in an animal model. *J Vasc Interv Radiol* 2004; **15**:

1111-1120 [PMID: 15466798 DOI: 10.1097/01.RVI.0000136031.91939.EC]
95 **Di Matteo F**, Grasso R, Pacella CM, Martino M, Pandolfi M, Rea R, Luppi G, Silvestri S, Zardi E, Costamagna G. EUS-guided Nd:YAG laser ablation of a hepatocellular carcinoma in the caudate lobe. *Gastrointest Endosc* 2011; **73**: 632-636 [PMID: 21030019 DOI: 10.1016/j.gie.2010.08.019]
96 **Jiang T**, Tian G, Bao H, Chen F, Deng Z, Li J, Chai W. EUS dating with laser ablation against the caudate lobe or left liver tumors: a win-win proposition? *Cancer Biol Ther* 2018; **19**: 145-152 [PMID: 29303406 DOI: 10.1080/15384047.2017.1414760]
97 **Carrara S**, Arcidiacono PG, Albarello L, Addis A, Enderle MD, Boemo C, Neugebauer A, Campagnol M, Doglioni C, Testoni PA. Endoscopic ultrasound-guided application of a new internally gas-cooled radiofrequency ablation probe in the liver and spleen of an animal model: a preliminary study. *Endoscopy* 2008; **40**: 759-763 [PMID: 18702032 DOI: 10.1055/s-2008-1077520]
98 **Pioche M**, Lafon C, Constanciel E, Vignot A, Birer A, Gincul R, Lépilliez V, Prat F, Roman S, Chapelon JY, Saurin JC, Ponchon T. High-intensity focused ultrasound liver destruction through the gastric wall under endoscopic ultrasound control: first experience in living pigs. *Endoscopy* 2012; **44** Suppl 2 UCTN: E376-E377 [PMID: 23012031 DOI: 10.1055/s-0032-1310061]
99 **Li T**, Khokhlova T, Maloney E, Wang YN, D'Andrea S, Starr F, Farr N, Morrison K, Keilman G, Hwang JH. Endoscopic high-intensity focused US: technical aspects and studies in an *in vivo* porcine model (with video). *Gastrointest Endosc* 2015; **81**: 1243-1250 [PMID: 25759124 DOI: 10.1016/j.gie.2014.12.019]
100 **Lah JJ**, Kuo JV, Chang KJ, Nguyen PT. EUS-guided brachytherapy. *Gastrointest Endosc* 2005; **62**: 805-808 [PMID: 16246706 DOI: 10.1016/j.gie.2005.07.019]
101 **Martínez-Monge R**, Subtil JC, López-Picazo JM. Transoesophageal endoscopic-ultrasonography-guided 125I permanent brachytherapy for unresectable mediastinal lymphadenopathy. *Lancet Oncol* 2006; **7**: 781-783 [PMID: 16945775 DOI: 10.1016/S1470-2045(06)70865-8]
102 **Sun S**, Xu H, Xin J, Liu J, Guo Q, Li S. Endoscopic ultrasound-guided interstitial brachytherapy of unresectable pancreatic cancer: results of a pilot trial. *Endoscopy* 2006; **38**: 399-403 [PMID: 16680642 DOI: 10.1055/s-2006-925253]
103 **Hu YH**, Tuo XP, Jin ZD, Liu Y, Guo Y, Luo L. Endoscopic ultrasound (EUS)-guided ethanol injection in hepatic metastatic carcinoma: a case report. *Endoscopy* 2010; **42** Suppl 2: E256-E257 [PMID: 20931470 DOI: 10.1055/s-0030-1255653]
104 **Samarasena JB**, Chang KJ. Endoscopic Ultrasound-Guided Interventions for the Measurement and Treatment of Portal Hypertension. *Gastrointest Endosc Clin N Am* 2019; **29**: 311-320 [PMID: 30846155 DOI: 10.1016/j.giec.2018.12.004]
105 **Helmy A**, Hayes PC. Review article: current endoscopic therapeutic options in the management of variceal bleeding. *Aliment Pharmacol Ther* 2001; **15**: 575-594 [PMID: 11328251 DOI: 10.1046/j.1365-2036.2001.00950.x]
106 **Lahoti S**, Catalano MF, Alcocer E, Hogan WJ, Geenen JE. Obliteration of esophageal varices using EUS-guided sclerotherapy with color Doppler. *Gastrointest Endosc* 2000; **51**: 331-333 [PMID: 10699783 DOI: 10.1016/s0016-5107(00)70363-4]
107 **de Paulo GA**, Ardengh JC, Nakao FS, Ferrari AP. Treatment of esophageal varices: a randomized controlled trial comparing endoscopic sclerotherapy and EUS-guided sclerotherapy of esophageal collateral veins. *Gastrointest Endosc* 2006; **63**: 396-402; quiz 463 [PMID: 16500386 DOI: 10.1016/j.gie.2005.10.039]
108 **Sarin SK**, Lahoti D, Saxena SP, Murthy NS, Makwana UK. Prevalence, classification and natural history of gastric varices: a long-term follow-up study in 568 portal hypertension patients. *Hepatology* 1992; **16**: 1343-1349 [PMID: 1446890 DOI: 10.1002/hep.1840160607]
109 **Garcia-Tsao G**, Sanyal AJ, Grace ND, Carey W; Practice Guidelines Committee of the American Association for the Study of Liver Diseases; Practice Parameters Committee of the American College of Gastroenterology. Prevention and management of gastroesophageal varices and variceal hemorrhage in cirrhosis. *Hepatology* 2007; **46**: 922-938 [PMID: 17879356 DOI: 10.1002/hep.21907]
110 **Iwase H**, Suga S, Morise K, Kuroiwa A, Yamaguchi T, Horiuchi Y. Color Doppler endoscopic ultrasonography for the evaluation of gastric varices and endoscopic obliteration with cyanoacrylate glue. *Gastrointest Endosc* 1995; **41**: 150-154 [PMID: 7721004 DOI: 10.1016/s0016-5107(05)80599-1]
111 **Romero-Castro R**, Pellicer-Bautista FJ, Jimenez-Saenz M, Marcos-Sanchez F, Caunedo-Alvarez A, Ortiz-Moyano C, Gomez-Parra M, Herrerias-Gutierrez JM. EUS-guided injection of cyanoacrylate in perforating feeding veins in gastric varices: results in 5 cases. *Gastrointest Endosc* 2007; **66**: 402-407 [PMID: 17643723 DOI: 10.1016/j.gie.2007.03.008]
112 **Lee YT**, Chan FK, Ng EK, Leung VK, Law KB, Yung MY, Chung SC, Sung JJ. EUS-guided injection of cyanoacrylate for bleeding gastric varices. *Gastrointest Endosc* 2000; **52**: 168-174 [PMID: 10922086 DOI: 10.1067/mge.2000.107911]
113 **Bhat YM**, Weilert F, Fredrick RT, Kane SD, Shah JN, Hamerski CM, Binmoeller KF. EUS-guided treatment of gastric fundal varices with combined injection of coils and cyanoacrylate glue: a large U.S. experience over 6 years (with video). *Gastrointest Endosc* 2016; **83**: 1164-1172 [PMID: 26452992 DOI: 10.1016/j.gie.2015.09.040]
114 **Robles-Medranda C**, Oleas R, Valero M, Puga-Tejada M, Baquerizo-Burgos J, Ospina J, Pitanga-Lukashok H. Endoscopic ultrasonography-guided deployment of embolization coils and cyanoacrylate injection in gastric varices *vs* coiling alone: a randomized trial. *Endoscopy* 2020; **52**: 268-275 [PMID: 32126576 DOI: 10.1055/a-1123-9054]

115 **Lôbo MRA**, Chaves DM, DE Moura DTH, Ribeiro IB, Ikari E, DE Moura EGH. Safety and efficacy of EUS-guided coil plus cyanoacrylate versus conventional cyanoacrylate technique in the treatment of gastric varices: a randomized controlled trial. *Arq Gastroenterol* 2019; **56**: 99-105 [PMID: 31141079 DOI: 10.1590/S0004-2803.201900000-08]

116 **McCarty TR**, Bazarbashi AN, Hathorn KE, Thompson CC, Ryou M. Combination therapy *versus* monotherapy for EUS-guided management of gastric varices: A systematic review and meta-analysis. *Endosc Ultrasound* 2020; **9**: 6-15 [PMID: 31417066 DOI: 10.4103/eus.eus_37_19]

117 **Irisawa A**, Shibukawa G, Hoshi K, Yamabe A, Sato A, Maki T, Yoshida Y, Yamamoto S, Obara K. Endoscopic ultrasound-guided coil deployment with sclerotherapy for isolated gastric varices: Case series of feasibility, safety, and long-term follow-up. *Dig Endosc* 2020; **32**: 1100-1104 [PMID: 32147871 DOI: 10.1111/den.13666]

118 **Bazarbashi AN**, Wang TJ, Thompson CC, Ryou M. Endoscopic ultrasound-guided treatment of gastric varices with coil embolization and absorbable hemostatic gelatin sponge: a novel alternative to cyanoacrylate. *Endosc Int Open* 2020; **8**: E221-E227 [PMID: 32010757 DOI: 10.1055/a-1027-6708]

119 **Frost JW**, Hebbar S. EUS-guided thrombin injection for management of gastric fundal varices. *Endosc Int Open* 2018; **6**: E664-E668 [PMID: 29868631 DOI: 10.1055/a-0599-0440]

120 **Carneiro FO**, Retes FA, Matuguma SE, Albers DV, Chaves DM, Dos Santos ME, Herman P, Chaib E, Sakai P, Carneiro D'Albuquerque LA, Maluf Filho F. Role of EUS evaluation after endoscopic eradication of esophageal varices with band ligation. *Gastrointest Endosc* 2016; **84**: 400-407 [PMID: 26905936 DOI: 10.1016/j.gie.2016.02.006]

121 **Kuramochi A**, Imazu H, Kakutani H, Uchiyama Y, Hino S, Urashima M. Color Doppler endoscopic ultrasonography in identifying groups at a high-risk of recurrence of esophageal varices after endoscopic treatment. *J Gastroenterol* 2007; **42**: 219-224 [PMID: 17380280 DOI: 10.1007/s00535-006-1992-x]

122 **Hashimoto R**, Chang KJ. Endoscopic ultrasound guided hepatic interventions. *Dig Endosc* 2021; **33**: 54-65 [PMID: 32145117 DOI: 10.1111/den.13661]

123 **Giday SA**, Clarke JO, Buscaglia JM, Shin EJ, Ko CW, Magno P, Kantsevoy SV. EUS-guided portal vein catheterization: a promising novel approach for portal angiography and portal vein pressure measurements. *Gastrointest Endosc* 2008; **67**: 338-342 [PMID: 18226699 DOI: 10.1016/j.gie.2007.08.037]

124 **Lai L**, Poneros J, Santilli J, Brugge W. EUS-guided portal vein catheterization and pressure measurement in an animal model: a pilot study of feasibility. *Gastrointest Endosc* 2004; **59**: 280-283 [PMID: 14745408 DOI: 10.1016/s0016-5107(03)02544-6]

125 **Magno P**, Ko CW, Buscaglia JM, Giday SA, Jagannath SB, Clarke JO, Shin EJ, Kantsevoy SV. EUS-guided angiography: a novel approach to diagnostic and therapeutic interventions in the vascular system. *Gastrointest Endosc* 2007; **66**: 587-591 [PMID: 17725951 DOI: 10.1016/j.gie.2007.01.011]

126 **Giday SA**, Ko CW, Clarke JO, Shin EJ, Magno P, Jagannath SB, Buscaglia JM, Kantsevoy SV. EUS-guided portal vein carbon dioxide angiography: a pilot study in a porcine model. *Gastrointest Endosc* 2007; **66**: 814-819 [PMID: 17905028 DOI: 10.1016/j.gie.2007.05.056]

127 **Buscaglia JM**, Dray X, Shin EJ, Magno P, Chmura KM, Surti VC, Dillon TE, Ducharme RW, Donatelli G, Thuluvath PJ, Giday SA, Kantsevoy SV. A new alternative for a transjugular intrahepatic portosystemic shunt: EUS-guided creation of an intrahepatic portosystemic shunt (with video). *Gastrointest Endosc* 2009; **69**: 941-947 [PMID: 19327481 DOI: 10.1016/j.gie.2008.09.051]

128 **Binmoeller KF**, Shah JN. Sa1428 EUS-guided transgastric intrahepatic portosystemic shunt using the axios stent. *Gastrointest Endosc* 2011; **73**: AB167

129 **Schulman AR**, Ryou M, Aihara H, Abidi W, Chiang A, Jirapinyo P, Sakr A, Ajeje E, Ryan MB, Thompson CC. EUS-guided intrahepatic portosystemic shunt with direct portal pressure measurements: a novel alternative to transjugular intrahepatic portosystemic shunting. *Gastrointest Endosc* 2017; **85**: 243-247 [PMID: 27468858 DOI: 10.1016/j.gie.2016.07.041]

130 **Chapman CG**, Waxman I. Portal-vein blood samples as a new diagnostic entity for pancreatic cancer. *Expert Rev Gastroenterol Hepatol* 2016; **10**: 665-667 [PMID: 27077275 DOI: 10.1080/17474124.2016.1176911]

131 **Catenacci DV**, Chapman CG, Xu P, Koons A, Konda VJ, Siddiqui UD, Waxman I. Acquisition of Portal Venous Circulating Tumor Cells From Patients With Pancreaticobiliary Cancers by Endoscopic Ultrasound. *Gastroenterology* 2015; **149**: 1794-1803.e4 [PMID: 26341722 DOI: 10.1053/j.gastro.2015.08.050]

132 **Liu X**, Li C, Li J, Yu T, Zhou G, Cheng J, Li G, Zhou Y, Lou W, Wang X, Gong G, Liu L, Chen Y. Detection of CTCs in portal vein was associated with intrahepatic metastases and prognosis in patients with advanced pancreatic cancer. *J Cancer* 2018; **9**: 2038-2045 [PMID: 29896289 DOI: 10.7150/jca.23989]

133 **Lai R**, Stephens V, Bardales R. Diagnosis and staging of hepatocellular carcinoma by EUS-FNA of a portal vein thrombus. *Gastrointest Endosc* 2004; **59**: 574-577 [PMID: 15044903 DOI: 10.1016/s0016-5107(04)00007-0]

134 **Storch I**, Gomez C, Contreras F, Schiff E, Ribeiro A. Hepatocellular carcinoma (HCC) with portal vein invasion, masquerading as pancreatic mass, diagnosed by endoscopic ultrasound-guided fine needle aspiration (EUS-FNA). *Dig Dis Sci* 2007; **52**: 789-791 [PMID: 17268833 DOI: 10.1007/s10620-006-9325-8]

135 **Moreno M**, Gimeno-García AZ, Corriente MM, Nicolás-Pérez D, Brito-García A, García-Castro C, Quintero E. EUS-FNA of a portal vein thrombosis in a patient with a hidden hepatocellular carcinoma: confirmation technique after contrast-enhanced ultrasound. *Endoscopy* 2014; **46** Suppl 1 UCTN: E590-E591 [PMID: 25502254 DOI: 10.1055/s-0034-1390734]

136 **Faigel DO**, Lake DF, Landreth TL, Kelman CC, Marler RJ. EUS-guided portal injection chemotherapy for treatment of hepatic metastases: feasibility in the acute porcine model. *Gastrointest Endosc* 2016; **83**: 444-446 [PMID: 26358330 DOI: 10.1016/j.gie.2015.08.064]

137 **Loffroy R**, Favelier S, Chevallier O, Estivalet L, Genson PY, Pottecher P, Gehin S, Krausé D, Cercueil JP. Preoperative portal vein embolization in liver cancer: indications, techniques and outcomes. *Quant Imaging Med Surg* 2015; **5**: 730-739 [PMID: 26682142 DOI: 10.3978/j.issn.2223-4292.2015.10.04]

138 **Matthes K**, Sahani D, Holalkere NS, Mino-Kenudson M, Brugge WR. Feasibility of endoscopic ultrasound-guided portal vein embolization with Enteryx. *Acta Gastroenterol Belg* 2005; **68**: 412-415 [PMID: 16432991]

139 **Park TY**, Seo DW, Kang HJ, Song TJ, Park DH, Lee SS, Lee SK, Kim MH. Feasibility and safety of EUS-guided selective portal vein embolization with a coil and cyanoacrylate in a live porcine model. *Endosc Ultrasound* 2018; **7**: 389-394 [PMID: 30246708 DOI: 10.4103/eus.eus_18_18]

140 **Park TY**, Seo DW, Kang HJ, Cho MK, Song TJ, Park DH, Lee SS, Lee SK, Kim MH. Endoscopic ultrasonography-guided placement of a transhepatic portal vein stent in a live porcine model. *Endosc Ultrasound* 2016; **5**: 315-319 [PMID: 27803904 DOI: 10.4103/2303-9027.191611]

141 **Chan HH**, Nishioka NS, Mino M, Lauwers GY, Puricelli WP, Collier KN, Brugge WR. EUS-guided photodynamic therapy of the pancreas: a pilot study. *Gastrointest Endosc* 2004; **59**: 95-99 [PMID: 14722560 DOI: 10.1016/s0016-5107(03)02361-7]

142 **Yusuf TE**, Matthes K, Brugge WR. EUS-guided photodynamic therapy with verteporfin for ablation of normal pancreatic tissue: a pilot study in a porcine model (with video). *Gastrointest Endosc* 2008; **67**: 957-961 [PMID: 18178203 DOI: 10.1016/j.gie.2007.08.020]

143 **Choi JH**, Oh D, Lee JH, Park JH, Kim KP, Lee SS, Lee YJ, Lim YS, Song TJ, Seo DW, Lee SK, Kim MH, Park DH. Initial human experience of endoscopic ultrasound-guided photodynamic therapy with a novel photosensitizer and a flexible laser-light catheter. *Endoscopy* 2015; **47**: 1035-1038 [PMID: 26070006 DOI: 10.1055/s-0034-1392150]

144 **DiMaio CJ**, Nagula S, Goodman KA, Ho AY, Markowitz AJ, Schattner MA, Gerdes H. EUS-guided fiducial placement for image-guided radiation therapy in GI malignancies by using a 22-gauge needle (with videos). *Gastrointest Endosc* 2010; **71**: 1204-1210 [PMID: 20598247 DOI: 10.1016/j.gie.2010.01.003]

145 **Varadarajulu S**, Trevino JM, Shen S, Jacob R. The use of endoscopic ultrasound-guided gold markers in image-guided radiation therapy of pancreatic cancers: a case series. *Endoscopy* 2010; **42**: 423-425 [PMID: 20232282 DOI: 10.1055/s-0029-1243989]

146 **Kuwahara T**, Hara K, Mizuno N, Haba S, Okuno N, Koda H, Miyano A, Fumihara D. Current status of artificial intelligence analysis for endoscopic ultrasonography. *Dig Endosc* 2021; **33**: 298-305 [PMID: 33098123 DOI: 10.1111/den.13880]

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OPINION REVIEW

Porta-caval fibrous connections — the lesser-known structure of intrahepatic connective-tissue framework: A unified view of liver extracellular matrix

Leila Patarashvili, Salome Gvidiani, Elza Azmaipharashvili, Keti Tsomaia, Marom Sareli, Dimitri Kordzaia, Ilia Chanukvadze

ORCID number: Leila Patarashvili 0000-0002-8397-6855; Salome Gvidiani 0000-0001-5814-8815; Elza Azmaipharashvili 0000-0002-0679-1558; Keti Tsomaia 0000-0002-2857-0115; Marom Sareli 0000-0002-1688-9217; Dimitri Kordzaia 0000-0002-4773-2896; Ilia Chanukvadze 0000-0001-6524-9112.

Author contributions: Patarashvili L, Azmaipharashvili E, Tsomaia K, Gvidiani S and Kordzaia D contributed equally to the research, data acquisition, and writing of the paper; Kordzaia D, Sareli M and Chanukvadze I elaborated the main concept of the opinion review and provided oversight of the works and manuscript appraisal; all authors agreed on the final version of the paper.

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Leila Patarashvili, Dimitri Kordzaia, Department of Clinical Anatomy and Operative Surgery, Ivane Javakhishvili Tbilisi State University, Tbilisi 0159, Georgia

Salome Gvidiani, Elza Azmaipharashvili, Faculty of Medicine, Ivane Javakhishvili Tbilisi State University, Tbilisi 0159, Georgia

Keti Tsomaia, Clinical Anatomy and Experimental Modeling, Institute of Morphology, Ivane Javakhishvili Tbilisi State University, Tbilisi 0159, Georgia

Marom Sareli, Department of Surgical Oncology (Surgery C), Chaim Sheba Medical Center at HaShomer, Ramat Gan, Tel Aviv 52621, Israel

Ilia Chanukvadze, Faculty of Medicine, Tbilisi State Medical University, Tbilsi 0177, Georgia

Corresponding author: Dimitri Kordzaia, DSc, MD, PhD, Dean, Professor, Clinical Anatomy and Operative Surgery, Ivane Javakhishvili Tbilisi State University, Beliashvili str. 78, Tbilisi 0159, Georgia. dimitri.kordzaia@tsu.ge

Abstract

Knowledge about the connective-tissue framework of the liver is not systematized, the terminology is inconsistent and some perspectives on the construction of the hepatic matrix components are contradictory. In addition, until the last two decades of the 20th century, the connective-tissue sheaths of the portal tracts and the hepatic veins were considered to be independent from each other in the liver and that they do not make contact with each other. The results of the research carried out by Professor Shalva Toidze and his colleagues started in the 1970s in the Department of Operative Surgery and Topographic Anatomy at the Tbilisi State Medical Institute have changed this perception. In particular, Chanukvadze I showed that in some regions where they intersect with each other, the connective tissue sheaths of the large portal complexes and hepatic veins fuse. The areas of such fusion are called porta-caval fibrous connections (PCFCs). This opinion review aims to promote a systematic understanding of the hepatic connective-tissue skeleton and to demonstrate the hitherto underappreciated PCFC as a genuine structure with high biological and clinical significance. The components of the liver connective-tissue framework — the capsules, plates,

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sheaths, covers — are described, and their intercommunication is discussed. The analysis of the essence of the PCFC and a description of its various forms are provided. It is also mentioned that analogs of different forms of PCFC are found in different mammals.

Key Words: Hepatic capsule; Hilar plate; Perivascular fibrous sheath; Glissonean pedicle; Portal tract; Caval port

Core Tip: In the places of spatial intersection of the Glissonean pedicles with the main hepatic veins, the fusion of their connective tissue sheaths is described. The sites of the above-mentioned fusion are called porta-caval fibrous connections. Various forms of porta-caval fibrous connections are discussed as well as their clinical and scientific implications.

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INTRODUCTION

The extracellular matrix — the connective-tissue framework of the liver — determines the shape of the organ and creates specialized compartments for the liver cell populations and blood and lymph circulations, the synergy of which determines the diverse functioning of the organ. The structure and components of the human liver extracellular matrix were comprehensively analyzed in a series of studies performed in the 1980s and the 90s[1,2].

The last five years saw a new wave of studies on hepatic connective-tissue structures. This "revisiting" is thanks to the introduction of new methods and computer technologies in morphological studies[3] and includes studies not only of the human liver but also of the liver of various animals and birds[4-7].

The emergence of endoscopic anatomic liver resections strengthened the need to specify the anatomy and interrelationship of the connective-tissue structures within the liver[8-11]. Additionally, the prospects for the use of human and animal liver matrices as scaffolds for the creation of bioartificial livers (thanks to the development of stem cells and bioengineering technologies)[11-14] also contribute to the resurgence of interests in the hepatic connective-tissue structures.

However, upon reviewing these studies, we noticed that knowledge on the connective-tissue skeleton of the liver were not systematized, the terminology was inconsistent, and the literature concerning the construction of one or another component of the hepatic matrix were sometimes contradictory[15].

Until the last two decades of the 20th century, the branches of the portal vein and the hepatic veins were considered to be independent from each other in the liver and that their connective-tissue sheaths did not make contact with each other[16-18]. Modern hepatology textbooks usually perpetuate this notion that the Glissonean portal pedicles and the main hepatic veins intersect spatially, but some liver parenchyma always remains between them. Thus, it was believed that they are anatomically independent of each other[19].

The results of the research carried out by Professor Shalva Toidze and his colleagues started in the 1970s in the Department of Operative Surgery, and the Topographic Anatomy of Tbilisi State Medical Institute changed this perception. In particular, Chanukvadze[20] showed that in some regions where they intersect with each other, the connective-tissue sheaths of the main portal complex and a hepatic vein fuse. The regions of such fusion he called porta-caval fibrous connections (PCFCs). Several forms of PCFC have been described. It has also been revealed that PCFC, as an

anatomical formation, develops in the 11th-12th weeks of gestation. Despite numerous publications, these data have not yet received proper acknowledgement in scientific discourse and, as a result, in clinical hepatology. This opinion review aims to promote a systematic understanding of the connective-tissue skeleton of the liver, standardize the definition and the nomenclature of its structural components, and highlight the importance of the hitherto underappreciated PCFC as a genuine structure.

Since the same connective-tissue structure of the liver is often referred to by different names, we have tried to standardize the terms used throughout this article. The following terms will be used in the ensuing discussion: (1) Liver capsule is the same as Laennec's capsule (but not Glisson's capsule); (2) Hilar plate is the same as Walaeus vasculo-biliary sheath (but not Glisson's plate); (3) Perivascular fibrous capsule is the same as Glisson's capsule; (4) Proper hepatic capsule (PHC) is the same as the intrahepatic part of Laennec's capsule covering the liver parenchyma; (5) Portal hilus is the same as portal port; (6) Caval port is the same as hepatic venous port (where the inferior vena cava adjoins to the liver and incorporates the hepatic veins); and (7) Glissonean pedicle is the same as the portal tract surrounded by Glisson's capsule.

DISCUSSION

Liver capsule and its derivatives

Laennec's capsule (liver capsule) covers the entire liver surface, including its bare area (aperitoneal area). In the portal hilus and venous port of the liver, Laennec's capsule around the Glissonean pedicles and the hepatic veins enters the hepatic parenchyma, covers it, and separates it from the portal tracts and hepatic vein tributaries[21].

In the hepatic hilus, the liver capsule directly touches the hilar plate (also known as Walaeus vascular-biliary sheath) covering the portal vein, the hepatic artery, and the bile ducts, while within the liver, the intrahepatic part of the liver capsule — PHC — covering the parenchyma, sets against the perivascular fibrous capsule (Glisson's capsule), which is a direct extension of the Walaeus sheath and envelops the lobar, sectoral, and segmental portal tracts[15,22]. These two fibrous fascial structures — PHC and Glisson's capsule — are separated by a narrow fissure[10] (Figure 1A and C). The individual fibers of the connective tissue (or their bundles) are located in this fissure and connect the outer side of Gleason's capsule with the PHC. On the other hand, soft collagen fibers (type I and III collagen) separate from the internal side of PHC and extend within the liver lobule (Disse's spaces), fusing to the intralobular matrix[3].

In the region of the thinner portal tracts (subsegmental, zonal), Glisson's capsule tapers off, and cross-banded collagen fibers from portal spaces are in continuity with similar fibers in the immediately adjacent lobular interstitium, which in turn are in continuity with those in central spaces; in this manner, collagen type I fibers and bundles form the structural scaffold of the liver lobule[2]. Meanwhile, the portal, extralobular and intralobular matrices of the liver are united by creating a complex labyrinth that represents the circulation area for tissue fluid and prelymph[23,24].

Laennec's capsule covering the liver parenchyma is related to the adventitia of the hepatic veins and their tributaries, represented by type I and type III collagen fibers and single muscle fibers, mainly running along the veins. Thick collagen fibers were found external to thin elastic fibers, which were intimately related to smooth muscle. The above-mentioned features are consistent with the observation that all veins of the infracardiac region in humans are mainly propulsive veins[25]. The increase in collagen content on the adventitial side of the interface may strengthen it and prevent rupture of the vein during extreme liver movements[26].

The PHC is often separated from the adventitia of the hepatic veins and their large tributaries by a narrow slit (similar to that described in relation to Glissonean pedicles), in which the tissue fluid and prelymph circulate[23,24] (Figure 1C). The average distance between the PHC and the Glissonean pedicle is 32 ± 8.7 μm, while that between the PHC and the hepatic veins is 26 ± 6.3 μm[8]. Some authors suggest that Laennec's capsule, Glisson's capsule and the sheath for the hepatic vein tributaries can be characterized by a high content of thin, wavy elastic fibers. The Waleaus vasculo-biliary sheath of the thick vessels and ducts does not contain elastic fibers[15]. However, some researchers believe that there is no fibrous sheath around the hepatic veins and that the adventitia of the hepatic veins is in direct contact with the PHC covering the liver parenchyma[27]. With the reduction of the diameter (caliber) of the tributaries of the hepatic veins, the adventitia of these veins thins out, PHC tapers off,

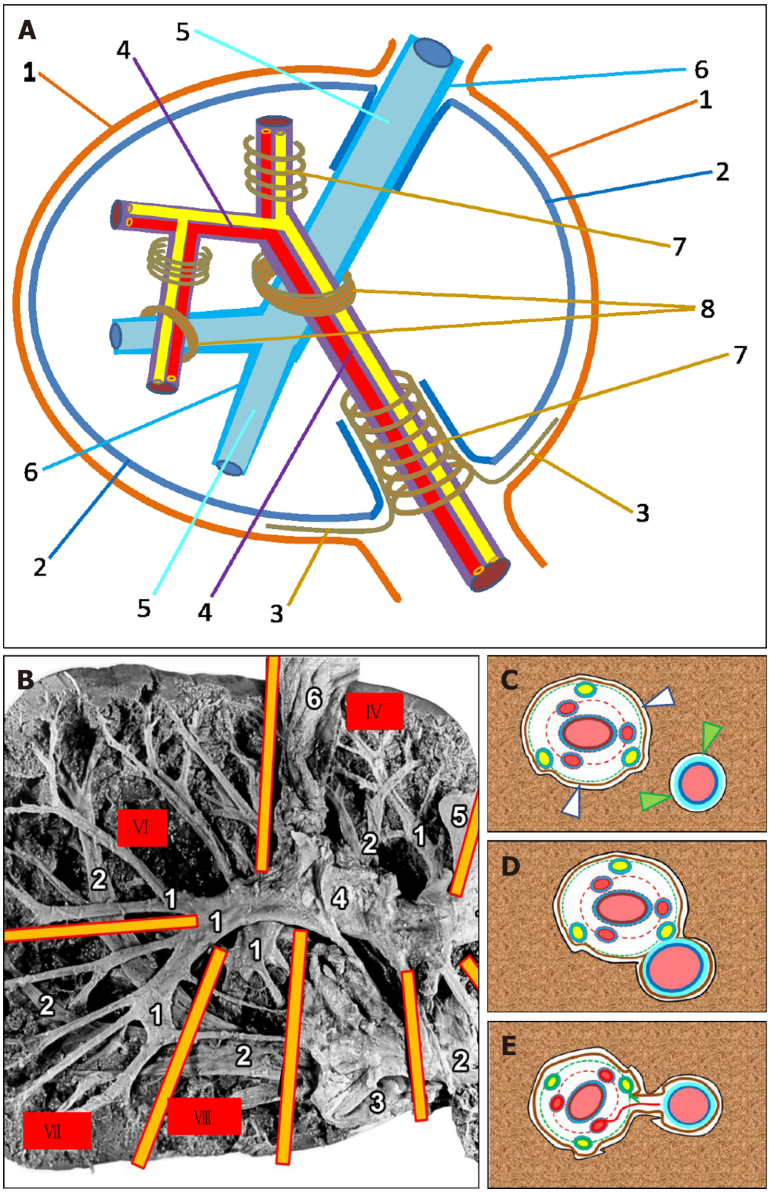

Figure 1 Connective tissue structures and their relationship in the liver. A: 1: Peritoneum; 2: Liver capsule (Laennec's capsule); 3: Hilar plate (Walaeus vasculo-biliary sheath); 4: Portal tract; 5: Hepatic vein and its tributaries; 6: Connective tissue sheath of a hepatic vein; 7: Portal tract surrounded by Glisson's capsule (Glissonean pedicle); 8: Porta-caval fibrous connection (PCFC); Arrowhead: the fissure among the Laennec's capsule (proper hepatic capsule, PHC) and the Glisson's capsule; B: Intrahepatic portal tracts and hepatic veins of the human liver after maceration from the visceral surface (preparation from the private archive of Professor Chanukvadze I); Intersection of portal tracts and the hepatic veins. Yellow lines show the borders among the liver segments enumeration of which is shown in red quadrats. 1: Portal tract; 2: Hepatic veins and their tributaries; 3: Inferior vena cava; 4: Walaeus vasculo-biliary sheath; 5: Round ligament; 6: Gallbladder; C: Section of liver tissue containing the portal tract and hepatic vein (scheme). White arrowhead: the fissure among the Laennec's capsule (PHC) and the Glisson's capsule; Green arrowhead: the fissure among the Laennec's capsule (PHC) and connective-tissue sheath surrounding the hepatic vein; D: Area of complete fusion of the Glisson's capsule and a connective-tissue sheath surrounding the hepatic vein (scheme); E: Plate-shaped PCFC (scheme).

and intralobular connective-tissue fibers connect directly to the connective-tissue fibers of adventitia of the small tributaries of the hepatic veins[2,28]. Such a relationship further reinforces the notion that the merger of the intralobular and extralobular connective-tissue fibers and that of the capsule covering the organ create a complex, yet well-regulated, structure of the extracellular matrix, which is the connective-tissue skeleton of the liver, coordinating the synergy between the cell populations and the neural and circulatory tubular structures. The PHC is mainly composed of reticular fibers (RFs) that cover the hepatic lobules. The ring of hepatocytes abutting the connective tissue of the portal region is called the periportal limiting plate. The RF bordering the hepatocytes constituting the limiting plate forms a capsule. This capsule covers the hepatic lobule from one side and abuts the perivascular fibrous capsule (Glisson's capsule) enveloping the portal tract, from another side[3].

Based on computer software analysis of liver specimens (histotopograms), the same authors distinguish loose fiber construction (and not the fissure described above) between Glisson's capsule and the PHC and called it the private hepatic ligament (PHL). The PHL is a structure in which collagen fibers have invaded from the portal region into the lattice-like or mesh-like RF that originally surrounded the lost hepatocytes[3]. However, it should be noted that the existence of such a formation has to be confirmed by additional studies.

There is a system of connective-tissue plates in the area of the hepatic port, whose origin and structure continue to be the subject of debate. This system includes a cystic plate, a round ligament plate, an Arantial plate, and a hilar plate (Walaeus vasculo-biliary sheath)[27,29].

The names of the plates are determined by their location: the gallbladder bed, round ligament gutter, Arantial ligament (obliterated venous duct) gutter, and hilus of the liver[30,31]. Several researchers have further described the caval plate, the connective-tissue sheath situated between the hepatic parenchyma and the adventitia of the hepatic part of the inferior vena cava[26,32]. Some researchers believe that these plates are derivatives of Laennec's capsule, which is attached to the liver capsule as an additional outer layer in the above-mentioned areas[30]. Other researchers indicate that the plate complexes, especially the hilar plate (which has special functional and clinical significance), is not an embryological derivative of Laennec's capsule and is connected with the fibrous part of the hepatoduodenal ligament and the connective tissues surrounding the blood vessels and bile ducts located in the portal area[27]. However, another group of researchers believes that the hilar plate does not exist at all as an independent entity; it is part of the liver capsule, which thickens in the area of the hepatic port due to a large number of thin-walled bile ducts (so-called "vaginal ductuli"). During surgery and dissection, it should be kept in mind that the hilar plate is likely to be artificially generated when, the surgeon unintentionally bundles collagenous fibers around the vaginal ductuli[15,29,33]. Taken together, the origin of the plates located on the visceral surface of the liver requires additional studies. Furthermore, we can state with confidence that the hilar plate (Walaeus vasculo-biliary sheath) covers the structures entering or exiting the liver at the hepatic port — the branches of the portal vein, hepatic artery, and bile ducts and accompanying lymphatic vessels and nerve cords. In combination with the accompanying connective-tissue fibers, afore-mentioned structures form the portal tracts that branch inside the liver. Large portal tracts, such as lobar, sectoral, segmental, and sometimes subsegmental tracts, are enveloped by a perivascular fibrous capsule (Glisson's capsule), which forms the so-called Glissonean pedicle[30]. Glisson's capsule is an intrahepatic extension of the hilar plate (Walaeus sheath). Thus, the portal tracts at the hepatic port are surrounded by the Walaeus sheath and inside the liver with Glisson's capsule. As mentioned above, Glisson's capsule is prominent around the large-caliber portal tracts but tapers off or completely disappears in thinner tracts[7].

Taking all of the above into consideration, Hu *et al*[8] concluded that the plate system represented a fibrous, thickened part of the Walaeus vasculo-biliary sheath and that Laennec's capsule had no continuity with the Glissonean pedicle. However, Laennec's capsule, which is dissociated from the main Glissonean capsule, extends to the peripheral portal tracts, where the structural integrity loosens and directly continues into the intralobular connective tissue fibers.

Laennec's capsule is the critical structure for understanding the comprehensive surgical anatomy of the liver and standardizing extrahepatic Glissonean pedicle isolation in anatomical liver resection[21]. Its precise understanding may rewrite the descriptions in the hepatology textbooks on the relationship between the hepatic capsule and intrahepatic and extrahepatic portal pedicle sheaths as follows: the connective tissue that constitutes the hepatic capsule wraps around the portal vein, hepatic artery, bile duct, lymphatics, and nerves that enter and exit the liver from the hilar part and then enters the liver where it is distributed as a skeleton in the parenchyma[34].

Portal tracts and their connective-tissue structures

The blood vessels, bile ducts and nerves located in the portal tracts are covered by their own fascial connective tissue. These structures are individually encased by a typical membrane containing laminin, collagen type IV, entactin, and heparan sulfate proteoglycan. The surrounding portal interstitium contains collagen types I, III, V, and VI, fibronectin and tenascin[2]. The fibrous covers are separated from the blood vessel walls by a space called the conceptual paravasal body[35].

In the liver hilus and adjacent proximal part of the hepatoduodenal ligament, the connective tissue cover of the portal vein is well distinguished. It surrounds the blood vessel in the form of a sheath, inside of which there is the aforementioned paravasal fissure, which contains connective tissue fibers running in different directions, connecting the portal vein adventitia with the inner surface of its fibrous cover. Likewise, in the same regions, the hepatic artery is also surrounded by a layer of fibrous connective tissue called the fibrous cover. It is separated from the blood vessel wall by a well-defined fissure containing the bundles of connective-tissue fibers connecting the inner wall of the fibrous cover with the adventitia of the artery[20,32].

The Brisbane Meeting of the International Society of Hepatobiliary-Pancreatic Surgery in 2000 formed a consensus on the uniform anatomical term/terminology classification to remedy the confusion that was present at that time. Their consensus was that first-order divisions of the elements of the portal triad were those that supplied the right and left halves of the liver, second-order divisions were those that supplied the liver sectors, and third-order divisions were those that supplied the segments[36].

The perivascular fibrous capsule abruptly appears in the area of the sectoral portal tract. It is dense and easily separates from the liver tissue, which in turn is covered by the PHC (the intrahepatic part of Laennec's capsule)[3].

The perivascular fibrous capsule is formed by collagen fibers running in various directions (elastic fibers are relatively rare). In addition, the outer layer of the capsule is denser. The relatively loose inner layer is contiguous to the connective tissue that surrounds the covers of individual elements of the portal triad. The thickness of the sectoral perivascular fibrous capsule is 45-110 μm (average 70-75 μm). Gradually, with the decrease in the caliber of the portal tract, the perivascular fibrous capsule also becomes thinner. The perivascular fibrous capsule of the 2-3 mm caliber subsegmental portal tract loses its sheath-like structure and transforms into loose connective tissue located between the individual elements of the portal triad.

The thickness of the proper cover of sectoral and segmental branches of the portal vein ranges from 50 μm to 150 μm (on average 90-100 μm) and it is directly proportional to the caliber of the blood vessel. The portal vein cover, within the subsegmental tract, gradually becomes thinner and looser. In addition, studies have shown that in 15% of cases, the identification of the connective tissue cover of the portal vein is hampered, even around the sectoral and segmental branches[32,37].

The number of bile ducts in sectoral and segmental portal tracts always exceeds three. Bile ducts are enveloped by the fibrous parabiliary sheath. The sheath has circularly oriented internal bundles, while the external bundles form septa oriented in various directions and connect closely to both the adjacent bile duct wall and the perivascular fibrous capsule. Bile ducts are accompanied by the peribiliary glands, which are connected to the ducts mainly along their opposite edges. The glands can be distinguished between intramural and extramural parts. The extramural part of the glands is several times larger in size than the intramural part. It is covered by the fibers of the fibrous parabiliary sheath, extends a considerable distance from the duct wall, is closely related to the connective tissue sheaths of other elements of the portal complex, and sometimes directly attaches to the perivascular fibrous capsule. Occasionally, the fibers covering the peribiliary glands and that of the internal surface of the perivascular capsule are so intertwined that no border can be identified between them[32,38-40].

The number of branches of the hepatic artery with a caliber larger than 1 mm varies from 2 to 5 in each sectoral and/or segmental portal tract. They are located more centrally (closer to the portal vein branch) than the bile ducts. The covers of the hepatic artery are not as distinct in sectoral and segmental tracts as in the hepatic hilus or hepatoduodenal ligament. The paravasal fissure is invisible as the adventitia is virtually contiguous with its own cover. The covers of the arteries at the peripheral edges of the blood vessels extend into the septa, which often interconnect and create the circular layer of para-arterial connective tissue located between the portal vein and the parabiliary fibrous sheath (Figure 1C). The degree of differentiation of the connective tissue covers of the arteries strongly depends on the caliber of the portal tract. In the small (subsegmental and thinner) portal tracts, the arteries have no connective tissue covers at all, and they are surrounded only by loose connective tissue that forms a bed for all elements of the portal triad[32,37]. Therefore, a combination of paravasal and parabiliary connective-tissue formations concentrated around the portal vein makes the skeletons of the hepatic portal tracts. The perivascular fibrous capsule, with adjacent parabiliary tissue with bile ducts and peribiliary glands, is located on the periphery of Glissonean pedicles[32,37].

PCFCs

In the liver, at the site of the spatial intersection of the main portal tracts and the hepatic veins, there is a little-known anatomical formation generated by the fusion of the connective-tissue fibrous sheaths of the portal tracts and the hepatic veins where these two structures come into contact with each other. The perivascular fibrous capsule extends from the portal complex to the wall of the hepatic vein and it becomes an additional element (Figure 1A, B, D and E). An anatomical formation created by the fusion of the sheaths of portal tracts and hepatic veins is called the intrahepatic PCFC [20,32].

Anatomical classification of PCFCs

Various forms of PCFC are distinguished.

Complete fusion: This type of porta-caval connection is characterized by the complete fusion of the surfaces of connective tissue sheaths of the portal tract and hepatic vein directed towards each other (Figure 1D and 2B). This type of connection is mainly found in segments II and III of the liver. The connective tissue sheaths of the hepatic veins are highly developed in the PCFC area, and its thickness reaches 90 μm. It represents a thick network of the collagen fibers running in various directions and the spiral bundles of elastic fibers and separate cellular elements are located between them. At the same time, irrespective of the density of the elements of the portal triad that merge with the hepatic veins in the area of the PCFC, there is always a narrow gap between them, filled with loose connective tissue. Small blood vessels (up to 1.5 mm in diameter), which are separated from the branches of the hepatic artery located in the portal tract, might pass through this place. They extend to the wall of the hepatic vein and supply it with blood.

Touching connection: This type of PCFC occurs when the perivascular fibrous capsule and the sheath of the hepatic vein merge only with the parts of the surface facing each other, while the rest of the space between them is filled with liver tissue. Similar to complete fusion, this form of PCFC also contains small blood vessels, but rarely the nerves or lymphatics. Touching PCFCs are often found within segments II, III, VI, and VII of the liver.

Fan-shaped connection: The fan-shaped connection, a special form of connection, is formed when the 2-5 mm caliber portal tract touches the wall of the inferior vena cava or large hepatic vein and immediately splits into thinner branches. The fan-shaped PCFC is constantly found within segment I (caudal lobe), including the inferior vena cava wall. The branches feeding the wall of the inferior vena cava or large caliber hepatic veins are separated from the arteries of the portal tract within this connection [20,32]. Within the complete fusion, touching and fan-shaped PCFCs, the hepatic vein is most often bordered by the bile ducts and their peribiliary glands. Such direct contacts may facilitate the spread of the inflammatory process from the bile ducts to the liver[32].

Plate and thread-shaped connections: The plate or thread-shaped PCFCs are represented by a fibrous plate or a cone that stretches between the perivascular fibrous capsule and the hepatic venous sheath. The plate may contain small blood and lymphatic vessels[20,32] (Figure 1E and 2A).

It should also be noted that the presence of various forms of PCFC has been confirmed in other mammals (pigs, sheep, dogs, rats). In the histological liver specimens of these animals, the sites of the crossing of different size portal tracts and hepatic vein tributaries with integration (fusion) of their connective-tissue sheaths were described. At the same time, in rat livers, the translocation of biliary structures from the portal tract toward hepatic veins was shown. This translocation causes the appearance of ductular profiles accompanying hepatic veins and their tributaries on histological specimens[32] (Figure 2C and D).

Clinical significance of PCFCs

Today, among the modern methods of surgical treatment of portal hypertension complicated by bleeding from varicose veins, the transjugular method of intrahepatic porta-caval anastomosis, which has a palliative effect, is widely used[41]. However, this method is often accompanied by complications; the most common ones are thrombotic or proliferative occlusion of the endoprosthetic shunt implanted between the branches of portal and hepatic veins, as well as stent migration-transposition[20]. This is exacerbated by the fact that the tubular shunt-prosthesis is often placed between the right branch of the portal vein and the right hepatic vein, which are

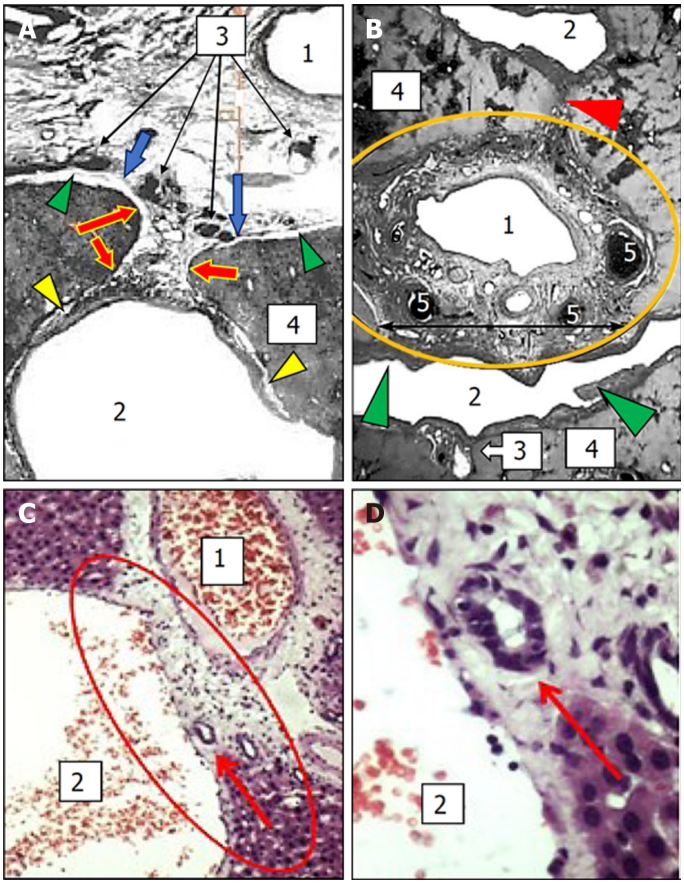

Figure 2 Porta-caval fibrous connections in humans and animals. A: Plate-shaped porta-caval fibrous connection (PCFC) (histotopogram of liver tissue): 1: Lumen of the portal vein; 2: Lumen of hepatic vein tributary; 3: Bile ducts and biliary glands (filled with Indian ink); 4: Liver parenchyma; Rad arrow: Proper hepatic capsule (PHC); Blue arrow: Perivascular fibrous capsule (Glisson's capsule); Green arrowhead: Fissure among the PHC and Glisson's capsule; Yellow arrowhead: Fissure among the PHC and perivenous connective-tissue sheath (preparation from the private archive of Professor Chanukvadze I); B: PCFCs (histotopogram of liver tissue): Large portal tract is surrounded by a yellow ellipse; 1: Lumen of the portal vein; 2: Lumen of hepatic vein tributaries; 3: Small portal tract; 4: Liver parenchyma; 5: Bile ducts (filled with Indian ink); Green arrowhead: The area of complete fusion; Red arrowhead: Thread-shaped PCFC (preparation from the private archive of Professor Ilya Chanukvadze); C: PCFC in rat liver (surrounded by a red ellipse). 1: Lumen of the portal vein; 2: Lumen of a hepatic vein; Red arrow: Bile ductule abutted to hepatic vein connective tissue sheath (preparation from the private archive of Professor Dimitri Kordzaia). Hematoxylin-eosin, Ob × 10, Oc × 10; D: Fragment of Figure C. Hematoxylin-eosin, Ob × 40, Oc × 10. C and D: Citation: Kordzaia D, Jangavadze M. Unknown bile ductuli accompanying hepatic vein tributaries (experimental study). Georgian Med News 2014: 121-129. Copyright ©Georgian Medical News 2014. Published by Georgian Medical News[43].

significantly separated from each other (from 2 cm to 9 cm). The longer the shunting prosthesis is, the higher the likelihood of thrombosis, suppression and/or transposition[41,42].

It is quite probable that the endovascular method may be more successful in developing portocaval anastomoses in the area of PCFCs, where parenchyma-free areas of direct contact between the walls of large branches (5 mm to 20 mm) of the hepatic and portal veins already exist. It is preferable to perform endovascular intervention on liver segments II and III, where the left hepatic vein passes below the main portal complex and is in direct contact with the portal vein branch, as well as between the right hepatic vein and the portal vein branch of segment VII. The various types of branching of the portal and caval veins determine a large variation in the number of PCFCs — from 4 to 20; however, despite this, the above-mentioned PCFCs in segments III and VII are characterized by high stability. In addition, the sites of integration within the connective-tissue sheaths of the large portal tracts and hepatic veins with the standard topography can be visualized by magnetic resonance imaging [20].

CONCLUSION

In the human liver where the portal tracts and hepatic veins spatially intersect (spatial crossing), the fusion of their connective-tissue sheaths develops an anatomical structural element in the form of a nodal fibrous connection — "porta-caval fibrous

connection" – allowing the hepatic vein to interact closely with the elements of the portal complex. The PCFC is a stable structure, whose formation begins at the 11th-12th week of embryogenic development. Based on the above discussion, intrahepatic PCFC can be considered an independent anatomical element of the liver, which deserves to be reflected in international anatomical nomenclature. Knowledge of the existence and features of PCFC enhance our understanding of the liver connective tissue framework and support the development of new surgical approaches for the treatment of various liver pathologies.

REFERENCES

1 **Rojkind M**, Ponce-Noyola P. The extracellular matrix of the liver. *Coll Relat Res* 1982; **2**: 151-175 [PMID: 7049553 DOI: 10.1016/s0174-173x(82)80031-9]
2 **Martinez-Hernandez A**, Amenta PS. The hepatic extracellular matrix. I. Components and distribution in normal liver. *Virchows Arch A Pathol Anat Histopathol* 1993; **423**: 1-11 [PMID: 8212529 DOI: 10.1007/BF01606425]
3 **Ikeda T**, Okano S, Hashimoto N, Kimura K, Kudo K, Tsutsumi R, Sasaki S, Kawasaki J, Miyashita Y, Wada H. Histomorphological investigation of intrahepatic connective tissue for surgical anatomy based on modern computer imaging analysis. *J Hepatobiliary Pancreat Sci* 2021; **28**: 76-85 [PMID: 32697892 DOI: 10.1002/jhbp.753]
4 **Lada E**, Anna M, Patrik M, Zbynek T, Miroslav J, Hynek M, Richard P, Sarah L, Vaclav L. Porcine Liver Anatomy Applied to Biomedicine. *J Surg Res* 2020; **250**: 70-79 [PMID: 32018145 DOI: 10.1016/j.jss.2019.12.038]
5 **Al-Samawy ER**, Waad SK, Hashim WS, Alabbas G. Comparative Histology of Human, Rats and Rabbits Liver. *Indian J Public Heal Res Dev* 2019; **10**: 1441 [DOI: 10.5958/0976-5506.2019.01134.3]
6 **Ota N**, Hirose H, Kato H, Maeda H, Shiojiri N. Immunohistological analysis on distribution of smooth muscle tissues in livers of various vertebrates with attention to different liver architectures. *Ann Anat* 2021; **233**: 151594 [PMID: 32911068 DOI: 10.1016/j.aanat.2020.151594]
7 **Patarashvili L**, Azmaipharashvili E, Jandieri K, Gvidiani S, Tsomaia K, Kikalishvili L, Sareli M, Chanukvadze I, Kordzaia D. Liver extracellular matrix peculiarities in mammals and avians. *Georgian Med News* 2021; 124-133 [PMID: 33658421]
8 **Hu Y**, Shi J, Wang S, Zhang W, Sun X, Sun B, Yu D. Laennec's approach for laparoscopic anatomic hepatectomy based on Laennec's capsule. *BMC Gastroenterol* 2019; **19**: 194 [PMID: 31752706 DOI: 10.1186/s12876-019-1107-9]
9 **Morimoto M**, Tomassini F, Berardi G, Mori Y, Shirata C, Abu Hilal M, Asbun HJ, Cherqui D, Gotohda N, Han HS, Kato Y, Rotellar F, Sugioka A, Yamamoto M, Wakabayashi G; Study group of Precision Anatomy for Minimally Invasive Hepato-Biliary-Pancreatic surgery (PAM-HBP surgery). Glissonean approach for hepatic inflow control in minimally invasive anatomic liver resection: A systematic review. *J Hepatobiliary Pancreat Sci* 2021 [PMID: 33528877 DOI: 10.1002/jhbp.908]
10 **Morimoto M**, Matsuo Y, Ueda G, Kato T, Aoyama Y, Hayashi Y, Omi K, Imafuji H, Saito K, Tsuboi K, Ogawa R. Exploring the Fine-Layer Structure Around a Glissonean Pedicle in Cadaveric Models. *Surg Gastroenterol Oncol* 2020; **25**: 67-72 [DOI: 10.21614/sgo-25-2-67]
11 **Navarro-Tableros V**, Herrera Sanchez MB, Figliolini F, Romagnoli R, Tetta C, Camussi G. Recellularization of rat liver scaffolds by human liver stem cells. *Tissue Eng Part A* 2015; **21**: 1929-1939 [PMID: 25794768 DOI: 10.1089/ten.TEA.2014.0573]
12 **Liu Y**, Yang R, He Z, Gao WQ. Generation of functional organs from stem cells. *Cell Regen* 2013; **2**: 1 [PMID: 25408873 DOI: 10.1186/2045-9769-2-1]
13 **Lee JS**, Shin J, Park HM, Kim YG, Kim BG, Oh JW, Cho SW. Liver extracellular matrix providing dual functions of two-dimensional substrate coating and three-dimensional injectable hydrogel platform for liver tissue engineering. *Biomacromolecules* 2014; **15**: 206-218 [PMID: 24350561 DOI: 10.1021/bm4015039]
14 **Felgendreff P**, Schindler C, Mussbach F, Xie C, Gremse F, Settmacher U, Dahmen U. Identification of tissue sections from decellularized liver scaffolds for repopulation experiments. *Heliyon* 2021; **7**: e06129 [PMID: 33644446 DOI: 10.1016/j.heliyon.2021.e06129]
15 **Yamamoto M**, Katagiri S, Ariizumi S, Kotera Y, Takahashi Y. Glissonean pedicle transection method for liver surgery (with video). *J Hepatobiliary Pancreat Sci* 2012; **19**: 3-8 [PMID: 21938411 DOI: 10.1007/s00534-011-0443-0]
16 **ELIAS H**, PETTY D. Gross anatomy of the blood vessels and ducts within the human liver. *Am J Anat* 1952; **90**: 59-111 [PMID: 14902689 DOI: 10.1002/aja.1000900104]
17 **Ostroverkhov**, G Zabrodskaya V. Surgical anatomy of the liver and biliary tract. In: Maksimenkov A. Surgical anatomy of the abdomen. Leningrad: 1972: 297-380
18 **Fegershanu N**, Ionescu-Bujar K, Aloman D, Albu A. Surgery of the liver and intrahepatic biliary tract. Bucharest: 1976
19 **Dancygier H**. Clinical Hepatology. Berlin, Heidelberg: Springer Berlin Heidelberg, 2010
20 **Chanukvadze I**. Portacaval Fibrous Connections: Little Known Anatomical Structures of Liver. *Rom Med J* 2017; **64**: 43-48

21 **Sugioka A**, Kato Y, Tanahashi Y. Systematic extrahepatic Glissonean pedicle isolation for anatomical liver resection based on Laennec's capsule: proposal of a novel comprehensive surgical anatomy of the liver. *J Hepatobiliary Pancreat Sci* 2017; **24**: 17-23 [PMID: 28156078 DOI: 10.1002/jhbp.410]

22 **Kawarada Y**, Das BC, Taoka H. Anatomy of the hepatic hilar area: the plate system. *J Hepatobiliary Pancreat Surg* 2000; **7**: 580-586 [PMID: 11180890 DOI: 10.1007/s005340070007]

23 **Poonkhum R**, Pisetpaisan K, Wang BJ, Anupunpisit V, Ohtani Y, Ohtani O. Origins and pathways of fluid entering sublobular lymphatic vessels in cat livers. *Arch Histol Cytol* 2003; **66**: 317-326 [PMID: 14692687 DOI: 10.1679/aohc.66.317]

24 **Ohtani O**, Ohtani Y. Lymph circulation in the liver. *Anat Rec (Hoboken)* 2008; **291**: 643-652 [PMID: 18484610 DOI: 10.1002/ar.20681]

25 **Medeiros de Mello J**, Orsi AM, Piffer CR, Torrejais MM. Architecture of the caudal vena cava wall of the dog at the level of the liver caudate lobe. *Ann Anat* 2000; **182**: 525-528 [PMID: 11125802 DOI: 10.1016/S0940-9602(00)80096-5]

26 **Karau PB**, Ogeng'o JA, Hassanali J, Odula PO. The histormorphological organization of the hepato-caval interface in the human. *J Morphol Sci* 2010; **27**: 148-151

27 **Takasaki K**, Yamamoto M. Surgical Anatomy of the Liver in the Glissonean Pedicle Approach: What We Need to Know. In: Madoff D, Makuuchi M, Nagino M, Vauthey JN. Venous Embolization of the Liver. London: Springer London, 2011: 23-27

28 **Crawford AR**, Lin XZ, Crawford JM. The normal adult human liver biopsy: a quantitative reference standard. *Hepatology* 1998; **28**: 323-331 [PMID: 9695993 DOI: 10.1002/hep.510280206]

29 **Hayashi S**, Murakami G, Ohtsuka A, Itoh M, Nakano T, Fukuzawa Y. Connective tissue configuration in the human liver hilar region with special reference to the liver capsule and vascular sheath. *J Hepatobiliary Pancreat Surg* 2008; **15**: 640-647 [PMID: 18987936 DOI: 10.1007/s00534-008-1336-8]

30 **Couinaud C**. Surgical anatomy of the liver revisited. Paris: Semantic Scholar, 1989: 29-39

31 **Couinaud C**. Le foie; études anatomiques et chirurgicales. Masson, 1957

32 **Kordzaia D**, Chanukvadze I, Jangavadze M. Functional Anatomy of Intrahepatic Biliary System (Clinical and Experimental Data). In: Miguel Ángel Mercado. Bile Duct: Functional Anatomy, Disease and Injury Classification and Surgical Management. Nova Science Publishers Inc, 2014: 1-87

33 **Guglielmi A**, Ruzzenente A, Iacono C. Surgical Anatomy of the Hepatic Hilus. In: Guglielmi A, Ruzzenente A, Iacono C. Surgical Treatment of Hilar and Intrahepatic Cholangiocarcinoma. Milano: Springer, 2008: 101-111

34 **Kuntz E**, Kuntz H-D. Hepatology Textbook and Atlas. *Hepatol Textb Atlas* 2008 [DOI: 10.1007/978-3-540-76839-5]

35 **Kovanov V**, Anikina M. Surgical anatomy of human paravasal connective tissue structures [in Russian]. *Moscow Med* 1985

36 **Strasberg SM**, Belghiti J, Clavien P-A, Gadzijev E, Garden JO, Lau W-Y, Makuuchi M, Strong RW. The Brisbane 2000 Terminology of Liver Anatomy and Resections. *HPB* 2000; **2**: 333-339 [DOI: 10.1016/s1365-182x(17)30755-4]

37 **Chanukvadze I**. Vasculo-fibrous architecture of the magistral portal complexes. *Rom Med J* 2016; **11**: 214-218

38 **de Jong IE**, van Leeuwen OB, Lisman T, Gouw AS, Porte RJ. Repopulating the biliary tree from the peribiliary glands. *Biochim Biophys Acta Mol Basis Dis* 2018; **1864**: 1524-1531

39 **Nakanuma Y**, Katayanagi K, Terada T, Saito K. Intrahepatic peribiliary glands of humans. I. Anatomy, development and presumed functions. *J Gastroenterol Hepatol* 1994; **9**: 75-79 [PMID: 8155872 DOI: 10.1111/j.1440-1746.1994.tb01220.x]

40 **Nakanuma Y**, Sasaki M, Terada T, Harada K. Intrahepatic peribiliary glands of humans. II. Pathological spectrum. *J Gastroenterol Hepatol* 1994; **9**: 80-86 [PMID: 8155873 DOI: 10.1111/j.1440-1746.1994.tb01221.x]

41 **Nolte W**, Manke H, Schndler C. Dopplerronographsche kurz und landzeitunterrsuchunger der patalen Hamodynamik nach transjugular intrahepatischem portosistemischem Stent-Shunt. *Ztsch fur Gastroenterol* 1998; 491-499

42 **Richter GM**, Roeren T, Brado M, Theilmann L, Sauer P, Kauffmann GW. [Portal hypertension and percutaneous transjugular portasystemic stent shunt]. *Chirurg* 1995; **66**: 555-565 [PMID: 7664584]

43 **Kordzaia D**, Jangavadze M. Unknown bile ductuli accompanying hepatic vein tributaries (experimental study). *Georgian Med News* 2014; 121-129 [PMID: 25341252]

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REVIEW

Promising diagnostic biomarkers of nonalcoholic fatty liver disease and nonalcoholic steatohepatitis: From clinical proteomics to microbiome

Carolina Castillo-Castro, Alexandro José Martagón-Rosado, Rocio Ortiz-Lopez, Luis Felipe Garrido-Treviño, Melissa Villegas-Albo, Francisco Javier Bosques-Padilla

ORCID number: Carolina Castillo-Castro 0000-0001-6541-3779; Alexandro José Martagón-Rosado 0000-0003-2135-6295; Rocio Ortiz-Lopez 0000-0002-7783-026X; Luis Felipe Garrido-Treviño 0000-0003-0786-6176; Melissa Villegas-Albo 0000-0003-3003-532X; Francisco Javier Bosques-Padilla 0000-0002-9795-7209.

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Carolina Castillo-Castro, Alexandro José Martagón-Rosado, Rocio Ortiz-Lopez, Luis Felipe Garrido-Treviño, Melissa Villegas-Albo, Francisco Javier Bosques-Padilla, Tecnológico de Monterrey, Escuela de Medicina y Ciencias de la Salud, Monterrey 64710, Mexico

Alexandro José Martagón-Rosado, Unidad de Investigación de Enfermedades Metabólicas, Instituto Nacional de Ciencias Médicas y Nutrición, Ciudad de México 14080, Mexico

Francisco Javier Bosques-Padilla, Centro Regional para el Estudio de las Enfermedades Digestivas, Servicio de Gastroenterología, Facultad de Medicina y Hospital Universitario Dr. José Eleuterio González, Universidad Autónoma de Nuevo León, Monterrey 64460, Mexico

Corresponding author: Francisco Javier Bosques-Padilla, PhD, Full Professor, Centro Regional para el Estudio de las Enfermedades Digestivas, Servicio de Gastroenterología, Facultad de Medicina y Hospital Universitario Dr. José Eleuterio González, Universidad Autónoma de Nuevo León, Ave. Madero y Gonzalitos S/N, Col. Mitras, Monterrey 64460, Mexico. fbosques58@hotmail.com

Abstract

Fatty liver has been present in the lives of patients and physicians for almost two centuries. Vast knowledge has been generated regarding its etiology and consequences, although a long path seeking novel and innovative diagnostic biomarkers for nonalcoholic fatty liver disease (NAFLD) and nonalcoholic steatohepatitis (NASH) is still envisioned. On the one hand, proteomics and lipidomics have emerged as potential noninvasive resources for NAFLD diagnosis. In contrast, metabolomics has been able to distinguish between NAFLD and NASH, even detecting degrees of fibrosis. On the other hand, genetic and epigenetic markers have been useful in monitoring disease progression, eventually functioning as target therapies. Other markers involved in immune dysregulation, oxidative stress, and inflammation are involved in the instauration and evolution of the disease. Finally, the fascinating gut microbiome is significantly involved in NAFLD and NASH. This review presents state-of-the-art biomarkers related to NAFLD and NASH and new promises that could eventually be positioned as diagnostic resources for this disease. As is evident, despite great advances in studying these biomarkers, there is still a long path before they translate into clinical benefits.

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Core Tip: Nonalcoholic fatty liver disease is increasing in prevalence worldwide. Liver biopsy is considered the gold standard for diagnosis, but it has several limitations. Given the burden on the healthcare system caused by liver fibrosis in a population with metabolic syndrome, there is a priority for noninvasive and accurate diagnostic biomarkers that differentiate patients with steatosis from those with nonalcoholic steatohepatitis, stage fibrosis, predict progression, and monitor treatment response. These biomarkers could assist clinicians in early interventions, avoiding complications and improving prognosis. Here, we summarize the current evidence and future directions.

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INTRODUCTION

Thomas Addison first described "fatty liver" in 1836 in England; however, it was not until 1885 when Bartholow made an association between obesity and fatty liver. In 1938, Charles Connor demonstrated a link between fatty liver and progression to cirrhosis in diabetic patients. Throughout the 1950s and up to the 1970s, pathologists reported similarities between alcoholic liver disease and hepatic histological changes in obese and diabetic patients. In 1980, Jurgen Ludwig[1] described patients who denied excessive alcohol consumption yet still had chronic liver disease and histological characteristics of alcoholic fatty liver disease. There was no name for the disease, so Ludwig coined the terms nonalcoholic fatty liver disease (NAFLD) and nonalcoholic steatohepatitis (NASH)[1].

As reported in the most recent guidelines, NAFLD is defined as the presence of steatosis in > 5% of hepatocytes in the absence of significant ongoing or recent alcohol consumption and other known causes of liver disease. While in 2005 it had a global prevalence of 15%, a rapid increase in sedentarism and excessive calorie intake independent of diet has pushed it to 24%, with the highest rates in South America (31%) and the Middle East (32%), followed by Asia (27%), the United States (24%), and Europe (23%)[2]. In persons with obesity or type 2 diabetes, it increases up to 70%-90% [3]. Although there is a significant difference between ethnicities within these populations, the exact explanation remains unknown[2].

NAFLD is a necessary and opportune diagnosis, given that 59% progress to NASH. From this stage, 41% continue to fibrosis, with 40% ending with cirrhosis, increasing their risk of a liver transplant, cardiovascular disease, and mortality if there are no interventions[4]. In our country, the Mexican population has several risk factors for the disease because there is a high incidence of overweight and obesity[5], making the NAFLD prevalence likely to surpass 50%. Up to 82% of obese patients who have undergone bariatric surgery present NAFLD, alongside 36% of women with obesity[6].

An international panel has now proposed to rename the disease metabolic dysfunction-associated fatty liver disease to represent the hepatic manifestation of a multisystemic disorder. Until now, the diagnosis was reached by the exclusion of other liver diseases; however, as the pathogenesis is better understood, it is now perceived as a distinct disease and requires a positive diagnosis, which is why it is proposed that the criteria be based on histological, imaging, or blood biomarker evidence of fat accumulation in the liver in addition to one of the following three: Overweight/obesity, type 2 diabetes mellitus, or evidence of metabolic dysregulation (at least two metabolic risk abnormalities)[1].

Today, the liver biopsy remains the gold standard for diagnosing and monitoring liver disease, with the disadvantage of being a costly and invasive procedure[7], which is why it is important to look into possible new noninvasive diagnostic tools, such as biomarkers, use of transcriptomics, proteomics, metabolomics, and now "glycomics" [8]. These should aid in predicting liver disease severity, progression, and response to lifestyle changes and pharmacological treatment[9]. The objective of this article is to review concisely and present the potential diagnostic biomarkers for NAFLD and NASH (Figure 1).

PROTEOMICS

The concentrations of several plasma components are determined in routine clinical practice, including electrolytes, molecules, and proteins. Plasma proteins, which constitute the plasma proteome, are released as a result of inflammation, apoptosis, and oxidative stress (OS)[10]. Mass spectrometry-based proteomics[9] and two-dimensional electrophoresis are powerful tools for studying differences[11] in the plasma proteome. There are differences in protein expression among patients with NAFLD and healthy controls. Proteomics technologies have gained relevance as potential non-invasive diagnostic methods for NAFLD.

Plasma proteomics

Plasma proteomics may be secreted by the liver or as a result of the response of the host to steatosis. Hemoglobin is currently the most replicated proteomic biomarker in NAFLD[12]. Studies have found that higher hemoglobin levels are associated with a higher incidence of NAFLD[12]. Circulating aminotransferase [aspartate aminotransferase (AST) and alanine aminotransferase (ALT)] levels are markers of several liver diseases, including NASH. Changes in these enzymes are one of the most commonly observed abnormalities[10].

Fibroblast growth factor 21 is another protein secreted in response to peroxisome proliferator-activated receptor (PPAR)-α activation, and several studies support its potential use as a biomarker for NAFLD[13,14]. The elevation of retinol-binding protein 4 has also been associated with liver fat accumulation[15]. Some glycoproteins like serum fucosylated haptoglobin and Mac-2 binding protein are predictors of hepatocyte ballooning and liver fibrosis[16].

Cytokeratin-18 fragments, such as CK18Asp396, are other proteins that have been extensively studied. These are produced during apoptosis (M30) or cell death (M65). CK18 is the most reviewed biomarker to evaluate liver inflammation[15], but current knowledge does not support its use in clinical practice[17] because of its modest accuracy[8].

Increased cytokeratin-18 levels have good predictive value for NASH *vs* normal livers but do not differentiate NASH *vs* simple steatosis[18,19]. Cytokeratin-18 serum levels decrease parallel with histological improvement, but its predictive value is not better than ALT in identifying histological responders[20].

Circulating concentrations of cytokeratin-18 fragments were proposed as the most reliable predictors of NASH in patients with NAFLD[21].

Circulating extracellular vesicles

Another important plasma component includes circulating extracellular vesicles (EVs), which are small cell-derived membrane-surrounded structures enclosed by a phospholipid bilayer, with a specific cargo of bioactive molecules of cell origin. There are three types according to their size: Exosomes (40-100 nm), microvesicles or microparticles (0.1-1 μm), and apoptotic bodies (1-4 μm)[22].

They can be detected in several body fluids and can serve several functions by delivering a variety of bioactive molecules, including non-coding RNAs, proteins, lipids, and nucleic acids[23]. Recent studies have provided insight on the bioavailability of circulating EVs in various fluids and, as a consequence, on their potential use as biomarkers for various diseases such as cancer[20,24,25], cardiovascular disease [26], renal disease[27], and liver disease[28,29].

Some authors consider them noninvasive "liquid biopsies" for NASH diagnosis, and studies suggest they can assess disease severity[30]. Serum levels of total and hepatocyte-derived EVs correlate with NASH clinical characteristics, and disease severity in experimental models of NASH, liver and blood levels of EVs are increased and correlate positively with changes in liver histology[31].

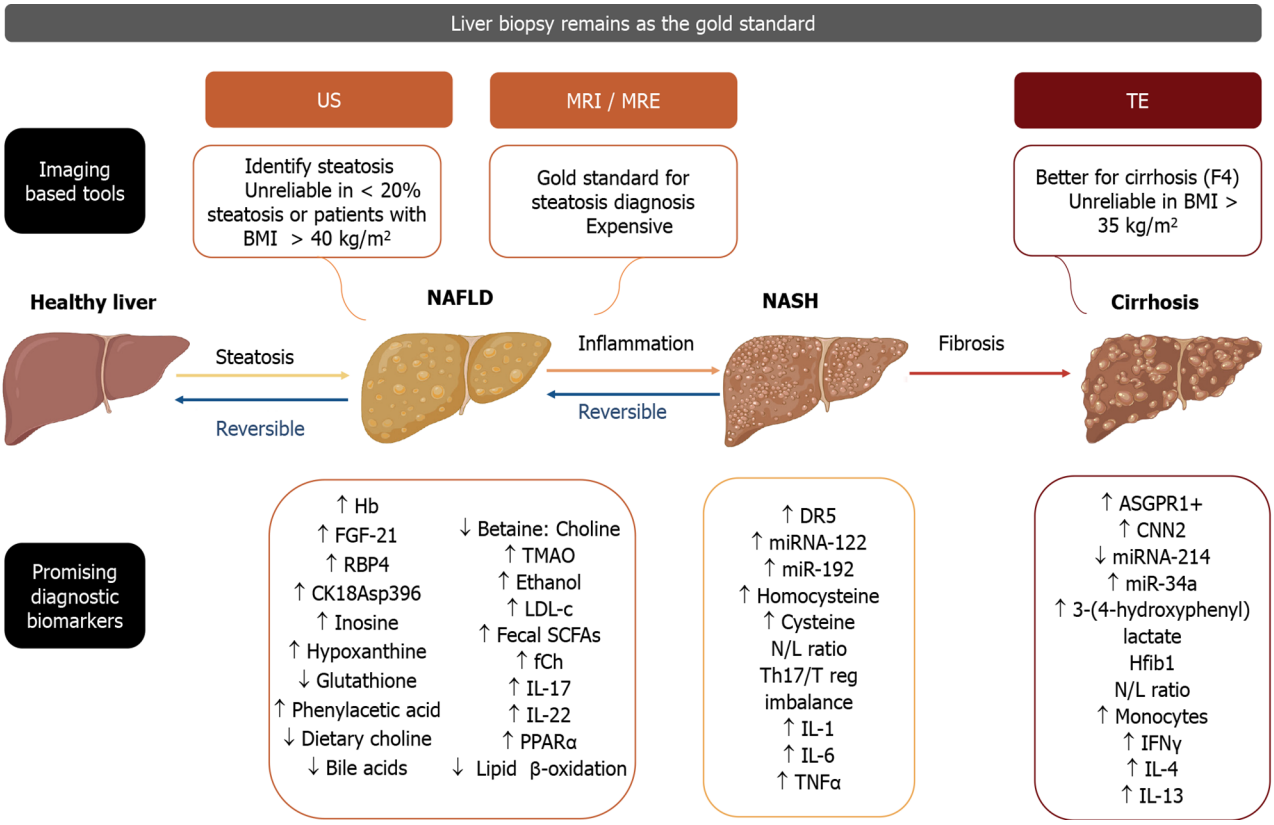

Figure 1 Although liver biopsy remains as the gold standard for the diagnosis of nonalcoholic fatty liver disease and nonalcoholic steatohepatitis, other current imaging studies are shown, along with promising diagnostic and/or monitoring biomarkers that may be present in each of the stages of hepatic pathology, ranging from reversible steatosis and inflammation to irreversible fibrosis and eventually cirrhosis (Figure 1 created with BioRender.com). US: Ultrasound; TE: Transient elastography; BMI: Body mass index; Hb: Hemoglobin; FGF-21: Fibroblast growth factor 21; RBP4: Retinol binding protein 4; CK18Asp396: Caspase cleaved cytokeratin-18 fragment; TMAO: Trimethylamine N-oxide; LDL-c: Low density lipoprotein cholesterol; Fecal SCFAs: Fecal Short chain fatty acids; fCh: Ferrochelatase; IL-17: Interleukin-17; IL-22: Interleukin-22; PPARα: Peroxisome proliferator-activated receptor α; DR5: Death receptor 5; miRNA-122: MicroRNA 122; miR-192: MicroRNA 192; N/L ratio: Neutrophil/lymphocyte ratio; Th17/Treg imbalance: T helper 17/T regulatory cells imbalance; IL-1: Interleukin-1; IL-6: Interleukin-6; TNFα: Tumor necrosis factor alpha; ASGPR1+: Asialoglycoprotein receptor 1; CNN2: Calponin 2; miRNA-214: MicroRNA 214; miR-34a: MicroRNA 34a; Hfib1: Hepatic fibrosis 1; N/L ratio: Neutrophil/lymphocyte ratio; IFNγ: Interferon γ; IL-4: Interleukin-4; IL-13: Interleukin-13.

Povero *et al*[30] performed a study isolating EVs from controls with histologically confirmed NASH without cirrhosis and patients with cirrhotic NASH[30]. After the characterization of EV structural features, they found that differences in the quantity and protein components of circulating EVs could be potentially useful for differentiating patients with NASH from controls and patients with pre-cirrhotic NASH from patients with cirrhotic NASH[30].

Notably, asialoglycoprotein receptor 1-positive hepatocyte-specific EVs may represent a surrogate noninvasive biomarker of portal hypertension in patients with cirrhotic NASH. If confirmed, these findings may support the clinical utility of asialoglycoprotein receptor 1-positive EVs (hepatocyte-specific EVs) as a potential alternative to an invasive hepatic venous pressure gradient[30].

Patients with NAFLD or NASH secrete increased levels of microvesicles derived from macrophages/monocytes [CD14(+)] and natural killer (NK) T cells; these levels correlate with NASH severity based on histology[28]. Hirsova *et al*[32] have demonstrated that lipids that stimulate death receptor 5 on hepatocytes also induce the release of hepatocyte EVs that activate an inflammatory phenotype in macrophages that lead to NASH[32].

However, a major problem in translating this research into clinically useful information is a lack of reproducibility and rigorous criteria for reporting these biomarkers. Proteomics analysis of EVs from patients with advanced NASH is currently limited.

Exosomes

Exosomes are a type of EVs secreted in most cells[22]. These nanovesicles of endocytic origin are present in nearly all-human fluids. Exosomes have several bioactive

molecules, including proteins, lipids, and genetic materials[33]. They are conduits for intracellular transfer, and their signals can induce fibrosis, macrophage activation, cytokine secretion, and remodeling extracellular matrix (ECM) production and inactivate hepatic stellate cells (HSC)[34]. Hepatocytes are exosome-secreting cells that are also regulated by hepatic and extrahepatic exosomes[33].

Koeck *et al*[35] found that exosomes from visceral adipose tissue were involved in the progression of NAFLD by inducing dysregulation of the transforming growth factor-beta (TGF-β) pathway in hepatocytes and HSCs *in vitro*[35]. Another study by Seo *et al*[36] detected that during liver injury, damaged hepatocytes produce exosomes that activate toll-like receptor 3, which exacerbates liver fibrosis by enhancing interleukin-17A (IL-17A) production by γδ T cells[36].

Liver fibrogenic pathways are primarily controlled by HSC, which produces and responds to fibrotic mediators such as connective tissue growth factor (CCN2)[37]. Tadokoro *et al*[29] found that CCN2 upregulation in fibrotic or steatotic livers is associated with the downregulation of microRNA-214 (miRNA-214). miR-214 levels increased in quiescent HSC-secreted exosomes compared with active HSC-released exosomes[29]. On the other hand, exosomal CCN2 may amplify fibrogenic signaling and might be useful for assessing hepatic fibrosis[37].

Chen *et al*[38] found that the miR-214 promoter binds to the basic helix-loop-helix transcription factor (Twist1), which drives miR-214 expression and results in CNN2 suppression. Twist 1 expression was suppressed during HSC activation. The amounts of Twist1, miR-214, or CCN2 in circulating exosomes from fibrotic mice reflected fibrosis-induced changes in the liver[38]. These findings suggest that during liver fibrosis, exosomes contain specific types of biomarkers, which could be helpful in the diagnosis and progression of liver diseases.

miRNA

Circulating microRNAs (miRNA) are RNA molecules that do not encode proteins but regulate gene expression in the body, binding to target mRNAs and interfering with their translation[22]. They are expressed in several liver cell types and may offer a biologically stable blood-based biomarker tool for the detection and stratification of liver disease[29].

Tadokoro *et al*[29] have suggested that serum/plasma miR-122 correlates with liver damage. They have also identified that miR-155 might serve as a liver inflammation biomarker. The one limitation found is that this miRNA cannot differentiate different liver damage etiologies[29].

Another study reported that miRNA-122 and miR-192 levels are dynamic and increase over time, closely correlating with the histopathological severity of NASH[31]. The miR-29 family (miR-29a, miR-29b, miR-29c) mediates the regulation of liver fibrosis through several cellular signaling pathways such as the nuclear transcription factor-kappa B pathway, TGF, and phosphatidylinositol 3-kinase/AKT signaling in HSC with upregulation of ECM genes for the progression of liver fibrosis[39].

Members of the miR-34 family (miR-34a, miR-34b, miR-34c) have pleiotropic roles in the cell cycle and promote the progression of hepatic fibrosis by activation of HSC[39]. miR-34a appears to have an important role in liver fibrosis by regulating the deposition of ECM[40]. miR-30c and miR-193 are also involved in fibrotic remodeling processes that modify the TGF-β-dependent regulation of ECM-related genes in HSCs [41].

The miR-15 family mainly regulates the TGF-β pathway. The activation of HSCs relates to miR15a and miR15b, and they are thought to be essential for apoptosis by targeting Bcl-2 and the caspase signaling pathway[42]. The miR-378 family (specially miR-378a-3p) suppresses the activation of HSCs by directly targeting Gli3[43]. miR-571 closely correlates with the liver cirrhosis stage, and it is upregulated in human hepatocytes and HSC[44]. miR-503 also acts on HSC activation and hepatic fibrosis through the TGF-β/SMAD pathway[45].

The miR-199 family and miR-200 family are responsible for ECM deposition and the release of profibrotic cytokines, which might play profibrotic or anti-fibrotic roles[39]. HSCs also have anti-fibrotic miRNAs, and these include miR-19b, miR-29, miR-30, miR-101, miR-122, miR-133a, miR-144, miR-146a, miR-150-5p, miR-155, miR-195, miR-200a, miR-214, miR-335, miR-370, miR-454, miR-483, *etc.* The latter are responsible for the maintenance of the quiescent phenotype of normal HSCs[46]. Thus, these studies evidence the role of microRNAs as potential biomarkers of liver damage in NAFLD.

METABOLOMICS

Technological advances in metabolomic analyses on feces, serum, plasma, urine, or liver biopsies led to identifying different metabolites in patients with NAFLD or NASH[47]. Recent studies have found that the severity of fibrosis is associated with serum metabolite changes[48-50].

Remarkably, some metabolites come from the host or the diet, but most need the participation of gut microbes. Notably, inosine and hypoxanthine are enriched in serum samples from patients with mild or moderate NAFLD[47]. Another study found that liver steatosis correlates with phenylacetic acid levels in humans[51]. Glutathione plasma concentration is significantly lower in subjects with liver steatosis, while in subjects with NASH, homocysteine and cysteine concentrations in plasma are higher [52].

Gut microbially-derived metabolomics

Choline, betaine, and circulating methylamines: Choline is an essential component of phosphatidylcholine (a precursor of acetylcholine), mostly obtained from the diet[53]. It is known that a reduction in dietary choline is related to an increase in liver fat. Mice fed with a choline deficient diet are identified as a characteristic model of NAFLD[54]. Choline can be oxidized to betaine, and it has been found that patients with increasing severity of NAFLD have a decreased betaine to choline ratio[55]. The gut microbiota metabolizes choline into trimethylamine (TMA), which is further metabolized into trimethylamine-N-oxide (TMAO) in the liver[56]. Studies suggest that NAFLD severity is associated with increased urinary levels of TMA and TMAO, while TMAO seems to be associated with NAFLD severity[47].

TMAO and bile acids: Gut microbiota regulates secondary bile acid metabolism and inhibits the liver synthesis of lipids by alleviating farnesoid X-activated receptor inhibition[57]. TMAO is a gut-dependent metabolite of choline. A decreased level of bile acids could be associated with TMAO production and NAFLD since it induces a decrease in the bile acid pool by inhibiting two key enzymes of bile acid metabolism: Cytochrome P450 (CYP)7A1 and CYP27A1[55]. Some studies have found adverse associations between the circulating TMAO levels and the presence and severity of NAFLD and a favorable betaine-NAFLD relationship in participants[55].

Three-(4-hydroxyphenyl) lactate: Three-(4-hydroxyphenyl) lactate is a derived product of amino acid metabolism. It was consistently associated with increased liver fibrosis severity in a test and validation cohort[48].

Ethanol: Gut microbiota leads to endogenous ethanol production, which might be a liver toxin involved in NAFLD and NASH development[47]. A study showed that *Klebsiella pneumoniae* can produce ethanol from glucose in the absence of alcohol consumption, and it might be associated with NAFLD[58].

LIPIDOMICS AND LIPOTOXICITY

Human serum and plasma are composed of lipids that play important roles in energy storage, metabolic regulation, signaling, *etc.*[10]. Technological advances have made possible the identification of specific alterations in lipids and metabolites in the feces, serum, plasma, urine, and liver of patients with NAFLD[47].

Choline is a dietary component metabolized in the liver, necessary for cell function. Epidemiological studies suggest that increased free choline levels are related to the degree of hepatic steatosis fibrosis[59].

Kalhan *et al*[60] have shown that plasma levels of triglycerides[60] and low-density lipoprotein cholesterol are higher in patients with NAFLD[52]; however, differences in this lipidomic profile are also observed in obesity. Therefore, this lack of specificity remains a limitation for their use. Barr *et al*[61] described a lipidomic signature associated with NAFLD progression to distinguish NASH from steatosis, depending on the body mass index in a large cohort of samples[61].

Gorden *et al*[62] described a panel of 20 lipids that differentiate patients with NASH and liver steatosis[62]. Later, Kimberly *et al*[63] identified the association between anandamide (endocannabinoid derived from arachidonic acid metabolism) and NAFLD severity[63]. Tokushige *et al*[64] reported 28 metabolites associated with liver fibrosis, showing a decrease of dehydroepiandrosterone sulfate and etiocholanolone-S with the progression of fibrosis[64].

Puri *et al*[65] analyzed plasma lipids and eicosanoid metabolites in NAFLD and NASH patients. They reported increased plasma monounsaturated fatty acids and primary palmitoleic and oleic acids and decreased linoleic acid. Plasmalogen levels were significantly decreased in NASH, and 11-HETE (a nonenzymatic product of arachidonic acid) was increased in NASH[65]. Loomba *et al*[66] assessed the lipidomic profile in NAFLD and NASH patients and reported that 11,12-dihydroxy- eicosatrienoic acid (11,12-diHETrE) was the best biomarker for differentiating NAFLD from NASH[66].

Short-chain fatty acids (SCFAs) are comprised of butyrate, acetate, and propionate. They are produced in the colon through microbial fermentation of dietary fiber and are a substrate that increases liver triglyceride levels[67]. They are also involved in fatty acid synthesis and gluconeogenesis[68]. Human studies have observed an increased fecal concentration of SCFAs in patients with NAFLD and/or NASH[69].

In NAFLD, lipid metabolism is disrupted, and lipotoxicity is a key mechanism for NAFLD progression. Lipidomic profiling might provide a novel biomarker for the noninvasive prediction of NASH.

GENETIC MARKERS

The role of genetic and epigenetic factors in the progression of liver fibrosis is well documented. It is known that key regulatory genes partially control the cell phenotype. Several genes are involved in the pathogenesis and histological stage of liver fibrosis, although the mechanisms underlying gene regulation are highly complex and need additional research[70].

Chromosome 15, designated Hfib1 (hepatic fibrogenic gene 1), affects the stage of liver fibrosis[71]. The core of risk genes that control fibrosis progression has been defined by quantitative trait locus analysis in mouse strains by genome-wide interval mapping, which identified several genomic loci related to fibrosis phenotypes on chromosomes 4, 5, 7, 12, and 17[72].

Bruschi *et al*[73] reported that PLPNA3 quantification correlates with the liver fibrosis stage. Expression of PLPNA3 in biopsies from NASH patients is increased during progression from mild to severe liver fibrosis. Carriers of the I148M single-nucleotide polymorphism (C>G) had higher PLPNA3 and serum liver enzyme (ALT/AST) levels, along with steatosis grade inflammation ballooning and NAFLD activity score, compared with non-polymorphism carriers[73]. On the other hand, Sharma *et al*[74] stated that neurocan is associated with NASH and liver fibrosis in patients of European ancestry. Another study found that patients of Indian descent with neurocan variations had higher ALT levels[74].

EPIGENETIC MARKERS

Epigenetics describes reversible gene expression changes that do not imply changes in the DNA sequence and are entirely cell type-specific. Epigenetic mechanisms initiate and sustain chromatin modifications by facilitating gene transcription, cell phenotype, and consequently, organ function. These mechanisms include DNA methylation, histone modifications, and noncoding RNAs mediating gene silencing[75].

Aberrant DNA methylation is associated with fibrosis. Komatsu *et al*[76] suggested that DNA hypomethylation in fibrogenic genes is crucial for the onset and progression of liver fibrosis[76]. Mann *et al*[77] confirmed this functional association of DNA methylation with liver fibrosis. The transdifferentiation of HSC to profibrogenic myofibroblast phenotype was suppressed *in vitro* by the DNMT inhibitor 5′-aza-deoxycytidine[77]. The development of fibrosis is also related to changes in the expression of enzymes that regulate DNA methylation and hydroxymethylation[78].

Epigenetic modulation on the PPAR-γ gene promoter is involved in HSC differentiation. Aberrant expression of a series of chemokines in HSCs aggravate inflammation and OS[79].

Small non-coding RNAs contribute to various pathologic states of liver disease, but miRNA has been previously reviewed. The detection of genetic and epigenetic markers may be helpful in the recognition and monitoring of disease evolution and can eventually be applied for targeted therapies.

IMMUNE DYSREGULATION

NASH pathology encompasses an intricate network of mechanisms. OS activates Kupffer cells (KC), and KC activation triggers an innate and adaptative immune response, including the release of cytokines and chemokines that activate NK T (NKT) cells and HSCs[80]. Besides, there is augmented infiltration of different immune cells, such as monocytes, T lymphocytes, and neutrophils, in the activation and *in situ* expansion of liver cells, like KC or stellate cells. Activated KC and NKT cells promote additional fat accumulation in the liver. KC, neutrophils, NKT cells, and inflammatory T cells [T helper (Th)1, Th17, CD8+ T cells] enhance liver inflammation and contribute to the development of fibrosis[81].

The neutrophil to lymphocyte ratio (N:L ratio) has been proposed as a novel noninvasive marker to predict NASH and advanced fibrosis in patients with NAFLD [82]. In patients with cirrhosis, these cells are functionally deficient, with impaired chemotaxis, phagocytosis, and intracellular killing. Their function correlates with 90-d survival[83].

On the other hand, monocytes are myeloid-derived cells that migrate to inflammation sites, phagocytose microbes, and secrete cytotoxins. They are spontaneously activated in patients with liver fibrosis. Cirrhotic patients have an increased peripheral frequency of monocytes, impaired phagocytosis, and reduced responses to stimulation [84].

Studies have reported that NK cells are dysregulated in liver diseases. One study found that IL-17- and IL-22- secreting iNKT cells are dominant at the beginning of liver steatosis, and IFNγ/IL-4/IL-13-secreting iNKT cells are prevalent at the most advanced course of the disease[85].

Notably, CD4+ T cells are reduced in patients with liver fibrosis. This finding could explain the increased risk of spontaneous bacterial peritonitis in these patients[86]. CD8+ T cells isolated from mice hepatic cells expressed an increased cytotoxic IL-10 phenotype and CD8+ T cell depletion[87].

Th17 cells and T regulatory cells (Treg) originate from naïve T cell precursors. Th17 cells are important for pathogen clearance and inflammation. Treg cells in patients with liver fibrosis are significant[88]. There is a Th17/Treg imbalance that positively correlates with NASH histological progression[89].

Innate lymphoid cells are lymphocytes that secrete cytokines and chemokines in response to pathogenic tissue damage. They have a role in inflammation and fibrogenesis that progresses with advancing chronic liver disease[90].

OS AND INFLAMMATION

Detoxification is a crucial hepatic activity. It is vulnerable to OS and inflammation. An increase in free fatty acids is critical for the elevation of reactive oxygen species (ROS). A balance between the ROS and antioxidant systems is necessary for adequate cell function[80]. OS causes liver damage by altering DNA molecules, proteins, and lipids and modulating pathways associated with gene transcription, protein expression, cell apoptosis, and HSC activation. Inflammation is manifested as inflammatory cell infiltration in the liver to fight pathogen invasion. When the stimuli are persistent, it can lead to cell injury and lipid accumulation associated with an increased risk of severe liver disease, including steatohepatitis and fibrosis[91].

In NASH, ROS are generated in several ways that can alter signaling pathways, such as cell kinases, phosphatases, and transcription factors, which impact cell proliferation, differentiation, and apoptosis. They can lead to cirrhosis *via* the rebuilding of stellate cells and ECM within the liver. Substantial hepatic ROS is produced by excessive angiotensin II and activated CYP2E1, resulting in impaired beta-oxidation and eventually fatty liver[91].

Lipotoxicity in NAFLD causes OS and induces organelle damage due to decreased antioxidant systems, mitochondrial dysfunction, and an increase in unfolded protein response by endoplasmic reticulum stress[80]. On the other hand, there is an impairment of α-oxidation due to a decrease in PPARα activity, which upturns hepatic lipid levels. Fatty acid overload is the major source of reducing equivalents responsible for increased ROS production. Also, TNF-α and lipid peroxidation products could induce mitochondrial dysfunction. Mitochondrial damage will result in secondary lipid α-oxidation inhibition and a further increase in the degree of steatosis[80].

Furthermore, inflammatory cytokines such as IL-1-β, TNF-α, and IL-17/20/33, chemokines, like monocyte chemoattractant protein-1 and C-X-C chemokine ligand 10,

and the toll-like receptor pathway are intensively involved in the regulation of hepatic fibrogenesis[91]. Macrophage activation and influx in the liver are important for the progression of NAFLD since hepatic macrophages promote NASH development *via* cytokines IL-1, IL-6, and TNF-α[92]. Liver failure causes an increase of TNF-α, IL-6, and angiotensin II[80].

OTHER NOVEL MARKERS

Gut permeability markers

The intestinal barrier is composed of chemical, physical, and immunological barriers. Maintaining a healthy barrier is essential to prevent microbial translocation and keep the liver safe to prevent systemic inflammation[93].

Differences in the taxonomic composition of the intestinal microbiome in NAFLD (an increased proportion of *Firmicutes* and a reduced proportion of *Bacteriodetes)* change metabolic function. The availability of bile acids, endogenous alcohols, and voltaic organic compounds increases. When these changes are combined with reduced SCFAs and choline, the integrity of the intestinal barrier is reduced[93].

Gut barrier disruption is recognized in patients with cirrhosis. The epithelial layers show structural abnormalities related to increased intestinal permeability or bacterial translocation[94]. Permeability can be measured by the urinary excretion of radiolabeled ^{51}chromium-ethylenediamine tetraacetic acid or by measuring volatile organic compounds formed by the fermentation of some dietary polysaccharides[95].

CTC–cardiotonic steroids

Cardiotonic steroids (CTS) are part of a group of specific ligands of Na^+, K^+-ATPase, a ubiquitously expressed enzyme responsible for the maintenance of electrochemical gradients across the cell membrane through active transport[96] that provokes a variety of cell signals[70]. In the last decades, studies have revealed the role of Na^+, K^+-ATPase and its signaling in various diseases, including inflammation and fibrosis[97].

CTS increase cholesterol synthesis in liver HepG2 cells, which augments the activity and expression of 3-hydroxy-3-methylglutaryl-coenzyme A reductase[98]. Disturbed cholesterol balance underlies cardiovascular disease and an increasing number of other diseases, such as neurodegenerative diseases, cancers, and liver disease[99].

Elevated CTS might encourage increased cholesterol levels in the liver and worsen liver fibrosis by activating HSCs[100] and other redox-inflammatory pathways[101]. This increase in cholesterol levels could precipitate hepatocyte injury and macrophage activation that could lead to liver fibrosis progression. However, even CTS seem to have an important role in hepatocyte lipotoxicity and fibrosis; to our knowledge, they have not been studied as biomarkers for liver disease progression.

GUT MICROBIOTA

A large community of viruses, bacteria, archaea, and fungi live in the gastrointestinal tract and composes the gut microbiota[102]. It has critical roles in digestion, immunity, and metabolism[103]. Recently, the characterization of gut microbiota has evolved rapidly due to the advances in sequencing technology, permitting the creation of a gut microbiota gene catalogue[102]. The collective genetic material of the microbiota is often referred to as the "gut microbiome". It encodes pathways that produce small bioactive molecules derived from dietary or metabolic precursors and may alter human health[104].

Thus, knowledge of microbiome characteristics in different metabolic diseases has increased in the past years. There has been great interest in dysbiosis (alterations in the composition and balance of microbiota[104]). Microbiota alterations are being studied as possible diagnostic biomarkers to improve personalized care. Animal studies have demonstrated a potential causal role of gut microbiota in NAFLD development[105]. However, extrapolating mouse model experimental information to humans has several limitations[106]. Consequently, signatures specific to liver alterations would be useful as NAFLD diagnostic biomarkers. However, discrepant microbiome signatures might be linked to the heterogeneity of diet, drugs, infections, environmental exposures, among others[104].

Bacterial microbiome

Alterations in the gut microbiome have been associated with the progression and severity of NAFLD[107]. Proteobacteria are enriched in steatosis[103,108,109]. Patients with NAFLD, compared with healthy individuals, also have significant changes at the phylum (increased Enterobacteriaceae[109] and decreased Rikenellaceae and Ruminococcaceae[109]) and genera level (increased *Escherichia*[109], *Dorea*, and *Peptoniphilus* and decreased *Anaerosporobacter, Coprococcus, Faecalibacterium,* and *Prevotella*)[103].

When comparing people with NASH *vs* healthy controls, some patterns are observed that also overlap with the NAFLD microbiome: Phylum (increased Proteobacteria[50,109-111]), family (increased Enterobacteriaceae[109,110] and decreased Ruminococcaceae[110-113] and Rikenellaceae[110]), and genera (increased *Dorea*[111] and decreased *Faecalibacterium*[110,113,114], *Coprococcus*[110,112,113], and *Anaerosporobacter*[112,114]).

Few projects have studied microbial composition as a function of fibrosis progression. *Bacteroides vulgatus* and *Escherichia coli* are the most abundant species in advanced fibrosis (F3–F4)[50]. Models have been proposed to use the microbiome as a reservoir for diagnostic signatures of NAFLD fibrosis[50], but further confirmation in independent cohorts and across geographical regions is necessary to assess their clinical relevance.

Microbial signatures of liver fibrosis are related to a severe shift in taxa conformation, leading to a growth in pathogenic taxa and a decline in metabolically beneficial taxa[115]. However, the evaluation of gut microbiota contribution to liver disease progression (from steatosis to NASH and NASH cirrhosis) is limited and bacterial markers are frequently identified in a given study yet not confirmed in independent cohorts.

Although some studies consider gut bacterial groups as promising markers of different stages of liver disease, if the microbiota is a causal factor and how it interacts with the complex pathophysiological processes driving disease progression from mild fibrosis to severe fibrosis is still under investigation[50,109].

Virome

Dense and complex populations of intestinal viruses reside in the gut and interact with other microorganisms and the human host[116,117]. Most intestinal viruses are bacteriophages (phages), viruses that can specifically infect bacteria[118]. Phages may serve as important microbiota genetic diversity reservoirs by acting as vehicles for the horizontal transfer of virulence, antibiotic resistance, and metabolic determinants among bacteria[119].

Lang *et al*[120] studied the fecal viromes from NAFLD patients and controls. They found associated histologic markers of NAFLD severity with significant decreases in viral diversity and proportion of bacteriophages[120]. The intestinal virome is specific for every individual, and viral diversity measures were the third and fifth most important variables following a higher AST and higher age. The most important viral species belonged to *Lactococcus* phages, and several *Lactococcus* phages were less present in patients with NAFLD and NASH.

Protozoa and fungi

Fungi and archaea are important components of the human microbiota. Recent findings have revealed that mycobiome (commensal fungi at barrier surfaces) can influence host immunity and the development and progression of human inflammatory diseases[121]. The human gut mycobiome is dominated by *Saccharomyces, Malassezia, Candida*, and *Cladosporium* and are an important modulator for local and peripheral immune responses. Patients with liver fibrosis have decreased fungal diversity and increased *Candida*[122]. Gut mycobiota disturbance might produce metabolites called mycotoxins (trichothecenes, zearalenone, fumonisins, ochratoxins, aflatoxins) that can alter gut health by compromising intestinal epithelia[123,124].

LIMITATION

The increasing burden of NAFLD worldwide has encouraged the search for novel biomarkers to detect liver diseases. Liver biopsy is currently the gold standard for diagnosis and staging, but it has several limitations, including sampling errors, invasiveness, inter-observer variability, and related procedure risks. Researchers have faced the challenge of developing novel biomarkers in past decades, and significant advances have been made. A promising biomarker should be liver-specific, accessible

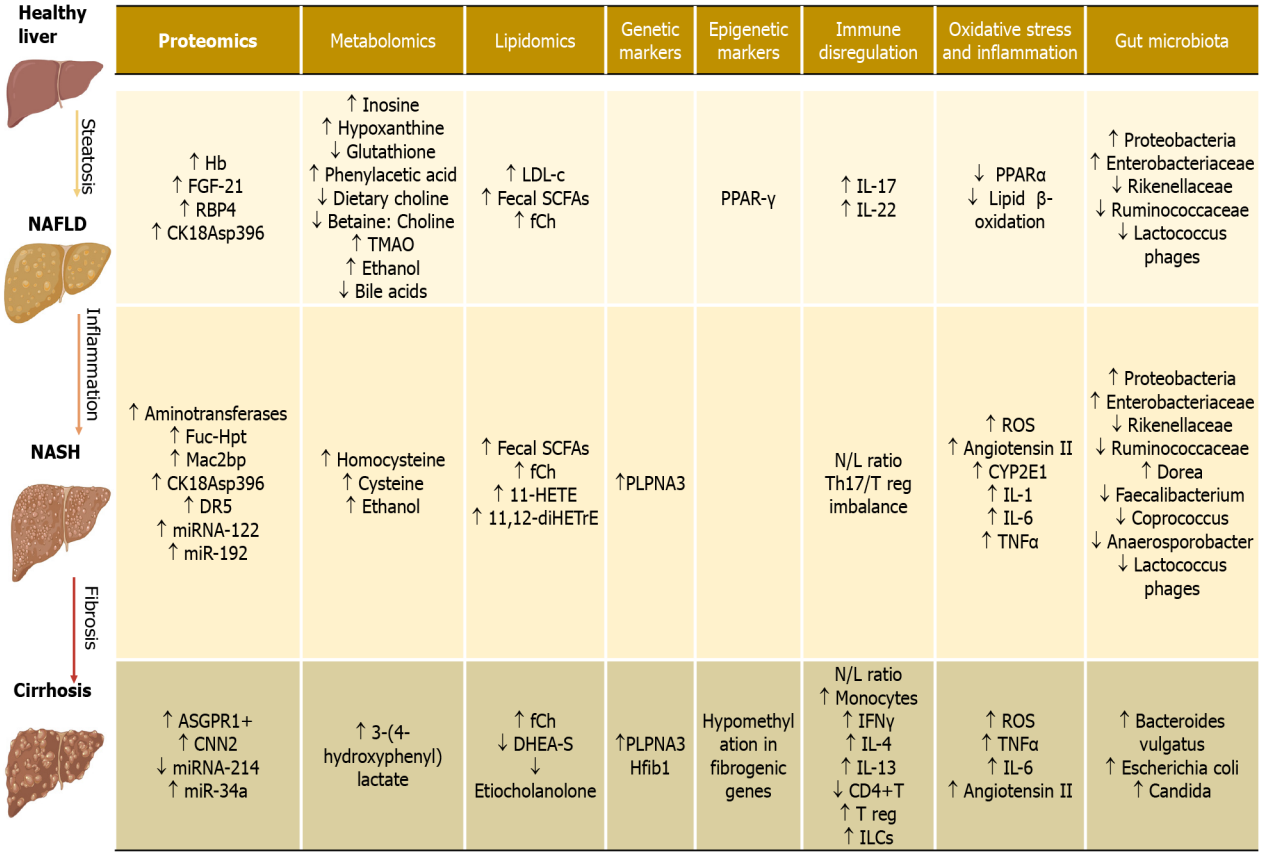

Figure 2 Potential biomarkers involved in hepatic pathophysiology. Hb: Hemoglobin; FGF-21: Fibroblast growth factor 21; RBP4: Retinol binding protein 4; CK18Asp396: Caspase cleaved cytokeratin-18 fragment (M30); Fuc-Hpt: Fucosylated haptoglobin; Mac2bp: Mac-2-binding protein; DR5: Death receptor 5; miRNA-122: MicroRNA 122; miR-192: MicroRNA 192; ASGPR1+: Asialoglycoprotein receptor 1; CNN2: Calponin 2; miRNA-214: MicroRNA 214; miR-34a: MicroRNA 34a; TMAO: Trimethylamine N-oxide; LDL-c: Low density lipoprotein cholesterol; Fecal SCFAs: Fecal Short chain fatty acids; fCh: Ferrochelatase; 11-HETE: 11-Hydroxyeicosatetraenoic Acid; 11,12-diHETrE: 11,12-dihydroxyicosatrienoic acid; DHEA-S: Dehydroepiandrosterone sulphate; PPAR-γ: Peroxisome proliferator-activated receptor γ; IL-17: Interleukin-17; IL-22: Interleukin-22; N/L ratio: Neutrophil/lymphocyte ratio; Th17/Treg imbalance: T helper 17/T regulatory cells imbalance; IFNγ: Interferon gamma; IL-4: Interleukin-4; IL-13: Interleukin-13; CD4+T: Cluster of differentiation 4, T helper cells; T reg: Regulatory T cells; ILCs: Innate lymphoid cells.

and accurate, replicable, and available in clinical laboratories. As summarized in this article, most studies have focused on proteomics, metabolomics, genome-wide association studies, microbiome, and inflammation markers. Still, some may be more specific for NAFLD while others for NASH, although the challenge for determining the etiology and staging the degree of severity remains a limitation (Figure 2).

The evaluation of future biomarkers for the assessment of liver fibrosis could greatly impact the health system. There is a priority for non-invasive diagnostic tools to fulfil medical needs, differentiate patients with steatosis from those with NASH and fibrosis, predict disease progression, and monitor patients to evaluate the therapeutic response. In the following years, it would be expected that a physician who faces a hepatic patient could suspect hepatic disease, perform imaging studies, and from there have a set of potential biomarkers that they may request to have a concrete and specific diagnosis. Some of these biomarkers have strong diagnostic performance, but current evidence shows a lack of reproducibility. Besides, the analytical, clinical validity of the methodology is lacking. Validity is necessary to translate basic research into real clinical application. Even if we perform this validation, it is unlikely that a single biomarker could fulfil this necessity. A combination of these biomarkers could soon be used to create a diagnostic panel. This panel, combined with the patient´s clinical history and clinical data, could certainly lead to a medical decision that results in an accurate diagnosis and treatment. This result must be the goal in the following years.

CONCLUSION

Through this review, we have shown that despite a wide range of potential biomarkers for the different stages of hepatic steatosis and fibrosis, there is still a long path to the translation of these resources. We provide evidence of the current absence of an efficient, non-invasive, and widely accessible test for NAFLD and NASH detection. Biomarkers are still in early stages. Rigorous, well-designed comprehensive studies are required to determine the actual benefit these may pose for determining the risk, diagnosis, and progression of the hepatic patient. In conclusion, our review compiles significant efforts to find new promising biomarkers for liver disease, still leaving great challenges. There is still a need to define normal reference levels in healthy individuals and the different stages of the disease and to determine the clinical sensitivity and specificity of biomarkers to develop a clinical diagnostic panel.

REFERENCES

1 **Fouad Y**, Waked I, Bollipo S, Gomaa A, Ajlouni Y, Attia D. What's in a name? *Liver Int* 2020; **40**: 1254-1261 [PMID: 32301554 DOI: 10.1111/liv.14478]

2 **Younossi Z**, Anstee QM, Marietti M, Hardy T, Henry L, Eslam M, George J, Bugianesi E. Global burden of NAFLD and NASH: trends, predictions, risk factors and prevention. *Nat Rev Gastroenterol Hepatol* 2018; **15**: 11-20 [PMID: 28930295 DOI: 10.1038/nrgastro.2017.109]

3 **Byrne CD**, Patel J, Scorletti E, Targher G. Tests for diagnosing and monitoring non-alcoholic fatty liver disease in adults. *BMJ* 2018; **362**: k2734 [PMID: 30002017 DOI: 10.1136/bmj.k2734]

4 **Younossi ZM**, Koenig AB, Abdelatif D, Fazel Y, Henry L, Wymer M. Global epidemiology of nonalcoholic fatty liver disease-Meta-analytic assessment of prevalence, incidence, and outcomes. *Hepatology* 2016; **64**: 73-84 [PMID: 26707365 DOI: 10.1002/hep.28431]

5 **Méndez-Sánchez N**, Villa AR, Chávez-Tapia NC, Ponciano-Rodriguez G, Almeda-Valdés P, González D, Uribe M. Trends in liver disease prevalence in Mexico from 2005 to 2050 through mortality data. *Ann Hepatol* 2005; **4**: 52-55 [PMID: 15798662]

6 **Bernal-Reyes R**, Castro-Narro G, Malé-Velázquez R, Carmona-Sánchez R, González-Huezo MS, García-Juárez I, Chávez-Tapia N, Aguilar-Salinas C, Aiza-Haddad I, Ballesteros-Amozurrutia MA, Bosques-Padilla F, Castillo-Barradas M, Chávez-Barrera JA, Cisneros-Garza L, Flores-Calderón J, García-Compeán D, Gutiérrez-Grobe Y, Higuera de la Tijera MF, Kershenobich-Stalnikowitz D, Ladrón de Guevara-Cetina L, Lizardi-Cervera J, López-Cossio JA, Martínez-Vázquez S, Márquez-Guillén E, Méndez-Sánchez N, Moreno-Alcantar R, Poo-Ramírez JL, Ramos-Martínez P, Rodríguez-Hernández H, Sánchez-Ávila JF, Stoopen-Rometti M, Torre-Delgadillo A, Torres-Villalobos G, Trejo-Estrada R, Uribe-Esquivel M, Velarde-Ruiz Velasco JA. The Mexican consensus on nonalcoholic fatty liver disease. *Rev Gastroenterol Mex (Engl Ed)* 2019; **84**: 69-99 [PMID: 30711302 DOI: 10.1016/j.rgmx.2018.11.007]

7 **Long MT**, Gandhi S, Loomba R. Advances in non-invasive biomarkers for the diagnosis and monitoring of non-alcoholic fatty liver disease. *Metabolism* 2020; **111S**: 154259 [PMID: 32387227 DOI: 10.1016/j.metabol.2020.154259]

8 **Giraudi PJ**, Stephenson AM, Tiribelli C, Rosso N. Novel high-throughput applications for NAFLD diagnostics and biomarker dis-covery. *Hepatoma Res* 2021; 2021 [DOI: 10.20517/2394-5079.2020.92]

9 **Geyer PE**, Holdt LM, Teupser D, Mann M. Revisiting biomarker discovery by plasma proteomics. *Mol Syst Biol* 2017; **13**: 942 [PMID: 28951502 DOI: 10.15252/msb.20156297]

10 **Iruarrizaga-Lejarreta M**, Bril F, Noureddin M, Ortiz P, Lu SC, Mato JM. Emerging circulating biomarkers for the diagnosis and assess-ment of treatment responses in patients with hepatic fat accumula-tion, nash and liver fibrosis. Translational Research Methods in Diabetes, Obesity, and Nonalcoholic Fatty Liver Disease: A Focus on Early Phase Clinical Drug Development. Gainesville: Springer, 2019: 423–448

11 **Rodriguez-Suarez E**, Mato JM, Elortza F. Proteomics analysis of human nonalcoholic fatty liver. *Methods Mol Biol* 2012; **909**: 241-258 [PMID: 22903720 DOI: 10.1007/978-1-61779-959-4_16]

12 **Yilmaz Y**. Serum proteomics for biomarker discovery in nonalcoholic fatty liver disease. *Clin Chim Acta* 2012; **413**: 1190-1193 [PMID: 22546610 DOI: 10.1016/j.cca.2012.04.019]

13 **Li H**, Dong K, Fang Q, Hou X, Zhou M, Bao Y, Xiang K, Xu A, Jia W. High serum level of fibroblast growth factor 21 is an independent predictor of non-alcoholic fatty liver disease: a 3-year prospective study in China. *J Hepatol* 2013; **58**: 557-563 [PMID: 23142063 DOI: 10.1016/j.jhep.2012.10.029]

14 **Seo JA**, Kim NH, Park SY, Kim HY, Ryu OH, Lee KW, Lee J, Kim DL, Choi KM, Baik SH, Choi DS, Kim SG. Serum retinol-binding protein 4 levels are elevated in non-alcoholic fatty liver disease. *Clin Endocrinol (Oxf)* 2008; **68**: 555-560 [PMID: 17941908 DOI: 10.1111/j.1365-2265.2007.03072.x]

15 **Leoni S**, Tovoli F, Napoli L, Serio I, Ferri S, Bolondi L. Current guidelines for the management of non-alcoholic fatty liver disease: A systematic review with comparative analysis. *World J*

Gastroenterol 2018; **24**: 3361-3373 [PMID: 30122876 DOI: 10.3748/wjg.v24.i30.3361]

16 **Kamada Y**, Akita M, Takeda Y, Yamada S, Fujii H, Sawai Y, Doi Y, Asazawa H, Nakayama K, Mizutani K, Yakushijin T, Miyazaki M, Ezaki H, Hiramatsu N, Yoshida Y, Kiso S, Imai Y, Kawada N, Takehara T, Miyoshi E. Serum Fucosylated Haptoglobin as a Novel Diagnostic Biomarker for Predicting Hepatocyte Ballooning and Nonalcoholic Steatohepatitis. *PLoS One* 2013; **8**: e66328 [PMID: 23805214 DOI: 10.1371/journal.pone.0066328]

17 **European Association for the Study of the Liver (EASL)**; European Association for the Study of Diabetes (EASD); European Association for the Study of Obesity (EASO). EASL-EASD-EASO Clinical Practice Guidelines for the management of non-alcoholic fatty liver disease. *Diabetologia* 2016; **59**: 1121-1140 [PMID: 27053230 DOI: 10.1007/s00125-016-3902-y]

18 **Shen J**, Chan HL, Wong GL, Chan AW, Choi PC, Chan HY, Chim AM, Yeung DK, Yu J, Chu WC, Wong VW. Assessment of non-alcoholic fatty liver disease using serum total cell death and apoptosis markers. *Aliment Pharmacol Ther* 2012; **36**: 1057-1066 [PMID: 23066946 DOI: 10.1111/apt.12091]

19 **Estep M**, Mehta R, Bratthauer G, Alaparthi L, Monge F, Ali S, Abdelatif D, Younoszai Z, Stepanova M, Goodman ZD, Younossi ZM. Hepatic sonic hedgehog protein expression measured by computer assisted morphometry significantly correlates with features of non-alcoholic steatohepatitis. *BMC Gastroenterol* 2019; **19**: 27 [PMID: 30744560 DOI: 10.1186/s12876-019-0951-y]

20 **Skog J**, Würdinger T, van Rijn S, Meijer DH, Gainche L, Sena-Esteves M, Curry WT Jr, Carter BS, Krichevsky AM, Breakefield XO. Glioblastoma microvesicles transport RNA and proteins that promote tumour growth and provide diagnostic biomarkers. *Nat Cell Biol* 2008; **10**: 1470-1476 [PMID: 19011622 DOI: 10.1038/ncb1800]

21 **Stefan N**, Häring HU, Cusi K. Non-alcoholic fatty liver disease: causes, diagnosis, cardiometabolic consequences, and treatment strategies. *Lancet Diabetes Endocrinol* 2019; **7**: 313-324 [PMID: 30174213 DOI: 10.1016/S2213-8587(18)30154-2]

22 **Shah R**, Patel T, Freedman JE. Circulating Extracellular Vesicles in Human Disease. *N Engl J Med* 2018; **379**: 958-966 [PMID: 30184457 DOI: 10.1056/NEJMra1704286]

23 **Maas SLN**, Breakefield XO, Weaver AM. Extracellular Vesicles: Unique Intercellular Delivery Vehicles. *Trends Cell Biol* 2017; **27**: 172-188 [PMID: 27979573 DOI: 10.1016/j.tcb.2016.11.003]

24 **Juan T**, Fürthauer M. Biogenesis and function of ESCRT-dependent extracellular vesicles. *Semin Cell Dev Biol* 2018; **74**: 66-77 [PMID: 28807885 DOI: 10.1016/j.semcdb.2017.08.022]

25 **Hornick NI**, Huan J, Doron B, Goloviznina NA, Lapidus J, Chang BH, Kurre P. Serum Exosome MicroRNA as a Minimally-Invasive Early Biomarker of AML. *Sci Rep* 2015; **5**: 11295 [PMID: 26067326 DOI: 10.1038/srep11295]

26 **Dickhout A**, Koenen RR. Extracellular Vesicles as Biomarkers in Cardiovascular Disease; Chances and Risks. *Front Cardiovasc Med* 2018; **5**: 113 [PMID: 30186839 DOI: 10.3389/fcvm.2018.00113]

27 **Erdbrügger U**, Le TH. Extracellular Vesicles in Renal Diseases: More than Novel Biomarkers? *J Am Soc Nephrol* 2016; **27**: 12-26 [PMID: 26251351 DOI: 10.1681/ASN.2015010074]

28 **Kornek M**, Lynch M, Mehta SH, Lai M, Exley M, Afdhal NH, Schuppan D. Circulating microparticles as disease-specific biomarkers of severity of inflammation in patients with hepatitis C or nonalcoholic steatohepatitis. *Gastroenterology* 2012; **143**: 448-458 [PMID: 22537612 DOI: 10.1053/j.gastro.2012.04.031]

29 **Tadokoro T**, Morishita A, Masaki T. Diagnosis and Therapeutic Management of Liver Fibrosis by MicroRNA. *Int J Mol Sci* 2021; **22** [PMID: 34360904 DOI: 10.3390/ijms22158139]

30 **Povero D**, Yamashita H, Ren W, Subramanian MG, Myers RP, Eguchi A, Simonetto DA, Goodman ZD, Harrison SA, Sanyal AJ, Bosch J, Feldstein AE. Characterization and Proteome of Circulating Extracellular Vesicles as Potential Biomarkers for NASH. *Hepatol Commun* 2020; **4**: 1263-1278 [PMID: 32923831 DOI: 10.1002/hep4.1556]

31 **Povero D**, Eguchi A, Li H, Johnson CD, Papouchado BG, Wree A, Messer K, Feldstein AE. Circulating extracellular vesicles with specific proteome and liver microRNAs are potential biomarkers for liver injury in experimental fatty liver disease. *PLoS One* 2014; **9**: e113651 [PMID: 25470250 DOI: 10.1371/journal.pone.0113651]

32 **Hirsova P**, Ibrahim SH, Krishnan A, Verma VK, Bronk SF, Werneburg NW, Charlton MR, Shah VH, Malhi H, Gores GJ. Lipid-Induced Signaling Causes Release of Inflammatory Extracellular Vesicles From Hepatocytes. *Gastroenterology* 2016; **150**: 956-967 [PMID: 26764184 DOI: 10.1053/j.gastro.2015.12.037]

33 **Cai S**, Cheng X, Pan X, Li J. Emerging role of exosomes in liver physiology and pathology. *Hepatol Res* 2017; **47**: 194-203 [PMID: 27539153 DOI: 10.1111/hepr.12794]

34 **Chen L**, Brenner DA, Kisseleva T. Combatting Fibrosis: Exosome-Based Therapies in the Regression of Liver Fibrosis. *Hepatol Commun* 2019; **3**: 180-192 [PMID: 30766956 DOI: 10.1002/hep4.1290]

35 **Koeck ES**, Iordanskaia T, Sevilla S, Ferrante SC, Hubal MJ, Freishtat RJ, Nadler EP. Adipocyte exosomes induce transforming growth factor beta pathway dysregulation in hepatocytes: a novel paradigm for obesity-related liver disease. *J Surg Res* 2014; **192**: 268-275 [PMID: 25086727 DOI: 10.1016/j.jss.2014.06.050]

36 **Seo W**, Eun HS, Kim SY, Yi HS, Lee YS, Park SH, Jang MJ, Jo E, Kim SC, Han YM, Park KG, Jeong WI. Exosome-mediated activation of toll-like receptor 3 in stellate cells stimulates interleukin-17 production by γδ T cells in liver fibrosis. *Hepatology* 2016; **64**: 616-631 [PMID: 27178735 DOI:

10.1002/hep.28644]
37 **Charrier A**, Chen R, Chen L, Kemper S, Hattori T, Takigawa M, Brigstock DR. Exosomes mediate intercellular transfer of pro-fibrogenic connective tissue growth factor (CCN2) between hepatic stellate cells, the principal fibrotic cells in the liver. *Surgery* 2014; **156**: 548-555 [PMID: 24882759 DOI: 10.1016/j.surg.2014.04.014]
38 **Chen L**, Chen R, Kemper S, Charrier A, Brigstock DR. Suppression of fibrogenic signaling in hepatic stellate cells by Twist1-dependent microRNA-214 expression: Role of exosomes in horizontal transfer of Twist1. *Am J Physiol Gastrointest Liver Physiol* 2015; **309**: G491-G499 [PMID: 26229009 DOI: 10.1152/ajpgi.00140.2015]
39 **Jiang XP**, Ai WB, Wan LY, Zhang YQ, Wu JF. The roles of microRNA families in hepatic fibrosis. *Cell Biosci* 2017; **7**: 34 [PMID: 28680559 DOI: 10.1186/s13578-017-0161-7]
40 **Yan G**, Li B, Xin X, Xu M, Ji G, Yu H. MicroRNA-34a Promotes Hepatic Stellate Cell Activation *via* Targeting ACSL1. *Med Sci Monit* 2015; **21**: 3008-3015 [PMID: 26437572 DOI: 10.12659/MSM.894000]
41 **Roy S**, Benz F, Vargas Cardenas D, Vucur M, Gautheron J, Schneider A, Hellerbrand C, Pottier N, Alder J, Tacke F, Trautwein C, Roderburg C, Luedde T. miR-30c and miR-193 are a part of the TGF-β-dependent regulatory network controlling extracellular matrix genes in liver fibrosis. *J Dig Dis* 2015; **16**: 513-524 [PMID: 26120970 DOI: 10.1111/1751-2980.12266]
42 **Guo CJ**, Pan Q, Li DG, Sun H, Liu BW. miR-15b and miR-16 are implicated in activation of the rat hepatic stellate cell: An essential role for apoptosis. *J Hepatol* 2009; **50**: 766-778 [PMID: 19232449 DOI: 10.1016/j.jhep.2008.11.025]
43 **Hyun J**, Wang S, Kim J, Rao KM, Park SY, Chung I, Ha CS, Kim SW, Yun YH, Jung Y. MicroRNA-378 limits activation of hepatic stellate cells and liver fibrosis by suppressing Gli3 expression. *Nat Commun* 2016; **7**: 10993 [PMID: 27001906 DOI: 10.1038/ncomms10993]
44 **Roderburg C**, Mollnow T, Bongaerts B, Elfimova N, Vargas Cardenas D, Berger K, Zimmermann H, Koch A, Vucur M, Luedde M, Hellerbrand C, Odenthal M, Trautwein C, Tacke F, Luedde T. Micro-RNA profiling in human serum reveals compartment-specific roles of miR-571 and miR-652 in liver cirrhosis. *PLoS One* 2012; **7**: e32999 [PMID: 22412969 DOI: 10.1371/journal.pone.0032999]
45 **Xie X**, Dou CY, Zhou Y, Zhou Q, Tang HB. MicroRNA-503 Targets Mothers Against Decapentaplegic Homolog 7 Enhancing Hepatic Stellate Cell Activation and Hepatic Fibrosis. *Dig Dis Sci* 2021; **66**: 1928-1939 [PMID: 32648079 DOI: 10.1007/s10620-020-06460-7]
46 **Ezhilarasan D**. MicroRNA interplay between hepatic stellate cell quiescence and activation. *Eur J Pharmacol* 2020; **885**: 173507 [PMID: 32858048 DOI: 10.1016/j.ejphar.2020.173507]
47 **Chu H**, Duan Y, Yang L, Schnabl B. Small metabolites, possible big changes: a microbiota-centered view of non-alcoholic fatty liver disease. *Gut* 2019; **68**: 359-370 [PMID: 30171065 DOI: 10.1136/gutjnl-2018-316307]
48 **Caussy C**, Loomba R. Gut microbiome, microbial metabolites and the development of NAFLD. *Nat Rev Gastroenterol Hepatol* 2018; **15**: 719-720 [PMID: 30158571 DOI: 10.1038/s41575-018-0058-x]
49 **Dong S**, Zhan ZY, Cao HY, Wu C, Bian YQ, Li JY, Cheng GH, Liu P, Sun MY. Urinary metabolomics analysis identifies key biomarkers of different stages of nonalcoholic fatty liver disease. *World J Gastroenterol* 2017; **23**: 2771-2784 [PMID: 28487615 DOI: 10.3748/wjg.v23.i15.2771]
50 **Loomba R**, Seguritan V, Li W, Long T, Klitgord N, Bhatt A, Dulai PS, Caussy C, Bettencourt R, Highlander SK, Jones MB, Sirlin CB, Schnabl B, Brinkac L, Schork N, Chen CH, Brenner DA, Biggs W, Yooseph S, Venter JC, Nelson KE. Gut Microbiome-Based Metagenomic Signature for Non-invasive Detection of Advanced Fibrosis in Human Nonalcoholic Fatty Liver Disease. *Cell Metab* 2017; **25**: 1054-1062.e5 [PMID: 28467925 DOI: 10.1016/j.cmet.2017.04.001]
51 **Hoyles L**, Fernández-Real JM, Federici M, Serino M, Abbott J, Charpentier J, Heymes C, Luque JL, Anthony E, Barton RH, Chilloux J, Myridakis A, Martinez-Gili L, Moreno-Navarrete JM, Benhamed F, Azalbert V, Blasco-Baque V, Puig J, Xifra G, Ricart W, Tomlinson C, Woodbridge M, Cardellini M, Davato F, Cardolini I, Porzio O, Gentileschi P, Lopez F, Foufelle F, Butcher SA, Holmes E, Nicholson JK, Postic C, Burcelin R, Dumas ME. Molecular phenomics and metagenomics of hepatic steatosis in non-diabetic obese women. *Nat Med* 2018; **24**: 1070-1080 [PMID: 29942096 DOI: 10.1038/s41591-018-0061-3]
52 **Engin A**. Non-Alcoholic Fatty Liver Disease. *Adv Exp Med Biol* 2017; **960**: 443-467 [PMID: 28585211 DOI: 10.1007/978-3-319-48382-5_19]
53 **Sherriff JL**, O'Sullivan TA, Properzi C, Oddo JL, Adams LA. Choline, Its Potential Role in Nonalcoholic Fatty Liver Disease, and the Case for Human and Bacterial Genes. *Adv Nutr* 2016; **7**: 5-13 [PMID: 26773011 DOI: 10.3945/an.114.007955]
54 **Sergeeva KV**. [Blood serum and liver phospholipids in rats with chronic choline-protein deficiency]. *Biull Eksp Biol Med* 1975; **79**: 47-49 [PMID: 1222249]
55 **Chen YM**, Liu Y, Zhou RF, Chen XL, Wang C, Tan XY, Wang LJ, Zheng RD, Zhang HW, Ling WH, Zhu HL. Associations of gut-flora-dependent metabolite trimethylamine-N-oxide, betaine and choline with non-alcoholic fatty liver disease in adults. *Sci Rep* 2016; **6**: 19076 [PMID: 26743949 DOI: 10.1038/srep19076]
56 **Zeisel SH**, daCosta KA, Youssef M, Hensey S. Conversion of dietary choline to trimethylamine and dimethylamine in rats: dose-response relationship. *J Nutr* 1989; **119**: 800-804 [PMID: 2723829 DOI: 10.1093/jn/119.5.800]

57 **Sayin SI**, Wahlström A, Felin J, Jäntti S, Marschall HU, Bamberg K, Angelin B, Hyötyläinen T, Orešič M, Bäckhed F. Gut microbiota regulates bile acid metabolism by reducing the levels of tauro-beta-muricholic acid, a naturally occurring FXR antagonist. *Cell Metab* 2013; **17**: 225-235 [PMID: 23395169 DOI: 10.1016/j.cmet.2013.01.003]

58 **Yuan J**, Chen C, Cui J, Lu J, Yan C, Wei X, Zhao X, Li N, Li S, Xue G, Cheng W, Li B, Li H, Lin W, Tian C, Zhao J, Han J, An D, Zhang Q, Wei H, Zheng M, Ma X, Li W, Chen X, Zhang Z, Zeng H, Ying S, Wu J, Yang R, Liu D. Fatty Liver Disease Caused by High-Alcohol-Producing Klebsiella pneumoniae. *Cell Metab* 2019; **30**: 675-688.e7 [PMID: 31543403 DOI: 10.1016/j.cmet.2019.08.018]

59 **Imajo K**, Fujita K, Yoneda M, Shinohara Y, Suzuki K, Mawatari H, Takahashi J, Nozaki Y, Sumida Y, Kirikoshi H, Saito S, Nakamuta M, Matsuhashi N, Wada K, Nakajima A. Plasma free choline is a novel non-invasive biomarker for early-stage non-alcoholic steatohepatitis: A multi-center validation study. *Hepatol Res* 2012; **42**: 757-766 [PMID: 22780848 DOI: 10.1111/j.1872-034X.2012.00976.x]

60 **Kalhan SC**, Guo L, Edmison J, Dasarathy S, McCullough AJ, Hanson RW, Milburn M. Plasma metabolomic profile in nonalcoholic fatty liver disease. *Metabolism* 2011; **60**: 404-413 [PMID: 20423748 DOI: 10.1016/j.metabol.2010.03.006]

61 **Barr J**, Caballería J, Martínez-Arranz I, Domínguez-Díez A, Alonso C, Muntané J, Pérez-Cormenzana M, García-Monzón C, Mayo R, Martín-Duce A, Romero-Gómez M, Lo Iacono O, Tordjman J, Andrade RJ, Pérez-Carreras M, Le Marchand-Brustel Y, Tran A, Fernández-Escalante C, Arévalo E, García-Unzueta M, Clement K, Crespo J, Gual P, Gómez-Fleitas M, Martínez-Chantar ML, Castro A, Lu SC, Vázquez-Chantada M, Mato JM. Obesity-dependent metabolic signatures associated with nonalcoholic fatty liver disease progression. *J Proteome Res* 2012; **11**: 2521-2532 [PMID: 22364559 DOI: 10.1021/pr201223p]

62 **Gorden DL**, Myers DS, Ivanova PT, Fahy E, Maurya MR, Gupta S, Min J, Spann NJ, McDonald JG, Kelly SL, Duan J, Sullards MC, Leiker TJ, Barkley RM, Quehenberger O, Armando AM, Milne SB, Mathews TP, Armstrong MD, Li C, Melvin WV, Clements RH, Washington MK, Mendonsa AM, Witztum JL, Guan Z, Glass CK, Murphy RC, Dennis EA, Merrill AH Jr, Russell DW, Subramaniam S, Brown HA. Biomarkers of NAFLD progression: a lipidomics approach to an epidemic. *J Lipid Res* 2015; **56**: 722-736 [PMID: 25598080 DOI: 10.1194/jlr.P056002]

63 **Kimberly WT**, O'Sullivan JF, Nath AK, Keyes M, Shi X, Larson MG, Yang Q, Long MT, Vasan R, Peterson RT, Wang TJ, Corey KE, Gerszten RE. Metabolite profiling identifies anandamide as a biomarker of nonalcoholic steatohepatitis. *JCI Insight* 2017; **2** [PMID: 28469090 DOI: 10.1172/jci.insight.92989]

64 **Tokushige K**, Hashimoto E, Kodama K, Tobari M, Matsushita N, Kogiso T, Taniai M, Torii N, Shiratori K, Nishizaki Y, Ohga T, Ohashi Y, Sato T. Serum metabolomic profile and potential biomarkers for severity of fibrosis in nonalcoholic fatty liver disease. *J Gastroenterol* 2013; **48**: 1392-1400 [PMID: 23478936 DOI: 10.1007/s00535-013-0766-5]

65 **Puri P**, Wiest MM, Cheung O, Mirshahi F, Sargeant C, Min HK, Contos MJ, Sterling RK, Fuchs M, Zhou H, Watkins SM, Sanyal AJ. The plasma lipidomic signature of nonalcoholic steatohepatitis. *Hepatology* 2009; **50**: 1827-1838 [PMID: 19937697 DOI: 10.1002/hep.23229]

66 **Loomba R**, Quehenberger O, Armando A, Dennis EA. Polyunsaturated fatty acid metabolites as novel lipidomic biomarkers for noninvasive diagnosis of nonalcoholic steatohepatitis. *J Lipid Res* 2015; **56**: 185-192 [PMID: 25404585 DOI: 10.1194/jlr.P055640]

67 **Samuel BS**, Shaito A, Motoike T, Rey FE, Backhed F, Manchester JK, Hammer RE, Williams SC, Crowley J, Yanagisawa M, Gordon JI. Effects of the gut microbiota on host adiposity are modulated by the short-chain fatty-acid binding G protein-coupled receptor, Gpr41. *Proc Natl Acad Sci U S A* 2008; **105**: 16767-16772 [PMID: 18931303 DOI: 10.1073/pnas.0808567105]

68 **den Besten G**, Lange K, Havinga R, van Dijk TH, Gerding A, van Eunen K, Müller M, Groen AK, Hooiveld GJ, Bakker BM, Reijngoud DJ. Gut-derived short-chain fatty acids are vividly assimilated into host carbohydrates and lipids. *Am J Physiol Gastrointest Liver Physiol* 2013; **305**: G900-G910 [PMID: 24136789 DOI: 10.1152/ajpgi.00265.2013]

69 **Rau M**, Rehman A, Dittrich M, Groen AK, Hermanns HM, Seyfried F, Beyersdorf N, Dandekar T, Rosenstiel P, Geier A. Fecal SCFAs and SCFA-producing bacteria in gut microbiome of human NAFLD as a putative link to systemic T-cell activation and advanced disease. *United European Gastroenterol J* 2018; **6**: 1496-1507 [PMID: 30574320 DOI: 10.1177/2050640618804444]

70 **Zehra M**, Curry JC, Pillai SS, Lakhani HV, Edwards CE, Sodhi K. Elucidating Potential Profibrotic Mechanisms of Emerging Biomarkers for Early Prognosis of Hepatic Fibrosis. *Int J Mol Sci* 2020; **21** [PMID: 32635162 DOI: 10.3390/ijms21134737]

71 **Haston CK**, Zhou X, Gumbiner-Russo L, Irani R, Dejournett R, Gu X, Weil M, Amos CI, Travis EL. Universal and radiation-specific loci influence murine susceptibility to radiation-induced pulmonary fibrosis. *Cancer Res* 2002; **62**: 3782-3788 [PMID: 12097289]

72 **Liebe R**, Hall RA, Williams RW, Dooley S, Lammert F. Systems genetics of hepatocellular damage in vivo and in vitro: identification of a critical network on chromosome 11 in mouse. *Physiol Genomics* 2013; **45**: 931-939 [PMID: 23943854 DOI: 10.1152/physiolgenomics.00078.2013]

73 **Bruschi FV**, Tardelli M, Herac M, Claudel T, Trauner M. Metabolic regulation of hepatic PNPLA3 expression and severity of liver fibrosis in patients with NASH. *Liver Int* 2020; **40**: 1098-1110 [PMID: 32043752 DOI: 10.1111/liv.14402]

74 **Sharma M**, Mitnala S, Vishnubhotla RK, Mukherjee R, Reddy DN, Rao PN. The Riddle of Nonalcoholic Fatty Liver Disease: Progression From Nonalcoholic Fatty Liver to Nonalcoholic Steatohepatitis. *J Clin Exp Hepatol* 2015; **5**: 147-158 [PMID: 26155043 DOI:

10.1016/j.jceh.2015.02.002]
75 **Moran-Salvador E**, Mann J. Epigenetics and Liver Fibrosis. *Cell Mol Gastroenterol Hepatol* 2017; **4**: 125-134 [PMID: 28593184 DOI: 10.1016/j.jcmgh.2017.04.007]
76 **Komatsu Y**, Waku T, Iwasaki N, Ono W, Yamaguchi C, Yanagisawa J. Global analysis of DNA methylation in early-stage liver fibrosis. *BMC Med Genomics* 2012; **5**: 5 [PMID: 22281153 DOI: 10.1186/1755-8794-5-5]
77 **Mann J**, Oakley F, Akiboye F, Elsharkawy A, Thorne AW, Mann DA. Regulation of myofibroblast transdifferentiation by DNA methylation and MeCP2: implications for wound healing and fibrogenesis. *Cell Death Differ* 2007; **14**: 275-285 [PMID: 16763620 DOI: 10.1038/sj.cdd.4401979]
78 **You H**, Sun YM. [Cellular mechanism in the fibrogenesis of liver fibrosis]. *Zhonghua Gan Zang Bing Za Zhi* 2012; **20**: 563-564 [PMID: 23227528]
79 **Zhao Q**, Qin CY, Zhao ZH, Fan YC, Wang K. Epigenetic modifications in hepatic stellate cells contribute to liver fibrosis. *Tohoku J Exp Med* 2013; **229**: 35-43 [PMID: 23238615 DOI: 10.1620/tjem.229.35]
80 **Gonzalez A**, Huerta-Salgado C, Orozco-Aguilar J, Aguirre F, Tacchi F, Simon F, Cabello-Verrugio C. Role of Oxidative Stress in Hepatic and Extrahepatic Dysfunctions during Nonalcoholic Fatty Liver Disease (NAFLD). *Oxid Med Cell Longev* 2020; **2020**: 1617805 [PMID: 33149804 DOI: 10.1155/2020/1617805]
81 **Nati M**, Haddad D, Birkenfeld AL, Koch CA, Chavakis T, Chatzigeorgiou A. The role of immune cells in metabolism-related liver inflammation and development of non-alcoholic steatohepatitis (NASH). *Rev Endocr Metab Disord* 2016; **17**: 29-39 [PMID: 26847547 DOI: 10.1007/s11154-016-9339-2]
82 **Alkhouri N**, Morris-Stiff G, Campbell C, Lopez R, Tamimi TA, Yerian L, Zein NN, Feldstein AE. Neutrophil to lymphocyte ratio: a new marker for predicting steatohepatitis and fibrosis in patients with nonalcoholic fatty liver disease. *Liver Int* 2012; **32**: 297-302 [PMID: 22097893 DOI: 10.1111/j.1478-3231.2011.02639.x]
83 **Irvine KM**, Ratnasekera I, Powell EE, Hume DA. Causes and Consequences of Innate Immune Dysfunction in Cirrhosis. *Front Immunol* 2019; **10**: 293 [PMID: 30873165 DOI: 10.3389/fimmu.2019.00293]
84 **Sargenti K**, Johansson Å, Bertilsson S, Mattsby-Baltzer I, Klintman D, Kalaitzakis E. Dysfunction of Circulating Polymorphonuclear Leukocytes and Monocytes in Ambulatory Cirrhotics Predicts Patient Outcome. *Dig Dis Sci* 2016; **61**: 2294-2302 [PMID: 27010544 DOI: 10.1007/s10620-016-4132-3]
85 **Maricic I**, Marrero I, Eguchi A, Nakamura R, Johnson CD, Dasgupta S, Hernandez CD, Nguyen PS, Swafford AD, Knight R, Feldstein AE, Loomba R, Kumar V. Differential Activation of Hepatic Invariant NKT Cell Subsets Plays a Key Role in Progression of Nonalcoholic Steatohepatitis. *J Immunol* 2018; **201**: 3017-3035 [PMID: 30322964 DOI: 10.4049/jimmunol.1800614]
86 **Confer BD**, Lopez R, Zein NN. CD4+ T-Cell function and the risk for developing spontaneous bacterial peritonitis in patients with cirrhosis. *J Clin Gastroenterol* 2013; **47**: 807-813 [PMID: 23507764 DOI: 10.1097/MCG.0b013e3182854969]
87 **Breuer DA**, Pacheco MC, Washington MK, Montgomery SA, Hasty AH, Kennedy AJ. $CD8^+$ T cells regulate liver injury in obesity-related nonalcoholic fatty liver disease. *Am J Physiol Gastrointest Liver Physiol* 2020; **318**: G211-G224 [PMID: 31709830 DOI: 10.1152/ajpgi.00040.2019]
88 **Huang CH**, Jeng WJ, Ho YP, Teng W, Chen WT, Chen YC, Lin SM, Chiu CT, Sheen IS, Lin CY. Increased regulatory T cells in patients with liver cirrhosis correlated with hyperbilirubinemia and predict bacterial complications. *J Gastroenterol Hepatol* 2015; **30**: 775-783 [PMID: 25250558 DOI: 10.1111/jgh.12781]
89 **Chackelevicius CM**, Gambaro SE, Tiribelli C, Rosso N. Th17 involvement in nonalcoholic fatty liver disease progression to non-alcoholic steatohepatitis. *World J Gastroenterol* 2016; **22**: 9096-9103 [PMID: 27895397 DOI: 10.3748/wjg.v22.i41.9096]
90 **Panda SK**, Colonna M. Innate Lymphoid Cells in Mucosal Immunity. *Front Immunol* 2019; **10**: 861 [PMID: 31134050 DOI: 10.3389/fimmu.2019.00861]
91 **Li S**, Hong M, Tan HY, Wang N, Feng Y. Insights into the Role and Interdependence of Oxidative Stress and Inflammation in Liver Diseases. *Oxid Med Cell Longev* 2016; **2016**: 4234061 [PMID: 28070230 DOI: 10.1155/2016/4234061]
92 **Shabangu CS**, Huang JF, Hsiao HH, Yu ML, Chuang WL, Wang SC. Liquid Biopsy for the Diagnosis of Viral Hepatitis, Fatty Liver Steatosis, and Alcoholic Liver Diseases. *Int J Mol Sci* 2020; **21** [PMID: 32466319 DOI: 10.3390/ijms21103732]
93 **Tranah TH**, Edwards LA, Schnabl B, Shawcross DL. Targeting the gut-liver-immune axis to treat cirrhosis. *Gut* 2021; **70**: 982-994 [PMID: 33060124 DOI: 10.1136/gutjnl-2020-320786]
94 **Such J**, Guardiola JV, de Juan J, Casellas JA, Pascual S, Aparicio JR, Solá-Vera J, Pérez-Mateo M. Ultrastructural characteristics of distal duodenum mucosa in patients with cirrhosis. *Eur J Gastroenterol Hepatol* 2002; **14**: 371-376 [PMID: 11943948 DOI: 10.1097/00042737-200204000-00006]
95 **Cho MS**, Kim SY, Suk KT, Kim BY. Modulation of gut microbiome in nonalcoholic fatty liver disease: pro-, pre-, syn-, and antibiotics. *J Microbiol* 2018; **56**: 855-867 [PMID: 30377993 DOI: 10.1007/s12275-018-8346-2]
96 **Orellana AM**, Kinoshita PF, Leite JA, Kawamoto EM, Scavone C. Cardiotonic Steroids as Modulators of Neuroinflammation. *Front Endocrinol (Lausanne)* 2016; **7**: 10 [PMID: 26909067

DOI: 10.3389/fendo.2016.00010]
97 **Xu Y**, Marck P, Huang M, Xie JX, Wang T, Shapiro JI, Cai L, Feng F, Xie Z. Biased Effect of Cardiotonic Steroids on Na/K-ATPase-Mediated Signal Transduction. *Mol Pharmacol* 2021; **99**: 217-225 [PMID: 33495275 DOI: 10.1124/molpharm.120.000101]
98 **Campia I**, Gazzano E, Pescarmona G, Ghigo D, Bosia A, Riganti C. Digoxin and ouabain increase the synthesis of cholesterol in human liver cells. *Cell Mol Life Sci* 2009; **66**: 1580-1594 [PMID: 19288057 DOI: 10.1007/s00018-009-9018-5]
99 **Luo J**, Yang H, Song BL. Mechanisms and regulation of cholesterol homeostasis. *Nat Rev Mol Cell Biol* 2020; **21**: 225-245 [PMID: 31848472 DOI: 10.1038/s41580-019-0190-7]
100 **Arguello G**, Balboa E, Arrese M, Zanlungo S. Recent insights on the role of cholesterol in non-alcoholic fatty liver disease. *Biochim Biophys Acta* 2015; **1852**: 1765-1778 [PMID: 26027904 DOI: 10.1016/j.bbadis.2015.05.015]
101 **Liu J**, Lilly MN, Shapiro JI. Targeting Na/K-ATPase Signaling: A New Approach to Control Oxidative Stress. *Curr Pharm Des* 2018; **24**: 359-364 [PMID: 29318961 DOI: 10.2174/1381612824666180110101052]
102 **Sharpton SR**, Ajmera V, Loomba R. Emerging Role of the Gut Microbiome in Nonalcoholic Fatty Liver Disease: From Composition to Function. *Clin Gastroenterol Hepatol* 2019; **17**: 296-306 [PMID: 30196156 DOI: 10.1016/j.cgh.2018.08.065]
103 **Aron-Wisnewsky J**, Vigliotti C, Witjes J, Le P, Holleboom AG, Verheij J, Nieuwdorp M, Clément K. Gut microbiota and human NAFLD: disentangling microbial signatures from metabolic disorders. *Nat Rev Gastroenterol Hepatol* 2020; **17**: 279-297 [PMID: 32152478 DOI: 10.1038/s41575-020-0269-9]
104 **Lynch SV**, Pedersen O. The Human Intestinal Microbiome in Health and Disease. *N Engl J Med* 2016; **375**: 2369-2379 [PMID: 27974040 DOI: 10.1056/NEJMra1600266]
105 **Henao-Mejia J**, Elinav E, Jin C, Hao L, Mehal WZ, Strowig T, Thaiss CA, Kau AL, Eisenbarth SC, Jurczak MJ, Camporez JP, Shulman GI, Gordon JI, Hoffman HM, Flavell RA. Inflammasome-mediated dysbiosis regulates progression of NAFLD and obesity. *Nature* 2012; **482**: 179-185 [PMID: 22297845 DOI: 10.1038/nature10809]
106 **Van Herck MA**, Vonghia L, Francque SM. Animal Models of Nonalcoholic Fatty Liver Disease-A Starter's Guide. *Nutrients* 2017; **9** [PMID: 28953222 DOI: 10.3390/nu9101072]
107 **Brandl K**, Schnabl B. Intestinal microbiota and nonalcoholic steatohepatitis. *Curr Opin Gastroenterol* 2017; **33**: 128-133 [PMID: 28257306 DOI: 10.1097/MOG.0000000000000349]
108 **Grabherr F**, Grander C, Effenberger M, Adolph TE, Tilg H. Gut Dysfunction and Non-alcoholic Fatty Liver Disease. *Front Endocrinol (Lausanne)* 2019; **10**: 611 [PMID: 31555219 DOI: 10.3389/fendo.2019.00611]
109 **Shen F**, Zheng RD, Sun XQ, Ding WJ, Wang XY, Fan JG. Gut microbiota dysbiosis in patients with non-alcoholic fatty liver disease. *Hepatobiliary Pancreat Dis Int* 2017; **16**: 375-381 [PMID: 28823367 DOI: 10.1016/S1499-3872(17)60019-5]
110 **Safari Z**, Gérard P. The links between the gut microbiome and non-alcoholic fatty liver disease (NAFLD). *Cell Mol Life Sci* 2019; **76**: 1541-1558 [PMID: 30683985 DOI: 10.1007/s00018-019-03011-w]
111 **Raman M**, Ahmed I, Gillevet PM, Probert CS, Ratcliffe NM, Smith S, Greenwood R, Sikaroodi M, Lam V, Crotty P, Bailey J, Myers RP, Rioux KP. Fecal microbiome and volatile organic compound metabolome in obese humans with nonalcoholic fatty liver disease. *Clin Gastroenterol Hepatol* 2013; **11**: 868-75.e1 [PMID: 23454028 DOI: 10.1016/j.cgh.2013.02.015]
112 **Wang B**, Jiang X, Cao M, Ge J, Bao Q, Tang L, Chen Y, Li L. Altered Fecal Microbiota Correlates with Liver Biochemistry in Nonobese Patients with Non-alcoholic Fatty Liver Disease. *Sci Rep* 2016; **6**: 32002 [PMID: 27550547 DOI: 10.1038/srep32002]
113 **de Faria Ghetti F**, Oliveira DG, de Oliveira JM, de Castro Ferreira LEVV, Cesar DE, Moreira APB. Influence of gut microbiota on the development and progression of nonalcoholic steatohepatitis. *Eur J Nutr* 2018; **57**: 861-876 [PMID: 28875318 DOI: 10.1007/s00394-017-1524-x]
114 **Tsai MC**, Liu YY, Lin CC, Wang CC, Wu YJ, Yong CC, Chen KD, Chuah SK, Yao CC, Huang PY, Chen CH, Hu TH, Chen CL. Gut Microbiota Dysbiosis in Patients with Biopsy-Proven Nonalcoholic Fatty Liver Disease: A Cross-Sectional Study in Taiwan. *Nutrients* 2020; **12** [PMID: 32204538 DOI: 10.3390/nu12030820]
115 **Bajaj JS**, Heuman DM, Hylemon PB, Sanyal AJ, White MB, Monteith P, Noble NA, Unser AB, Daita K, Fisher AR, Sikaroodi M, Gillevet PM. Altered profile of human gut microbiome is associated with cirrhosis and its complications. *J Hepatol* 2014; **60**: 940-947 [PMID: 24374295 DOI: 10.1016/j.jhep.2013.12.019]
116 **Lima M**, Andrade A, Oliveira G, Nicoli J, Martins F, Kroon E, Abrahão J. Virus and microbiota relationships in humans and other mammals: An evolutionary view. *Hum Micro J* 2019; **11**: 100050 [DOI: 10.1016/j.humic.2018.11.001]
117 **Carding SR**, Davis N, Hoyles L. Review article: the human intestinal virome in health and disease. *Aliment Pharmacol Ther* 2017; **46**: 800-815 [PMID: 28869283 DOI: 10.1111/apt.14280]
118 **Schmelcher M**, Loessner MJ. Bacteriophage endolysins: applications for food safety. *Curr Opin Biotechnol* 2016; **37**: 76-87 [PMID: 26707470 DOI: 10.1016/j.copbio.2015.10.005]
119 **Canchaya C**, Fournous G, Chibani-Chennoufi S, Dillmann ML, Brüssow H. Phage as agents of lateral gene transfer. *Curr Opin Microbiol* 2003; **6**: 417-424 [PMID: 12941415 DOI: 10.1016/s1369-5274(03)00086-9]

120 **Lang S**, Demir M, Martin A, Jiang L, Zhang X, Duan Y, Gao B, Wisplinghoff H, Kasper P, Roderburg C, Tacke F, Steffen HM, Goeser T, Abraldes JG, Tu XM, Loomba R, Stärkel P, Pride D, Fouts DE, Schnabl B. Intestinal Virome Signature Associated With Severity of Nonalcoholic Fatty Liver Disease. *Gastroenterology* 2020; **159**: 1839-1852 [PMID: 32652145 DOI: 10.1053/j.gastro.2020.07.005]

121 **Li XV**, Leonardi I, Iliev ID. Gut Mycobiota in Immunity and Inflammatory Disease. *Immunity* 2019; **50**: 1365-1379 [PMID: 31216461 DOI: 10.1016/j.immuni.2019.05.023]

122 **Jiang L**, Stärkel P, Fan JG, Fouts DE, Bacher P, Schnabl B. The gut mycobiome: a novel player in chronic liver diseases. *J Gastroenterol* 2021; **56**: 1-11 [PMID: 33151407 DOI: 10.1007/s00535-020-01740-5]

123 **Liew WP**, Mohd-Redzwan S. Mycotoxin: Its Impact on Gut Health and Microbiota. *Front Cell Infect Microbiol* 2018; **8**: 60 [PMID: 29535978 DOI: 10.3389/fcimb.2018.00060]

124 **BioRender**. Adapted from "Nonalcoholic Fatty Liver Disease (NAFLD) Spec-trum. [cited 10 January 2021]. Available from: https://app.biorender.com/biorender-templates

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REVIEW

Fatty acid metabolism and acyl-CoA synthetases in the *liver-gut axis*

Yunxia Ma, Miljana Nenkov, Yuan Chen, Adrian T Press, Elke Kaemmerer, Nikolaus Gassler

ORCID number: Yunxia Ma 0000-0001-7409-4001; Miljana Nenkov 0000-0001-7976-2611; Yuan Chen 0000-0002-4752-9222; Adrian T Press 0000-0002-6089-6764; Elke Kaemmerer 0000-0002-9640-9358; Nikolaus Gassler 0000-0002-7351-258X.

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Yunxia Ma, Miljana Nenkov, Yuan Chen, Nikolaus Gassler, Section Pathology, Institute of Forensic Medicine, Jena University Hospital, Friedrich Schiller University Jena, Jena 07747, Germany

Adrian T Press, Department of Anesthesiology and Intensive Care Medicine and Center for Sepsis Control and Care, Jena University Hospital, Friedrich Schiller University Jena, Jena 07747, Germany

Elke Kaemmerer, Department of Pediatrics, Jena University Hospital, Friedrich Schiller University Jena, Jena 07747, Germany

Corresponding author: Nikolaus Gassler, MA, MD, Professor, Section Pathology, Institute of Forensic Medicine, Jena University Hospital, Friedrich Schiller University Jena, Am Klinikum 1, Jena 07747, Germany. nikolaus.gassler@med.uni-jena.de

Abstract

Fatty acids are energy substrates and cell components which participate in regulating signal transduction, transcription factor activity and secretion of bioactive lipid mediators. The acyl-CoA synthetases (ACSs) family containing 26 family members exhibits tissue-specific distribution, distinct fatty acid substrate preferences and diverse biological functions. Increasing evidence indicates that dysregulation of fatty acid metabolism in the *liver-gut axis*, designated as the bidirectional relationship between the gut, microbiome and liver, is closely associated with a range of human diseases including metabolic disorders, inflammatory disease and carcinoma in the gastrointestinal tract and liver. In this review, we depict the role of ACSs in fatty acid metabolism, possible molecular mechanisms through which they exert functions, and their involvement in hepatocellular and colorectal carcinoma, with particular attention paid to long-chain fatty acids and small-chain fatty acids. Additionally, the *liver-gut* communication and the liver and gut intersection with the microbiome as well as diseases related to microbiota imbalance in the *liver-gut axis* are addressed. Moreover, the development of potentially therapeutic small molecules, proteins and compounds targeting ACSs in cancer treatment is summarized.

Key Words: Long-chain fatty acids; Short-chain fatty acids; Acyl-CoA synthetases; Microbiota; *Liver-gut axis*

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Core Tip: To understand the role of acyl-CoA synthetases (ACSs) in the fatty acid metabolism, it is necessary to explore the biological function, gene interactions/regulations and signal pathways in physiological and pathological conditions. Growing evidence demonstrates that the control of microbial balance plays an important role in maintaining homeostasis and normal functions of the *liver-gut* axis, and the bidirectional communication in turn affects microbial communities. As novel therapeutic targets, miRNAs are receiving more and more attention, together with other compounds targeting ACSs.

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INTRODUCTION

Lipids, one of three main nutrients, are mainly composed of fatty acids (FAs), triglycerides (TGs), phospholipid and cholesterol. Lipid metabolites are involved in various biological functions and physiological processes, ranging from energy storage and degradation and structural composition to molecule signaling as well as signal transduction cascade[1].

The *liver-gut axis* plays a critical role in the homeostasis of lipid metabolism in the human body during the feed-fast cycle. Free FAs are absorbed by enterocyte and intestine-derived products released into portal blood which is directed to the liver; in turn, the liver responds by secreting bile acids (BAs) to the intestine *via* the biliary tract. BAs are transported back to the liver *via* enterohepatic circulation. Since the Volta group identified the important role of microorganisms in the *liver-gut axis* for the first time[2], a number of studies have confirmed that gut microbiota, described as an invisible metabolic 'organ', has a tight and coordinated connection with the gut and liver[3,4]. The intestinal mucosal barrier either acts as a physical barrier or lives in symbiosis with microbiota. Once the balance of symbiosis is disrupted, microbiota responds to this imbalance, microbiota metabolites (short-chain fatty acids, SCFAs) are modified and circulated into the liver. Aberrant lipid metabolism in the *liver-gut axis* has been linked with intestinal bowel diseases and diverse liver diseases[5].

Around 95% of dietary lipids absorbed are TGs, mainly composed of long-chain fatty acids (LCFAs)[6]. Fatty acid metabolism takes place mainly in intestinal enterocytes and hepatocytes, further assisted by adipocytes and other cell types. To become further involved in both anabolic and catabolic pathways, FAs must be taken up and activated by thioesterification. This ATP-mediated coupling reaction of FAs with coenzyme A is catalyzed by the enzymes called acyl-CoA synthetases (ACSs). ACSs are classified into five groups according to the fatty acid chain length: short-chain, medium-chain, bubblegum-chain, long-chain and very-long-chain acyl CoA synthetases (ACSVLs)[7]. ACSVLs as membrane channel proteins have been identified as a major enzyme responsible for LCFA uptake and activation[8]. Long-chain acyl-CoA synthetases (ACSLs) are responsible for the catalyzation of intracellular free LCFAs which are transported by other transport proteins, such as fatty acid translocase (CD36) and fatty acid binding proteins (FABPs)[9]. Short-chain acyl-CoA synthetases (ACSSs) are involved in the activation of microbiota-derived SCFAs, such as acetate and propionate[10] (Table 1).

In this review, we will summarize the functional role of ACSs in fatty acid metabolism, focusing on LCFAs and SCFAs, as well as potential therapeutic targets of ACSs. Furthermore, we will explore the influence of dietary diversity on microbiota and the microbial metabolites, and their bidirectional communication in the *liver-gut* axis.

Table 1 miRNA and compounds targeting acyl-CoA synthetases

Type	Name	Target	Mechanism	Ref.
miRNA	miR-205	ACSL4/ACSL1	Inhibition of ACSL4/ACSL1 in hepatocellular carcinoma	[155,171]
	miR-211-5p	ACSL4	Inhibition of ACSL4 in hepatocellular carcinoma	[172]
	miR-19b-1	ACSL1/ACSL4/SCD1	Inhibition of ACSL1/ACSL4/SCD1 axis in colorectal cancer	[173]
	miR-142-3p	ACSL1/ACSL4/SCD1	Inhibition of ACSL1/ACSL4/SCD1 axis in colorectal cancer	[173,174]
	miR-34c	ACSL1	Inhibition of ACSL1 and induction of liver fibrogenesis	[175]
	miR-497-5p	ACSL5	Inhibition of ACSL5 in colon cancer	[170]
Compounds	Triacsin C	ACSL1/ACSL3/ACSL4 and ACSL5[1]	Inhibition of ACSL1/ACSL3/ACSL4 and ACSL5[1]	[177,178]
	Roglitazone Pioglitazone Troglitazone	ACSL4	Inhibition of ACSL4	[179-181]
	Lipofermata	FATP2	Inhibition of FATP2	[191,192]
	Grassofermata	FATP2	Inhibition of FATP2	[191,193,194]
	Ursodiol chenodiol	FATP5	Inhibition of FATP5 in liver	[195]
	Fenofibrate	PPARα	Indirect activation of FATP in liver predominantly	[196,197]

[1]Triacsin C is also competitive inhibitor of ACSL5 when used in higher concentration.

FATTY ACID METABOLISM MEDIATED BY ACYL-COA SYNTHETASES IN THE *LIVER-GUT AXIS*

Circulation of fatty acids and bile acids in the liver-gut axis

Intestinal absorption of FAs is a multistep process that includes digestion, uptake and absorption and needs to cooperate with large numbers of enzymes secreted by series of organs in the gastrointestinal tract[11]. TGs are first released from a fatty diet after digestion with lingual and gastric lipase in the stomach, and released TGs are further hydrolyzed by pancreatic lipase to produce 2-monoacylglycerides and free FAs[12]. Sequentially those digested FAs mix with BAs and emulsify to form spherical water-soluble droplets, called micelles (MCs). With intestinal peristalsis, MCs are transported to the small intestinal lumen and further translocated into the apical membrane of enterocytes.

In intestinal enterocytes, absorbed LCFAs experience a series of catabolic metabolisms for energy supply for massive biological activities, and anabolic metabolism to reconstitute lipids. Newly synthesized lipids are incorporated into transport vehicles, chylomicrons (CMs), that are later liberated from enterocytes, and then transported to the liver through the hepatic portal vein. The liver is the major processing factory of FAs and regulates and balances lipid homeostasis systemically in the *liver-gut axis*. Fatty acid uptake and metabolism occur in hepatocytes. During feeding, hepatocytes take up the influx of FAs and get rid of FAs *via* β-oxidation to produce energy, and reformed TGs integrated into CMs partition into two pathways: (1) Secreted into bloodstream; and (2) transported and stored in adipose tissue. During fasting or starvation, hepatocytes recycle TGs from lipid droplets and adipose tissue, and initiate *de novo* lipogenesis by using other energy sources in the liver, such as carbohydrates [13,14]. Therefore, the pool of FAs is always in dynamic equilibrium between dietary absorption in the enterocytes, process and lipogenesis in the liver and liver feedback regulation *via* BAs during the feed-fast cycle.

As previously mentioned, BAs are involved not only in facilitating MC formation, but also as signaling molecules and metabolic regulators of lipid/glucose metabolism, energy homeostasis and inflammation in the *liver-gut axis*[15]. It has been demonstrated that a higher level of BAs can be detected in the tissues of the *liver-gut axis* compared to peripheral blood[16]. Primary BAs are synthesized in the hepatocytes and secreted into the small intestine; most of them are reabsorbed in the ileum. A small

number of unabsorbed BAs are taken up by microbiota and metabolized into secondary BAs[17]. In enterocytes BAs are reabsorbed through the apical sodium-dependent BA transporter (ASBT), carried by the intestinal bile acid-binding protein (FABP6) and released into portal blood *via* heterodimeric transporter OSTα/OSTβ. BA activation of the nuclear farnesoid X receptor (FXR) also upregulates FABP6, OSTα/OSTβ and fibroblast growth factor 19 (FGF19), which further inhibits BAs synthesis. In hepatocytes, the transport of BAs is mediated by sodium-taurocholate cotransporting polypeptide (NTCP) and organic anion transporters (OATPs). BAs acting as an activator of hepatic FXR regulate the expression of genes involved in bile acid transport and synthesis. This enterohepatic circulation of BAs plays a critical role in maintaining the BAs pool in the *liver-gut axis*[18,19].

Long-chain fatty acid transport to enterocytes and hepatocytes

Free fatty acid uptake is requested across the phospholipid bilayer in the mammalian membrane. It is widely known that LCFAs can be taken up into cells *via* flip-flop diffusion with rate limiting[20,21]. High permeability of LCFA transport is mediated by several membrane-associated transport proteins including FA transport proteins (FATPs), FABPs, CD36 and caveolin (CAV)[9].

FATP1-6 (fatp in mice, also called ACSVL1-6) is a group of enzymatic proteins with double capabilities of transport and activation. FATP can trap and activate a broad range of LCFA and VLCFA to form acyl-CoA[9,22]. Different FATP family members have tissue-specific expression patterns[23]. In the intestine, FATP4 (ACSVL5) is strongly expressed in intestinal villi but not in crypts, which plays an important role in fatty acid absorption[24]. Fapt4-null mice display an embryonic lethality with a defective epidermal barrier. Fapt4 depletion alters the ceramide fatty acid composition significantly, especially in saturated VLCFA substitutes C26:0 and C26:0-OH[25]. FAPT5 (ACSVL6) mainly transports BAs but also LCFAs, is only expressed in the liver and particularly in the basal membrane of hepatocytes[8,26]. Fapt5 knockout mice showed this defective bile acid conjugation, indicating that Fapt5 is essential for fatty acid uptake by hepatocytes and maintenance of the lipid balance which further regulates body weight[27]. With the discovery of the topological structure of murine FAPT1 containing one transmembrane domain and a large cytoplasm domain[28], different mechanisms of FATP1 transporting exogenous FAs into cells have been proposed, one of which is vectorial transport or flipase function[29]. Moreover, BAs acting as a FATP5 antagonist dramatically decrease hepatic fatty acid uptake as well as liver triglyceride synthesis[30].

FABP 1-9 (fabp in mice) are a fatty acid binding protein superfamily that binds to FAs, cholesterol or other non-esterified FAs, facilitate fatty acid uptake and lipid metabolism[31]. FABP appears in two distinct forms depending on localization: one is peripheral membrane protein (FABPpm) and the other is intracellular/cytoplasmic protein (FABPc)[32]. Like FATP, different family members of FABPs exhibit organ-specific expression. FABP2 (Intestinal-FABP, I-FABP) encodes the intestinal form which is only expressed in the small intestine, and FABP-1(Liver-FABP, L-FABP) is only expressed in the liver[33]. I-FABP and L-FABP are all cytoplasmic proteins, but it is reported that they deliver FAs through different mechanisms of L-FABP in diffusion and I-FABP in collision[34]. L-fabp-null mice showed a reduced uptake of LCFAs as well as new biosynthesis for lipid storage or secretion, suggesting the important role of L-fabp in fatty acid esterification at endoplasmic reticulum (ER)[35]. Furthermore, L-FABP depletion suppresses lipid catabolism in mitochondria and downregulates the transcription of oxidative enzymes through inhibition of peroxisome proliferator-activated receptor (PPARα) transcriptor in the nucleus[36,37].

CD36, officially designated as scavenger receptor B2 (SR-B2), is a transmembrane glycoprotein which has a broad range of binding profiles including LCFAs, plasma lipoproteins, phospholipids, collagen[38]. CD36 whole body knockout mice showed significantly decreased fatty acid uptake in the heart and skeletal muscle[39]. In the intestine, CD36 is only detected in the duodenal and jejunal parts and plays a critical role for fatty acid and cholesterol uptake in the small intestine[40]. Although CD36 has a very low expression level in the liver, CD36 liver-specific knockout in the steatosis model indicated that CD36 deletion reduces lipid content and inflammation and improves insulin sensitivity[41].

CAV 1-3 (cav in mice) are intramembrane proteins which are responsible for caveolae formation. CAV1 as a cholesterol-binding protein is implicated in cholesterol trafficking and absorption[42]. However, Cav1 knockout mice did not show a compensatory mechanism to increase other family members, such as Cav2 and Cav3, and cholesterol absorption and sterol excretion were also not changed in the intestine [43]. Additionally, CAV1 also acts as a cytosolic intermediate form involved in

lipogenesis and lipid body formation during liver regeneration[44].

It is widely recognized that several fatty acid transport proteins cooperate synergistically to accomplish the process of fatty acid transport (Figure 1). Due to the tissue-specific expression pattern, FATP4, FABPpm, FABP-I, CD36 are main types in the intestine and FATP5, FABPpm, FABP-L, CD36 are major types in the liver. Partial LCFAs are activated during transport *via* FATP. The rest of the LCFAs are grabbed by FABPpm and presented to CD36. Free cytosolic LCFAs is not only activated by ACSLs for esterification of acyl-CoA but also trapped by FABPc for subcellular function. Generated acyl-CoA as a raw material initiates the subsequent metabolism pathway to produce energy or synthesize diverse complex lipids. In addition, acyl-CoA can be deactivated to free FAs and CoA, and this process is mediated by acyl-CoA thioesterases (ACOTs). ACSLs and ACOTs are two critical enzymes helping to control the dynamic balance between acyl-CoA and free FAs.

Long-chain fatty acid activation in enterocytes and hepatocytes

As mentioned previously, most of the abundant dietary FAs are LCFAs so ACSLs are addressed in more details here. In humans and rodents there are five existing ACSL isoforms namely ACSL1, ACSL3, ACSL4, ACSL5 and ACSL6 (acsl in mice), each one coded by the different gene containing several splice variants[45]. Due to the differences in the 5'UTRs, the first coding exon, alternative coding exons and exchangeable motifs, different variants of each ACSL isoform are available[46]. The ACSL isoforms have two motifs: ATP binding and fatty acid binding[47]. The fatty acid binding tunnel located at the N-terminal domain has been linked to the substrate specificity of each ACSL isoform[48]. Since the N-terminal domain varies between the different ACSL isoforms, it contributes to the substrate preference of each family member and its different subcellular localization which is essential for vectorial acylation[49].

ACSL1 is predominantly located in the liver. Knockout of ACSL1 in the liver demonstrated a reduction in total ACSL activity of up to 50%, together with a decrease in the hepatic amount of acyl-CoA and a decreased level of oleic acid-derived TG[1, 50]. Acsl1 deficient mice showed a 50% reduction in the amount of long-chain acyl-carnitines, leading to the conclusion that the loss of Acsl1 impaired partitioning of its products into TG synthesis and oxidation pathways[1]. Due to its both endoplasmic and mitochondrial localization, ACSL1 directs its metabolites to both the anabolic (TG synthesis) and catabolic (β-oxidation) pathway[1].

ACSL3 Localization is linked to the lipid droplets and ER in the liver and other tissue. The increase in fatty acid uptake causes a transition of ACSL3 from ER to the lipid droplets, suggesting its role in neutral lipid synthesis[1]. Knockdown of ACSL3 reduced the activity of transcription factors including PPARγ, ChREBP, SREBP1C and Liver X receptor and their target genes involved in hepatic lipogenesis[1]. ACSL3 activates FAs incorporated into phospholipids, which are used for very-low density lipoprotein (VLDL) production[50]. As revealed by Yan *et al*, ACSL3 knockdown decreased the level of VLDL in hepatic cells[50]. Besides its role in the activation of FAs, overexpression of ACSL3 was found to be able to induce cellular fatty acid uptake[51].

ACSL4 is mostly expressed in adrenal glands and steroid-producing organs[52,53]. The role of ACSL4 is related to the activation of polyunsaturated FAs in steroidogenic tissue. ACSL4 has a preference for the arachidonic acid which is involved in the eicosanoid synthesis.

The nuclear-coded ACSL5 is prominent in both the mitochondria and ER of the intestinal mucosa and liver[50]. Highest expression was detected in the jejunum and ACSL5 was assumed to be involved in dietary fatty acid absorption. However, studies in acsl5 null mice showed no alteration in dietary fatty acid absorption but a significant decrease in total ACSL activity[1]. In the liver, ACSL5 activates LCFAs mostly of C18 carbon atoms, which are further incorporated into TGs, phospholipids and cholesterol esters. According to previous reports, ACSL5 plays a role in the metabolism of dietary FAs, but not in *de novo* synthetized ones[50,54,55]. Since ACSL5 is localized on the mitochondrial outer membrane, the activity was initially attributed to β-oxidation. Some studies with ectopic expression of ACSL5 failed to prove this, but the increased synthesis of TGs and diglycerides was observed in the liver[54]. ACSL5 is a dominant activator of dietary LCFAs and displayed an 80% lower activity in total acsl of the jejunum in acsl5 knockout mice[56]. ACSL5 is strongly expressed by enterocytes in an ascending gradient along the *crypt-villus* axis with the highest expression level at the villus tip; however, nuclear β-catenin, a hallmark of Wnt activation, is expressed in a descending gradient along the *crypt-villus* axis[57], suggesting an interplay between ACSL5 and Wnt activity during enterocyte differen-

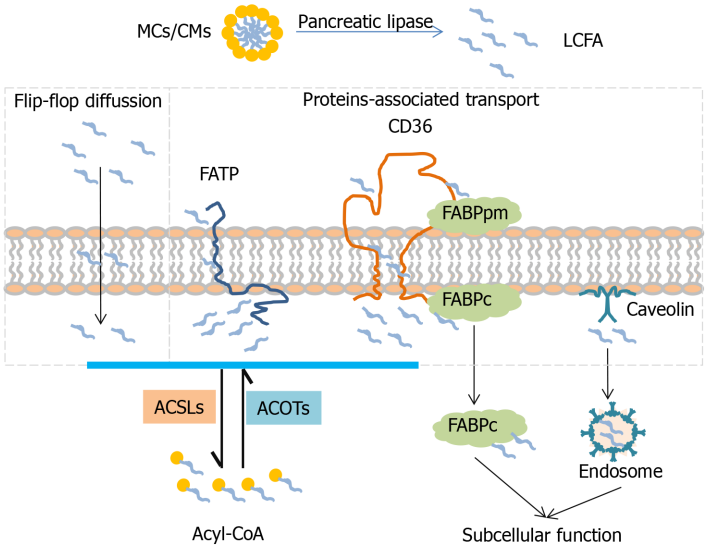

Figure 1 Mechanism of long-chain fatty acid transport across the lipid raft. LCFAs are taken up into cell in two different ways. One is passive transport by a flip-flop with rate limiting. The other is active transport, which is mediated with transport-associated proteins (FATPs, CD36, FABPs and Caveolin). FATPs with tissue-specific distribution integrating both transport and activation functions are responsible for LCFAs uptake. Free FAs trapped by the FABPpm present to CD36 and are transported into cells. Consequently released free FAs bind with FABPc and CAV channel into different organelles and are activated by different subcellular expression of ACSLs into acyl-CoA. In addition, acyl-CoA can be deactivated to free FA and CoA which is mediated by ACOTs. Liver-specific proteins: FATP5, FABP-L, ACSL1; Intestine-specific proteins: FATP4, FABP-I, ACSL5; ACSL: Acyl-CoA synthetase, ACOT: Acyl-CoA thioesterase; MCs: Micelles, CMs: Chylomicrons

tiation and maturation[58].

ACSL6 is highly expressed in the brain where it plays a role in phospholipid synthesis during neurite outgrowth. ACSL expression is controlled by the level of intracellular FAs in physiological conditions[1].

Short-chain fatty acid transport and activation in enterocytes and hepatocytes

Microbiota-derived SCFAs cross the lipid membrane *via* different mechanisms: non-ionized diffusion, Na^+/H^+-dependent gradient exchange[59,60]. Intracellular SCFAs can shuttle between cytosol, nucleus and mitochondria *via* a diffusion mechanism[10,60]. SCFA activation by ACSSs is the first step in utilizing the energy source. ACSS 1-3 (acss in mice) are encoded and designated in humans. ACSS1 and ACSS3 are localized at the mitochondria matrix, while ACSS2 is a nuclear-cytosolic enzyme. ACSS1 and ACSS2 activate acetate to thioester into acetyl-CoA, but ACSS3 favors propionate[10].

In humans, mitochondrial ACSS1 is most highly expressed in the brain, blood, testis and intestine, also to a certain level in the heart, muscle and kidney, but not in the liver or spleen[61]. In mice, ACSS1 is strongly expressed in the heart, kidney, skeletal muscle and brown adipose tissue, which all need high energy expenditure[62]. Acss1 knockout mice showed a remarkably decreased acetate oxidation in the whole body during fasting compared with the wild type, however, no histological changes were detected in multiple tissues including the intestine and liver[63]. ACSS3 displays the character of propionyl-CoA synthetase as well as the highest expression in the liver. Knockdown of ACSS3 in hepG2 significantly decreases the activity of propionyl-CoA synthetase. During fasting, ACSS3 is upregulated, which is probably linked to ketogenesis, and ACSS2 is downregulated[64].

ACSS2 is most highly expressed in the liver and kidney[64,65]. Moffet *et al*[10] introduced the concept that the expression of ACSS2 in different cell types is based on the different physiological conditions to utilize acetate. Therefore, the liver is supposed to be the main organ for processing acetate. With the feature of localization, ACSS2 catalyzes acetate into acetyl-CoA which is correlated with fatty acid biosynthesis in cytosol, and retains acetate released from histone in the nucleus[66]. Acss2-deficient mice with high-fat feeding can lighten fat deposition in the liver by regulating many genes involved in lipid metabolism, suggesting that Acss2 acts as a transcription regulator during lipogenesis[67].

The expression and localization pattern of ACSS 1-3 suggests that ACSS1 and ACSS3 are responsible for energy production by using acetate in the intestine and liver respectively. The majority of acetate is taken up by the liver, ACSS2 in cytoplasm is involved in lipogenesis and is distributed to other organs in ketone bodies through systemic circulation. Acetyl-CoA as a central metabolite can go into either energy

production or lipid biosynthesis. ACSS1-3 plays a key role in regulating the level of acetyl-CoA in the nucleus, mitochondria and cytoplasm (Figure 2).

MICROBIOTA UTILIZATION OF DIET, MICROBIOTA METABOLITES AND THE ROLE OF MICROBIOTA IN THE *LIVER-GUT AXIS*

Dietary structure shapes the composition of microbiota

Gut microbiota, a diverse microbial community with approximately 100 trillion microorganisms, is colonized in the gastrointestinal tract. In human adults, five families microbiota are mainly Firmicutes, Bacteroidetes, Actinobacteria, Proteobacteria and Verrucomicrobia, while phylum Firmicutes and Bacteroidetes make up approximately 80% of all species[68].

A high-fiber intake population has higher diversity microbiota and more SCFAs production than a high-calorie diet population, and two populations showed distinct diet favor microbiota[69]. *Bacteroides* and *Prevotella* are two dominant groups which are highly enriched in a high-protein/fat diet population and high-fiber population respectively[70,71]. Moreover, the composition of fecal microbiota varies by age, geography and lifestyle due to the behavior of microbiota dietary preferences[72]. The term microbiota-accessible carbohydrates (MACs) introduced by Sonnenburg *et al* refers to microbiota favorable-carbohydrates that cannot be digested by the host. Mice feeding on a long-term low-MACs diet display a remarkably reduced diversity of microflora containing mostly *Bacteroildales* and *Clostridiales*. Although the microbiota composition cannot be restored after refeeding with a high-MAC diet, it increases again mainly in *Bacteroidales* upon reintroduction of fecal microbiota[73].

SCFAs are metabolic end-products from specialized bacteria utilizing with undigested dietary polysaccharides in human small intestine. The most abundant SCFAs in the intestine are acetate (C2), propionate (C3) and butyrate (C4). The phylum Bacteroidetes, the most abundant gram-negative bacteria with a high flexibility to adapt the environment, are associated with acetate production[74]. Phylum Bacteroidetes and Negativicutes (*Akkermansia muciniphila*, family Veillonellacear and phylum Firmicutes) are dominantly responsible for production of propionate by the succinate pathway, small bacterial genera from phylum Firmicutes have been identified to form propionate through the acrylate pathway, and distant Lachnospiraceae are known to produce propionate by utilizing the propanediol pathway[75]. Several species from families Lachnospiraceae, Ruminococcaceae and Erysipelotrichacear (Phylum Firmicutes) produce butyrate *via* butyrate kinase route and butyryl-CoA:acetate CoA-transferase route[76]. Diverse composition of microbiota has distinct SCFAs profiles, and additionally, SCFAs-metabolic network is a cross-feeding microbial system between different bacterial species[77].

In all, a high intake of MACs is pivotal in shaping the diversity and composition of microbiota. Diverse microbiota-generated SCFAs reversely influence the microbial communities and further act as a mediator is strongly involved in host-microbiota cross-talk.

Utilization of long-chain fatty acid in microbiota

Microbiota can also employ luminal unabsorbed LCFAs directly as energy source once there is a fermentable fiber deficiency[78]. LCFAs cross the cellular envelope in bacteria and yeast, unlike in mammalian cells. In bacteria, FadL transports exogenous LCFAs from outer membrane to periplasm, FadD (role as ACSLs) extracts LCFAs into the cytoplasmic membrane and activates to form acyl-CoA. In yeast, Fat1p and Faa1p/Faa4p are required for LCFAs transport and activation respectively[29]. Moreover, LCFAs can also permeate the bilayers *via* the TolC channel in *E. coli*[79,80].

Subsequently activated acyl-CoA is degraded to acetyl-CoA *via* β-oxidation. Acetyl-CoA is located at the crossroads of central metabolism[81]. During bacterial overgrowth, acetyl-CoA is not only necessary only for energy generation *via* entering citric acid cycle and respiratory chain, but also synthesizes new cell material *via* the glyoxylate cycle. Moreover, the conversion from acetyl-CoA to acetate and ethanol takes place through anaerobic fermentation due to oxidant deficiency[82].

In addition to being a nutrient, LCFAs serve as an environmental factor which guides a series of gram-negative bacteria to colonize and invade intestinal lumen by repressing the expression of the strain-specific pathogenicity island. A pathogenicity island has been reported as a transcriptional activator which is mandatory for tissue invasion, such as *Salmonella* PI1/hilA[80], the *Vibril cholera* AraC/Xyls family ToxT

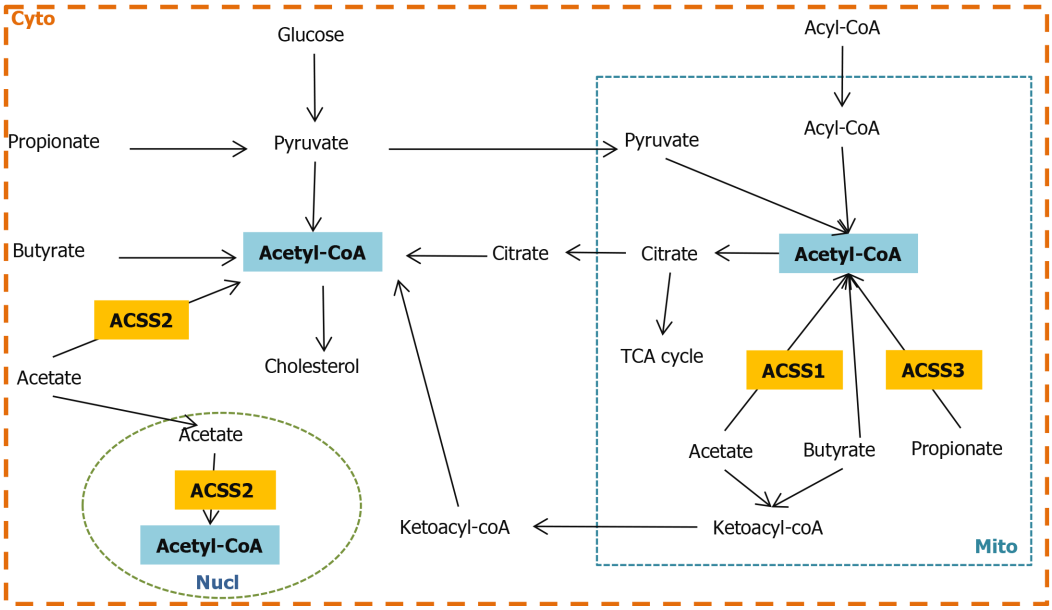

Figure 2 The crosslink between acyl-CoA synthetases and short-chain fatty acids. In mitochondria, acetyl-CoA is generated either from fatty acid β-oxidation and glucose *via* pyruvate or SCFAs through ACSS1 and ACSS3; acetyl-CoA is directed into energy production through the TCA cycle and electron respiration chain, as well as reflux into cytosol *via* citrate and again synthesizes acetyl-CoA. In addition, excessive acetate and butyrate synthesize into ketone bodies and are released into cytosol. In cytosol, acetyl-CoA is produced from pyruvate which is from both glucose and propionate; the source of acetyl-CoA can be converted from butyrate and acetate *via* butyryl-CoA/acetate CoA-transferase and ACSS2 respectively; cytosolic ketone bodies can also either produce acetyl-CoA or enter the blood circulation in the whole body. On the other hand, acetyl-CoA is involved in cholesterol biosynthesis. In the nucleus, acetate synthesizes acetyl-CoA *via* ACSS2 which is responsible for chromosome stability through histone acylation regulation. Cyto: Cytoplasma; Mito: mitochondria; Nucl: Nucleus; TCA: tricarboxylic acid cycle.

[83], *Yersinia enterocolitica* VirF and enterotoxigenic *E. coli* Rns[84].

Microbiota-derived short-chain fatty acids

Microbiota-derived SCFAs make up almost all SCFAs due to the lower level of SCFAs in human blood[85]. SCFAs as the basic substance sources play an important role in regulating lipid metabolism as well as maintaining the host energy homeostasis. In part, SCFAs can be absorbed directly as an energy source by enterocytes or transported to the liver *via* the portal vein; in part, SCFAs are reassigned by the liver and released into bloodstream for the systemic circulation through the whole body[10, 86]. SCFAs are mainly composed of acetate, butyrate and propionate which comprise 60%, 20% and 20% respectively[87]. SCFAs are transported and taken up into cells *via* non-ionized and ionized diffusion. The *liver-gut axis* plays a key role in the absorption, metabolism and systemic circulation of SCFAs[88].

Acetate, which is produced from pyruvate *via* acetyl-CoA and the wood-Ljungdahl pathway in microbiota, is the most abundant SCFA. Acetate is activated by ACSS1-3 to form acetyl-CoA and metabolized for energy production. However, the majority of acetate reaches and is processed in the liver. In cytosol, acetyl-CoA can synthesize cholesterol[89]; in the nucleus, acetate and acetyl-CoA are involved in regulating DNA histone acetylation and deacetylation[90]; in mitochondria, acetyl-CoA can be either for energy supply or ketogenesis in case of glucose deficiency, ketone bodies enter blood circulation for peripheral tissues usages[91]. Moreover, acetate can cross the blood-brain barrier freely and is an energy source for glial cells[92]. Acetate has a direct role in appetite regulation. Acetate is metabolized to generate more adenosine triphosphate, and inhibits adenosine monophosphate-active protein kinase (AMPK), as well as upregulating anorectic neuropeptide POMC and downregulating orexigenic neuropeptide AgRP[93].

Of the SCFAs which are mainly composed of acetate, butyrate and propionate, butyrate is the most widely studied. Butyrate is generated through the butyrate kinase or butyryl-CoA/acetate CoA-transferase route. Butyrate is a major SCFA in the large intestine. In enterocytes, the majority of butyrate is converted into acetyl-CoA that further participates in catabolism for host energy supply[94]; a small amount of butyrate is delivered to the liver and incorporated into ketone bodies (β-hydroxybutyrate) in mitochondrial for ATP production[95]. Butyrate plays a key role in maturating the intestinal barrier function in premature infants[96]. *In vivo* studies

showed that butyrate administration has favorable therapeutic effects on normal colonic health in a safe dose[86]. In a mouse model with globin chain synthesis disorder, the application of a high dose of butyrate resulted in striking neuropathological changes and multiorgan system failure due to harmful systemic concentrations [97]. Therefore, mechanisms underlying the dosage-dependent effects on the intestinal barrier are controversial, but reasonable. A low dose promotes restitution of intestinal epithelial lumen and a high dose impairs the intestinal barrier function with regulation of permeability by inducing apoptosis[98]. The selective paracellular permeability is determined by junction proteins including tight junction, adherence junction and desmosomes[99]. Excessive SCFA accumulation downregulates the expression of junction protein and further impairs the integrity of the membrane, leading to a leaky gut[100]. Moreover, increased intestinal permeability has been linked to inflammatory bowel disease[101].

Propionate is produced *via* the succinate, acrylate and propanediol pathway in microbiota. Propionate is activated by ACSS3 in mitochondria of hepatocytes. The concentration of dietary propionate regulates the balance between lipid and glucose metabolism[102]. Propionate reduces cancer cell proliferation through activation of G-protein-coupled receptors 43 GPR43) in mice liver[103].

In view of the biosynthesis of SCFAs, acetate, butyrate and propionate have crosslinks through acetyl-CoA, pyruvate, oxaloacetate, some of which can be converted between them to meet the physiological need of microbiota[104]. SCFAs as key microbiota metabolites are closely correlated with host health and disease conditions through regulation of diverse physiological processes. Two major signaling pathways related to SCFAs including G-protein-coupled receptors (GPCRs) and histone deacetylases have been characterized[105]. GPCRs, also named free fatty acid receptors (FFAR) are activated by SCFAs. Two SCFA receptors, GPR41 (FFAR3) and GPR43 (FFAR2) have been reported. FFAR2 has preference to acetate and propionate, and FFAR3 has a specificity in butyrate[106]. FFAR2 is expressed along the entire gastrointestinal tract. FFAR2 can be upregulated by propionate during adipocyte differentiation[107]. In addition, FFAR2 activated by SCFAs releases glucagon-like peptide 1(GLP-1) and peptide YY (PYY) in enteroendocrine L cells, GLP-1 and PYY, are involved in gut motility, glucose tolerance and regulation of appetite[108]. Moreover, Butyrate plays a role in anti-inflammation through inhibition of pro-inflammatory mediators/adipokines, adhesion molecules, metalloproteinase production as well as inflammatory signaling pathways (NFκB, MAPKinase, AMPK-α, and PI3K/Akt). However, the anti-inflammatory activity of butyrate was eliminated by FFRA3 knockdown[109]. Supplementation of SCFAs significantly improved hepatic metabolic actiity in FFAR3-dificient mice, but not FFAR-2 deficient mice[110].

SCFAs are also considered a promising supplementary treatment for active intestinal bowel disease[111]. Moreover, SCFAs, as inhibitors of histone deacetylases, show potential anti-inflammatory activity[112,113]. It is demonstrated that three SCFAs alone or in combination protect the intestinal barrier *via* stimulation of tight junction formation and repression of NLRP3 inflammasome and autophagy in the colon cancer cell model[114]. Apart from this, a high-fiber intake, fecal microbiota transplant, prebiotics and probiotics are suggested to have a beneficial effect on colonic health by increasing the level of SCFAs.

Microbiota-imbalance-related diseases in the liver-gut axis

Gut microbiota exert multifunction in maintaining the host homeostasis, including defensing against pathogens, affecting immune system, mediating digestion and metabolism, involving in insulin regulation and maintaining the intestinal epithelial cell renewal[115]. Gut microbiota interact with host through producing a serial of metabolites, particularly SCFAs. Imbalance in diversity and composition as well as alterations in the function of gut microbiota is associated with the pathogenesis of diverse gastrointestinal tract diseases, such as small intestinal bacterial overgrowth (SIBO), intestinal bowel disease (IBD), and a serial of liver diseases[116].

SIBO takes place in short bowel syndrome (SBS) and causes variable signs and symptoms resulting in nutrient malabsorption[117]. SIBO is characterized with the small intestinal excessive numbers and types of bacteria overgrowth exceeding 10^5 organisms/mL, which are mainly colonic type with predominantly gram-negative aerobic species (*Streptococcus*, *Escherichia coli, staphylococcus*) and anaerobic species (*Lactobacillus, bacteroides, clostridium and veillonella*)[118]. Enterotoxins expressing in the outer membrane of germ-negative species can damage the intestinal mucosa barrier by stimulation of fluid secretion in enterocytes, and further affect the absorptive function [119]. SIBO is associated with irritable bowel syndrome (IBS), celiac disease (CD) as well as IBD[120], and also involved in the development of nonalcoholic fatty liver

disease[121].

IBD occurs due to the imbalance between the host immune system and gut microbiota in digestive tract and is becoming an increasing health problem. Crohn's disease and ulcerative colitis are the two prevailing types. The worldwide epidemiologic data shows that the higher incidence and prevalence of IBD is associated with industrialization[122]. Differences in dietary habits highly influence the composition of microbiota; a high-fat diet induces microbiota dysbiosis which alters the intestinal permeability[123].

Additionally, the disruption of bacterial colonization with dysbiosis and an exaggerated inflammatory response has been linked with the pathological process of necrotizing enterocolitis (NEC) in preterm infants[124]. In NEC cases, an increased proportion of Proteobacteria and Actinobacteria, a decreased numbers of Bifidobacteria and Bacteroidetes were detected before NEC diagnosis. Moreover, a type of bacteria related to Klebsiella pneumoniae has been strongly correlated with the NEC development later stage[125].

Although the mechanism involved in diverse gastrointestinal tract diseases is still not completely understood, an impaired intestinal mucosal barrier is common feature among them. In addition, Paneth cells located in the crypts of the small intestine are very important for providing a sterile inner mucus layer and maintaining mucosal barrier integrity against microbiota by secreting antibiotic peptides containing α-defensin, angiogenin, lysozyme and lectins[126]. α-defensin 5/6 are the most abundant components. α-defensin 5 can be digested into fragments which exert specific antibiotic activity[127]. However, α-defensin 6 prevents invasion by bacterial pathogens through self-assembly to form fibrils and nanonets[128]. Diminished expression of Paneth cell defensins regulated by the Wnt factor is associated with Crohn's disease (also called Paneth's disease)[129,130]. Paneth-cell-deficient mice showed a dysbiosis in favor of an E. coli expansion and further weakening of the intestinal mucosal barrier with a visceral hypersensitivity[131]. Moreover, active Crohn's disease is accompanied by bile acid malabsorption due to altered expression of the major bile acid transporter[132].

As a consequence of intestinal mucosal barrier disruption, microbial/pathogen-associated molecular patterns (MAMPs/PAMPs) pass through lumen and mucosa to induce the inflammatory signaling nuclear factor kappa B (NFκB) *via* toll-like receptors (TLRs) and nod-like receptors (NLRs). Activation of this signaling induces the release of cytokines and chemokines into portal circulation[133,134].

Both bacterial components and metabolites reach the liver *via* the portal vein to induce hepatocytes damage. Additionally if dysbiosis occurs, secondary BAs including deoxycholic and lithocholic acid, which are toxic for both intestine and liver, are produced more than usual in microbiota[135]. Hepatocytes are damaged due a high level of secondary BAs, bacterial components and metabolites. High lipid peroxides and PAMPs derived from damaged hepatocytes induce liver microphage activation and initiate an immune response through NFκB, p-38/c-Jun-N-terminal kinase, TGF-β 1 and other inflammation cytokines[136]. A macrophage-mediated immune response is a major player in liver fibrogenesis. Chronic liver injury leads to hepatic stellate cells to transition into myofibroblast-like cells which produce an extracellular matrix and further contribute to the progression of fibrosis[137,138]. Moreover, chronic liver inflammation is significantly involved in the pathogenesis of liver fibrosis/cirrhosis and probably contributes to carcinogenesis.

POTENTIAL THERAPEUTIC APPLICATION TARGETING ACYL-COA SYNTHETASES

Long-chain acyl-CoA synthetases and cancer

Alteration in a fatty acid metabolism with a higher fatty acid synthesis and lipid deposition is a major player in the pathogenesis of metabolic disorders and cancer [139]. Deregulation of metabolism is known as a hallmark of cancer[140]**.** The Warburg effect, one of the hallmarks of cancer, first introduced by Otto Warburg, has been used to describe the deregulated metabolism of cancer cells characterized by increased conversion of glucose into lactate even in the presence of oxygen[141]. Many cancer cells are highly dependent on aerobic glycolysis for their growth and division[142]. Recently, several studies have shown that some cancers, including colon cancer, rather synthetize ATP by oxidative phosphorylation, which has been called the reverse Warburg effect[143-146]. In addition to previously reported abnormalities of glucose and glutamine metabolism in cancers, abnormal lipid metabolism was also found in

different cancer types[143]. Highly proliferative cancer cells are dependent not only on glucose but also on other metabolites including glutamine, serine and FAs[147-151]. It was reported that many cancer cells are characterized by an increased level of *de novo* fatty acid synthesis[152,153]. Upregulation of processes as fatty acid synthesis and FA release from lipid storage on the one hand, and downregulation of β-oxidation of FAs and their reesterification on the other, leads to an increased level of fatty acid in cancer cells. The fatty acid level was reported as a prognostic marker in several types of cancers including colorectal carcinoma (CRC)[7]. A high level of FA is considered a cancer biomarker and is associated with a worse prognosis and survival[7].

There is some evidence from mice with genetic inactivation of the *Muc2* gene that in adenocarcinoma arising in both the small and large intestine, alterations of the glucose metabolism induce expression of genes linked to *de novo* lipogenesis[154]. However, a systematic comparative analysis of adenocarcinomas arising in different locations of the intestinal tract with lipidomics is not available at present. Increased expression of ACSL1 was reported in several cancers, including colon[155,156] and liver[157,158], related to a poor clinical outcome[159]; ACSL4 was also upregulated in multiple cancer types, including colon[155,160] and liver[161-163]. Poorer patient survival in stage II colon cancer was correlated with the expression of ACSL4 and expression of stearoyl CoA desaturase 1 (SCD1)[156]. Concomitant overexpression of ACSL1, ACSL4 and SCD1 was found to induce epithelial-mesenchymal transtion in colorectal cancer [155]. ACSL3 and ACSL4 were upregulated in hepatocellular carcinoma (HCC)[164]. Deregulated expression of both ACSL3 and ACSL4 is associated with disease and especially with cancer[7]. ACSL3 drives tumor growth by increasing both fatty acid β-oxidation[165] and arachidonic acid conversion into prostaglandin[166]. As previously reported, ACSL4 indirectly stabilizes c-Myc by acting on the *ERK/FBW7* axis and driving oncogenesis *via* c-Myc-oncogenic signaling in HCC[167]. ACSL4 expression is highly linked to the cell sensitivity for ferroptosis, known as an iron-mediated non-apoptotic cell death[168]. Reported roles of ACSL4 include metabolic signaling resulting in drug resistance and the activation of intracellular, pro-oncogenic signaling pathways[139]. Impaired expression of ACSL5 is associated with coeliac disease and sporadic colorectal adenocarcinomas[169] and overexpression of ACSL5 induces apoptosis[170] and suppresses proliferation by inhibiting the activation of the Wnt/β-catenin signaling pathway in colon cancer[57].

ACSS1 and ACSS2 are overexpressed in HCC[171]. Both are key players in acetate metabolism which is shown to be highly taken up by several types of cancers, including liver. Gao *et al*[171] reported a role of acetate in epigenetic regulation (Histone acetylation) of a promoter region of FASN. Induction of lipid synthesis driven by increased FASN expression supports tumor cell survival and growth[171].

miRNAs targeting of long-chain acyl-CoA synthetases

Micro RNAs (miRNAs) are non-coding single stranded RNAs which regulate transcription of messenger RNA *via* binding to their 3'-untranslated region[172]. Cancer cells evolved a regulatory mechanism to control the mRNA stability of ACSLs by targeting their 3'-untranslated regions (3'UTR). For example, it was reported that miR-205 was decreased in liver cancer[173]. Negative correlation between miR-205 and ACSL4 expression was reported in human HCC patients[173]. The miR-205 targeting site is reported at the 3'UTR region of ACSL4-mRNA[173]. In addition, it is known that miR-205 binds to the 3'UTR of ACSL1 and induces its degradation[157]. The role of miR-211-5p as a tumor suppressor was reported in HCC[174]. This tumor-suppressive role was accomplished by downregulation of ACSL4 which is highly expressed in HCC[174]. miR-19b-1 showed an inhibitory effect on the *ACSL1/ACSL4/SCD1* axis by downregulating the Wnt/β-catenin pathway[175]. *ACSL/SCD* increases GSK3β phosphorylation, activating Wnt signaling and EMT, therefore, downregulation of β-catenin signaling by miR-19b-1 can be beneficial in colon cancer[175]. miR-142-3p has been reported to target cancer stem cell markers, such as the Wnt target and *LGR5* in colorectal cancer cells[176], in agreement with its action on the *ACSL/SCD* network cancer stem cell feature generation[175,176]. miR-34c was reported to be involved in hepatic fibrogenesis, miR-34c increases lipid droplet formation and hepatic stellate cell activation by downregulating ACSL1 in the liver[177]. miR-497-5p was reported to induce death in colon cancer cells by targeting ACSL5, suggesting its therapeutic potential in colon cancer[172].

Pharmacological targeting of long-chain acyl-CoA synthetases

Triacsin C, a fungal metabolite and a potent competitive inhibitor of ACSs activity[178, 179], competes with FAs for the catalytic domain. It inhibits ACSL1, ACSL3 and ACSL4, and in higher concentration proves effective against ACSL5[179,180]. It is

worth highlighting that triacsin C has a high toxicity (IC50) and consequently normal cells can be damaged[7].

Thiazolidinediones, also known as glitazones, are used for the therapy of diabetes II. Troglitazone and rosiglitazone are PPARγ agonists; interestingly they inhibit ACSL4 *via* PPARγ indirect mechanism[181,182]. Some of these drugs (Troglitazone, Ciglitazone) showed a protective effect against diabetes-promoted cancer[183].

Pharmacological targeting of very-long-chain acyl-CoA synthetases

FATP1 and FATP4 inhibitors were detected using high-throughput screening[184-186]. However, these compounds were not effective as revealed by *in vivo* studies. Screening compounds that specifically target domains involved in fatty acid transport, rather than the ACSL activity domain, might help to discover more effective compounds which could inhibit fatty acid transport. FATP2/ACSVL1, expressed mostly in the liver and intestine, acts as a transport protein and ACS[187]. FATP2 might be considered as an early marker for the development of overweight disorder after a high-fat diet[188]. A high-fat diet significantly upregulated fatp2 expression in the intestine of mice[188,189] It has a role in hepatic long-chain fatty acid uptake[190]. Due to its important role in fatty acid transport, FATP2 can be a promising pharmacological target in diseases which are characterized by an abnormal accumulation of intracellular FAs and lipids which may eventually result in irreversible hepatic cirrhosis[191,192]. Lipofermata and Grassofermata are selected FATP2 inhibitors which show specificity toward attenuating transport of LCFAs and VLCFAs. Lipofermata (5'-bromo-5-phenyl-spiro[3H-1,3,4-thiadiazole-2,3'-indoline]-2'-one) inhibits the function of FATP2 as a transport protein, without compromising its function as an ACS[193,194]. Grassofermata (2-benzyl-3-(4-chlorophenyl)-5-(4-nitrophenyl) pyrazolo[1,5-a] pyrimidin-7(4H)-one) suppresses palmitic acid mediated lipotoxicity[193,195,196]. Both of them reduce intestinal fat absorption of 13C labeled oleate[186]. In addition to its contribution to the development of metabolic liver diseases, FATP2 promotes the growth of cancer cells and induces their resistance to targeted therapies[190]. A study by Veglia *et al*[194] demonstrated that lipofermata abrogated the activity of polymorphonuclear myeloid-derived suppressor cells (PMN-MDSCs) and substantially delayed tumor progression in colon cancer cell line CT26 tumor-bearing mice. STAT5 signaling induced by granulocyte-macrophage colony stimulating factor (GM-CSF) upregulated the FATP2 in these cells. FATP2 overexpression in these PMN-MDSCs cells induced PGE2 synthesis and its immunosuppressive effect on CD8+ T cell[194]. Interestingly in this study, it was found that lipofermata elevated the therapeutical effect of immune checkpoint inhibitor therapy (anti-PD-1 and anti-CTLA-4) as well as macrophage targeted therapy (anti CSF-1R)[194].

FATP5 can be exclusively found in liver, at the basal plasma membrane of hepatocytes[197]. Both its location and role in long-chain fatty acid uptake make it an attractive target for treatment of metabolic disorders. Interestingly, screening of potential compounds revealed the potential of BAs including the primary BAs produced by the liver and the secondary BA secreted by intestinal bacteria (microbiota) to attenuate specifically FATP5 function without affecting FATP4[197]. The following BAs showed potential for FATP5 inhibition: chenodiol, primary BA, produced by the liver and ursodiol, secondary BA, which is metabolically produced by intestinal bacteria[197].

Experimental *in vivo* studies in rats showed induction of FATP mRNA expression, finding the highest upregulation in the liver. In the intestine, there was an increase in the FATP mRNA level but two times less than in the liver[198], suggesting that fenofibrates show specificity towards liver FATPs. Fibrates are known as PPARα activators, their hypolipidemic effect is accomplished *via* FATP activation, induction of β-oxidation and consequently reduction in triglyceride synthesis[198]. The indirect activation of FATP by the fenofibrate is mediated *via* PPARα[199].

Targeting of short-chain acyl-CoA synthetases

As reported by Bjorson *et al*[200], mitochondrial acetate appears to be the main metabolic energy source under hypoxia in HCC patients. Upregulation of ACSS1 Led to an enhanced level of mitochondrial acetate in HCC, which is associated with several metabolic alterations including decreased fatty acid oxidation, glutamine utilization, gluconeogenesis and increased glycolysis[200] This finding suggests a potential of ACSS1 as a target in cancer treatment. Indeed, the ACSS1 inhibitor showed a growth inhibitory effect on glioma[201].

CONCLUSION

LCFAs and SCFAs are the most abundant energy sources from dietary lipid intake and microbiota-derived fermentation products. Members of ACSs play a critical role in lipid metabolism, participating in fatty acid transport and activation. Abnormal expression of ACSs is closely associated with lipid metabolic disorders and carcinogenesis. Research on ACSs will shed further light on their biological functions and molecular mechanisms in fatty acid metabolism and eventually lead to the development of therapeutic drugs targeting ACSs in the treatment of human metabolic diseases.

REFERENCES

1 **Grevengoed TJ**, Klett EL, Coleman RA. Acyl-CoA metabolism and partitioning. *Annu Rev Nutr* 2014; **34**: 1-30 [PMID: 24819326 DOI: 10.1146/annurev-nutr-071813-105541]
2 **Volta U**, Bonazzi C, Bianchi FB, Baldoni AM, Zoli M, Pisi E. IgA antibodies to dietary antigens in liver cirrhosis. *Ric Clin Lab* 1987; **17**: 235-242 [PMID: 3671996 DOI: 10.1007/BF02912537]
3 **Guinane CM**, Cotter PD. Role of the gut microbiota in health and chronic gastrointestinal disease: understanding a hidden metabolic organ. *Therap Adv Gastroenterol* 2013; **6**: 295-308 [PMID: 23814609 DOI: 10.1177/1756283X13482996]
4 **Konturek PC**, Harsch IA, Konturek K, Schink M, Konturek T, Neurath MF, Zopf Y. Gut–Liver Axis: How Do Gut Bacteria Influence the Liver? *Med Sci (Basel)* 2018; **6** [PMID: 30227645 DOI: 10.3390/medsci6030079]
5 **Park W**. Gut microbiomes and their metabolites shape human and animal health. *J Microbiol* 2018; **56**: 151-153 [PMID: 29492871 DOI: 10.1007/s12275-018-0577-8]
6 **Niot I**, Poirier H, Tran TT, Besnard P. Intestinal absorption of long-chain fatty acids: evidence and uncertainties. *Prog Lipid Res* 2009; **48**: 101-115 [PMID: 19280719 DOI: 10.1016/j.plipres.2009.01.001]
7 **Rossi Sebastiano M**, Konstantinidou G. Targeting Long Chain Acyl-CoA Synthetases for Cancer Therapy. *Int J Mol Sci* 2019; **20** [PMID: 31344914 DOI: 10.3390/ijms20153624]
8 **Anderson CM**, Stahl A. SLC27 fatty acid transport proteins. *Mol Aspects Med* 2013; **34**: 516-528 [PMID: 23506886 DOI: 10.1016/j.mam.2012.07.010]
9 **Hamilton JA**. New insights into the roles of proteins and lipids in membrane transport of fatty acids. *Prostaglandins Leukot Essent Fatty Acids* 2007; **77**: 355-361 [PMID: 18032007 DOI: 10.1016/j.plefa.2007.10.020]
10 **Moffett JR**, Puthillathu N, Vengilote R, Jaworski DM, Namboodiri AM. Acetate Revisited: A Key Biomolecule at the Nexus of Metabolism, Epigenetics and Oncogenesis-Part 1: Acetyl-CoA, Acetogenesis and Acyl-CoA Short-Chain Synthetases. *Front Physiol* 2020; **11**: 580167 [PMID: 33281616 DOI: 10.3389/fphys.2020.580167]
11 **Wang TY**, Liu M, Portincasa P, Wang DQ. New insights into the molecular mechanism of intestinal fatty acid absorption. *Eur J Clin Invest* 2013; **43**: 1203-1223 [PMID: 24102389 DOI: 10.1111/eci.12161]
12 **Lowe ME**. The triglyceride lipases of the pancreas. *J Lipid Res* 2002; **43**: 2007-2016 [PMID: 12454260 DOI: 10.1194/jlr.r200012-jlr200]
13 **Alves-Bezerra M**, Cohen DE. Triglyceride Metabolism in the Liver. *Compr Physiol* 2017; **8**: 1-8 [PMID: 29357123 DOI: 10.1002/cphy.c170012]
14 **Hardie DG**. Organismal carbohydrate and lipid homeostasis. *Cold Spring Harb Perspect Biol* 2012; **4** [PMID: 22550228 DOI: 10.1101/cshperspect.a006031]
15 **Chiang JY**. Bile acid metabolism and signaling. *Compr Physiol* 2013; **3**: 1191-1212 [PMID: 23897684 DOI: 10.1002/cphy.c120023]
16 **Zhang YK**, Guo GL, Klaassen CD. Diurnal variations of mouse plasma and hepatic bile acid concentrations as well as expression of biosynthetic enzymes and transporters. *PLoS One* 2011; **6**: e16683 [PMID: 21346810 DOI: 10.1371/journal.pone.0016683]
17 **Ramírez-Pérez O**, Cruz-Ramón V, Chinchilla-López P, Méndez-Sánchez N. The Role of the Gut Microbiota in Bile Acid Metabolism. *Ann Hepatol* 2017; **16**: s15-s20 [PMID: 29080339 DOI: 10.5604/01.3001.0010.5494]
18 **de Aguiar Vallim TQ**, Tarling EJ, Edwards PA. Pleiotropic roles of bile acids in metabolism. *Cell Metab* 2013; **17**: 657-669 [PMID: 23602448 DOI: 10.1016/j.cmet.2013.03.013]
19 **Li T**, Chiang JY. Nuclear receptors in bile acid metabolism. *Drug Metab Rev* 2013; **45**: 145-155 [PMID: 23330546 DOI: 10.3109/03602532.2012.740048]
20 **Massey JB**, Bick DH, Pownall HJ. Spontaneous transfer of monoacyl amphiphiles between lipid and protein surfaces. *Biophys J* 1997; **72**: 1732-1743 [PMID: 9083677 DOI: 10.1016/S0006-3495(97)78819-2]
21 **Kamp F**, Hamilton JA. How fatty acids of different chain length enter and leave cells by free diffusion. *Prostaglandins Leukot Essent Fatty Acids* 2006; **75**: 149-159 [PMID: 16829065 DOI: 10.1016/j.plefa.2006.05.003]
22 **Schaffer JE**, Lodish HF. Expression cloning and characterization of a novel adipocyte long chain

fatty acid transport protein. *Cell* 1994; **79**: 427-436 [PMID: 7954810 DOI: 10.1016/0092-8674(94)90252-6]

23 **Pohl J**, Ring A, Hermann T, Stremmel W. Role of FATP in parenchymal cell fatty acid uptake. *Biochim Biophys Acta* 2004; **1686**: 1-6 [PMID: 15522816 DOI: 10.1016/j.bbalip.2004.06.004]

24 **Stahl A**, Hirsch DJ, Gimeno RE, Punreddy S, Ge P, Watson N, Patel S, Kotler M, Raimondi A, Tartaglia LA, Lodish HF. Identification of the major intestinal fatty acid transport protein. *Mol Cell* 1999; **4**: 299-308 [PMID: 10518211 DOI: 10.1016/s1097-2765(00)80332-9]

25 **Herrmann T**, van der Hoeven F, Grone HJ, Stewart AF, Langbein L, Kaiser I, Liebisch G, Gosch I, Buchkremer F, Drobnik W, Schmitz G, Stremmel W. Mice with targeted disruption of the fatty acid transport protein 4 (Fatp 4, Slc27a4) gene show features of lethal restrictive dermopathy. *J Cell Biol* 2003; **161**: 1105-1115 [PMID: 12821645 DOI: 10.1083/jcb.200207080]

26 **Doege H**, Baillie RA, Ortegon AM, Tsang B, Wu Q, Punreddy S, Hirsch D, Watson N, Gimeno RE, Stahl A. Targeted deletion of FATP5 reveals multiple functions in liver metabolism: alterations in hepatic lipid homeostasis. *Gastroenterology* 2006; **130**: 1245-1258 [PMID: 16618416 DOI: 10.1053/j.gastro.2006.02.006]

27 **Hubbard B**, Doege H, Punreddy S, Wu H, Huang X, Kaushik VK, Mozell RL, Byrnes JJ, Stricker-Krongrad A, Chou CJ, Tartaglia LA, Lodish HF, Stahl A, Gimeno RE. Mice deleted for fatty acid transport protein 5 have defective bile acid conjugation and are protected from obesity. *Gastroenterology* 2006; **130**: 1259-1269 [PMID: 16618417 DOI: 10.1053/j.gastro.2006.02.012]

28 **Lewis SE**, Listenberger LL, Ory DS, Schaffer JE. Membrane topology of the murine fatty acid transport protein 1. *J Biol Chem* 2001; **276**: 37042-37050 [PMID: 11470793 DOI: 10.1074/jbc.M105556200]

29 **Black PN**, DiRusso CC. Transmembrane movement of exogenous long-chain fatty acids: proteins, enzymes, and vectorial esterification. *Microbiol Mol Biol Rev* 2003; **67**: 454-472, table of contents [PMID: 12966144 DOI: 10.1128/MMBR.67.3.454-472.2003]

30 **Nie B**, Park HM, Kazantzis M, Lin M, Henkin A, Ng S, Song S, Chen Y, Tran H, Lai R, Her C, Maher JJ, Forman BM, Stahl A. Specific bile acids inhibit hepatic fatty acid uptake in mice. *Hepatology* 2012; **56**: 1300-1310 [PMID: 22531947 DOI: 10.1002/hep.25797]

31 **Chmurzyńska A**. The multigene family of fatty acid-binding proteins (FABPs): function, structure and polymorphism. *J Appl Genet* 2006; **47**: 39-48 [PMID: 16424607 DOI: 10.1007/BF03194597]

32 **Glatz JF**, van der Vusse GJ. Cellular fatty acid-binding proteins: their function and physiological significance. *Prog Lipid Res* 1996; **35**: 243-282 [PMID: 9082452 DOI: 10.1016/s0163-7827(96)00006-9]

33 **Sweetser DA**, Birkenmeier EH, Klisak IJ, Zollman S, Sparkes RS, Mohandas T, Lusis AJ, Gordon JI. The human and rodent intestinal fatty acid binding protein genes. A comparative analysis of their structure, expression, and linkage relationships. *J Biol Chem* 1987; **262**: 16060-16071 [PMID: 2824476]

34 **Mansbach CM**, Abumrad NA. Chapter 60 - Enterocyte Fatty Acid Handling Proteins and Chylomicron Formation. In: Johnson LR, Ghishan FK, Kaunitz JD, Merchant JL, Said HM, Wood JD, editors. Physiology of the Gastrointestinal Tract (Fifth Edition). Boston: Academic Press, 2012: 1625-1641 [DOI: 10.1016/b978-0-12-382026-6.00060-9]

35 **Newberry EP**, Xie Y, Kennedy S, Han X, Buhman KK, Luo J, Gross RW, Davidson NO. Decreased hepatic triglyceride accumulation and altered fatty acid uptake in mice with deletion of the liver fatty acid-binding protein gene. *J Biol Chem* 2003; **278**: 51664-51672 [PMID: 14534295 DOI: 10.1074/jbc.M309377200]

36 **Atshaves BP**, McIntosh AM, Lyuksyutova OI, Zipfel W, Webb WW, Schroeder F. Liver fatty acid-binding protein gene ablation inhibits branched-chain fatty acid metabolism in cultured primary hepatocytes. *J Biol Chem* 2004; **279**: 30954-30965 [PMID: 15155724 DOI: 10.1074/jbc.M313571200]

37 **Huang H**, Starodub O, McIntosh A, Atshaves BP, Woldegiorgis G, Kier AB, Schroeder F. Liver fatty acid-binding protein colocalizes with peroxisome proliferator activated receptor alpha and enhances ligand distribution to nuclei of living cells. *Biochemistry* 2004; **43**: 2484-2500 [PMID: 14992586 DOI: 10.1021/bi0352318]

38 **Glatz JF**, Luiken JJ. From fat to FAT (CD36/SR-B2): Understanding the regulation of cellular fatty acid uptake. *Biochimie* 2017; **136**: 21-26 [PMID: 28013071 DOI: 10.1016/j.biochi.2016.12.007]

39 **Coburn CT**, Knapp FF Jr, Febbraio M, Beets AL, Silverstein RL, Abumrad NA. Defective uptake and utilization of long chain fatty acids in muscle and adipose tissues of CD36 knockout mice. *J Biol Chem* 2000; **275**: 32523-32529 [PMID: 10913136 DOI: 10.1074/jbc.M003826200]

40 **Lobo MV**, Huerta L, Ruiz-Velasco N, Teixeiro E, de la Cueva P, Celdrán A, Martín-Hidalgo A, Vega MA, Bragado R. Localization of the lipid receptors CD36 and CLA-1/SR-BI in the human gastrointestinal tract: towards the identification of receptors mediating the intestinal absorption of dietary lipids. *J Histochem Cytochem* 2001; **49**: 1253-1260 [PMID: 11561009 DOI: 10.1177/002215540104901007]

41 **Wilson CG**, Tran JL, Erion DM, Vera NB, Febbraio M, Weiss EJ. Hepatocyte-Specific Disruption of CD36 Attenuates Fatty Liver and Improves Insulin Sensitivity in HFD-Fed Mice. *Endocrinology* 2016; **157**: 570-585 [PMID: 26650570 DOI: 10.1210/en.2015-1866]

42 **Murata M**, Peränen J, Schreiner R, Wieland F, Kurzchalia TV, Simons K. VIP21/caveolin is a cholesterol-binding protein. *Proc Natl Acad Sci U S A* 1995; **92**: 10339-10343 [PMID: 7479780 DOI: 10.1073/pnas.92.22.10339]

43 **Valasek MA**, Weng J, Shaul PW, Anderson RG, Repa JJ. Caveolin-1 is not required for murine intestinal cholesterol transport. *J Biol Chem* 2005; **280**: 28103-28109 [PMID: 15919660 DOI: 10.1074/jbc.M504609200]

44 **Pol A**, Martin S, Fernandez MA, Ferguson C, Carozzi A, Luetterforst R, Enrich C, Parton RG. Dynamic and regulated association of caveolin with lipid bodies: modulation of lipid body motility and function by a dominant negative mutant. *Mol Biol Cell* 2004; **15**: 99-110 [PMID: 14528016 DOI: 10.1091/mbc.e03-06-0368]

45 **Mashek DG**, Bornfeldt KE, Coleman RA, Berger J, Bernlohr DA, Black P, DiRusso CC, Farber SA, Guo W, Hashimoto N, Khodiyar V, Kuypers FA, Maltais LJ, Nebert DW, Renieri A, Schaffer JE, Stahl A, Watkins PA, Vasiliou V, Yamamoto TT. Revised nomenclature for the mammalian long-chain acyl-CoA synthetase gene family. *J Lipid Res* 2004; **45**: 1958-1961 [PMID: 15292367 DOI: 10.1194/jlr.E400002-JLR200]

46 **Soupene E**, Kuypers FA. Mammalian long-chain acyl-CoA synthetases. *Exp Biol Med (Maywood)* 2008; **233**: 507-521 [PMID: 18375835 DOI: 10.3181/0710-MR-287]

47 **Watkins PA**, Maiguel D, Jia Z, Pevsner J. Evidence for 26 distinct acyl-coenzyme A synthetase genes in the human genome. *J Lipid Res* 2007; **48**: 2736-2750 [PMID: 17762044 DOI: 10.1194/jlr.M700378-JLR200]

48 **Hisanaga Y**, Ago H, Nakagawa N, Hamada K, Ida K, Yamamoto M, Hori T, Arii Y, Sugahara M, Kuramitsu S, Yokoyama S, Miyano M. Structural basis of the substrate-specific two-step catalysis of long chain fatty acyl-CoA synthetase dimer. *J Biol Chem* 2004; **279**: 31717-31726 [PMID: 15145952 DOI: 10.1074/jbc.M400100200]

49 **Li LO**, Klett EL, Coleman RA. Acyl-CoA synthesis, lipid metabolism and lipotoxicity. *Biochim Biophys Acta* 2010; **1801**: 246-251 [PMID: 19818872 DOI: 10.1016/j.bbalip.2009.09.024]

50 **Yan S**, Yang XF, Liu HL, Fu N, Ouyang Y, Qing K. Long-chain acyl-CoA synthetase in fatty acid metabolism involved in liver and other diseases: an update. *World J Gastroenterol* 2015; **21**: 3492-3498 [PMID: 25834313 DOI: 10.3748/wjg.v21.i12.3492]

51 **Poppelreuther M**, Rudolph B, Du C, Großmann R, Becker M, Thiele C, Ehehalt R, Füllekrug J. The N-terminal region of acyl-CoA synthetase 3 is essential for both the localization on lipid droplets and the function in fatty acid uptake. *J Lipid Res* 2012; **53**: 888-900 [PMID: 22357706 DOI: 10.1194/jlr.M024562]

52 **Coleman RA**, Lewin TM, Van Horn CG, Gonzalez-Baró MR. Do long-chain acyl-CoA synthetases regulate fatty acid entry into synthetic vs degradative pathways? *J Nutr* 2002; **132**: 2123-2126 [DOI: 10.1093/jn/132.8.2123]

53 **Ellis JM**, Frahm JL, Li LO, Coleman RA. Acyl-coenzyme A synthetases in metabolic control. *Curr Opin Lipidol* 2010; **21**: 212-217 [PMID: 20480548 DOI: 10.1097/mol.0b013e32833884bb]

54 **Mashek DG**, McKenzie MA, Van Horn CG, Coleman RA. Rat long chain acyl-CoA synthetase 5 increases fatty acid uptake and partitioning to cellular triacylglycerol in McArdle-RH7777 cells. *J Biol Chem* 2006; **281**: 945-950 [PMID: 16263710 DOI: 10.1074/jbc.M507646200]

55 **Bu SY**, Mashek MT, Mashek DG. Suppression of long chain acyl-CoA synthetase 3 decreases hepatic de novo fatty acid synthesis through decreased transcriptional activity. *J Biol Chem* 2009; **284**: 30474-30483 [PMID: 19737935 DOI: 10.1074/jbc.M109.036665]

56 **Meller N**, Morgan ME, Wong WP, Altemus JB, Sehayek E. Targeting of Acyl-CoA synthetase 5 decreases jejunal fatty acid activation with no effect on dietary long-chain fatty acid absorption. *Lipids Health Dis* 2013; **12**: 88 [PMID: 23767941 DOI: 10.1186/1476-511X-12-88]

57 **Klaus C**, Kaemmerer E, Reinartz A, Schneider U, Plum P, Jeon MK, Hose J, Hartmann F, Schnölzer M, Wagner N, Kopitz J, Gassler N. TP53 status regulates ACSL5-induced expression of mitochondrial mortalin in enterocytes and colorectal adenocarcinomas. *Cell Tissue Res* 2014; **357**: 267-278 [PMID: 24770931 DOI: 10.1007/s00441-014-1826-8]

58 **Klaus C**, Jeon MK, Kaemmerer E, Gassler N. Intestinal acyl-CoA synthetase 5: activation of long chain fatty acids and behind. *World J Gastroenterol* 2013; **19**: 7369-7373 [PMID: 24259967 DOI: 10.3748/wjg.v19.i42.7369]

59 **Paroder V**, Spencer SR, Paroder M, Arango D, Schwartz S Jr, Mariadason JM, Augenlicht LH, Eskandari S, Carrasco N. Na(+)/monocarboxylate transport (SMCT) protein expression correlates with survival in colon cancer: molecular characterization of SMCT. *Proc Natl Acad Sci U S A* 2006; **103**: 7270-7275 [PMID: 16670197 DOI: 10.1073/pnas.0602365103]

60 **Rowe WA**, Lesho MJ, Montrose MH. Polarized Na+/H+ exchange function is pliable in response to transepithelial gradients of propionate. *Proc Natl Acad Sci U S A* 1994; **91**: 6166-6170 [PMID: 8016132 DOI: 10.1073/pnas.91.13.6166]

61 **Castro LF**, Lopes-Marques M, Wilson JM, Rocha E, Reis-Henriques MA, Santos MM, Cunha I. A novel Acetyl-CoA synthetase short-chain subfamily member 1 (Acss1) gene indicates a dynamic history of paralogue retention and loss in vertebrates. *Gene* 2012; **497**: 249-255 [PMID: 22313524 DOI: 10.1016/j.gene.2012.01.013]

62 **Fujino T**, Kondo J, Ishikawa M, Morikawa K, Yamamoto TT. Acetyl-CoA synthetase 2, a mitochondrial matrix enzyme involved in the oxidation of acetate. *J Biol Chem* 2001; **276**: 11420-11426 [PMID: 11150295 DOI: 10.1074/jbc.M008782200]

63 **Sakakibara I**, Fujino T, Ishii M, Tanaka T, Shimosawa T, Miura S, Zhang W, Tokutake Y, Yamamoto J, Awano M, Iwasaki S, Motoike T, Okamura M, Inagaki T, Kita K, Ezaki O, Naito M, Kuwaki T, Chohnan S, Yamamoto TT, Hammer RE, Kodama T, Yanagisawa M, Sakai J. Fasting-induced hypothermia and reduced energy production in mice lacking acetyl-CoA synthetase 2. *Cell*

Metab 2009; **9**: 191-202 [PMID: 19187775 DOI: 10.1016/j.cmet.2008.12.008]

64 **Yoshimura Y**, Araki A, Maruta H, Takahashi Y, Yamashita H. Molecular cloning of rat acss3 and characterization of mammalian propionyl-CoA synthetase in the liver mitochondrial matrix. *J Biochem* 2017; **161**: 279-289 [PMID: 28003429 DOI: 10.1093/jb/mvw067]

65 **Luong A**, Hannah VC, Brown MS, Goldstein JL. Molecular characterization of human acetyl-CoA synthetase, an enzyme regulated by sterol regulatory element-binding proteins. *J Biol Chem* 2000; **275**: 26458-26466 [PMID: 10843999 DOI: 10.1074/jbc.M004160200]

66 **Bulusu V**, Tumanov S, Michalopoulou E, van den Broek NJ, MacKay G, Nixon C, Dhayade S, Schug ZT, Vande Voorde J, Blyth K, Gottlieb E, Vazquez A, Kamphorst JJ. Acetate Recapturing by Nuclear Acetyl-CoA Synthetase 2 Prevents Loss of Histone Acetylation during Oxygen and Serum Limitation. *Cell Rep* 2017; **18**: 647-658 [PMID: 28099844 DOI: 10.1016/j.celrep.2016.12.055]

67 **Huang Z**, Zhang M, Plec AA, Estill SJ, Cai L, Repa JJ, McKnight SL, Tu BP. ACSS2 promotes systemic fat storage and utilization through selective regulation of genes involved in lipid metabolism. *Proc Natl Acad Sci U S A* 2018; **115**: E9499-E9506 [PMID: 30228117 DOI: 10.1073/pnas.1806635115]

68 **Rios-Covian D**, Salazar N, Gueimonde M, de Los Reyes-Gavilan CG. Shaping the Metabolism of Intestinal *Bacteroides* Population through Diet to Improve Human Health. *Front Microbiol* 2017; **8**: 376 [PMID: 28326076 DOI: 10.3389/fmicb.2017.00376]

69 **De Filippo C**, Cavalieri D, Di Paola M, Ramazzotti M, Poullet JB, Massart S, Collini S, Pieraccini G, Lionetti P. Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa. *Proc Natl Acad Sci U S A* 2010; **107**: 14691-14696 [PMID: 20679230 DOI: 10.1073/pnas.1005963107]

70 **Wu GD**, Chen J, Hoffmann C, Bittinger K, Chen YY, Keilbaugh SA, Bewtra M, Knights D, Walters WA, Knight R, Sinha R, Gilroy E, Gupta K, Baldassano R, Nessel L, Li H, Bushman FD, Lewis JD. Linking long-term dietary patterns with gut microbial enterotypes. *Science* 2011; **334**: 105-108 [PMID: 21885731 DOI: 10.1126/science.1208344]

71 **Arumugam M**, Raes J, Pelletier E, Le Paslier D, Yamada T, Mende DR, Fernandes GR, Tap J, Bruls T, Batto JM, Bertalan M, Borruel N, Casellas F, Fernandez L, Gautier L, Hansen T, Hattori M, Hayashi T, Kleerebezem M, Kurokawa K, Leclerc M, Levenez F, Manichanh C, Nielsen HB, Nielsen T, Pons N, Poulain J, Qin J, Sicheritz-Ponten T, Tims S, Torrents D, Ugarte E, Zoetendal EG, Wang J, Guarner F, Pedersen O, de Vos WM, Brunak S, Doré J; MetaHIT Consortium, Antolín M, Artiguenave F, Blottiere HM, Almeida M, Brechot C, Cara C, Chervaux C, Cultrone A, Delorme C, Denariaz G, Dervyn R, Foerstner KU, Friss C, van de Guchte M, Guedon E, Haimet F, Huber W, van Hylckama-Vlieg J, Jamet A, Juste C, Kaci G, Knol J, Lakhdari O, Layec S, Le Roux K, Maguin E, Mérieux A, Melo Minardi R, M'rini C, Muller J, Oozeer R, Parkhill J, Renault P, Rescigno M, Sanchez N, Sunagawa S, Torrejon A, Turner K, Vandemeulebrouck G, Varela E, Winogradsky Y, Zeller G, Weissenbach J, Ehrlich SD, Bork P. Enterotypes of the human gut microbiome. *Nature* 2011; **473**: 174-180 [PMID: 21508958 DOI: 10.1038/nature09944]

72 **Yatsunenko T**, Rey FE, Manary MJ, Trehan I, Dominguez-Bello MG, Contreras M, Magris M, Hidalgo G, Baldassano RN, Anokhin AP, Heath AC, Warner B, Reeder J, Kuczynski J, Caporaso JG, Lozupone CA, Lauber C, Clemente JC, Knights D, Knight R, Gordon JI. Human gut microbiome viewed across age and geography. *Nature* 2012; **486**: 222-227 [PMID: 22699611 DOI: 10.1038/nature11053]

73 **Sonnenburg ED**, Smits SA, Tikhonov M, Higginbottom SK, Wingreen NS, Sonnenburg JL. Diet-induced extinctions in the gut microbiota compound over generations. *Nature* 2016; **529**: 212-215 [PMID: 26762459 DOI: 10.1038/nature16504]

74 **Salonen A**, Lahti L, Salojärvi J, Holtrop G, Korpela K, Duncan SH, Date P, Farquharson F, Johnstone AM, Lobley GE, Louis P, Flint HJ, de Vos WM. Impact of diet and individual variation on intestinal microbiota composition and fermentation products in obese men. *ISME J* 2014; **8**: 2218-2230 [PMID: 24763370 DOI: 10.1038/ismej.2014.63]

75 **Reichardt N**, Duncan SH, Young P, Belenguer A, McWilliam Leitch C, Scott KP, Flint HJ, Louis P. Phylogenetic distribution of three pathways for propionate production within the human gut microbiota. *ISME J* 2014; **8**: 1323-1335 [PMID: 24553467 DOI: 10.1038/ismej.2014.14]

76 **Louis P**, Flint HJ. Formation of propionate and butyrate by the human colonic microbiota. *Environ Microbiol* 2017; **19**: 29-41 [PMID: 27928878 DOI: 10.1111/1462-2920.13589]

77 **Giri S**, Oña L, Waschina S, Shitut S, Yousif G, Kaleta C, Kost C. Metabolic dissimilarity determines the establishment of cross-feeding interactions in bacteria. bioRxiv 2020: 2020.2010.2009.333336 [DOI: 10.1101/2020.10.09.333336]

78 **Wall R**, Ross RP, Shanahan F, O'Mahony L, O'Mahony C, Coakley M, Hart O, Lawlor P, Quigley EM, Kiely B, Fitzgerald GF, Stanton C. Metabolic activity of the enteric microbiota influences the fatty acid composition of murine and porcine liver and adipose tissues. *Am J Clin Nutr* 2009; **89**: 1393-1401 [PMID: 19357220 DOI: 10.3945/ajcn.2008.27023]

79 **Lennen RM**, Politz MG, Kruziki MA, Pfleger BF. Identification of transport proteins involved in free fatty acid efflux in Escherichia coli. *J Bacteriol* 2013; **195**: 135-144 [PMID: 23104810 DOI: 10.1128/JB.01477-12]

80 **Golubeva YA**, Ellermeier JR, Cott Chubiz JE, Slauch JM. Intestinal Long-Chain Fatty Acids Act as a Direct Signal To Modulate Expression of the Salmonella Pathogenicity Island 1 Type III Secretion System. *mBio* 2016; **7**: e02170-e02115 [PMID: 26884427 DOI: 10.1128/mBio.02170-15]

81 **Wolfe AJ**. The acetate switch. *Microbiol Mol Biol Rev* 2005; **69**: 12-50 [PMID: 15755952 DOI:

10.1128/MMBR.69.1.12-50.2005]
82 **Clark DP**, Cronan JE. Two-Carbon Compounds and Fatty Acids as Carbon Sources. *EcoSal Plus* 2005; **1** [PMID: 26443509 DOI: 10.1128/ecosalplus.3.4.4]
83 **Childers BM**, Cao X, Weber GG, Demeler B, Hart PJ, Klose KE. N-terminal residues of the Vibrio cholerae virulence regulatory protein ToxT involved in dimerization and modulation by fatty acids. *J Biol Chem* 2011; **286**: 28644-28655 [PMID: 21673111 DOI: 10.1074/jbc.M111.258780]
84 **Cruite JT**, Kovacikova G, Clark KA, Woodbrey AK, Skorupski K, Kull FJ. Structural basis for virulence regulation in *Vibrio cholerae* by unsaturated fatty acid components of bile. *Commun Biol* 2019; **2**: 440 [PMID: 31815195 DOI: 10.1038/s42003-019-0686-x]
85 **Ktsoyan ZA**, Mkrtchyan MS, Zakharyan MK, Mnatsakanyan AA, Arakelova KA, Gevorgyan ZU, Sedrakyan AM, Hovhannisyan AI, Arakelyan AA, Aminov RI. Systemic Concentrations of Short Chain Fatty Acids Are Elevated in Salmonellosis and Exacerbation of Familial Mediterranean Fever. *Front Microbiol* 2016; **7**: 776 [PMID: 27252692 DOI: 10.3389/fmicb.2016.00776]
86 **van der Beek CM**, Bloemen JG, van den Broek MA, Lenaerts K, Venema K, Buurman WA, Dejong CH. Hepatic Uptake of Rectally Administered Butyrate Prevents an Increase in Systemic Butyrate Concentrations in Humans. *J Nutr* 2015; **145**: 2019-2024 [PMID: 26156796 DOI: 10.3945/jn.115.211193]
87 **den Besten G**, van Eunen K, Groen AK, Venema K, Reijngoud DJ, Bakker BM. The role of short-chain fatty acids in the interplay between diet, gut microbiota, and host energy metabolism. *J Lipid Res* 2013; **54**: 2325-2340 [PMID: 23821742 DOI: 10.1194/jlr.R036012]
88 **Zambell KL**, Fitch MD, Fleming SE. Acetate and butyrate are the major substrates for de novo lipogenesis in rat colonic epithelial cells. *J Nutr* 2003; **133**: 3509-3515 [PMID: 14608066 DOI: 10.1093/jn/133.11.3509]
89 **Sunami Y**, Rebelo A, Kleeff J. Lipid Metabolism and Lipid Droplets in Pancreatic Cancer and Stellate Cells. *Cancers (Basel)* 2017; **10** [PMID: 29295482 DOI: 10.3390/cancers10010003]
90 **Sidoli S**, Trefely S, Garcia BA, Carrer A. Integrated Analysis of Acetyl-CoA and Histone Modification *via* Mass Spectrometry to Investigate Metabolically Driven Acetylation. *Methods Mol Biol* 2019; **1928**: 125-147 [PMID: 30725455 DOI: 10.1007/978-1-4939-9027-6_9]
91 **Puchalska P**, Crawford PA. Multi-dimensional Roles of Ketone Bodies in Fuel Metabolism, Signaling, and Therapeutics. *Cell Metab* 2017; **25**: 262-284 [PMID: 28178565 DOI: 10.1016/j.cmet.2016.12.022]
92 **Wyss MT**, Magistretti PJ, Buck A, Weber B. Labeled acetate as a marker of astrocytic metabolism. *J Cereb Blood Flow Metab* 2011; **31**: 1668-1674 [PMID: 21654698 DOI: 10.1038/jcbfm.2011.84]
93 **Frost G**, Sleeth ML, Sahuri-Arisoylu M, Lizarbe B, Cerdan S, Brody L, Anastasovska J, Ghourab S, Hankir M, Zhang S, Carling D, Swann JR, Gibson G, Viardot A, Morrison D, Louise Thomas E, Bell JD. The short-chain fatty acid acetate reduces appetite *via* a central homeostatic mechanism. *Nat Commun* 2014; **5**: 3611 [PMID: 24781306 DOI: 10.1038/ncomms4611]
94 **Hamer HM**, Jonkers D, Venema K, Vanhoutvin S, Troost FJ, Brummer RJ. Review article: the role of butyrate on colonic function. *Aliment Pharmacol Ther* 2008; **27**: 104-119 [PMID: 17973645 DOI: 10.1111/j.1365-2036.2007.03562.x]
95 **Liu H**, Wang J, He T, Becker S, Zhang G, Li D, Ma X. Butyrate: A Double-Edged Sword for Health? *Adv Nutr* 2018; **9**: 21-29 [PMID: 29438462 DOI: 10.1093/advances/nmx009]
96 **Rouwet EV**, Heineman E, Buurman WA, ter Riet G, Ramsay G, Blanco CE. Intestinal permeability and carrier-mediated monosaccharide absorption in preterm neonates during the early postnatal period. *Pediatr Res* 2002; **51**: 64-70 [PMID: 11756641 DOI: 10.1203/00006450-200201000-00012]
97 **Blau CA**, Constantoulakis P, Shaw CM, Stamatoyannopoulos G. Fetal hemoglobin induction with butyric acid: efficacy and toxicity. *Blood* 1993; **81**: 529-537 [PMID: 8422469]
98 **Peng L**, He Z, Chen W, Holzman IR, Lin J. Effects of butyrate on intestinal barrier function in a Caco-2 cell monolayer model of intestinal barrier. *Pediatr Res* 2007; **61**: 37-41 [PMID: 17211138 DOI: 10.1203/01.pdr.0000250014.92242.f3]
99 **Suzuki T**. Regulation of intestinal epithelial permeability by tight junctions. *Cell Mol Life Sci* 2013; **70**: 631-659 [PMID: 22782113 DOI: 10.1007/s00018-012-1070-x]
100 **Turner JR**. Intestinal mucosal barrier function in health and disease. *Nat Rev Immunol* 2009; **9**: 799-809 [PMID: 19855405 DOI: 10.1038/nri2653]
101 **Bach Knudsen KE**, Lærke HN, Hedemann MS, Nielsen TS, Ingerslev AK, Gundelund Nielsen DS, Theil PK, Purup S, Hald S, Schioldan AG, Marco ML, Gregersen S, Hermansen K. Impact of Diet-Modulated Butyrate Production on Intestinal Barrier Function and Inflammation. *Nutrients* 2018; **10** [PMID: 30322146 DOI: 10.3390/nu10101499]
102 **Weitkunat K**, Schumann S, Nickel D, Kappo KA, Petzke KJ, Kipp AP, Blaut M, Klaus S. Importance of propionate for the repression of hepatic lipogenesis and improvement of insulin sensitivity in high-fat diet-induced obesity. *Mol Nutr Food Res* 2016; **60**: 2611-2621 [PMID: 27467905 DOI: 10.1002/mnfr.201600305]
103 **Bindels LB**, Porporato P, Dewulf EM, Verrax J, Neyrinck AM, Martin JC, Scott KP, Buc Calderon P, Feron O, Muccioli GG, Sonveaux P, Cani PD, Delzenne NM. Gut microbiota-derived propionate reduces cancer cell proliferation in the liver. *Br J Cancer* 2012; **107**: 1337-1344 [PMID: 22976799 DOI: 10.1038/bjc.2012.409]
104 **Koh A**, De Vadder F, Kovatcheva-Datchary P, Bäckhed F. From Dietary Fiber to Host Physiology: Short-Chain Fatty Acids as Key Bacterial Metabolites. *Cell* 2016; **165**: 1332-1345 [PMID: 27259147 DOI: 10.1016/j.cell.2016.05.041]

105 **He J**, Zhang P, Shen L, Niu L, Tan Y, Chen L, Zhao Y, Bai L, Hao X, Li X, Zhang S, Zhu L. Short-Chain Fatty Acids and Their Association with Signalling Pathways in Inflammation, Glucose and Lipid Metabolism. *Int J Mol Sci* 2020; **21** [PMID: 32887215 DOI: 10.3390/ijms21176356]

106 **Milligan G**, Stoddart LA, Smith NJ. Agonism and allosterism: the pharmacology of the free fatty acid receptors FFA2 and FFA3. *Br J Pharmacol* 2009; **158**: 146-153 [PMID: 19719777 DOI: 10.1111/j.1476-5381.2009.00421.x]

107 **Hong YH**, Nishimura Y, Hishikawa D, Tsuzuki H, Miyahara H, Gotoh C, Choi KC, Feng DD, Chen C, Lee HG, Katoh K, Roh SG, Sasaki S. Acetate and propionate short chain fatty acids stimulate adipogenesis *via* GPCR43. *Endocrinology* 2005; **146**: 5092-5099 [PMID: 16123168 DOI: 10.1210/en.2005-0545]

108 **Forbes S**, Stafford S, Coope G, Heffron H, Real K, Newman R, Davenport R, Barnes M, Grosse J, Cox H. Selective FFA2 Agonism Appears to Act *via* Intestinal PYY to Reduce Transit and Food Intake but Does Not Improve Glucose Tolerance in Mouse Models. *Diabetes* 2015; **64**: 3763-3771 [DOI: 10.2337/db15-0481]

109 **Pirozzi C**, Francisco V, Guida FD, Gómez R, Lago F, Pino J, Meli R, Gualillo O. Butyrate Modulates Inflammation in Chondrocytes *via* GPR43 Receptor. *Cell Physiol Biochem* 2018; **51**: 228-243 [PMID: 30448827 DOI: 10.1159/000495203]

110 **Shimizu H**, Masujima Y, Ushiroda C, Mizushima R, Taira S, Ohue-Kitano R, Kimura I. Dietary short-chain fatty acid intake improves the hepatic metabolic condition *via* FFAR3. *Sci Rep* 2019; **9**: 16574 [PMID: 31719611 DOI: 10.1038/s41598-019-53242-x]

111 **Parada Venegas D**, De la Fuente MK, Landskron G, González MJ, Quera R, Dijkstra G, Harmsen HJM, Faber KN, Hermoso MA. Short Chain Fatty Acids (SCFAs)-Mediated Gut Epithelial and Immune Regulation and Its Relevance for Inflammatory Bowel Diseases. *Front Immunol* 2019; **10**: 277 [PMID: 30915065 DOI: 10.3389/fimmu.2019.00277]

112 **Fellows R**, Denizot J, Stellato C, Cuomo A, Jain P, Stoyanova E, Balázsi S, Hajnády Z, Liebert A, Kazakevych J, Blackburn H, Corrêa RO, Fachi JL, Sato FT, Ribeiro WR, Ferreira CM, Perée H, Spagnuolo M, Mattiuz R, Matolcsi C, Guedes J, Clark J, Veldhoen M, Bonaldi T, Vinolo MAR, Varga-Weisz P. Microbiota derived short chain fatty acids promote histone crotonylation in the colon through histone deacetylases. *Nat Commun* 2018; **9**: 105 [PMID: 29317660 DOI: 10.1038/s41467-017-02651-5]

113 **Halili MA**, Andrews MR, Sweet MJ, Fairlie DP. Histone deacetylase inhibitors in inflammatory disease. *Curr Top Med Chem* 2009; **9**: 309-319 [PMID: 19355993 DOI: 10.2174/156802609788085250]

114 **Feng Y**, Huang Y, Wang Y, Wang P, Song H, Wang F. Antibiotics induced intestinal tight junction barrier dysfunction is associated with microbiota dysbiosis, activated NLRP3 inflammasome and autophagy. *PLoS One* 2019; **14**: e0218384 [PMID: 31211803 DOI: 10.1371/journal.pone.0218384]

115 **Hasan N**, Yang H. Factors affecting the composition of the gut microbiota, and its modulation. *PeerJ* 2019; **7**: e7502 [PMID: 31440436 DOI: 10.7717/peerj.7502]

116 **Vrancken G**, Gregory AC, Huys GRB, Faust K, Raes J. Synthetic ecology of the human gut microbiota. *Nat Rev Microbiol* 2019; **17**: 754-763 [PMID: 31578461 DOI: 10.1038/s41579-019-0264-8]

117 **Lakhani SV**, Shah HN, Alexander K, Finelli FC, Kirkpatrick JR, Koch TR. Small intestinal bacterial overgrowth and thiamine deficiency after Roux-en-Y gastric bypass surgery in obese patients. *Nutr Res* 2008; **28**: 293-298 [PMID: 19083422 DOI: 10.1016/j.nutres.2008.03.002]

118 **Bouhnik Y**, Alain S, Attar A, Flourié B, Raskine L, Sanson-Le Pors MJ, Rambaud JC. Bacterial populations contaminating the upper gut in patients with small intestinal bacterial overgrowth syndrome. *Am J Gastroenterol* 1999; **94**: 1327-1331 [PMID: 10235214 DOI: 10.1111/j.1572-0241.1999.01016.x]

119 **Walker WA**. Role of the mucosal barrier in toxin/microbial attachment to the gastrointestinal tract. *Ciba Found Symp* 1985; **112**: 34-56 [PMID: 3891256 DOI: 10.1002/9780470720936.ch3]

120 **Pyleris E**, Giamarellos-Bourboulis EJ, Tzivras D, Koussoulas V, Barbatzas C, Pimentel M. The prevalence of overgrowth by aerobic bacteria in the small intestine by small bowel culture: relationship with irritable bowel syndrome. *Dig Dis Sci* 2012; **57**: 1321-1329 [PMID: 22262197 DOI: 10.1007/s10620-012-2033-7]

121 **Schnabl B**, Brenner DA. Interactions between the intestinal microbiome and liver diseases. *Gastroenterology* 2014; **146**: 1513-1524 [PMID: 24440671 DOI: 10.1053/j.gastro.2014.01.020]

122 **Molodecky NA**, Soon IS, Rabi DM, Ghali WA, Ferris M, Chernoff G, Benchimol EI, Panaccione R, Ghosh S, Barkema HW, Kaplan GG. Increasing incidence and prevalence of the inflammatory bowel diseases with time, based on systematic review. *Gastroenterology* 2012; **142**: 46-54.e42; quiz e30 [PMID: 22001864 DOI: 10.1053/j.gastro.2011.10.001]

123 **Chelakkot C**, Ghim J, Ryu SH. Mechanisms regulating intestinal barrier integrity and its pathological implications. *Exp Mol Med* 2018; **50**: 1-9 [PMID: 30115904 DOI: 10.1038/s12276-018-0126-x]

124 **Grishin A**, Papillon S, Bell B, Wang J, Ford HR. The role of the intestinal microbiota in the pathogenesis of necrotizing enterocolitis. *Semin Pediatr Surg* 2013; **22**: 69-75 [PMID: 23611609 DOI: 10.1053/j.sempedsurg.2013.01.002]

125 **Torrazza RM**, Ukhanova M, Wang X, Sharma R, Hudak ML, Neu J, Mai V. Intestinal microbial ecology and environmental factors affecting necrotizing enterocolitis. *PLoS One* 2013; **8**: e83304 [PMID: 24386174 DOI: 10.1371/journal.pone.0083304]

126 **Wehkamp J**, Stange EF. An Update Review on the Paneth Cell as Key to Ileal Crohn's Disease. *Front Immunol* 2020; **11**: 646 [PMID: 32351509 DOI: 10.3389/fimmu.2020.00646]

127 **Ehmann D**, Wendler J, Koeninger L, Larsen IS, Klag T, Berger J, Marette A, Schaller M, Stange EF, Malek NP, Jensen BAH, Wehkamp J. Paneth cell α-defensins HD-5 and HD-6 display differential degradation into active antimicrobial fragments. *Proc Natl Acad Sci U S A* 2019; **116**: 3746-3751 [PMID: 30808760 DOI: 10.1073/pnas.1817376116]

128 **Chu H**, Pazgier M, Jung G, Nuccio SP, Castillo PA, de Jong MF, Winter MG, Winter SE, Wehkamp J, Shen B, Salzman NH, Underwood MA, Tsolis RM, Young GM, Lu W, Lehrer RI, Bäumler AJ, Bevins CL. Human α-defensin 6 promotes mucosal innate immunity through self-assembled peptide nanonets. *Science* 2012; **337**: 477-481 [PMID: 22722251 DOI: 10.1126/science.1218831]

129 **Wehkamp J**, Wang G, Kübler I, Nuding S, Gregorieff A, Schnabel A, Kays RJ, Fellermann K, Burk O, Schwab M, Clevers H, Bevins CL, Stange EF. The Paneth cell alpha-defensin deficiency of ileal Crohn's disease is linked to Wnt/Tcf-4. *J Immunol* 2007; **179**: 3109-3118 [PMID: 17709525 DOI: 10.4049/jimmunol.179.5.3109]

130 **Wehkamp J**, Stange EF. Paneth's disease. *J Crohns Colitis* 2010; **4**: 523-531 [PMID: 21122555 DOI: 10.1016/j.crohns.2010.05.010]

131 **Riba A**, Olier M, Lacroix-Lamandé S, Lencina C, Bacquié V, Harkat C, Gillet M, Baron M, Sommer C, Mallet V, Salvador-Cartier C, Laurent F, Théodorou V, Ménard S. Paneth Cell Defects Induce Microbiota Dysbiosis in Mice and Promote Visceral Hypersensitivity. *Gastroenterology* 2017; **153**: 1594-1606.e2 [PMID: 28865734 DOI: 10.1053/j.gastro.2017.08.044]

132 **Jahnel J**, Fickert P, Hauer AC, Högenauer C, Avian A, Trauner M. Inflammatory bowel disease alters intestinal bile acid transporter expression. *Drug Metab Dispos* 2014; **42**: 1423-1431 [PMID: 24965812 DOI: 10.1124/dmd.114.058065]

133 **Yiu JH**, Dorweiler B, Woo CW. Interaction between gut microbiota and toll-like receptor: from immunity to metabolism. *J Mol Med (Berl)* 2017; **95**: 13-20 [PMID: 27639584 DOI: 10.1007/s00109-016-1474-4]

134 **Milosevic I**, Vujovic A, Barac A, Djelic M, Korac M, Radovanovic Spurnic A, Gmizic I, Stevanovic O, Djordjevic V, Lekic N, Russo E, Amedei A. Gut-Liver Axis, Gut Microbiota, and Its Modulation in the Management of Liver Diseases: A Review of the Literature. *Int J Mol Sci* 2019; **20** [PMID: 30658519 DOI: 10.3390/ijms20020395]

135 **Payne CM**, Weber C, Crowley-Skillicorn C, Dvorak K, Bernstein H, Bernstein C, Holubec H, Dvorakova B, Garewal H. Deoxycholate induces mitochondrial oxidative stress and activates NF-kappaB through multiple mechanisms in HCT-116 colon epithelial cells. *Carcinogenesis* 2007; **28**: 215-222 [PMID: 16887864 DOI: 10.1093/carcin/bgl139]

136 **Ohtani N**, Kawada N. Role of the Gut-Liver Axis in Liver Inflammation, Fibrosis, and Cancer: A Special Focus on the Gut Microbiota Relationship. *Hepatol Commun* 2019; **3**: 456-470 [PMID: 30976737 DOI: 10.1002/hep4.1331]

137 **Tsuchida T**, Friedman SL. Mechanisms of hepatic stellate cell activation. *Nat Rev Gastroenterol Hepatol* 2017; **14**: 397-411 [PMID: 28487545 DOI: 10.1038/nrgastro.2017.38]

138 **Wandzioch E**, Kolterud A, Jacobsson M, Friedman SL, Carlsson L. Lhx2-/- mice develop liver fibrosis. *Proc Natl Acad Sci U S A* 2004; **101**: 16549-16554 [PMID: 15536133 DOI: 10.1073/pnas.0404678101]

139 **Tang Y**, Zhou J, Hooi SC, Jiang YM, Lu GD. Fatty acid activation in carcinogenesis and cancer development: Essential roles of long-chain acyl-CoA synthetases. *Oncol Lett* 2018; **16**: 1390-1396 [PMID: 30008815 DOI: 10.3892/ol.2018.8843]

140 **Hanahan D**, Weinberg RA. Hallmarks of cancer: the next generation. *Cell* 2011; **144**: 646-674 [PMID: 21376230 DOI: 10.1016/j.cell.2011.02.013]

141 **Warburg O**, Wind F, Negelein E. THE METABOLISM OF TUMORS IN THE BODY. *J Gen Physiol* 1927; **8**: 519-530 [PMID: 19872213 DOI: 10.1085/jgp.8.6.519]

142 **Vander Heiden MG**, Cantley LC, Thompson CB. Understanding the Warburg effect: the metabolic requirements of cell proliferation. *Science* 2009; **324**: 1029-1033 [PMID: 19460998 DOI: 10.1126/science.1160809]

143 **Pakiet A**, Kobiela J, Stepnowski P, Sledzinski T, Mika A. Changes in lipids composition and metabolism in colorectal cancer: a review. *Lipids Health Dis* 2019; **18**: 29 [PMID: 30684960 DOI: 10.1186/s12944-019-0977-8]

144 **Chekulayev V**, Mado K, Shevchuk I, Koit A, Kaldma A, Klepinin A, Timohhina N, Tepp K, Kandashvili M, Ounpuu L, Heck K, Truu L, Planken A, Valvere V, Kaambre T. Metabolic remodeling in human colorectal cancer and surrounding tissues: alterations in regulation of mitochondrial respiration and metabolic fluxes. *Biochem Biophys Rep* 2015; **4**: 111-125 [PMID: 29124194 DOI: 10.1016/j.bbrep.2015.08.020]

145 **Satoh K**, Yachida S, Sugimoto M, Oshima M, Nakagawa T, Akamoto S, Tabata S, Saitoh K, Kato K, Sato S, Igarashi K, Aizawa Y, Kajino-Sakamoto R, Kojima Y, Fujishita T, Enomoto A, Hirayama A, Ishikawa T, Taketo MM, Kushida Y, Haba R, Okano K, Tomita M, Suzuki Y, Fukuda S, Aoki M, Soga T. Global metabolic reprogramming of colorectal cancer occurs at adenoma stage and is induced by MYC. *Proc Natl Acad Sci U S A* 2017; **114**: E7697-E7706 [PMID: 28847964 DOI: 10.1073/pnas.1710366114]

146 **Fu Y**, Liu S, Yin S, Niu W, Xiong W, Tan M, Li G, Zhou M. The reverse Warburg effect is likely to be an Achilles' heel of cancer that can be exploited for cancer therapy. *Oncotarget* 2017; **8**: 57813-57825 [PMID: 28915713 DOI: 10.18632/oncotarget.18175]

147 **DeBerardinis RJ**, Mancuso A, Daikhin E, Nissim I, Yudkoff M, Wehrli S, Thompson CB. Beyond aerobic glycolysis: transformed cells can engage in glutamine metabolism that exceeds the requirement for protein and nucleotide synthesis. *Proc Natl Acad Sci U S A* 2007; **104**: 19345-19350 [PMID: 18032601 DOI: 10.1073/pnas.0709747104]

148 **Locasale JW**, Grassian AR, Melman T, Lyssiotis CA, Mattaini KR, Bass AJ, Heffron G, Metallo CM, Muranen T, Sharfi H, Sasaki AT, Anastasiou D, Mullarky E, Vokes NI, Sasaki M, Beroukhim R, Stephanopoulos G, Ligon AH, Meyerson M, Richardson AL, Chin L, Wagner G, Asara JM, Brugge JS, Cantley LC, Vander Heiden MG. Phosphoglycerate dehydrogenase diverts glycolytic flux and contributes to oncogenesis. *Nat Genet* 2011; **43**: 869-874 [PMID: 21804546 DOI: 10.1038/ng.890]

149 **Possemato R**, Marks KM, Shaul YD, Pacold ME, Kim D, Birsoy K, Sethumadhavan S, Woo HK, Jang HG, Jha AK, Chen WW, Barrett FG, Stransky N, Tsun ZY, Cowley GS, Barretina J, Kalaany NY, Hsu PP, Ottina K, Chan AM, Yuan B, Garraway LA, Root DE, Mino-Kenudson M, Brachtel EF, Driggers EM, Sabatini DM. Functional genomics reveal that the serine synthesis pathway is essential in breast cancer. *Nature* 2011; **476**: 346-350 [PMID: 21760589 DOI: 10.1038/nature10350]

150 **Le A**, Lane AN, Hamaker M, Bose S, Gouw A, Barbi J, Tsukamoto T, Rojas CJ, Slusher BS, Zhang H, Zimmerman LJ, Liebler DC, Slebos RJ, Lorkiewicz PK, Higashi RM, Fan TW, Dang CV. Glucose-independent glutamine metabolism *via* TCA cycling for proliferation and survival in B cells. *Cell Metab* 2012; **15**: 110-121 [PMID: 22225880 DOI: 10.1016/j.cmet.2011.12.009]

151 **Carracedo A**, Cantley LC, Pandolfi PP. Cancer metabolism: fatty acid oxidation in the limelight. *Nat Rev Cancer* 2013; **13**: 227-232 [PMID: 23446547 DOI: 10.1038/nrc3483]

152 **Counihan JL**, Grossman EA, Nomura DK. Cancer Metabolism: Current Understanding and Therapies. *Chem Rev* 2018; **118**: 6893-6923 [PMID: 29939018 DOI: 10.1021/acs.chemrev.7b00775]

153 **Ookhtens M**, Kannan R, Lyon I, Baker N. Liver and adipose tissue contributions to newly formed fatty acids in an ascites tumor. *Am J Physiol* 1984; **247**: R146-R153 [PMID: 6742224 DOI: 10.1152/ajpregu.1984.247.1.R146]

154 **Tadesse S**, Corner G, Dhima E, Houston M, Guha C, Augenlicht L, Velcich A. MUC2 mucin deficiency alters inflammatory and metabolic pathways in the mouse intestinal mucosa. *Oncotarget* 2017; **8**: 71456-71470 [PMID: 29069719 DOI: 10.18632/oncotarget.16886]

155 **Sánchez-Martínez R**, Cruz-Gil S, García-Álvarez MS, Reglero G, Ramírez de Molina A. Complementary ACSL isoforms contribute to a non-Warburg advantageous energetic status characterizing invasive colon cancer cells. *Sci Rep* 2017; **7**: 11143 [PMID: 28894242 DOI: 10.1038/s41598-017-11612-3]

156 **Sánchez-Martínez R**, Cruz-Gil S, Gómez de Cedrón M, Álvarez-Fernández M, Vargas T, Molina S, García B, Herranz J, Moreno-Rubio J, Reglero G, Pérez-Moreno M, Feliu J, Malumbres M, Ramírez de Molina A. A link between lipid metabolism and epithelial-mesenchymal transition provides a target for colon cancer therapy. *Oncotarget* 2015; **6**: 38719-38736 [PMID: 26451612 DOI: 10.18632/oncotarget.5340]

157 **Cui M**, Wang Y, Sun B, Xiao Z, Ye L, Zhang X. MiR-205 modulates abnormal lipid metabolism of hepatoma cells *via* targeting acyl-CoA synthetase long-chain family member 1 (ACSL1) mRNA. *Biochem Biophys Res Commun* 2014; **444**: 270-275 [PMID: 24462768 DOI: 10.1016/j.bbrc.2014.01.051]

158 **Cui M**, Xiao Z, Wang Y, Zheng M, Song T, Cai X, Sun B, Ye L, Zhang X. Long noncoding RNA HULC modulates abnormal lipid metabolism in hepatoma cells through an miR-9-mediated RXRA signaling pathway. *Cancer Res* 2015; **75**: 846-857 [PMID: 25592151 DOI: 10.1158/0008-5472.CAN-14-1192]

159 **Vargas T**, Moreno-Rubio J, Herranz J, Cejas P, Molina S, Mendiola M, Burgos E, Custodio AB, De Miguel M, Martín-Hernández R, Reglero G, Feliu J, Ramírez de Molina A. 3'UTR Polymorphism in ACSL1 Gene Correlates with Expression Levels and Poor Clinical Outcome in Colon Cancer Patients. *PLoS One* 2016; **11**: e0168423 [PMID: 27992526 DOI: 10.1371/journal.pone.0168423]

160 **Cao Y**, Dave KB, Doan TP, Prescott SM. Fatty acid CoA ligase 4 is up-regulated in colon adenocarcinoma. *Cancer Res* 2001; **61**: 8429-8434 [PMID: 11731423]

161 **Xia H**, Lee KW, Chen J, Kong SN, Sekar K, Deivasigamani A, Seshachalam VP, Goh BKP, Ooi LL, Hui KM. Simultaneous silencing of ACSL4 and induction of GADD45B in hepatocellular carcinoma cells amplifies the synergistic therapeutic effect of aspirin and sorafenib. *Cell Death Discov* 2017; **3**: 17058 [PMID: 28900541 DOI: 10.1038/cddiscovery.2017.58]

162 **Sung YK**, Hwang SY, Park MK, Bae HI, Kim WH, Kim JC, Kim M. Fatty acid-CoA ligase 4 is overexpressed in human hepatocellular carcinoma. *Cancer Sci* 2003; **94**: 421-424 [PMID: 12824887 DOI: 10.1111/j.1349-7006.2003.tb01458.x]

163 **Hu C**, Chen L, Jiang Y, Li Y, Wang S. The effect of fatty acid-CoA ligase 4 on the growth of hepatic cancer cells. *Cancer Biol Ther* 2008; **7**: 131-134 [PMID: 18059177 DOI: 10.4161/cbt.7.1.5198]

164 **Ndiaye H**, Liu JY, Hall A, Minogue S, Morgan MY, Waugh MG. Immunohistochemical staining reveals differential expression of ACSL3 and ACSL4 in hepatocellular carcinoma and hepatic gastrointestinal metastases. *Biosci Rep* 2020; **40** [PMID: 32286604 DOI: 10.1042/BSR20200219]

165 **Padanad MS**, Konstantinidou G, Venkateswaran N, Melegari M, Rindhe S, Mitsche M, Yang C, Batten K, Huffman KE, Liu J, Tang X, Rodriguez-Canales J, Kalhor N, Shay JW, Minna JD,

McDonald J, Wistuba II, DeBerardinis RJ, Scaglioni PP. Fatty Acid Oxidation Mediated by Acyl-CoA Synthetase Long Chain 3 Is Required for Mutant KRAS Lung Tumorigenesis. *Cell Rep* 2016; **16**: 1614-1628 [PMID: 27477280 DOI: 10.1016/j.celrep.2016.07.009]

166 **Saliakoura M**, Reynoso-Moreno I, Pozzato C, Rossi Sebastiano M, Galié M, Gertsch J, Konstantinidou G. The ACSL3-LPIAT1 signaling drives prostaglandin synthesis in non-small cell lung cancer. *Oncogene* 2020; **39**: 2948-2960 [PMID: 32034305 DOI: 10.1038/s41388-020-1196-5]

167 **Chen J**, Ding C, Chen Y, Hu W, Lu Y, Wu W, Zhang Y, Yang B, Wu H, Peng C, Xie H, Zhou L, Wu J, Zheng S. ACSL4 promotes hepatocellular carcinoma progression *via* c-Myc stability mediated by ERK/FBW7/c-Myc axis. *Oncogenesis* 2020; **9**: 42 [PMID: 32350243 DOI: 10.1038/s41389-020-0226-z]

168 **Doll S**, Proneth B, Tyurina YY, Panzilius E, Kobayashi S, Ingold I, Irmler M, Beckers J, Aichler M, Walch A, Prokisch H, Trümbach D, Mao G, Qu F, Bayir H, Füllekrug J, Scheel CH, Wurst W, Schick JA, Kagan VE, Angeli JP, Conrad M. ACSL4 dictates ferroptosis sensitivity by shaping cellular lipid composition. *Nat Chem Biol* 2017; **13**: 91-98 [PMID: 27842070 DOI: 10.1038/nchembio.2239]

169 **Gassler N**, Schneider A, Kopitz J, Schnölzer M, Obermüller N, Kartenbeck J, Otto HF, Autschbach F. Impaired expression of acyl-CoA-synthetase 5 in epithelial tumors of the small intestine. *Hum Pathol* 2003; **34**: 1048-1052 [PMID: 14608540 DOI: 10.1053/s0046-8177(03)00431-3]

170 **Gassler N**, Roth W, Funke B, Schneider A, Herzog F, Tischendorf JJ, Grund K, Penzel R, Bravo IG, Mariadason J, Ehemann V, Sykora J, Haas TL, Walczak H, Ganten T, Zentgraf H, Erb P, Alonso A, Autschbach F, Schirmacher P, Knüchel R, Kopitz J. Regulation of enterocyte apoptosis by acyl-CoA synthetase 5 splicing. *Gastroenterology* 2007; **133**: 587-598 [PMID: 17681178 DOI: 10.1053/j.gastro.2007.06.005]

171 **Gao X**, Lin SH, Ren F, Li JT, Chen JJ, Yao CB, Yang HB, Jiang SX, Yan GQ, Wang D, Wang Y, Liu Y, Cai Z, Xu YY, Chen J, Yu W, Yang PY, Lei QY. Acetate functions as an epigenetic metabolite to promote lipid synthesis under hypoxia. *Nat Commun* 2016; **7**: 11960 [PMID: 27357947 DOI: 10.1038/ncomms11960]

172 **Gharib E**, Nasri Nasrabadi P, Reza Zali M. miR-497-5p mediates starvation-induced death in colon cancer cells by targeting acyl-CoA synthetase-5 and modulation of lipid metabolism. *J Cell Physiol* 2020; **235**: 5570-5589 [PMID: 32012265 DOI: 10.1002/jcp.29488]

173 **Cui M**, Xiao Z, Sun B, Wang Y, Zheng M, Ye L, Zhang X. Involvement of cholesterol in hepatitis B virus X protein-induced abnormal lipid metabolism of hepatoma cells *via* up-regulating miR-205-targeted ACSL4. *Biochem Biophys Res Commun* 2014; **445**: 651-655 [PMID: 24576478 DOI: 10.1016/j.bbrc.2014.02.068]

174 **Qin X**, Zhang J, Lin Y, Sun XM, Zhang JN, Cheng ZQ. Identification of MiR-211-5p as a tumor suppressor by targeting ACSL4 in Hepatocellular Carcinoma. *J Transl Med* 2020; **18**: 326 [PMID: 32859232 DOI: 10.1186/s12967-020-02494-7]

175 **Cruz-Gil S**, Sanchez-Martinez R, Gomez de Cedron M, Martin-Hernandez R, Vargas T, Molina S, Herranz J, Davalos A, Reglero G, Ramirez de Molina A. Targeting the lipid metabolic axis *ACSL/SCD* in colorectal cancer progression by therapeutic miRNAs: miR-19b-1 role. *J Lipid Res* 2018; **59**: 14-24 [PMID: 29074607 DOI: 10.1194/jlr.M076752]

176 **Shen WW**, Zeng Z, Zhu WX, Fu GH. MiR-142-3p functions as a tumor suppressor by targeting CD133, ABCG2, and Lgr5 in colon cancer cells. *J Mol Med (Berl)* 2013; **91**: 989-1000 [PMID: 23619912 DOI: 10.1007/s00109-013-1037-x]

177 **Li B**, Liu J, Xin X, Zhang L, Zhou J, Xia C, Zhu W, Yu H. MiR-34c promotes hepatic stellate cell activation and Liver Fibrogenesis by suppressing ACSL1 expression. *Int J Med Sci* 2021; **18**: 615-625 [PMID: 33437196 DOI: 10.7150/ijms.51589]

178 **Tomoda H**, Igarashi K, Omura S. Inhibition of acyl-CoA synthetase by triacsins. *Biochim Biophys Acta* 1987; **921**: 595-598 [PMID: 3117118]

179 **Kaemmerer E**, Peuscher A, Reinartz A, Liedtke C, Weiskirchen R, Kopitz J, Gassler N. Human intestinal acyl-CoA synthetase 5 is sensitive to the inhibitor triacsin C. *World J Gastroenterol* 2011; **17**: 4883-4889 [PMID: 22171129 DOI: 10.3748/wjg.v17.i44.4883]

180 **Van Horn CG**, Caviglia JM, Li LO, Wang S, Granger DA, Coleman RA. Characterization of recombinant long-chain rat acyl-CoA synthetase isoforms 3 and 6: identification of a novel variant of isoform 6. *Biochemistry* 2005; **44**: 1635-1642 [PMID: 15683247 DOI: 10.1021/bi0477211]

181 **Kim JH**, Lewin TM, Coleman RA. Expression and characterization of recombinant rat Acyl-CoA synthetases 1, 4, and 5. Selective inhibition by triacsin C and thiazolidinediones. *J Biol Chem* 2001; **276**: 24667-24673 [PMID: 11319222 DOI: 10.1074/jbc.M010793200]

182 **Askari B**, Kanter JE, Sherrid AM, Golej DL, Bender AT, Liu J, Hsueh WA, Beavo JA, Coleman RA, Bornfeldt KE. Rosiglitazone inhibits acyl-CoA synthetase activity and fatty acid partitioning to diacylglycerol and triacylglycerol *via* a peroxisome proliferator-activated receptor-gamma-independent mechanism in human arterial smooth muscle cells and macrophages. *Diabetes* 2007; **56**: 1143-1152 [PMID: 17259370 DOI: 10.2337/db06-0267]

183 **Weng JR**, Chen CY, Pinzone JJ, Ringel MD, Chen CS. Beyond peroxisome proliferator-activated receptor gamma signaling: the multi-facets of the antitumor effect of thiazolidinediones. *Endocr Relat Cancer* 2006; **13**: 401-413 [PMID: 16728570 DOI: 10.1677/erc.1.01182]

184 **Matsufuji T**, Ikeda M, Naito A, Hirouchi M, Kanda S, Izumi M, Harada J, Shinozuka T. Arylpiperazines as fatty acid transport protein 1 (FATP1) inhibitors with improved potency and pharmacokinetic properties. *Bioorg Med Chem Lett* 2013; **23**: 2560-2565 [PMID: 23528296 DOI:

10.1016/j.bmcl.2013.02.116]
185 **Blackburn C**, Guan B, Brown J, Cullis C, Condon SM, Jenkins TJ, Peluso S, Ye Y, Gimeno RE, Punreddy S, Sun Y, Wu H, Hubbard B, Kaushik V, Tummino P, Sanchetti P, Yu Sun D, Daniels T, Tozzo E, Balani SK, Raman P. Identification and characterization of 4-aryl-3,4-dihydropyrimidin-2(1H)-ones as inhibitors of the fatty acid transporter FATP4. *Bioorg Med Chem Lett* 2006; **16**: 3504-3509 [PMID: 16644217 DOI: 10.1016/j.bmcl.2006.03.102]
186 **Black PN**, Ahowesso C, Montefusco D, Saini N, DiRusso CC. Fatty Acid Transport Proteins: Targeting FATP2 as a Gatekeeper Involved in the Transport of Exogenous Fatty Acids. *Medchemcomm* 2016; **7**: 612-622 [PMID: 27446528 DOI: 10.1039/C6MD00043F]
187 **Uchiyama A**, Aoyama T, Kamijo K, Uchida Y, Kondo N, Orii T, Hashimoto T. Molecular cloning of cDNA encoding rat very long-chain acyl-CoA synthetase. *J Biol Chem* 1996; **271**: 30360-30365 [PMID: 8939997 DOI: 10.1074/jbc.271.48.30360]
188 **Wiśniewski JR**, Friedrich A, Keller T, Mann M, Koepsell H. The impact of high-fat diet on metabolism and immune defense in small intestine mucosa. *J Proteome Res* 2015; **14**: 353-365 [PMID: 25285821 DOI: 10.1021/pr500833v]
189 **van den Bosch HM**, Bünger M, de Groot PJ, van der Meijde J, Hooiveld GJ, Müller M. Gene expression of transporters and phase I/II metabolic enzymes in murine small intestine during fasting. *BMC Genomics* 2007; **8**: 267 [PMID: 17683626 DOI: 10.1186/1471-2164-8-267]
190 **Qiu P**, Wang H, Zhang M, Peng R, Zhao Q, Liu J. FATP2-targeted therapies - A role beyond fatty liver disease. *Pharmacol Res* 2020; **161**: 105228 [PMID: 33027714 DOI: 10.1016/j.phrs.2020.105228]
191 **Leamy AK**, Egnatchik RA, Young JD. Molecular mechanisms and the role of saturated fatty acids in the progression of non-alcoholic fatty liver disease. *Prog Lipid Res* 2013; **52**: 165-174 [PMID: 23178552 DOI: 10.1016/j.plipres.2012.10.004]
192 **Arias-Barrau E**, Dirusso CC, Black PN. Methods to monitor Fatty Acid transport proceeding through vectorial acylation. *Methods Mol Biol* 2009; **580**: 233-249 [PMID: 19784603 DOI: 10.1007/978-1-60761-325-1_13]
193 **Sandoval A**, Chokshi A, Jesch ED, Black PN, Dirusso CC. Identification and characterization of small compound inhibitors of human FATP2. *Biochem Pharmacol* 2010; **79**: 990-999 [PMID: 19913517 DOI: 10.1016/j.bcp.2009.11.008]
194 **Veglia F**, Tyurin VA, Blasi M, De Leo A, Kossenkov AV, Donthireddy L, To TKJ, Schug Z, Basu S, Wang F, Ricciotti E, DiRusso C, Murphy ME, Vonderheide RH, Lieberman PM, Mulligan C, Nam B, Hockstein N, Masters G, Guarino M, Lin C, Nefedova Y, Black P, Kagan VE, Gabrilovich DI. Fatty acid transport protein 2 reprograms neutrophils in cancer. *Nature* 2019; **569**: 73-78 [PMID: 30996346 DOI: 10.1038/s41586-019-1118-2]
195 **Calder PC**. Polyunsaturated fatty acids and inflammation. *Biochem Soc Trans* 2005; **33**: 423-427 [PMID: 15787620 DOI: 10.1042/BST0330423]
196 **Saini N**, Black PN, Montefusco D, DiRusso CC. Fatty acid transport protein-2 inhibitor Grassofermata/CB5 protects cells against lipid accumulation and toxicity. *Biochem Biophys Res Commun* 2015; **465**: 534-541 [PMID: 26284975 DOI: 10.1016/j.bbrc.2015.08.055]
197 **Zhou W**, Madrid P, Fluitt A, Stahl A, Xie XS. Development and validation of a high-throughput screening assay for human long-chain fatty acid transport proteins 4 and 5. *J Biomol Screen* 2010; **15**: 488-497 [PMID: 20448275 DOI: 10.1177/1087057110369700]
198 **Martin G**, Schoonjans K, Lefebvre AM, Staels B, Auwerx J. Coordinate regulation of the expression of the fatty acid transport protein and acyl-CoA synthetase genes by PPARalpha and PPARgamma activators. *J Biol Chem* 1997; **272**: 28210-28217 [PMID: 9353271 DOI: 10.1074/jbc.272.45.28210]
199 **Schoonjans K**, Watanabe M, Suzuki H, Mahfoudi A, Krey G, Wahli W, Grimaldi P, Staels B, Yamamoto T, Auwerx J. Induction of the acyl-coenzyme A synthetase gene by fibrates and fatty acids is mediated by a peroxisome proliferator response element in the C promoter. *J Biol Chem* 1995; **270**: 19269-19276 [PMID: 7642600 DOI: 10.1074/jbc.270.33.19269]
200 **Björnson E**, Mukhopadhyay B, Asplund A, Pristovsek N, Cinar R, Romeo S, Uhlen M, Kunos G, Nielsen J, Mardinoglu A. Stratification of Hepatocellular Carcinoma Patients Based on Acetate Utilization. *Cell Rep* 2015; **13**: 2014-2026 [PMID: 26655911 DOI: 10.1016/j.celrep.2015.10.045]
201 **Min W**, Li Y, Zhang Y, Dai D, Cao Y, Yue Z, Liu J. Role of the anti-glioma drug AT13148 in the inhibition of Notch signaling pathway. *Gene* 2015; **573**: 153-159 [PMID: 26187072 DOI: 10.1016/j.gene.2015.07.033]

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REVIEW

Liver involvement in inflammatory bowel disease: What should the clinician know?

Giuseppe Losurdo, Irene Vita Brescia, Chiara Lillo, Martino Mezzapesa, Michele Barone, Mariabeatrice Principi, Enzo Ierardi, Alfredo Di Leo, Maria Rendina

ORCID number: Giuseppe Losurdo 0000-0001-7038-3287; Irene Vita Brescia 0000-0001-6291-7517; Chiara Lillo 0000-0002-4866-2316; Martino Mezzapesa 0000-0003-3917-8300; Michele Barone 0000-0001-8284-5127; Mariabeatrice Principi 0000-0003-0545-5656; Enzo Ierardi 0000-0001-7275-5080; Alfredo Di Leo 0000-0003-2026-1200; Maria Rendina 0000-0003-0077-6629.

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Giuseppe Losurdo, Irene Vita Brescia, Chiara Lillo, Martino Mezzapesa, Michele Barone, Mariabeatrice Principi, Enzo Ierardi, Alfredo Di Leo, Maria Rendina, Section of Gastroenterology, Department of Emergency and Organ Transplantation, University of Bari, Bari 70124, Italy

Corresponding author: Giuseppe Losurdo, MD, Doctor, Section of Gastroenterology, Department of Emergency and Organ Transplantation, University of Bari, Pzza Giulio Cesare, Bari 70124, Italy. giuseppelos@alice.it

Abstract

Inflammatory bowel disease (IBD) may show a wide range of extraintestinal manifestations. In this context, liver involvement is a focal point for both an adequate management of the disease and its prognosis, due to possible serious comorbidity. The association between IBD and primary sclerosing cholangitis is the most known example. This association is relevant because it implies an increased risk of both colorectal cancer and cholangiocarcinoma. Additionally, drugs such as thiopurines or biologic agents can cause drug-induced liver damage; therefore, this event should be considered when planning IBD treatment. Additionally, particular consideration should be given to the evidence that IBD patients may have concomitant chronic viral hepatitis, such as hepatitis B and hepatitis C. Chronic immunosuppressive regimens may cause a hepatitis flare or reactivation of a healthy carrier state, therefore careful monitoring of these patients is necessary. Finally, the spread of obesity has involved even IBD patients, thus increasing the risk of non-alcoholic fatty liver disease, which has already proven to be more common in IBD patients than in the non-IBD population. This phenomenon is considered an emerging issue, as it will become the leading cause of liver cirrhosis.

Key Words: Inflammatory bowel disease; Liver; Primary sclerosing cholangitis; Viral hepatitis; Immunosuppression; Non-alcoholic fatty liver disease

Core Tip: In the present article, several aspects of liver involvement of inflammatory bowel disease (IBD) have been highlighted. Co-occurrence of primary sclerosing

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cholangitis is one of the most well-known comorbidities and deserves more attention by the clinician. Liver damage due to drugs used to cure IBD is also a relevant issue. Finally, some emerging topics such as the spread of liver steatosis or the implications of chronic viral hepatitis have been analyzed.

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INTRODUCTION

Inflammatory bowel disease (IBD) consists of two separate disease entities, ulcerative colitis (UC) and Crohn's disease (CD), affecting the gastrointestinal tract[1]. However, IBD does not exclusively affect the gut. The gut-liver axis refers to the bidirectional relationship between the gut and its microbiota, and the liver, resulting from the integration of signals generated by dietary, genetic and environmental factors[2]. Therefore, a perturbation of this axis may mirror pathologic conditions both in the gut and the liver. Based on this consideration, the relationships between IBD and liver disorders are noteworthy and should always be considered by the clinician. The association between IBD and primary sclerosing cholangitis (PSC) is the most known and studied model, as it has several implications, the most important ones are the increased risk of both colorectal cancer and cholangiocarcinoma. Additionally, hepatotoxicity due to drugs such as thiopurines or biologic drugs is a relevant issue that should also be taken into account when planning IBD treatment[3]. It should not be forgotten that IBD patients may have concomitant chronic viral hepatitis, such as hepatitis B (HBV) and hepatitis C (HCV)[3]. Chronic immunosuppressive regimens may cause a hepatitis flare or reactivation of a healthy carrier state; therefore, careful monitoring of these patients is necessary. Finally, the obesity epidemic has involved even IBD patients, thus increasing the risk of non-alcoholic fatty liver disease (NAFLD), which has already proven to be higher than the control population in IBD patients[3]. This phenomenon is considered an emerging issue, as it will become the leading cause of liver cirrhosis.

Therefore, we aimed to perform a narrative review describing the main interactions between IBD and corresponding liver involvement, with a particular focus on PSC and other autoimmune liver disorders, drug-induced hepatitis, HBV, HCV and NAFLD (Table 1).

IBD AND PRIMARY SCLEROSING CHOLANGITIS

IBD and PSC are two pathologic entities that can occur alone or in combination. In this case they create a phenotypically different disease known as PSC-IBD. PSC-IBD prevalence is uncertain and differs in several studies, but it is agreed that it is very low (0.024%-0.041%)[4-6]. PSC and IBD may occur simultaneously or sequentially. Indeed, PSC patients develop IBD in 20%-70% of cases, with a stronger association with UC (80%) than with CD (10%) and indeterminate colitis (IC) (10%)[7]. Conversely only 5% of patients with UC show concomitant PSC.

Primary Sclerosing Cholangitis and Ulcerative Colitis

UC represents the underlying IBD in most cases of PSC-IBD. In patients with PSC and UC (PSC-UC), UC characteristically tends to be mild, quiescent and may even appear endoscopically normal (in this case, the diagnosis is based simply on histological analysis)[8]. Therefore, random biopsies during the first colonoscopy should always be performed to reveal an underlying UC in patients with PSC. Similarly, PSC may be underdiagnosed in patients with UC, as it can be asymptomatic. Thus, liver function tests, including cholestatic and hepatocellular damage markers, should always be recommended in the follow-up of UC. If a patient with UC is found to have hepatocellular injury or a cholestatic pattern, magnetic resonance cholangiopancreatography

Table 1 Main liver comorbidities associated with inflammatory bowel disease

Associated diseases	Prevalence in IBD (%)	Notes
PSC	0.024-0.041	Higher risk of cholangiocarcinoma and colorectal cancer; IBD shows less severe lesions than IBD alone
NAFLD	20-30	Associated with the use of corticosteroids, long disease duration, severe disease course; Associated with metabolic syndrome
Viral hepatitis	1-9	More common in the elderly; Association with advanced liver fibrosis; Need for anti-viral treatment before starting immunosuppressive drugs; HBV vaccine recommended

HBV: Hepatitis B virus; NAFLD: Non-alcoholic fatty liver disease; PSC: Primary sclerosing cholangitis; IBD: Inflammatory bowel disease.

(MRCP) should be performed to confirm the diagnosis[9]. The onset of the two disorders may vary. Typically, UC occurs first, with a median time interval of 10 years [10]. Nevertheless, in a minority of cases, UC may appear some years after the diagnosis of PSC, even after orthotopic liver transplantation[11]. The degree and the extension of colorectal inflammation in PSC-UC differ from UC alone. Indeed, the incidence of pancolitis appears increased in PSC-UC patients when compared with UC-only patients, as shown by Boonstra *et al*[12] In their series, PSC-UC patients were affected by pancolitis in 94% of cases, while pancolitis was demonstrated only in 62% of patients affected by UC alone. Patients with PSC-UC usually have a greater prevalence of backwash ileitis and rectal sparing (51% and 52%, respectively) than controls with UC alone (7% and 6%, respectively)[13]. However, the mild degree of colitis and the low rate of endoscopically visible inflammation may overestimate rectal sparing, when random biopsies are not performed[12,14]. Even though, the extension of colitis tends to be more diffuse, and in PSC-UC the severity of the mucosal inflammation seems less pronounced. Patients with PSC-UC have less significant bowel symptoms, a lower need for steroids and undergo fewer hospitalizations than patients with UC alone[15].

Primary Sclerosing Cholangitis and Crohn's Disease

Similar to patients affected by PSC-UC, patients with PSC and CD (PSC-CD) have a phenotypical and clinical pattern that sharply differs from patients with CD alone. Indeed, isolated ileal involvement, which occurs in about 30% of patients affected with CD, is rare in patients with PSC-CD (2%-5%)[12,16]. As shown with PSC-UC, the degree of endoscopically visible inflammation is milder in patient with PSC-CD than in those affected by UC. Likewise, the incidence of CD complications seems low in PSC-CD[12,16,17].

Main characteristics of PSC in IBD

While IBD in PSC-IBD has specific phenotypical patterns as listed above, PSC does not show significant differences in terms of histologic findings such as periductal fibrosis, inflammation and portal edema or fibrosis[18]. From a clinical point of view, according to Yanai *et al*[19] PSC outcomes, including cirrhosis incidence and transplant-free survival, did not differ in PSC-IBD compared with PSC alone patients. Conversely, Fevery *et al*[20] reported higher rates of liver-related death and malignancies in patients with PSC-UC when compared to patients with PSC-CD. Interestingly, Nordenvall *et al*[21] found that patients with PSC-UC who underwent colectomy, seemed to have a lower risk of mortality, morbidity and the need for liver transplantation.

Risk of colorectal cancer (CRC) and hepatobiliary carcinomas in PSC-IBD

Although both PSC and IBD patients do not have a general higher risk of malignancies than the general population, patients with PSC-IBD show a significantly more marked risk of developing colorectal carcinoma (CRC) and cholangiocarcinoma (CCA), and hepatocellular carcinoma (HCC). In a meta-analysis, Zengh *et al*[22] found that patients with PSC-IBD have a strikingly higher risk for the development of CRC than patients with IBD alone. In detail, the stratification by IBD type showed a three-fold increased risk for the development of CRC and colorectal dysplasia in patients with PSC-UC compared to those with UC alone. A non-significant increase in the risk of neoplasia was shown in patients with PSC-CD, in contrast to that found in patients with CD alone. For these reasons, patients with PSC-IBD (especially those with PSC-

UC) require close colorectal neoplasia endoscopic surveillance. Major American and European Societies recommend that annual CRC screening should be started at the time of PSC-IBD diagnosis. In PSC-IBD patients an increased risk of hepatobiliary malignancies such as CCA, gallbladder carcinoma (GBC), and HCC has been demonstrated. Gulamhusein *et al*[23] demonstrated that prolonged duration of IBD is associated with an increased risk of CCA in patients with PSC-IBD. They also observed that the risk of CCA was not modified after colectomy, thus suggesting that colonic resection itself does not reduce the risk of CCA. European and American Societies recommend that CA 19-9 and biliary imaging should be completed every year for these patients[24,25]. IBD could be an additional risk factor that further increases the hazard of CCA in PSC. In particular, a long duration of IBD is associated with CCA with a hazard ratio of 1.37[23].

There are no studies demonstrating an increased risk of GBC in PSC-IBD patients, even if that risk is demonstrated in PSC-alone patients[26]. Said *et al*[27] found in their cohort of patients affected with PSC, that 6% had gallbladder masses, of which 56% were malignant. The American Association for the Study of Liver Disease (AASLD) guidelines support cholecystectomy for polyps of any size in these patients, given the high likelihood of malignancy[28]. HCC seems to be a rare malignancy in PSC-IBD. Zanouzi *et al*[29] analyzed a cohort of PSC-cirrhosis patients and found no cases of HCC. However, in the same cohort of patients, IBD was found in 65%.

As both CCA and CRC are likely to occur in PSC-IBD patients, a chemopreventive strategy could be proposed. A meta-analysis[30] showed that low dose ursodeoxycholic acid may have a protective effect on both CRC and colonic dysplastic lesions, with an odds ratio of 0.19. However, the studies were performed on small populations in tertiary centers, and were often retrospective, therefore the strength of evidence is not high[31]. Even mesalazine has demonstrated, *in vitro* and in animal models, an anti-proliferative effect as well as the ability to inhibit the Wnt/β-Catenin pathway and epithelial growth factor receptor activation; therefore, it may be a promising agent for CRC prevention, despite the chemopreventive effect of mesalazine only being documented for patients with UC alone so far[32]. Unfortunately, no effective approach for CCA chemoprevention has emerged, therefore surveillance remains the mainstay for early CCA detection in PSC patients.

Therapeutic perspectives

The pathogenetic mechanisms underlying PSC-IBD remain unknown, even though many hypotheses have been proposed. Understanding the basis of the disease could lead to the identification of a new targeted therapy. One of the most interesting assumptions suggests that intestinal mucosal lymphocytes may migrate to the liver following activation in the bowel of IBD patients, thus promoting liver inflammation [33]. It has been shown that adhesion molecules and chemokine receptors normally expressed only in the gut can be aberrantly expressed within the liver to promote the homing of gut-associated lymphocytes. One of these adhesion molecules is α4β7 integrin. A monoclonal antibody directed against α4β7, vedolizumab, has been approved for the treatment of IBD. It was hypothesized that vedolizumab could provide hepatic anti-inflammatory benefits. Nevertheless, Christensen *et al* found that, after treatment with vedolizumab, symptoms and intestinal clinical activity were significantly decreased, but the Mayo PSC Risk Score and liver damage biomarkers were only slightly improved[34].

Aberrant microbiota epitope recognition and gut dysbiosis seem to have a role in the pathogenesis of PSC-IBD, while genetics, gut mucosal permeability and autoimmune mechanisms have a controversial role[35]. Further studies are needed to improve our knowledge on the pathogenesis of PSC-IBD in order to provide new and efficient therapeutic strategies.

When PSC causes end-stage liver disease, liver transplantation is the only curative treatment. Regarding this point, some studies found that IBD does not worsen survival in patients who undergo liver transplantation for PSC. Only exposure to azathioprine seems to increase post-transplant mortality, while IBD per se increases the risk of cytomegalovirus infection[36].

PRIMARY BILIARY CHOLANGITIS AND AUTOIMMUNE HEPATITIS IN IBD

PBC is an autoimmune liver disease characterized by inflammatory cell infiltration of intralobular biliary ducts, with consequent biliary duct damage, which can progress towards fibrosis. Currently, there is no solid link between IBD and PBC, as only a few

case reports have been published. The most consistent case series involving six PBC patients in a cohort of IBD subjects during the period 2006-2016 (3 CD and 3 UC), who were diagnosed with PBC by liver biopsy responded to ursodeoxycholic acid therapy [37]. In a genetic association study, it was found that TNFSF15 and ICOSLG-CXCR5 might be a shared pathogenic pathway in the development of PBC and CD[38].

Similarly, only some case reports on the association between IBD and autoimmune hepatitis (AIH) have been published. A systematic review found approximately 109 cases, which were mostly overlap syndrome with PBC. The authors reported that jaundice was the most common onset sign and that response to steroids was good, with a low mortality rate[39]. Interestingly, a case report of AIH onset after starting adalimumab has been described, which underlines the possibility that an immunogenic drug may alter an equilibrium in the immune system[40].

HEPATIC STEATOSIS IN IBD

Hepatic steatosis is defined as intrahepatic fat accumulation of at least 5% of liver weight. Prolonged hepatic lipid storage may lead to liver metabolic dysfunction, inflammation, and advanced forms of NAFLD. Non-alcoholic hepatic steatosis is associated with obesity, type 2 diabetes and dyslipidemia. Several mechanisms are involved in the accumulation of intrahepatic fat, including increased flux of fatty acids to the liver, increased *de novo* lipogenesis, and/or reduced clearance through β-oxidation or very-low-density lipoprotein secretion[41,42] in the absence of secondary causes of lipid overload such as significant alcohol intake.

A link between hepatic steatosis and IBD has been studied since 1873, when Thomas [43] described for the first time the association between "ulceration of the colon" and a "much enlarged fatty liver". In recent years, due to the spread of obesity in the context of IBD[44], fatty liver disease has been increasingly recognized in IBD. The intestinal inflammatory state and gut barrier perturbation secondary to IBD might increase toxin and bacterial constituents translocation from the gut to the portal vein; this event has been recognized as a possible pathophysiologic mechanism underlying NAFLD[45]. Moreover, diets poor in high fiber foods, such as fruits and vegetables, frequently consumed by IBD subjects to avoid intestinal symptoms, could lead to a great prevalence of NAFLD[46]. Moreover, food components and alimentary habits with high proteins and fats, excessive sugar intake and less vegetables and fiber can influence the composition of the intestinal microbiome, and play a role in driving IBD pathogenesis and fat metabolism leading tog NAFLD onset[47].

A recent meta-analysis showed that the overall pooled prevalence of NAFLD in IBD patients was 27.5%[48]. NAFLD, in particular, was more common among patients with features of severe IBD, such as longer disease duration or a history of abdominal surgery.

Another study by Bessisow *et al*[49] showed a frequency of NAFLD in IBD of 33.6% and demonstrated that disease activity, duration of IBD and prior surgery were predictors of NAFLD development.

Conversely, in a Japanese study[50], the ultrasonographic prevalence of NAFLD in CD was 21.8% and this was the only study in which NAFLD was identified as an independent predictor of a negative C-reactive protein level and higher rate of remission, so NAFLD might offer a protective effect in patients with CD.

Nevertheless, most studies did not include non-IBD patients as a control group.

Glassner *et al*[51] examined 3 groups of patients: IBD + NAFLD, IBD alone, and NAFLD alone. A total of 168 patients were evaluated, 56 patients in each group. They found an overall NAFLD prevalence of 13.3% in IBD patients. IBD patients with NAFLD had longer IBD disease duration and developed NAFLD even in the absence of metabolic risk factors when compared to patients with NAFLD alone.

A study performed in 2018 by Principi *et al*[52] included 465 IBD patients and 223 non-IBD patients. The prevalence of NAFLD was higher in IBD than in non-IBD patients (28.0% *vs* 20.1% respectively, $P = 0.04$); furthermore, younger age was observed in NAFLD-IBD than in non-IBD individuals, whereas no other differences were found between these two subgroups. Regarding risk factors, diabetes and fasting blood glucose were associated with development of NAFLD in IBD, without any difference in the populations without IBD, with only a higher waist circumference in IBD compared to non-IBD patients. No IBD-related variable was associated with NAFLD.

There are no studies on the progression of NASH in IBD. However, since IBD may induce gut barrier perturbation and an increase in toxin and bacterial translocation, it

is possible that in patients with NAFLD, the coexistence of IBD can trigger the progression from simple steatosis to NASH. A single study, on the other hand, has shown that progression of fibrosis, estimated by the NAFLD fibrosis score, is quite rare in IBD[53].

In conclusion, NAFLD is common in patients with IBD. Screening, prevention, and early treatment of NAFLD might be recommended in IBD patients. However, a better understanding of the underlying mechanism of the coexistence of IBD and NAFLD is necessary to improve management. The treatment of NAFLD in IBD does not differ from other cases. In particular, so far only diet and physical exercise have been proved to be effective[54].

CHRONIC VIRAL HEPATITIS IN IBD

Chronic viral hepatitis, in particular HBV and HCV-related, is a very common infection and a worldwide health issue. It is estimated that over 350 million people in the world have chronic HBV infection and over 250 million people have chronic HCV infection, with a mean prevalence of 5% and 2% for HBV and HCV, respectively[55, 56].

With regard to the prevalence of chronic hepatitis B (CHB) and chronic hepatitis C (CHC) in IBD, recent evidence[57-61] shows that it was comparable to a control population, ranging from 1% to 9%. A recent Italian study by Losurdo *et al*[62] on 807 IBD patients and 189 controls, found a prevalence of 3.4% for CHC and 0.9% for CHB, a result which agrees with recent literature reports[57,58,61]. This analysis demonstrated that advanced age was independently associated with increased risk of CHB/CHC. It is possible that surgery performed before the diffusion of presurgical hepatitis screening could explain this result, also taking into account that CHC was more common in patients operated before 1990. Indeed, the introduction of the HBV vaccine and HCV routine detection led to an improvement in the prevention measures against viral hepatitis transmission during surgery or blood donation, thus reducing the risk of infection in young generations[62].

As the treatment of IBD is based in selected cases on immunosuppressive agents (thiopurines and biologic drugs such as monoclonal antibodies), an accurate clinical and laboratory assessment is preliminarily required to look for chronic infections that may have a severe flare under biologic drugs[57,63]. Among these, chronic viral hepatitis and in particular CHB and CHC, are advised to be investigated by the guidelines before starting immunosuppressive treatment[64].

According to the guidelines, all IBD patients should be tested for HBV (HBsAg, anti-HBs, anti-HBc) at diagnosis of IBD to determine HBV status. In patients with positive HBsAg, viremia (HBV-DNA) should also be quantified. Moreover, HBV vaccination is recommended in all HBV anti-HBc seronegative patients with IBD. All HBsAg positive subjects should start anti-viral agents before undergoing biologic treatment to prevent potentially serious hepatitis B flares[64,65]. A number of case series and study cohorts suggest that nucleotide/nucleoside analogues are safe and effective in IBD patients on immunomodulator treatment[66]. Entecavir and tenofovir are preferred for IBD patients due to their rapid onset of action, high anti-viral potency and low incidence of resistance. On the other hand, patients with HBsAg positive (chronic HBV infection) should receive anti-viral agents before, during and for at least 12 mo after immunomodulator treatment has ceased[64]. Additionally, HBV vaccination is strongly advised by the guidelines, possibly before starting any immunosuppressive treatment and preferably at the moment of diagnosis, if anti-HBs level is not protective. This approach should be followed in any region, irrespective of HBV prevalence.

With regard to CHC, present knowledge shows in some cases mild liver dysfunction and an amplified detrimental effect by the simultaneous presence of other viruses (HBV/HIV) in relation to immunomodulator assumption[67,68]; therefore, HCV antibody testing and HCV-RNA should be investigated. Immunomodulators are not contraindicated but should be used with caution. The decision depends on the severity of IBD and the stage of liver disease. In the past years, an interferon-based treatment for HCV infection in CD has generally not been recommended, as it could worsened the intestinal disorder; however, this aspect remains controversial[69]. Conversely, in UC, interferon therapy did not appear to have an adverse effect[70]. In addition, the administration of ribavirin plus interferon or triple anti-viral therapy (interferon, ribavirin and protease inhibitors) could have increased the toxicity of drugs used for IBD maintenance (for example azathioprine, methotrexate)[64]. Therefore, the risk that anti-viral therapy or drug interactions with IBD therapy might

exacerbate IBD should assessed cautiously when considering the need for HCV treatment[64]. However, over the last years, concomitant IBD and HCV infection management has completely changed due to the recent introduction of direct-acting anti-virals (DAAs). Recently published data on DAAs are very encouraging also in IBD patients[71]. There are three possible timing strategies for administration in patients requiring biological therapies: (1) Sequential strategy, meaning the choice of treating firstly the active IBD with biologics and then, once the acute phase has been controlled, treating the HCV infection; (2) Concomitant strategy, that is the contemporaneous initiation of DAAs and biologic drug administration; and (3) Inverted sequential strategy, *i.e.*, the administration of anti-viral therapy before biologics. The timing strategy could depend on several factors, including IBD activity and patient comorbidity. This means that a case-by-case decision could be the best choice[72]. The opportunity to eradicate HCV should always be taken into account, as it has demonstrated that a sustained viral response may reduce liver stiffness in these patients[73].

IBD AND DRUG-INDUCED LIVER INJURY

In the last decade, treatment options for IBD have included new molecules acting at different target levels. Usually, as new drugs are introduced, their side effects should also be considered, and liver toxicity is one of the most meaningful among these.

Drug-induced liver injury (DILI) caused by these drugs can be classified into three forms: hepatocellular, cholestatic or a mixed pattern. Moreover, some forms of drug-induced AIH should also be considered. This issue leads to a schedule of specific screening before starting therapy for IBD, and a follow-up to monitor liver enzymes is necessary[74,75].

In Table 2, we summarize the main knowledge on DILI in IBD patients.

Thiopurines

Thiopurines, in particular azathioprine (AZA) and 6-mercaptopurine (6-MP) are used for induction and maintenance of remission in IBD. Studies have shown that AZA/6-MP as add-on to infliximab can reduce the development of antibodies against infliximab. Thiopurines act as DNA synthesis inhibitors by incorporating purine analogues into DNA with cytotoxic and immunosuppressive effects. AZA is metabolized in the liver to 6-MP, which is metabolized by three enzymes, including thiopurine S-methyltransferase (TMPT) to 6-methylmercaptopurine (6-MMP). AZA and 6-MP are prodrugs of 6-thioguanine (6-TGN), the real effective metabolite. Some studies have suggested that some TMPT polymorphisms could cause a rise in 6-MMP level, thereby amplifying hepatotoxicity. In a cohort study of 270 patients treated with 6-MP, 47 patients showed evidence of altered liver function tests (LFT) in the first 20 weeks of treatment and > 80% of these patients had elevated levels of 6-MMP in the first week[76]. Another study proved that patients with high concentrations of 6-MMP had not only a strong risk of side effects but also a reduction in therapeutic response [77]. Conversely, Dong *et al*[78] found that the presence of TMPT polymorphisms increased bone marrow toxicity but not hepatotoxicity. A recent meta-analysis of 10 studies (recruiting 1875 patients) proved that TMPT polymorphisms were not linked with liver injury. The physiopathology of liver injury due to thiopurine is still unclear.

The prevalence of thiopurine-induced liver toxicity can vary between 0% and 17%. In a systematic review of 34 studies with 3485 patients, the prevalence of hepatotoxicity induced by AZA/6-MP was 3.4% with no differences between the two drugs [79]. Additionally, Chaparro *et al*[80] in a study of 3931 patients with IBD treated with thiopurine reported that hepatotoxicity was one of the most common side effects, with a prevalence of 4%. CD, smoking and preexisting NAFLD seemed to be risk factors, while the prevalence was lower in females. In a study by Shroder, who analyzed 259 patients undergoing immunosuppressive treatment with AZA, 6MP and MTX, liver steatosis was found in 28.2% of them, and patients with steatosis also had a higher risk of having elevated alanine transaminase (ALT) blood levels[81].

On the other hand, dose independent, idiosyncratic liver reactions have been described for thiopurines. Acute dose-independent toxicity is caused by an idiosyncratic cholestatic reaction accompanied by fever, rash, lymphadenopathy and hepatomegaly with increased alkaline phosphatase level. The median onset time of hepatotoxicity is 110 days, and in most cases is self-limiting with a good prognosis.

Another atypical, long-term liver injury caused by thiopurines is characterized by vascular endothelial lesions. Nodular regenerative hyperplasia (NRH), is the most

Table 2 Main features of drug-induced liver injury in inflammatory bowel disease

Drug	Characteristics of drug induced liver injury
Aminosalicylates	Increases in LFT; Cholestatic pattern; Rarely eosinophilia
Thiopurines	Influenced by TMPT polymorphisms > increase in 6-MMP, the hepatotoxic molecule; Increases in LFT; Idiosyncratic cholestatic reaction; Fever, rash, lymphadenopathy and hepatomegaly; Nodular regenerative hyperplasia
Anti-TNF	Idiosyncratic reaction > dose-dependent mechanism; Hepatocellular injury > cholestasis; Autoimmune phenomena
Anti-integrins	Rare; Asymptomatic LFT increase
Anti IL12/23	Mild LFT increase

LFT: Liver function test; TMPT: Thiopurine S-methyltransferase; TNF: Tumor necrosis factor.

frequent of these lesions, while peliosis hepatis and sinusoidal obstruction syndrome (SOS) are less common. NRH is frequently asymptomatic. The mechanism underlying NRH is still unknown, it is possible that hepatocyte atrophy and portal venules destruction could be involved; risk factors seem to be male sex, CD with stricturing behavior and previous small bowel resection. In a large French study, NRH was found in 37 cases, with a cumulative risk of 0.5% at five years and a median onset time of 48 mo[82]. A recent study observed a similar prevalence of NRH between patients treated with thiopurines and patients thiopurine-naïve[83]. On the other hand, it was found that thiopurines are associated with NRH when the dose is high (tioguanine > 40 mg/day) or in male patients with small bowel resection > 50 cm[84,85]. The evolution of NRH after stopping thiopurine therapy is still unclear.

There is no agreement on thiopurine toxicity management. In a large study with a long-term follow-up only 3.6% of patients needed to discontinue therapy[86]. In another study, 90% of patients had normalization of LFT by reducing thiopurine doses [87]. It is unclear whether the frequency of hepatotoxicity is the same for AZA and 6-MP treatment: a study of 135 patients reported that 6-MP was well tolerated in 71% patients who had shown liver toxicity with AZA[88]. Coadministration of allopurinol (a xanthine-oxidase inhibitor) seems to reduce 6-MMP levels as it leads to a higher concentration of 6-MP converted to 6-TGN. However, since allopurinol is a xanthine-oxidase inhibitor, the AZA dose should be reduced. A retrospective cohort study of 105 patients reported that coadministration of allopurinol allowed long-lasting therapy and transaminase normalization[89]. Also, in another study by Krejineof, among 211 patients with liver toxicity, 86% experienced an improvement by lowering the dose of thiopurines in association with allopurinol[90]. A larger study by Vasuvedan analyzed 767 patients on thiopurine therapy and demonstrated that allopurinol should be started to reduce side effects, as 94% of patients who had hepatotoxicity achieved resolution by changing to co-therapy[91]. As TMPT polymorphisms are likely to be involved in hepatotoxicity, some authors have proposed that these polymorphisms should be identified before starting therapy, but a review by the American Gastroenterological Association Institute stated that the benefits of these tests were low[92]. On the contrary, a consensus guideline by the British Society of Gastroenterology focused on TMPT activity and recommended the administration of a half-dose of thiopurines to patients with low TMPT activity[93].

LFT should be monitored routinely, but there is no agreement on their timing. Mottet *et al*[93] recommended LTF every wk for the first mo, then twice a mo during the second mo and then once every 3 mo.

Sulfasalazine and mesalamine

Sulfasalazine is used for mild UC. It has been associated with acute hepatitis, cholestatic hepatitis, granulomatous hepatitis and rarely with acute liver failure[94]. The incidence of hepatotoxicity is low: A review by Ransford *et al* who analyzed 4.7 million prescriptions in the period from 1991 and 1998, reported only 9 cases of hepatitis caused by sulfasalazine[95].

Mesalamine (oral and rectal) is approved for mild UC. Authors in the last three years have demonstrated that the prevalence of liver toxicity caused by mesalamine is low, between 0% and 4%. The use of mesalamine may be associated with asymptomatic elevations in LFT, hepatitis and cholestatic hepatitis[96]. A recent review reported that LTF should be monitored every year and therapy should be stopped in the case of abnormal increases, while treatment with corticosteroids should be considered if fever, rash, or eosinophilia are observed. The same review

demonstrated that most cases of hepatotoxicity quickly reversed with drug withdrawal[97].

Methotrexate

Low doses of methotrexate (MTX) are used for mild CD, and it is widely used for rheumatologic disease; therefore, in this field its hepatotoxicity has been more extensively studied. The underlying mechanism is still not clear; several polymorphisms of enzymes involved in folic acid metabolism are thought to be involved. Two systematic reviews on this topic reported opposite results: the first review found an association between MTX hepatotoxicity and C677T polymorphism of methylenetetrahydrofolate reductase (MTHFR) gene, while the second review did not confirm this result[98,99]. MTX can cause different histological liver findings according to the Roenigk's classification including: (1) Normal; (2) Mild fatty infiltration, nuclear alterations or portal inflammation; (3) Moderate to severe fatty infiltration, nuclear alterations, or portal infiltration and mild fibrosis; (4) Moderate to severe fibrosis; and (5) cirrhosis[100].

Some studies reported that the prevalence of abnormal LTF in these patients ranged from 15 to 50%, while most recent evidence demonstrated a lower prevalence. A meta-analysis of patients with IBD treated with MTX reported a rate of abnormal LTF (defined as ALT higher than normal values but less than x2 upper normal limit (ULN)) of 1.4 per 100 person-month and a rate of hepatotoxicity (defined as ALT higher than two times normal values) of 0.9 per 100 person-month[101]. It should be noted that, in CD, methotrexate is given *i.m.*, with a dose of 25 mg/wk at induction and 15 mg/wk for maintaining remission. Considering that this dose is higher than in rheumatologic patients, this could explain the more frequent liver adverse events.

Before starting MTX treatment, patients should be screened for preexisting medical conditions, such as alcohol intake, viral hepatitis, steatosis and family history of liver disease. Rheumatological consensus guidelines recommend monitoring LFT every two wk for the first 2 mo, then every 2 or 3 mo[102]. Liver biopsy should be considered in some cases, such as when liver laboratory tests remain abnormal despite dose reduction or when there are high blood levels of drug in patients with known risk factors for hepatotoxicity. Treatment should be stopped in the case of severe fibrosis or cirrhosis and daily doses should be reduced in the case of LFT elevation. Co-administration with folic acid or folinic acid seems to reduce the frequency of serum transaminase elevation[103]. Elastography (Fibroscan) and laboratory tests are emerging tools to diagnose fibrosis as reported by *Labadie et al*[104]. Furthermore, in a case control study of 518 patients treated with MTX, 8.5% showed Fibroscan and FibroTest abnormalities, *i.e.*, severe fibrosis[105]. A multivariate analysis reported that elastography should be used mainly in patients with an alcohol habit or obesity, or affected by NAFLD. Similar results were reported in a study by Herfath *et al*[106].

Tumor necrosis factor alpha inhibiting agents

Currently several molecules belonging to this class have been approved to treat IBD: infliximab (IFX), adalimumab (ADA), golimumab and certolizumab pegol. Few data are available on the hepatotoxicity of golimumab and certolizumab, while most of the literature reports DILI by IFX and ADA.

The Food and Drug Administration (FDA) in 2004 after 130 cases of liver injury in patients treated with IFX and etanercept (which has no indication in IBD), issued an alarm statement of severe hepatic adverse reactions, including acute liver failure, autoimmune hepatitis (AIH) and cholestatic hepatitis during IFX therapy[107]. In an Icelandic study by Bjornsson that included patients with IBD, rheumatological and dermatological disorders, the occurrence of DILI in patients treated with IFX or ADA was 1:120 and 1:270, respectively[108]. Shelton *et al*[109] in a retrospective study analyzed 1753 patients under anti-TNF therapy (1170 IFX, 575 ADA, 8 certolizumab), and found that 102 patients had high blood levels of ALT, but in 54 of these patients, additional risk factors for liver injury were found and, of the remaining 48 patients (45 IFX, 3 ADA), only 4 were considered to be affected by anti-TNF induced liver injury. Koller *et al*[110] in a recent observational study of 251 patients with IBD, monitored liver injury in 163 receiving IFX. Twenty-six patients (16%) showed a grade 1 liver injury (ALT < x3 ULN), 4 patients (2.5%) a grade 2 (ALT > x3 ULN); grade 1 alkaline phosphatase elevation was seen in 11 patients (6.7%) and grade 2 alkaline phosphatase elevation (> x2.5 ULN) in none. Liver injury in these patients was associated with high BMI, hepatic steatosis and longer duration of IBD[110]. In an Australian retrospective cohort study of adult patients with IBD treated with IFX (IDLE STUDY), out of 175 patients (149 with CD and 26 with UC), 57 showed abnormal liver laboratory tests. In this study, the authors used the Roussel Uclaf Causality Assessment Method

(RUCAM) score to predict the risk of hepatic injury caused by drugs. A score of 0 rules out DILI, 1-2 means unlikely DILI, 3-5 possible DILI, 6-8 probable DILI, and > 8 highly probable DILI. Eleven patients had a RUCAM score > 3, but just one patient had a score > 8. Usually, liver injury due to IFX occurs after multiple infusions and a mean latency of 14-18 wk from induction. In this context, the RUCAM score is not a diagnostic test, but it is useful to predict DILI relying on LFT, timing of drug initiation and cessation, and on liver biopsy, when performed[111].

Although IFX, ADA and etanercept are anti-TNF drugs, they are structurally different. This explains the different responses to these agents and the different capacity to induce liver injury. Some authors have described how patients tolerate successful treatment with another molecule after a prior DILI episode induced by an anti-TNF agent. This suggests a lack of cross-toxicity within this class of drugs.

The pathogenetic mechanism underlying anti-TNF hepatotoxicity is still unknown. As liver injury can occur after a singular infusion it seems more an idiosyncratic injury rather than a dose-dependent one[107]. A genetic predisposition may be considered. Another hypothesis is that anti-TNF agents may trigger a pre-existing autoimmune disorder or generate autoantibodies: the binding of IFX to the transmembrane TNF-alpha can lead to apoptosis of monocytes and T-lymphocytes with exposure of nucleosomal autoantigens and the production of autoantibodies[112,114]. Another possibility is that anti-TNF drugs inhibit T-lymphocytes activity, thus suppressing auto-reactive B cells; this may lead to increased humoral autoimmunity[114]. However, there are several cases without evidence of autoimmunity, in which direct liver injury is involved.

DILI caused by anti-TNF agents can show different patterns: Hepatocellular injury in 75% cases, but also a mixed pattern, most rarely with cholestasis, while few cases of acute liver failure have been described. Colina *et al*[115] reported histological necroinflammation caused by IFX, with bridging and massive necrosis in the most severe cases and some features of autoimmune injury with piecemeal necrosis in the periportal interface and prominent plasma cells infiltration. Liver injury caused by anti-TNF drugs is associated with the presence of autoimmunity markers in some patients: anti-nucleus, anti-DsDNA and anti-smooth muscle actin positivity and/or histologic features of AIH are described for IFX, ADA and etanercept. In a study analyzing 34 patients undergoing anti-TNF treatment with DILI, 22 were positive for such antibodies and showed higher levels of ALT than seronegative patients. Fifteen out of 22 subjects underwent liver biopsy that revealed clear features of autoimmunity [116]. Indeed, it is difficult to distinguish between AIH and drug-induced AIH, since these conditions may have similar clinical, biochemical, serological and histological features. Actually, IFX-induced AIH is rare in IBD patients and is described more often in rheumatology patients. In several studies, autoimmunity features were treated with corticosteroids, achieving in some cases a reduction or disappearance of autoantibodies titer; this suggests an immune-mediated DILI rather than an anti-TNF induced AIH. Ierardi *et al*[117] reported a case of acute liver injury after a single IFX administration. Analogously, Adar *et al*[118] described the first case of AIH caused by ADA that resolved after treatment cessation and corticosteroid therapy.

There is still a lack of consensus on the management of DILI induced by anti-TNF agents. The prognosis is usually favorable with normalization of LFT without cessation of anti-TNF therapy. Liver enzymes should be monitored before starting treatment and then monitored periodically, especially during the first 3 mo. If ALT remains < x3 ULN, anti-TNF can be continued until resolution; if ALT is persistently elevated > x3 ULN or in the case of jaundice, corticosteroids and liver biopsy should be considered. If a DILI is documented, anti-TNF withdrawal is still controversial. Also, the necessity to obtain an autoimmune panel before starting anti-TNF treatment is debated: several studies demonstrated that this practice does not predict the risk of developing drug-induced AIH and that anti-TNF therapy could be continued in the presence of asymptomatic anti-nucleus positivity[102].

Anti-Integrins

Natalizumab and vedolizumab were approved some years ago for the treatment of IBD. Both drugs have shown a good safety profile, but in the post-marketing phase, 6 cases of significant DILI associated with natalizumab were reported to the FDA[119].

Liver injury caused by natalizumab is rare with a 5% rate of asymptomatic liver enzymes elevation and it can manifest with both the hepatocellular and cholestatic pattern and can be associated with jaundice. Some cases with autoimmune features (autoantibodies positive) have also been described[120]. The guidelines recommend monitoring LFT before starting the treatment and then every 3 or 6 mo[121]. Nevertheless, the use of natalizumab is quite rare in IBD due to possible severe

neurologic complications such as progressive multifocal leukoencephalopathy[122].

Similar to natalizumab, liver injury associated with vedolizumab is rare, less than 2% in clinical trials, with both the hepatocellular or cholestatic pattern[123]. Similar to natalizumab, the guidelines recommend monitoring liver enzymes every 3-6 mo.

Anti IL12/23

Ustekimumab was approved for CD treatment in 2016 and UC treatment in 2019. Most of the data regarding hepatotoxicity induced by ustekimumab comes from dermatologic studies. In PHOENIX 1 and 2, both studies evaluated the efficacy and safety of ustekimumab in patients with psoriasis, and the rate of liver enzymes abnormalities was low (between 0.5% and 2%) and similar between the case and control group[124,125]. A small retrospective study including 44 patients with psoriasis treated with ustekimumab described cases of mild elevation of liver enzymes and no cases of severe DILI[126]. Some case reports described spontaneous regression of liver injury after ustekinumab withdrawal[127].

Small molecules

Tofacitinib was approved for UC treatment in 2018. Liver enzymes elevation with a hepatocellular pattern has been rarely described[128]. One case of possible AIH was reported, but liver injury due to other drugs could not be excluded[129]. Monitoring liver enzymes periodically during tofacitinib treatment is recommended.

Ozanimod is a new molecule introduced for IBD treatment. Aspartate transaminase increases 32 wk after drug exposure were described in 2% and 1% of patients treated with 0.5 mg and 1 mg of ozanimod, respectively. Preliminary data suggest a low rate of hepatotoxicity associated with these new therapeutic approaches[102].

PORTAL VEIN THROMBOSIS

Portal vein thrombosis (PVT) is a common event in IBD. Indeed, IBD patients have a high risk of thromboembolism due to systemic inflammation and alterations in the concentrations of some coagulation factors, such as high factor V and VIII or low antithrombin III[130].

In a retrospective study, the incidence of thromboembolic events in patients with IBD rose from 5.65% in 2000 to 7.17% by 2009[131]. In particular, the prevalence of PVT in IBD has been estimated to be about 0.17%[132]. There are several causes of PVT, including inflammation, immobilization, major extent of colon disease, disease severity, surgery, use of corticosteroids and smoking. For that reason, the guidelines recommend starting heparin when facing an acute flare of UC, for PVT prophylaxis [133].

After the onset of PVT, complications such as portal hypertension, bleeding or even death are not common, but early anticoagulation is safe and associated with a better outcome, and the use of novel direct oral anticoagulants was associated with particularly favorable outcomes in this setting[134].

CONCLUSIONS

In conclusion, the scenario of liver involvement of IBD patients is quite extensive. The relationship between IBD and PSC is the most studied. PSC is a disease that currently has no effective medical therapy; therefore, research on drugs that may be effective for both hepatic and intestinal disorders is required. Moreover, the strategies for early neoplasia screening (both CCA and CCR) in these patients are not sufficiently efficient at present, and this is a pitfall that needs to be resolved.

NAFLD in IBD is another focal issue, as this novel comorbidity may complicate the management of IBD patients due to its multifaceted aspects.

As viral hepatitis may soon become a thing of the past, due to the advent of drugs with very high success rates, some patients will still require careful monitoring, especially when immunosuppression for IBD is required.

Among the drugs currently in use to treat IBD, thiopurines, mesalazine derivatives and methotrexate are the most studied, and periodic assessment of LFT is still required. However, the field of DILI is expected to expand quickly, as several novel molecules for the treatment of IBD (tyrosine kinase inhibitors, small molecules and others) have been developed, and their possible hepatotoxicity will be a matter of

debate.

REFERENCES

1 **Jairath V**, Feagan BG. Global burden of inflammatory bowel disease. *Lancet Gastroenterol Hepatol* 2020; **5**: 2-3 [PMID: 31648974 DOI: 10.1016/S2468-1253(19)30358-9]

2 **Bibbò S**, Ianiro G, Dore MP, Simonelli C, Newton EE, Cammarota G. Gut Microbiota as a Driver of Inflammation in Nonalcoholic Fatty Liver Disease. *Mediators Inflamm* 2018; **2018**: 9321643 [PMID: 29563854 DOI: 10.1155/2018/9321643]

3 **Fousekis FS**, Theopistos VI, Katsanos KH, Tsianos EV, Christodoulou DK. Hepatobiliary Manifestations and Complications in Inflammatory Bowel Disease: A Review. *Gastroenterology Res* 2018; **11**: 83-94 [PMID: 29707074 DOI: 10.14740/gr990w]

4 **Lunder AK**, Hov JR, Borthne A, Gleditsch J, Johannesen G, Tveit K, Viktil E, Henriksen M, Hovde Ø, Huppertz-Hauss G, Høie O, Høivik ML, Monstad I, Solberg IC, Jahnsen J, Karlsen TH, Moum B, Vatn M, Negård A. Prevalence of Sclerosing Cholangitis Detected by Magnetic Resonance Cholangiography in Patients With Long-term Inflammatory Bowel Disease. *Gastroenterology* 2016; **151**: 660-669.e4 [PMID: 27342213 DOI: 10.1053/j.gastro.2016.06.021]

5 **Lakatos L**, Kiss LS, David G, Pandur T, Erdelyi Z, Mester G, Balogh M, Szipocs I, Molnar C, Komaromi E, Lakatos PL. Incidence, disease phenotype at diagnosis, and early disease course in inflammatory bowel diseases in Western Hungary, 2002-2006. *Inflamm Bowel Dis* 2011; **17**: 2558-2565 [PMID: 22072315 DOI: 10.1002/ibd.21607]

6 **Burisch J**, Jess T, Martinato M, Lakatos PL; ECCO -EpiCom. The burden of inflammatory bowel disease in Europe. *J Crohns Colitis* 2013; **7**: 322-337 [PMID: 23395397 DOI: 10.1016/j.crohns.2013.01.010]

7 **Loftus EV Jr**, Harewood GC, Loftus CG, Tremaine WJ, Harmsen WS, Zinsmeister AR, Jewell DA, Sandborn WJ. PSC-IBD: a unique form of inflammatory bowel disease associated with primary sclerosing cholangitis. *Gut* 2005; **54**: 91-96 [PMID: 15591511 DOI: 10.1136/gut.2004.046615]

8 **de Vries AB**, Janse M, Blokzijl H, Weersma RK. Distinctive inflammatory bowel disease phenotype in primary sclerosing cholangitis. *World J Gastroenterol* 2015; **21**: 1956-1971 [PMID: 25684965 DOI: 10.3748/wjg.v21.i6.1956]

9 **Dave M**, Elmunzer BJ, Dwamena BA, Higgins PD. Primary sclerosing cholangitis: meta-analysis of diagnostic performance of MR cholangiopancreatography. *Radiology* 2010; **256**: 387-396 [PMID: 20656832 DOI: 10.1148/radiol.10091953]

10 **Sørensen JØ**, Nielsen OH, Andersson M, Ainsworth MA, Ytting H, Bélard E, Jess T. Inflammatory bowel disease with primary sclerosing cholangitis: A Danish population-based cohort study 1977-2011. *Liver Int* 2018; **38**: 532-541 [PMID: 28796371 DOI: 10.1111/liv.13548]

11 **Riley TR**, Schoen RE, Lee RG, Rakela J. A case series of transplant recipients who despite immunosuppression developed inflammatory bowel disease. *Am J Gastroenterol* 1997; **92**: 279-282 [PMID: 9040206]

12 **Boonstra K**, van Erpecum KJ, van Nieuwkerk KM, Drenth JP, Poen AC, Witteman BJ, Tuynman HA, Beuers U, Ponsioen CY. Primary sclerosing cholangitis is associated with a distinct phenotype of inflammatory bowel disease. *Inflamm Bowel Dis* 2012; **18**: 2270-2276 [PMID: 22407885 DOI: 10.1002/ibd.22938]

13 **Fousekis FS**, Theopistos VI, Mitselos IV, Skamnelos A, Kavvadias A, Katsanos KH, Christodoulou DK. Specific Features of Patients With Inflammatory Bowel Disease and Primary Sclerosing Cholangitis. *J Clin Med Res* 2019; **11**: 81-88 [PMID: 30700999 DOI: 10.14740/jocmr3680]

14 **Ricciuto A**, Fish J, Carman N, Walters TD, Church PC, Hansen BE, Crowley E, Siddiqui I, Nguyen GC, Kamath BM, Griffiths AM. Symptoms Do Not Correlate With Findings From Colonoscopy in Children With Inflammatory Bowel Disease and Primary Sclerosing Cholangitis. *Clin Gastroenterol Hepatol* 2018; **16**: 1098-1105.e1 [PMID: 29378308 DOI: 10.1016/j.cgh.2018.01.020]

15 **Sano H**, Nakazawa T, Ando T, Hayashi K, Naitoh I, Okumura F, Miyabe K, Yoshida M, Takahashi S, Ohara H, Joh T. Clinical characteristics of inflammatory bowel disease associated with primary sclerosing cholangitis. *J Hepatobiliary Pancreat Sci* 2011; **18**: 154-161 [PMID: 20740366 DOI: 10.1007/s00534-010-0319-8]

16 **Halliday JS**, Djordjevic J, Lust M, Culver EL, Braden B, Travis SP, Chapman RW. A unique clinical phenotype of primary sclerosing cholangitis associated with Crohn's disease. *J Crohns Colitis* 2012; **6**: 174-181 [PMID: 22325171 DOI: 10.1016/j.crohns.2011.07.015]

17 **Lindström L**, Lapidus A, Ost A, Bergquist A. Increased risk of colorectal cancer and dysplasia in patients with Crohn's colitis and primary sclerosing cholangitis. *Dis Colon Rectum* 2011; **54**: 1392-1397 [PMID: 21979184 DOI: 10.1097/DCR.0b013e31822bbcc1]

18 **Rabinovitz M**, Gavaler JS, Schade RR, Dindzans VJ, Chien MC, Van Thiel DH. Does primary sclerosing cholangitis occurring in association with inflammatory bowel disease differ from that occurring in the absence of inflammatory bowel disease? *Hepatology* 1990; **11**: 7-11 [PMID: 2295474 DOI: 10.1002/hep.1840110103]

19 **Yanai H**, Matalon S, Rosenblatt A, Awadie H, Berdichevski T, Snir Y, Kopylov U, Katz L, Stein A, Mlynarsky L, Tulchinsky H, Konikoff FM, Horin SB, Braun M, Ben-Ari Z, Chowers Y, Baruch Y, Shibolet O, Dotan I. Prognosis of primary sclerosing cholangitis in israel is independent of

coexisting inflammatory bowel Disease. *J Crohns Colitis* 2015; **9**: 177-184 [PMID: 25518055 DOI: 10.1093/ecco-jcc/jju013]

20 **Fevery J**, Van Steenbergen W, Van Pelt J, Laleman W, Hoffman I, Geboes K, Vermeire S, Nevens F. Patients with large-duct primary sclerosing cholangitis and Crohn's disease have a better outcome than those with ulcerative colitis, or without IBD. *Aliment Pharmacol Ther* 2016; **43**: 612-620 [PMID: 26748470 DOI: 10.1111/apt.13516]

21 **Nordenvall C**, Olén O, Nilsson PJ, von Seth E, Ekbom A, Bottai M, Myrelid P, Bergquist A. Colectomy prior to diagnosis of primary sclerosing cholangitis is associated with improved prognosis in a nationwide cohort study of 2594 PSC-IBD patients. *Aliment Pharmacol Ther* 2018; **47**: 238-245 [PMID: 29064110 DOI: 10.1111/apt.14393]

22 **Zheng HH**, Jiang XL. Increased risk of colorectal neoplasia in patients with primary sclerosing cholangitis and inflammatory bowel disease: a meta-analysis of 16 observational studies. *Eur J Gastroenterol Hepatol* 2016; **28**: 383-390 [PMID: 26938805 DOI: 10.1097/MEG.0000000000000576]

23 **Gulamhusein AF**, Eaton JE, Tabibian JH, Atkinson EJ, Juran BD, Lazaridis KN. Duration of Inflammatory Bowel Disease Is Associated With Increased Risk of Cholangiocarcinoma in Patients With Primary Sclerosing Cholangitis and IBD. *Am J Gastroenterol* 2016; **111**: 705-711 [PMID: 27002801 DOI: 10.1038/ajg.2016.55]

24 **Lindor KD**, Kowdley KV, Harrison ME; American College of Gastroenterology. ACG Clinical Guideline: Primary Sclerosing Cholangitis. *Am J Gastroenterol* 2015; **110**: 646-59; quiz 660 [PMID: 25869391 DOI: 10.1038/ajg.2015.112]

25 **Harbord M**, Annese V, Vavricka SR, Allez M, Barreiro-de Acosta M, Boberg KM, Burisch J, De Vos M, De Vries AM, Dick AD, Juillerat P, Karlsen TH, Koutroubakis I, Lakatos PL, Orchard T, Papay P, Raine T, Reinshagen M, Thaci D, Tilg H, Carbonnel F; European Crohn's and Colitis Organisation. The First European Evidence-based Consensus on Extra-intestinal Manifestations in Inflammatory Bowel Disease. *J Crohns Colitis* 2016; **10**: 239-254 [PMID: 26614685 DOI: 10.1093/ecco-jcc/jjv213]

26 **Lewis JT**, Talwalkar JA, Rosen CB, Smyrk TC, Abraham SC. Prevalence and risk factors for gallbladder neoplasia in patients with primary sclerosing cholangitis: evidence for a metaplasia-dysplasia-carcinoma sequence. *Am J Surg Pathol* 2007; **31**: 907-913 [PMID: 17527079 DOI: 10.1097/01.pas.0000213435.99492.8a]

27 **Said K**, Glaumann H, Bergquist A. Gallbladder disease in patients with primary sclerosing cholangitis. *J Hepatol* 2008; **48**: 598-605 [PMID: 18222013 DOI: 10.1016/j.jhep.2007.11.019]

28 **Chapman R**, Fevery J, Kalloo A, Nagorney DM, Boberg KM, Shneider B, Gores GJ; American Association for the Study of Liver Diseases. Diagnosis and management of primary sclerosing cholangitis. *Hepatology* 2010; **51**: 660-678 [PMID: 20101749 DOI: 10.1002/hep.23294]

29 **Zenouzi R**, Weismüller TJ, Hübener P, Schulze K, Bubenheim M, Pannicke N, Weiler-Normann C, Lenzen H, Manns MP, Lohse AW, Schramm C. Low risk of hepatocellular carcinoma in patients with primary sclerosing cholangitis with cirrhosis. *Clin Gastroenterol Hepatol* 2014; **12**: 1733-1738 [PMID: 24530461 DOI: 10.1016/j.cgh.2014.02.008]

30 **Singh S**, Khanna S, Pardi DS, Loftus EV Jr, Talwalkar JA. Effect of ursodeoxycholic acid use on the risk of colorectal neoplasia in patients with primary sclerosing cholangitis and inflammatory bowel disease: a systematic review and meta-analysis. *Inflamm Bowel Dis* 2013; **19**: 1631-1638 [PMID: 23665966 DOI: 10.1097/MIB.0b013e318286fa61]

31 **Carey EJ**, Lindor KD. Chemoprevention of colorectal cancer with ursodeoxycholic acid: cons. *Clin Res Hepatol Gastroenterol* 2012; **36** Suppl 1: S61-S64 [PMID: 23141896 DOI: 10.1016/S2210-7401(12)70023-2]

32 **Stolfi C**, Pallone F, Monteleone G. Colorectal cancer chemoprevention by mesalazine and its derivatives. *J Biomed Biotechnol* 2012; **2012**: 980458 [PMID: 22701310 DOI: 10.1155/2012/980458]

33 **Tsaitas C**, Semertzidou A, Sinakos E. Update on inflammatory bowel disease in patients with primary sclerosing cholangitis. *World J Hepatol* 2014; **6**: 178-187 [PMID: 24799986 DOI: 10.4254/wjh.v6.i4.178]

34 **Christensen B**, Micic D, Gibson PR, Yarur A, Bellaguarda E, Corsello P, Gaetano JN, Kinnucan J, Rao VL, Reddy S, Singh S, Pekow J, Rubin DT. Vedolizumab in patients with concurrent primary sclerosing cholangitis and inflammatory bowel disease does not improve liver biochemistry but is safe and effective for the bowel disease. *Aliment Pharmacol Ther* 2018; **47**: 753-762 [DOI: 10.1111/apt.14525]

35 **Shah A**, Macdonald GA, Morrison M, Holtmann G. Targeting the Gut Microbiome as a Treatment for Primary Sclerosing Cholangitis: A Conceptional Framework. *Am J Gastroenterol* 2020; **115**: 814-822 [PMID: 32250997 DOI: 10.14309/ajg.0000000000000604]

36 **Irlès-Depé M**, Roullet S, Neau-Cransac M, Dumortier J, Dharancy S, Houssel-Debry P, Boillot O, Chiche L, Laurent C, Laharie D, De Lédinghen V. Impact of Preexisting Inflammatory Bowel Disease on the Outcome of Liver Transplantation for Primary Sclerosing Cholangitis. *Liver Transpl* 2020; **26**: 1477-1491 [PMID: 32603007 DOI: 10.1002/lt.25838]

37 **Liberal R**, Gaspar R, Lopes S, Macedo G. Primary biliary cholangitis in patients with inflammatory bowel disease. *Clin Res Hepatol Gastroenterol* 2020; **44**: e5-e9 [PMID: 31171469 DOI: 10.1016/j.clinre.2019.05.002]

38 **Aiba Y**, Yamazaki K, Nishida N, Kawashima M, Hitomi Y, Nakamura H, Komori A, Fuyuno Y,

Takahashi A, Kawaguchi T, Takazoe M, Suzuki Y, Motoya S, Matsui T, Esaki M, Matsumoto T, Kubo M, Tokunaga K, Nakamura M. Disease susceptibility genes shared by primary biliary cirrhosis and Crohn's disease in the Japanese population. *J Hum Genet* 2015; **60**: 525-531 [PMID: 26084578 DOI: 10.1038/jhg.2015.59]

39 **Ballotin VR**, Bigarella LG, Riva F, Onzi G, Balbinot RA, Balbinot SS, Soldera J. Primary sclerosing cholangitis and autoimmune hepatitis overlap syndrome associated with inflammatory bowel disease: A case report and systematic review. *World J Clin Cases* 2020; **8**: 4075-4093 [PMID: 33024765 DOI: 10.12998/wjcc.v8.i18.4075]

40 **Miranda-Bautista J**, Menchén L. Adalimumab-induced autoimmune hepatitis in a patient with Crohn's disease. *Gastroenterol Hepatol* 2019; **42**: 306-307 [PMID: 30029925 DOI: 10.1016/j.gastrohep.2018.06.006]

41 **Nassir F**, Rector RS, Hammoud GM, Ibdah JA. Pathogenesis and Prevention of Hepatic Steatosis. *Gastroenterol Hepatol (N Y)* 2015; **11**: 167-175 [PMID: 27099587]

42 **Sunny NE**, Parks EJ, Browning JD, Burgess SC. Excessive hepatic mitochondrial TCA cycle and gluconeogenesis in humans with nonalcoholic fatty liver disease. *Cell Metab* 2011; **14**: 804-810 [PMID: 22152305 DOI: 10.1016/j.cmet.2011.11.004]

43 C. Ulceration of the colon with a much enlarged fatty liver. *Trans Pathol Soc Philadelphia* 1873; **4**: 87-88 [DOI: 10.1056/nejm187304100881506]

44 **Losurdo G**, La Fortezza RF, Iannone A, Contaldo A, Barone M, Ierardi E, Di Leo A, Principi M. Prevalence and associated factors of obesity in inflammatory bowel disease: A case-control study. *World J Gastroenterol* 2020; **26**: 7528-7537 [PMID: 33384552 DOI: 10.3748/wjg.v26.i47.7528]

45 **Miele L**, Valenza V, La Torre G, Montalto M, Cammarota G, Ricci R, Mascianà R, Forgione A, Gabrieli ML, Perotti G, Vecchio FM, Rapaccini G, Gasbarrini G, Day CP, Grieco A. Increased intestinal permeability and tight junction alterations in nonalcoholic fatty liver disease. *Hepatology* 2009; **49**: 1877-1887 [PMID: 19291785 DOI: 10.1002/hep.22848]

46 **Principi M**, Losurdo G, Iannone A, Contaldo A, Deflorio V, Ranaldo N, Pisani A, Ierardi E, Di Leo A, Barone M. Differences in dietary habits between patients with inflammatory bowel disease in clinical remission and a healthy population. *Ann Gastroenterol* 2018; **31**: 469-473 [PMID: 29991892 DOI: 10.20524/aog.2018.0273]

47 **Ruemmele FM**. Role of Diet in Inflammatory Bowel Disease. *Ann Nutr Metab* 2016; **68** Suppl 1: 33-41 [PMID: 27355913 DOI: 10.1159/000445392]

48 **Zou ZY**, Shen B, Fan JG. Systematic Review With Meta-analysis: Epidemiology of Nonalcoholic Fatty Liver Disease in Patients With Inflammatory Bowel Disease. *Inflamm Bowel Dis* 2019; **25**: 1764-1772 [PMID: 30918952 DOI: 10.1093/ibd/izz043]

49 **Bessissow T**, Le NH, Rollet K, Afif W, Bitton A, Sebastiani G. Incidence and Predictors of Nonalcoholic Fatty Liver Disease by Serum Biomarkers in Patients with Inflammatory Bowel Disease. *Inflamm Bowel Dis* 2016; **22**: 1937-1944 [PMID: 27379445 DOI: 10.1097/MIB.0000000000000832]

50 **Sagami S**, Ueno Y, Tanaka S, Fujita A, Hayashi R, Oka S, Hyogo H, Chayama K. Significance of non-alcoholic fatty liver disease in Crohn's disease: A retrospective cohort study. *Hepatol Res* 2017; **47**: 872-881 [PMID: 27737498 DOI: 10.1111/hepr.12828]

51 **Glassner K**, Malaty HM, Abraham BP. Epidemiology and Risk Factors of Nonalcoholic Fatty Liver Disease Among Patients with Inflammatory Bowel Disease. *Inflamm Bowel Dis* 2017; **23**: 998-1003 [PMID: 28511199 DOI: 10.1097/MIB.0000000000001085]

52 **Principi M**, Iannone A, Losurdo G, Mangia M, Shahini E, Albano F, Rizzi SF, La Fortezza RF, Lovero R, Contaldo A, Barone M, Leandro G, Ierardi E, Di Leo A. Nonalcoholic Fatty Liver Disease in Inflammatory Bowel Disease: Prevalence and Risk Factors. *Inflamm Bowel Dis* 2018; **24**: 1589-1596 [PMID: 29688336 DOI: 10.1093/ibd/izy051]

53 **Ritaccio G**, Stoleru G, Abutaleb A, Cross RK, Shetty K, Sakiani S, Wong U. Nonalcoholic Fatty Liver Disease Is Common in IBD Patients However Progression to Hepatic Fibrosis by Noninvasive Markers Is Rare. *Dig Dis Sci* 2020 [DOI: 10.1007/s10620-020-06588-6]

54 **Franco I**, Bianco A, Mirizzi A, Campanella A, Bonfiglio C, Sorino P, Notarnicola M, Tutino V, Cozzolongo R, Giannuzzi V, Aballay LR, Buongiorno C, Bruno I, Osella AR. Physical Activity and Low Glycemic Index Mediterranean Diet: Main and Modification Effects on NAFLD Score. Results from a Randomized Clinical Trial. *Nutrients* 2020; **13** [PMID: 33379253 DOI: 10.3390/nu13010066]

55 **Hou JK**, Velayos F, Terrault N, Mahadevan U. Viral hepatitis and inflammatory bowel disease. *Inflamm Bowel Dis* 2010; **16**: 925-932 [PMID: 20480515 DOI: 10.1002/ibd.21284]

56 **Basnayake SK**, Easterbrook PJ. Wide variation in estimates of global prevalence and burden of chronic hepatitis B and C infection cited in published literature. *J Viral Hepat* 2016; **23**: 545-559 [PMID: 27028545 DOI: 10.1111/jvh.12519]

57 **Sansone S**, Guarino M, Castiglione F, Rispo A, Auriemma F, Loperto I, Rea M, Caporaso N, Morisco F. Hepatitis B and C virus reactivation in immunosuppressed patients with inflammatory bowel disease. *World J Gastroenterol* 2014; **20**: 3516-3524 [PMID: 24707134 DOI: 10.3748/wjg.v20.i13.3516]

58 **Loras C**, Saro C, Gonzalez-Huix F, Mínguez M, Merino O, Gisbert JP, Barrio J, Bernal A, Gutiérrez A, Piqueras M, Calvet X, Andreu M, Abad A, Ginard D, Bujanda L, Panés J, Torres M, Fernández-Bañares F, Viver JM, Esteve M; GETECCu (Grupo Español de Enfermedades de Crohn y Colitis Ulcerosa). Prevalence and factors related to hepatitis B and C in inflammatory bowel disease patients

in Spain: a nationwide, multicenter study. *Am J Gastroenterol* 2009; **104**: 57-63 [PMID: 19098850 DOI: 10.1038/ajg.2008.4]

59 **Chevaux JB**, Bigard MA, Bensenane M, Oussalah A, Jarlot S, Belle A, Nani A, Bronowicki JP, Peyrin-Biroulet L. Inflammatory bowel disease and hepatitis B and C. *Gastroenterol Clin Biol* 2009; **33**: 1082-1093 [PMID: 19896313 DOI: 10.1016/j.gcb.2009.03.021]

60 **Morisco F**, Castiglione F, Rispo A, Stroffolini T, Sansone S, Vitale R, Guarino M, Biancone L, Caruso A, D'Inca R, Marmo R, Orlando A, Riegler G, Donnarumma L, Camera S, Zorzi F, Renna S, Bove V, Tontini G, Vecchi M, Caporaso N. Effect of immunosuppressive therapy on patients with inflammatory bowel diseases and hepatitis B or C virus infection. *J Viral Hepat* 2013; **20**: 200-208 [PMID: 23383659 DOI: 10.1111/j.1365-2893.2012.01643.x]

61 **Papa A**, Felice C, Marzo M, Andrisani G, Armuzzi A, Covino M, Mocci G, Pugliese D, De Vitis I, Gasbarrini A, Rapaccini GL, Guidi L. Prevalence and natural history of hepatitis B and C infections in a large population of IBD patients treated with anti-tumor necrosis factor-α agents. *J Crohns Colitis* 2013; **7**: 113-119 [PMID: 22464811 DOI: 10.1016/j.crohns.2012.03.001]

62 **Losurdo G**, Iannone A, Contaldo A, Barone M, Ierardi E, Di Leo A, Principi M. Chronic Viral Hepatitis in a Cohort of Inflammatory Bowel Disease Patients from Southern Italy: A Case-Control Study. *Pathogens* 2020; **9** [PMID: 33113974 DOI: 10.3390/pathogens9110870]

63 **Borman ZA**, Côté-Daigneault J, Colombel JF. The risk for opportunistic infections in inflammatory bowel disease with biologics: an update. *Expert Rev Gastroenterol Hepatol* 2018; **12**: 1101-1108 [PMID: 30277409 DOI: 10.1080/17474124.2018.1530983]

64 **Rahier JF**, Magro F, Abreu C, Armuzzi A, Ben-Horin S, Chowers Y, Cottone M, de Ridder L, Doherty G, Ehehalt R, Esteve M, Katsanos K, Lees CW, Macmahon E, Moreels T, Reinisch W, Tilg H, Tremblay L, Veereman-Wauters G, Viget N, Yazdanpanah Y, Eliakim R, Colombel JF; European Crohn's and Colitis Organisation (ECCO). Second European evidence-based consensus on the prevention, diagnosis and management of opportunistic infections in inflammatory bowel disease. *J Crohns Colitis* 2014; **8**: 443-468 [PMID: 24613021 DOI: 10.1016/j.crohns.2013.12.013]

65 **Lok AS**, McMahon BJ. Chronic hepatitis B. *Hepatology* 2007; **45**: 507-539 [PMID: 17256718 DOI: 10.1002/hep.21513]

66 **Katsanos KH**, Tsianos VE, Zois CD, Zioga H, Vagias I, Zervou E, Christodoulou DK, Tsianos EV; Northwest Greece IBD Study Group. Inflammatory bowel disease and hepatitis B and C in Western Balkans: a referral centre study and review of the literature. *J Crohns Colitis* 2010; **4**: 450-465 [PMID: 21122543 DOI: 10.1016/j.crohns.2010.03.001]

67 **Magro F**, Gionchetti P, Eliakim R, Ardizzone S, Armuzzi A, Barreiro-de Acosta M, Burisch J, Gecse KB, Hart AL, Hindryckx P, Langner C, Limdi JK, Pellino G, Zagórowicz E, Raine T, Harbord M, Rieder F; European Crohn's and Colitis Organisation [ECCO]. Third European Evidence-based Consensus on Diagnosis and Management of Ulcerative Colitis. Part 1: Definitions, Diagnosis, Extra-intestinal Manifestations, Pregnancy, Cancer Surveillance, Surgery, and Ileo-anal Pouch Disorders. *J Crohns Colitis* 2017; **11**: 649-670 [PMID: 28158501 DOI: 10.1093/ecco-jcc/jjx008]

68 **Brunasso AM**, Puntoni M, Gulia A, Massone C. Safety of anti-tumour necrosis factor agents in patients with chronic hepatitis C infection: a systematic review. *Rheumatology (Oxford)* 2011; **50**: 1700-1711 [PMID: 21690185 DOI: 10.1093/rheumatology/ker190]

69 **Scherzer TM**, Staufer K, Novacek G, Steindl-Munda P, Schumacher S, Hofer H, Ferenci P, Vogelsang H. Efficacy and safety of antiviral therapy in patients with Crohn's disease and chronic hepatitis C. *Aliment Pharmacol Ther* 2008; **28**: 742-748 [PMID: 19145730 DOI: 10.1111/j.1365-2036.2008.03779.x]

70 **Tilg H**, Vogelsang H, Ludwiczek O, Lochs H, Kaser A, Colombel JF, Ulmer H, Rutgeerts P, Krüger S, Cortot A, D'Haens G, Harrer M, Gasche C, Wrba F, Kuhn I, Reinisch W. A randomised placebo controlled trial of pegylated interferon alpha in active ulcerative colitis. *Gut* 2003; **52**: 1728-1733 [PMID: 14633951 DOI: 10.1136/gut.52.12.1728]

71 **Koff RS**. Review article: the efficacy and safety of sofosbuvir, a novel, oral nucleotide NS5B polymerase inhibitor, in the treatment of chronic hepatitis C virus infection. *Aliment Pharmacol Ther* 2014; **39**: 478-487 [PMID: 24387618 DOI: 10.1111/apt.12601]

72 **Imperatore N**, Castiglione F, Rispo A, Sessa A, Caporaso N, Morisco F. Timing Strategies of Direct-Acting Antivirals and Biologics Administration in HCV-Infected Subjects with Inflammatory Bowel Diseases. *Front Pharmacol* 2017; **8**: 867 [PMID: 29209223 DOI: 10.3389/fphar.2017.00867]

73 **Losurdo G**, Iannone A, Contaldo A, Barone M, Ierardi E, Di Leo A, Principi M. Trends of Liver Stiffness in Inflammatory Bowel Disease with Chronic Hepatitis C. *Diagnostics (Basel)* 2020; **10** [PMID: 33276638 DOI: 10.3390/diagnostics10121037]

74 **Andrade RJ**, Chalasani N, Björnsson ES, Suzuki A, Kullak-Ublick GA, Watkins PB, Devarbhavi H, Merz M, Lucena MI, Kaplowitz N, Aithal GP. Drug-induced liver injury. *Nat Rev Dis Primers* 2019; **5**: 58 [PMID: 31439850 DOI: 10.1038/s41572-019-0105-0]

75 **Yaccob A**, Mari A. Practical clinical approach to the evaluation of hepatobiliary disorders in inflammatory bowel disease. *Frontline Gastroenterol* 2019; **10**: 309-315 [PMID: 31281626 DOI: 10.1136/flgastro-2018-101037]

76 **Wong DR**, Coenen MJ, Derijks LJ, Vermeulen SH, van Marrewijk CJ, Klungel OH, Scheffer H, Franke B, Guchelaar HJ, de Jong DJ, Engels LG, Verbeek AL, Hooymans PM; TOPIC Recruitment Team. Early prediction of thiopurine-induced hepatotoxicity in inflammatory bowel disease. *Aliment*

Pharmacol Ther 2017; **45**: 391-402 [PMID: 27943397 DOI: 10.1111/apt.13879]
77 **Marinaki AM**, Arenas-Hernandez M. Reducing risk in thiopurine therapy. *Xenobiotica* 2020; **50**: 101-109 [PMID: 31682552 DOI: 10.1080/00498254.2019.1688424]
78 **Dong X**, Zheng Q, Zhu M, Tong JL, Ran ZH. Thiopurine Smethyltransferase polymorphisms and thiopurine toxicity in treatment of inflammatory bowel disease. *World J Gastroenterol* 2010; **16**: 3187-3195 [DOI: 10.3748/wjg.v16.i25.3187]
79 **Gisbert JP**, González-Lama Y, Maté J. Thiopurine-induced liver injury in patients with inflammatory bowel disease: a systematic review. *Am J Gastroenterol* 2007; **102**: 1518-1527 [PMID: 17391318 DOI: 10.1111/j.1572-0241.2007.01187.x]
80 **Chaparro M**, Ordás I, Cabré E, Garcia-Sanchez V, Bastida G, Peñalva M, Gomollón F, García-Planella E, Merino O, Gutiérrez A, Esteve M, Márquez L, Garcia-Sepulcre M, Hinojosa J, Vera I, Muñoz F, Mendoza JL, Cabriada JL, Montoro MA, Barreiro-de Acosta M, Ceña G, Saro C, Aldeguer X, Barrio J, Maté J, Gisbert JP. Safety of thiopurine therapy in inflammatory bowel disease: long-term follow-up study of 3931 patients. *Inflamm Bowel Dis* 2013; **19**: 1404-1410 [PMID: 23665964 DOI: 10.1097/MIB.0b013e318281f28f]
81 **Schröder T**, Schmidt KJ, Olsen V, Möller S, Mackenroth T, Sina C, Lehnert H, Fellermann K, Büning J. Liver steatosis is a risk factor for hepatotoxicity in patients with inflammatory bowel disease under immunosuppressive treatment. *Eur J Gastroenterol Hepatol* 2015; **27**: 698-704 [PMID: 25923946 DOI: 10.1097/MEG.0000000000000350]
82 **Romagnuolo J**, Sadowski DC, Lalor E, Jewell L, Thomson AB. Cholestatic hepatocellular injury with azathioprine: a case report and review of the mechanisms of hepatotoxicity. *Can J Gastroenterol* 1998; **12**: 479-483 [PMID: 9812167 DOI: 10.1155/1998/294752]
83 **Seinen ML**, van Asseldonk DP, de Boer NK, Bouma G, van Nieuwkerk CM, Mulder CJ, Bloemena E, van Bodegraven AA. Nodular Regenerative Hyperplasia of the Liver in Patients with IBD Treated with Allopurinol-Thiopurine Combination Therapy. *Inflamm Bowel Dis* 2017; **23**: 448-452 [PMID: 28151736 DOI: 10.1097/MIB.0000000000001036]
84 **Musumba CO**. Review article: the association between nodular regenerative hyperplasia, inflammatory bowel disease and thiopurine therapy. *Aliment Pharmacol Ther* 2013; **38**: 1025-1037 [PMID: 24099468 DOI: 10.1111/apt.12490]
85 **Seksik P**, Mary JY, Beaugerie L, Lémann M, Colombel JF, Vernier-Massouille G, Cosnes J. Incidence of nodular regenerative hyperplasia in inflammatory bowel disease patients treated with azathioprine. *Inflamm Bowel Dis* 2011; **17**: 565-572 [PMID: 20848502 DOI: 10.1002/ibd.21330]
86 **Gisbert JP**, Luna M, González-Lama Y, Pousa ID, Velasco M, Moreno-Otero R, Maté J. Liver injury in inflammatory bowel disease: long-term follow-up study of 786 patients. *Inflamm Bowel Dis* 2007; **13**: 1106-1114 [PMID: 17455203 DOI: 10.1002/ibd.20160]
87 **Shaye OA**, Yadegari M, Abreu MT, Poordad F, Simon K, Martin P, Papadakis KA, Ippoliti A, Vasiliauskas E, Tran TT. Hepatotoxicity of 6-mercaptopurine (6-MP) and Azathioprine (AZA) in adult IBD patients. *Am J Gastroenterol* 2007; **102**: 2488-2494 [PMID: 17764490 DOI: 10.1111/j.1572-0241.2007.01515.x]
88 **Hindorf U**, Johansson M, Eriksson A, Kvifors E, Almer SH. Mercaptopurine treatment should be considered in azathioprine intolerant patients with inflammatory bowel disease. *Aliment Pharmacol Ther* 2009; **29**: 654-661 [PMID: 19183142 DOI: 10.1111/j.1365-2036.2008.03925.x]
89 **Meijer B**, Seinen ML, van Egmond R, Bouma G, Mulder CJJ, van Bodegraven AA, de Boer NKH. Optimizing Thiopurine Therapy in Inflammatory Bowel Disease Among 2 Real-life Intercept Cohorts: Effect of Allopurinol Comedication? *Inflamm Bowel Dis* 2017; **23**: 2011-2017 [PMID: 28617756 DOI: 10.1097/MIB.0000000000001168]
90 **Kreijne JE**, de Veer RC, de Boer NK, Dijkstra G, West R, Moorsel SAW, de Jong DJ, van der Woude CJ, de Vries AC; of the Dutch Initiative on Crohn, Colitis (ICC). Real-life study of safety of thiopurine-allopurinol combination therapy in inflammatory bowel disease: myelotoxicity and hepatotoxicity rarely affect maintenance treatment. *Aliment Pharmacol Ther* 2019; **50**: 407-415 [PMID: 31359480 DOI: 10.1111/apt.15402]
91 **Vasudevan A**, Beswick L, Friedman AB, Moltzen A, Haridy J, Raghunath A, Sparrow M, van Langenberg D. Low-dose thiopurine with allopurinol co-therapy overcomes thiopurine intolerance and allows thiopurine continuation in inflammatory bowel disease. *Dig Liver Dis* 2018; **50**: 682-688 [PMID: 29525182 DOI: 10.1016/j.dld.2018.02.001]
92 **Vande Casteele N**, Herfarth H, Katz J, Falck-Ytter Y, Singh S. American Gastroenterological Association Institute Technical Review on the Role of Therapeutic Drug Monitoring in the Management of Inflammatory Bowel Diseases. *Gastroenterology* 2017; **153**: 835-857.e6 [PMID: 28774547 DOI: 10.1053/j.gastro.2017.07.031]
93 **Mottet C**, Schoepfer AM, Juillerat P, Cosnes J, Froehlich F, Kessler-Brondolo V, Seibold F, Rogler G, Vavricka SR, Michetti P. Experts Opinion on the Practical Use of Azathioprine and 6-Mercaptopurine in Inflammatory Bowel Disease. *Inflamm Bowel Dis* 2016; **22**: 2733-2747 [PMID: 27760078 DOI: 10.1097/MIB.0000000000000923]
94 **Khokhar OS**, Lewis JH. Hepatotoxicity of agents used in the management of inflammatory bowel disease. *Dig Dis* 2010; **28**: 508-518 [PMID: 20926880 DOI: 10.1159/000320410]
95 **Ransford RA**, Langman MJ. Sulphasalazine and mesalazine: serious adverse reactions re-evaluated on the basis of suspected adverse reaction reports to the Committee on Safety of Medicines. *Gut* 2002; **51**: 536-539 [PMID: 12235076 DOI: 10.1136/gut.51.4.536]
96 **Sehgal P**, Colombel JF, Aboubakr A, Narula N. Systematic review: safety of mesalazine in

ulcerative colitis. *Aliment Pharmacol Ther* 2018; **47**: 1597-1609 [PMID: 29722441 DOI: 10.1111/apt.14688]

97 **Food and Drug Administration**. Highlights of prescribing information: Delzicol. 2015. [cited 20 February 2021]. Available from: https://www.accessdata.fda.gov/drugsatfda_docs/label/2015204412s006lbl.pdf

98 **Fisher MC**, Cronstein BN. Metaanalysis of methylenetetrahydrofolate reductase (MTHFR) polymorphisms affecting methotrexate toxicity. *J Rheumatol* 2009; **36**: 539-545 [PMID: 19208607 DOI: 10.3899/jrheum.080576]

99 **Cáliz R**, del Amo J, Balsa A, Blanco F, Silva L, Sanmarti R, Martínez FG, Collado MD, Ramirez Mdel C, Tejedor D, Artieda M, Pascual-Salcedo D, Oreiro N, Andreu JL, Graell E, Simon L, Martínez A, Mulero J. The C677T polymorphism in the MTHFR gene is associated with the toxicity of methotrexate in a Spanish rheumatoid arthritis population. *Scand J Rheumatol* 2012; **41**: 10-14 [PMID: 22044028 DOI: 10.3109/03009742.2011.617312]

100 **Berends MA**, van Oijen MG, Snoek J, van de Kerkhof PC, Drenth JP, Han van Krieken J, de Jong EM. Reliability of the Roenigk classification of liver damage after methotrexate treatment for psoriasis: a clinicopathologic study of 160 liver biopsy specimens. *Arch Dermatol* 2007; **143**: 1515-1519 [PMID: 18087000 DOI: 10.1001/archderm.143.12.1515]

101 **Khan N**, Abbas AM, Whang N, Balart LA, Bazzano LA, Kelly TN. Incidence of liver toxicity in inflammatory bowel disease patients treated with methotrexate: a meta-analysis of clinical trials. *Inflamm Bowel Dis* 2012; **18**: 359-367 [PMID: 21751301 DOI: 10.1002/ibd.21820]

102 **Tran-Minh ML**, Sousa P, Maillet M, Allez M, Gornet JM. Hepatic complications induced by immunosuppressants and biologics in inflammatory bowel disease. *World J Hepatol* 2017; **9**: 613-626 [PMID: 28539989 DOI: 10.4254/wjh.v9.i13.613]

103 **Saibeni S**, Bollani S, Losco A, Michielan A, Sostegni R, Devani M, Lupinacci G, Pirola L, Cucino C, Meucci G, Basilisco G, D'Incà R, Bruno S. The use of methotrexate for treatment of inflammatory bowel disease in clinical practice. *Dig Liver Dis* 2012; **44**: 123-127 [PMID: 22051323 DOI: 10.1016/j.dld.2011.09.015]

104 **Labadie JG**, Jain M. Noninvasive Tests to Monitor Methotrexate-Induced Liver Injury. *Clin Liver Dis (Hoboken)* 2019; **13**: 67-71 [PMID: 30988939 DOI: 10.1002/cld.765]

105 **Laharie D**, Seneschal J, Schaeverbeke T, Doutre MS, Longy-Boursier M, Pellegrin JL, Chabrun E, Villars S, Zerbib F, de Lédinghen V. Assessment of liver fibrosis with transient elastography and FibroTest in patients treated with methotrexate for chronic inflammatory diseases: a case-control study. *J Hepatol* 2010; **53**: 1035-1040 [PMID: 20801541 DOI: 10.1016/j.jhep.2010.04.043]

106 **Herfarth HH**, Kappelman MD, Long MD, Isaacs KL. Use of Methotrexate in the Treatment of Inflammatory Bowel Diseases. *Inflamm Bowel Dis* 2016; **22**: 224-233 [PMID: 26457382 DOI: 10.1097/MIB.0000000000000589]

107 **Food and Drug Administration**. Approved Drug Products. [cited 20 February 2021]. Available from: http://www.accessdata.fda.gov/scripts/cder/drugsatfda/index.cfm?fuseaction=Search.Label_ApprovalHistory#apphist

108 **Björnsson ES**, Gunnarsson BI, Gröndal G, Jonasson JG, Einarsdottir R, Ludviksson BR, Gudbjörnsson B, Olafsson S. Risk of drug-induced liver injury from tumor necrosis factor antagonists. *Clin Gastroenterol Hepatol* 2015; **13**: 602-608 [PMID: 25131534 DOI: 10.1016/j.cgh.2014.07.062]

109 **Shelton E**, Chaudrey K, Sauk J, Khalili H, Masia R, Nguyen DD, Yajnik V, Ananthakrishnan AN. New onset idiosyncratic liver enzyme elevations with biological therapy in inflammatory bowel disease. *Aliment Pharmacol Ther* 2015; **41**: 972-979 [PMID: 25756190 DOI: 10.1111/apt.13159]

110 **Koller T**, Galambosova M, Filakovska S, Kubincova M, Hlavaty T, Toth J, Krajcovicova A, Payer J. Drug-induced liver injury in inflammatory bowel disease: 1-year prospective observational study. *World J Gastroenterol* 2017; **23**: 4102-4111 [PMID: 28652663 DOI: 10.3748/wjg.v23.i22.4102]

111 **Worland T**, Chin KL, van Langenberg D, Garg M, Nicoll A. Retrospective study of idiosyncratic drug-induced liver injury from infliximab in an inflammatory bowel disease cohort: the IDLE study. *Ann Gastroenterol* 2020; **33**: 162-169 [PMID: 32127737 DOI: 10.20524/aog.2020.0453]

112 **Van den Brande JM**, Braat H, van den Brink GR, Versteeg HH, Bauer CA, Hoedemaeker I, van Montfrans C, Hommes DW, Peppelenbosch MP, van Deventer SJ. Infliximab but not etanercept induces apoptosis in lamina propria T-lymphocytes from patients with Crohn's disease. *Gastroenterology* 2003; **124**: 1774-1785 [PMID: 12806611 DOI: 10.1016/s0016-5085(03)00382-2]

113 **Cantaert T**, De Rycke L, Mavragani CP, Wijbrandts CA, Niewold TB, Niers T, Vandooren B, Veys EM, Richel D, Tak PP, Crow MK, Baeten D. Exposure to nuclear antigens contributes to the induction of humoral autoimmunity during tumour necrosis factor alpha blockade. *Ann Rheum Dis* 2009; **68**: 1022-1029 [PMID: 18625621 DOI: 10.1136/ard.2008.093724]

114 **Gershov D**, Kim S, Brot N, Elkon KB. C-Reactive protein binds to apoptotic cells, protects the cells from assembly of the terminal complement components, and sustains an antiinflammatory innate immune response: implications for systemic autoimmunity. *J Exp Med* 2000; **192**: 1353-1364 [PMID: 11067883 DOI: 10.1084/jem.192.9.1353]

115 **Colina F**, Molero A, Casís B, Martínez-Montiel P. Infliximab-related hepatitis: a case study and literature review. *Dig Dis Sci* 2013; **58**: 3362-3367 [PMID: 23645381 DOI: 10.1007/s10620-013-2698-6]

116 **Ghabril M**, Bonkovsky HL, Kum C, Davern T, Hayashi PH, Kleiner DE, Serrano J, Rochon J, Fontana RJ, Bonacini M; US Drug-Induced Liver Injury Network. Liver injury from tumor necrosis

factor-α antagonists: analysis of thirty-four cases. *Clin Gastroenterol Hepatol* 2013; **11**: 558-564.e3 [PMID: 23333219 DOI: 10.1016/j.cgh.2012.12.025]

117 **Ierardi E**, Della Valle N, Nacchiero MC, De Francesco V, Stoppino G, Panella C. Infliximab single administration followed by acute liver injury. *Inflamm Bowel Dis* 2006; **12**: 1089-1091 [PMID: 17075352 DOI: 10.1097/01.mib.0000246783.75753.9f]

118 **Adar T**, Mizrahi M, Pappo O, Scheiman-Elazary A, Shibolet O. Adalimumab-induced autoimmune hepatitis. *J Clin Gastroenterol* 2010; **44**: e20-e22 [PMID: 19593165 DOI: 10.1097/MCG.0b013e3181a745e7]

119 **Bezabeh S**, Flowers CM, Kortepeter C, Avigan M. Clinically significant liver injury in patients treated with natalizumab. *Aliment Pharmacol Ther* 2010; **31**: 1028-1035 [PMID: 20163378 DOI: 10.1111/j.1365-2036.2010.04262.x]

120 **Lisotti A**, Azzaroli F, Brillanti S, Mazzella G. Severe acute autoimmune hepatitis after natalizumab treatment. *Dig Liver Dis* 2012; **44**: 356-357 [PMID: 22154948 DOI: 10.1016/j.dld.2011.11.003]

121 **Lamb CA**, Kennedy NA, Raine T, Hendy PA, Smith PJ, Limdi JK, Hayee B, Lomer MCE, Parkes GC, Selinger C, Barrett KJ, Davies RJ, Bennett C, Gittens S, Dunlop MG, Faiz O, Fraser A, Garrick V, Johnston PD, Parkes M, Sanderson J, Terry H; IBD guidelines eDelphi consensus group, Gaya DR, Iqbal TH, Taylor SA, Smith M, Brookes M, Hansen R, Hawthorne AB. British Society of Gastroenterology consensus guidelines on the management of inflammatory bowel disease in adults. *Gut* 2019; **68**: s1-s106 [PMID: 31562236 DOI: 10.1136/gutjnl-2019-318484]

122 **Bellizzi A**, Barucca V, Fioriti D, Colosimo MT, Mischitelli M, Anzivino E, Chiarini F, Pietropaolo V. Early years of biological agents therapy in Crohn's disease and risk of the human polyomavirus JC reactivation. *J Cell Physiol* 2010; **224**: 316-326 [PMID: 20432445 DOI: 10.1002/jcp.22146]

123 **Food and Drug Administration**. Highlights of prescribing information: Entyvio (Vedolizumab). 2014. [cited 20 February 2021]. Available from: https://www.accessdata.fda.gov/drugsatfda_docs/label/2014/125476s000lbl.pdf

124 **Leonardi CL**, Kimball AB, Papp KA, Yeilding N, Guzzo C, Wang Y, Li S, Dooley LT, Gordon KB; PHOENIX 1 study investigators. Efficacy and safety of ustekinumab, a human interleukin-12/23 monoclonal antibody, in patients with psoriasis: 76-wk results from a randomised, double-blind, placebo-controlled trial (PHOENIX 1). *Lancet* 2008; **371**: 1665-1674 [PMID: 18486739 DOI: 10.1016/S0140-6736(08)60725-4]

125 **Papp KA**, Langley RG, Lebwohl M, Krueger GG, Szapary P, Yeilding N, Guzzo C, Hsu MC, Wang Y, Li S, Dooley LT, Reich K; PHOENIX 2 study investigators. Efficacy and safety of ustekinumab, a human interleukin-12/23 monoclonal antibody, in patients with psoriasis: 52-wk results from a randomised, double-blind, placebo-controlled trial (PHOENIX 2). *Lancet* 2008; **371**: 1675-1684 [PMID: 18486740 DOI: 10.1016/S0140-6736(08)60726-6]

126 **Llamas-Velasco M**, Concha-Garzón MJ, García-Diez A, Daudén E. Liver Injury in Psoriasis Patients Receiving Ustekinumab: A Retrospective Study of 44 Patients Treated in the Clinical Practice Setting. *Actas Dermosifiliogr* 2015; **106**: 470-476 [PMID: 25912374 DOI: 10.1016/j.ad.2015.02.002]

127 **Lovero R**, Losurdo G, Mastromauro M, Castellaneta NM, Mongelli A, Gentile A, Di Leo A, Principi M. A Case of Severe Transaminase Elevation Following a Single Ustekinumab Dose with Remission After Drug Withdrawal. *Curr Drug Saf* 2018; **13**: 221-223 [PMID: 30027852 DOI: 10.2174/1574886313666180719165212]

128 **Valenzuela F**, Korman NJ, Bissonnette R, Bakos N, Tsai TF, Harper MK, Ports WC, Tan H, Tallman A, Valdez H, Gardner AC. Tofacitinib in patients with moderate-to-severe chronic plaque psoriasis: long-term safety and efficacy in an open-label extension study. *Br J Dermatol* 2018; **179**: 853-862 [PMID: 29782642 DOI: 10.1111/bjd.16798]

129 **Wollenhaupt J**, Silverfield J, Lee EB, Curtis JR, Wood SP, Soma K, Nduaka CI, Benda B, Gruben D, Nakamura H, Komuro Y, Zwillich SH, Wang L, Riese RJ. Safety and efficacy of tofacitinib, an oral janus kinase inhibitor, for the treatment of rheumatoid arthritis in open-label, longterm extension studies. *J Rheumatol* 2014; **41**: 837-852 [PMID: 24692527 DOI: 10.3899/jrheum.130683]

130 **Rojas-Feria M**, Castro M, Suárez E, Ampuero J, Romero-Gómez M. Hepatobiliary manifestations in inflammatory bowel disease: the gut, the drugs and the liver. *World J Gastroenterol* 2013; **19**: 7327-7340 [PMID: 24259964 DOI: 10.3748/wjg.v19.i42.7327]

131 **Kuy S**, Dua A, Chappidi R, Seabrook G, Brown KR, Lewis B, Rossi PJ, Lee CJ. The increasing incidence of thromboembolic events among hospitalized patients with inflammatory bowel disease. *Vascular* 2015; **23**: 260-264 [PMID: 24986868 DOI: 10.1177/1708538114541799]

132 **Maconi G**, Bolzacchini E, Dell'Era A, Russo U, Ardizzone S, de Franchis R. Portal vein thrombosis in inflammatory bowel diseases: a single-center case series. *J Crohns Colitis* 2012; **6**: 362-367 [PMID: 22405175 DOI: 10.1016/j.crohns.2011.10.003]

133 **Zezos P**, Kouklakis G, Saibil F. Inflammatory bowel disease and thromboembolism. *World J Gastroenterol* 2014; **20**: 13863-13878 [PMID: 25320522 DOI: 10.3748/wjg.v20.i38.13863]

134 **Naymagon L**, Tremblay D, Zubizarreta N, Moshier E, Naymagon S, Mascarenhas J, Schiano T. The Natural History, Treatments, and Outcomes of Portal Vein Thrombosis in Patients With Inflammatory Bowel Disease. *Inflamm Bowel Dis* 2021; **27**: 215-223 [PMID: 32185400 DOI: 10.1093/ibd/izaa053]

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REVIEW

Chelation therapy in liver diseases of childhood: Current status and response

Jayendra Seetharaman, Moinak Sen Sarma

ORCID number: Jayendra Seetharaman 0000-0001-7991-6975; Moinak Sen Sarma 0000-0003-2015-4069.

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Jayendra Seetharaman, Moinak Sen Sarma, Department of Pediatric Gastroenterology, Sanjay Gandhi Post-graduate Institute of Medical Sciences, Lucknow 226014, Uttar Pradesh, India

Corresponding author: Moinak Sen Sarma, MD, DM Associate Professor, Department of Pediatric Gastroenterology, Sanjay Gandhi Postgraduate Institute of Medical Sciences, Raebareli Road, Lucknow 226014, Uttar Pradesh, India. moinaksen@gmail.com

Abstract

Chelation is the mainstay of therapy in certain pediatric liver diseases. Copper and iron related disorders require chelation. Wilson's disease (WD), one of the common causes of cirrhosis in children is treated primarily with copper chelating agents like D-penicillamine and trientine. D-Penicillamine though widely used due its high efficacy in hepatic WD is fraught with frequent adverse effects resulting discontinuation. Trientine, an alternative drug has comparable efficacy in hepatic WD but has lower frequency of adverse effects. The role of ammonium tetra-thiomolybdate is presently experimental in hepatic WD. Indian childhood cirrhosis is related to excessive copper ingestion, rarely seen in present era. D-Penicillamine is effective in the early part of this disease with reversal of clinical status. Iron chelators are commonly used in secondary hemochromatosis of liver in hemolytic anemias. There are strict chelation protocols during bone marrow transplant. The role of iron chelation in neonatal hemochromatosis is presently not in vogue due to its poor efficacy and availability of other modalities of therapy. Hereditary hemochromatosis is rare in children and the use of iron chelators in this condition is limited.

Key Words: Wilson's disease; D-Penicillamine; Trientine; Indian childhood cirrhosis; Deferoxamine; Deferasirox; Hemochromatosis

Core Tip: Chelation forms the most important part of management of certain liver diseases in children. In Wilson's disease and secondary hemochromatosis related to transfusion, chelation is well established treatment modality with proven efficacy. In other diseases like copper associated childhood cirrhosis and neonatal hemochromatosis the role of chelation is doubtful. In hereditary hemochromatosis, chelation is

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recommended as alternative therapy. The selection of chelating agents for treatment depends on the efficacy, feasibility and risk of adverse effects known from literature. The review discusses the concepts of chelation and reviews the literature to assess the role of chelation in treatment of various pediatric liver diseases.

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INTRODUCTION

Chelation is a process in which a synthetic compound is administered to remove an excess mineral or heavy metal from the body. There are various liver diseases that are caused by excess deposition of various heavy metals such as copper, iron and arsenic. Some of these are genetic-metabolic, others are due to environmental exposure. In the landmarks of chelation therapy in hepatology, Walshe documented cupriuresis after administering dimethyl cysteine (penicillamine) in Wilson's disease (WD) in 1956[1]. Chelation was thereafter used in non-Wilsonian liver diseases. In the subsequent years newer chelators such as trientine and ammonium tetra thiomolybdate were identified for WD. From the 1970s, transfusion-related liver siderosis of hemolytic anemias was revolutionized by the use of deferoxamine[2]. The use of iron chelators was attempted in gestational alloimmune liver disease and hereditary hemochromatosis. This review explores the rationale and outcome of chelation therapy in various pediatric liver diseases.

MECHANISM OF CHELATION

Metal ion (M) complexes with cheating agent (L) through an equilibrium reaction to form metal-ligand complex (ML) or chelate. The concentration of the chelate in the solution is directly proportional to the concentration of metal ion [M] and the ligand [L].

$$M + L \leftrightharpoons ML$$

$$[M][L] \propto [ML]$$

$$[M][L]k = [ML]$$

Where k is the effective stability constant. Value k denotes the affinity of the chelating agent. High k values suggest high affinity of the chelating agent. The value of k depends on the nature of the chelating agent, temperature, pH of the solution[3]. The *in-vivo* milieu is not similar to the *in-vitro* chemical reaction. The presence of weak acids in the body fluids like glutamate, sulfate, citrate, amino acids, albumin, macroglobulin *etc*. affect the chelation. These are called biological ligands. Chelating agent binds to the biological ligands and the effective concentration in the body fluid is lowered. Hence the equation becomes.

$$[Mt][Lt]k = [ML]$$

Where Mt, Lt is the total concentration of the metal ion and chelating agent respectively which is very difficult to assess in the clinical setting[4].

Effective chelation occurs when concentration of M and/or L is high, when affinity of the chelator (k) is high or when the concentration of the chelate [ML] is low. The metal ion concentration [M] in the body depends on the severity of the disease. For example, in a WD presenting as acute liver failure, serum copper (Cu) levels are usually very high. The concentration of chelating agent [L] is increased by increasing the dosing and/or frequency as tolerated by the patient. For the chelation to progress, urinary excretion of chelate [ML] is very important as it effectively reduces the concentration[3]. Ideal chelating agents must have good oral absorption, acceptable bioavailability, high affinity to metal ions, low toxicity at appropriate plasma concentration, undergo rapid elimination or detoxification after combining with metal ions and more

importantly should be available in affordable price[5].

CHELATION IN WD

WD is an autosomal recessive disorder caused by mutation of ATP7B gene that encodes for a protein P-type ATPase which transports copper into trans Golgi network and for biliary excretion of copper. In lysosomes, copper is incorporated into ceruloplasmin. In WD, due to defect in ATPase transport protein, ceruloplasmin formation is defective and biliary excretion of copper is impaired[6,7]. This causes excess accumulation of intracellular copper subsequently increasing the levels in blood causing accumulation in extra-hepatic organs (Figure 1).

Chelating drugs

D-Penicillamine (3, 3-dimethylcysteine) is the most commonly used medication for WD worldwide. The L-isomer of this drug is not advised for treatment due to its neurotoxicity. The chelation property of DPA is due to the presence of thiol (-SH), which is responsible for its high affinity towards divalent metal ions such as copper. The mechanism of action of D-Penicillamine (DPA) is by inducing cuprieuresis, inducing hepatic metallothionine synthesis, reducing fibrosis (by preventing collagen formation). DPA also has an anti-inflammatory property[8]. It is rapidly absorbed in proximal intestine but only 40%-70% are absorbed[9]. The peak plasma concentration occurs after 1-3 h after ingestion. It circulates in the plasma predominantly by binding to albumin (80%), while the rest of the compound is present as free or disulphide forms. DPA is metabolized in the liver by conjugation with sulfide or by methylation (phase II reaction) and excreted in urine with almost 80% being eliminated within 10 h of ingestion. After discontinuation of therapy, the drug is eliminated in about 3-6 d [10]. Food, antacids, iron and zinc preparations reduce the bioavailability by almost 50%. Plasma concentration reduces significantly when the drug is taken with food[11]. It is recommended to give the drug either 1- hour before or 2- h after food. The drug is given in the dose of 20 mg/kg per day (up to 1500 mg) rounded to nearest 250 mg in 2-4 divided doses and can be maintained at 1000 mg/d once the disease is in remission [12]. As DPA causes pyridoxine deficiency, pyridoxine should be supplemented at 25-50 mg/d. In case of neurological WD, to prevent paradoxical neurological worsening, the drug is started at low dose (125-250 mg) and slowly increased (125-250 mg every week) to reach the desired dose by 4-6 wk[13].

Trientine (triethylenetetramine) is an alternative chelating agent in WD. It is a derivative of spermine and putrescine and binds to copper in the ratio 1:1 to form a stable complex, which is eliminated in the urine. Trientine dihydrochloride is the oral ingestible form requiring storage at 2-8 degree Celsius to maintain stability. 10% of the trientine is absorbed in the proximal small intestine and achieves its peak concentration 1.5-4 h after ingestion. Trientine is extensively metabolized in tissues by acetylation but the enzyme responsible for it is not identified. 1% of ingested trientine and 8% trientine metabolite acetyltrien, appears in the urine. Plasma concentration of the trientine significantly reduces when given with food due to its affinity to dietary copper in the lumen thereby compromising the removal of tissue copper and the other reason could be due to the physiological polyamines secreted during food intake inhibits effective trientine absorption[14]. Trientine is not to be given with iron as it forms toxic complexes. The dose recommended is 20 mg/kg per day with the maximum of 1500 mg/d rounded to nearest 250 mg (300 mg capsules in North America) and maintenance dose of 1000 mg/d. Similar to DPA, trientine also should be ingested 1 h before or 2 h after food intake[12,15]. The decoppering efficacy of any chelating agent is evident from the effective stability constant (k) which denotes copper affinity. The comparison of k-value of DPA (2.38×10^{-16}) and trientine (1.74×10^{-16}) suggests the decoppering efficacy of DPA is much higher than trientine[16].

Efficacy of chelation

Improvement in symptoms and biochemical parameters in WD takes around 2-6 mo in hepatic forms whereas in isolated neurological forms it may take up to 12-24 mo[12]. DPA in WD children shows an efficacy of almost 70%-90%[17-20]. The response depends on whether it is hepatic or neurological form and severity of the disease at presentation. Long term of follow up of WD (median duration- 15.1 years) studied by Bruha *et al*[19] showed the response to DPA to hepatic forms is 82% compared to 69% for neurological forms. One of the largest series of WD patients (n = 327) from Euro Wilson consortium, showed hepatic forms had 91% response compared to only 68% in

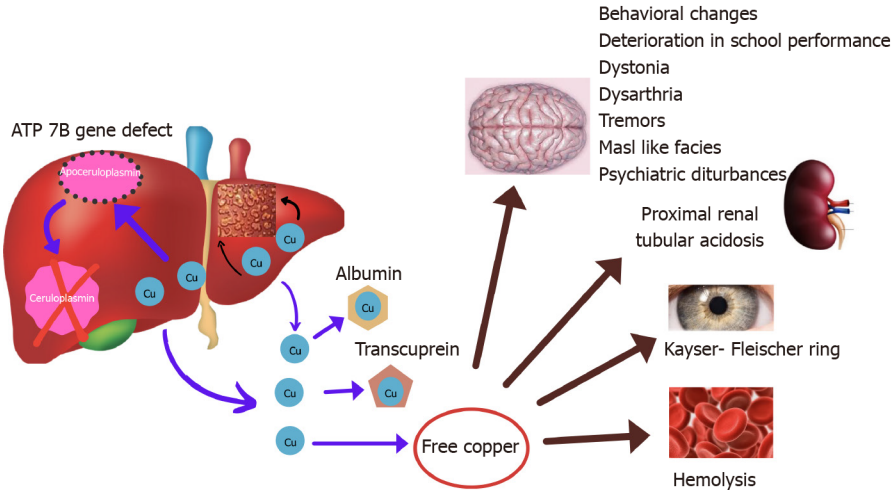

Figure 1 Pathophysiology of Wilson's disease. Due to mutation in *ATP 7B* gene, P type ATPase is defective and copper is not incorporated in ceruloplasmin. Free copper increases in blood and is deposited in liver and extrahepatic sites (brain, kidneys, bones, cornea, RBC).

neurological forms after a median follow up duration of 13.3 years[20]. In most series, trientine is used as a second line either due to poor response or due to toxicity to DPA. Hence, there are no head-to-head randomized trials comparing the efficacy of DPA and trientine. Overall efficacy of trientine is reported to be 80%-92%[21,22]. Retrospective analysis of efficacy of the two drugs by Hölscher *et al*[23] showed response in hepatic forms with DPA was 92% compared to 84% response with trientine after a median follow up duration of 13.3 years. In neurological forms, DPA fares significantly better (68%) than trientine (48%, P = 0.008)[23]. In Euro Wilson consortium, the response of both the DPA and trientine were comparable when used as a first line in both hepatic (90.7% *vs* 92.6%, P = 0.98) and neurological forms (67.5% *vs*55%, P = 0.76). However when used as a second line therapy, trientine *vs* DPA showed similar response in hepatic form (75% *vs* 68.9%, P = 0.76) but better response in neurological form (51% *vs* 23.1%, P = 0.01)[20].

Adverse effects of copper chelators

Adverse effects of DPA are always a major concern with up to 30% of the patients develop one or more adverse effects (Table 1)[20,24,25]. Adverse effect can be early onset (less than 3 wk of therapy) or late (more than 3 wk to up to 2-3 years of initiation of therapy). Early adverse effects like fever, rash, arthralgia, lymphadenopathy, pancytopenia are predominantly immune mediated[26]. Nephropathy, the most common late adverse effect of DPA is seen in 5%-30%. Presentations include proteinuria, glomerulonephritis, nephrotic syndrome less commonly as Good Pasture's syndrome[27-29]. More than 90% of the nephropathy occurs within 12 mo of therapy. High doses of DPA, decompensated liver disease, intrinsic renal diseases or presence of HLA-B8/DR3 are probable risk factors of nephropathy[30]. Eighty percent are membranous glomerulonephritis on renal biopsy. In a study by Hall *et al*[27] of 33 patients with DPA nephropathy, one-third each showed resolution at 6, 12 and 18 mo respectively, after drug discontinuation. There are no clear recommendations as to whether the drug can be rechallenged after resolution of nephropathy. However, in such situations, it is prudent to continue the patient on an alternative drug such as trientine or zinc. DPA related myelotoxicity occur in up to 7% patients undergoing chelation with DPA[31-33]. Two types of myelotoxicity are known to occur, idiosyncratic (usually with in 1 year of therapy) or dose dependent (more than after 1 year therapy)[34]. Though, there are no definite guidelines for monitoring and treatment of myelotoxicity, European society of Pediatric Gastroenterology Hepatology and Nutrition (ESPGHAN) suggests weekly blood counts initially, 1-3 mo till remission and 3-6 monthly thereafter[35]. If two or more values of total leukocyte count less than 3.5×10^3 per cubic mm, drug is to be discontinued. Bone marrow examination and reticulocyte counts differentiates this condition if concomitant hypersplenism is present[36,37]. Blood products, colony stimulating factor and anti-thymocyte globulin may improve the counts. Usual time of spontaneous recovery is 4-12 wk. Rarely hematopoietic stem cell transplantation may be required in refractory and prolonged cases. Once bone marrow toxicity has ensued, the drug should not be re-challenged. Adverse effects of DPA related to skin may be due to either acute hypersensitivity

Table 1 Adverse effects of copper chelating drugs

Name of the drug	Side effects
D-Penicillamine	Early (1-3 wk): Fever, rash, arthralgia, cytopenia, proteinuriaLate: (1) Skin: degenerative dermatoses elastosis perforans serpingosa, cutis laxa, pseudoxanthoma elasticum, bullous dermatoses, psoriasiform dermatoses, lichen planus, seborrheic dermatitis alopecia, aphthous ulcerations, hair loss; (2) Connective tissue disorders: Lupus like syndrome, arthralgia, Rheumatoid arthritis, polymyositis; (3) Renal: proteinuria, hematuria, glomerulonephritis, nephrotic syndrome, renal vasculitis, Goodpasture's syndrome; (4) Nervous system: paradoxical neurological worsening, neuropathies, myasthenia, hearing abnormalities, serous retinitis; (5) Gastrointestinal: Nausea, vomiting, diarrhea, elevated transaminases, cholestasis, hepatic siderosis; (6) Respiratory: pneumonitis, pulmonary fibrosis, pleural effusion; (7) Hematological: cytopenia, agranulocytosis, aplastic anemia, hemolytic anemia; and (8) Others: Immunoglobulin deficiency, breast enlargement, pyridoxine deficiency
Trientine	Paradoxical neurological worsening (10%-50%), sideroblastic anemia, bone marrow suppression, gastritis, skin rash, arthralgia, myalgia, hirsutism
Ammonium tetra thiomolybdate	Neurological dysfunction (rare), hepatotoxicity, bone marrow suppression

reaction presenting as morbilliform rash, urticaria, degenerative dermatoses (cutis laxa or elastosis perforans serpingosa) or an autoimmune phenomenon (pemphigus, scleroderma or lichen planus[38]. Rare musclar adverse effects of DPA include myasthenia (1%-2%) and ptosis. Anti- nicotinic acetyl choline receptor or Anti- MuSK (Anti- Muscle Specific tyrosine Kinase) is present in up to 70%[39]. Systemic lupus erythematosus can occur within 6-12 mo after the onset of DPA therapy presenting as pleurisy, arthritis, rash with or without presence of anti-nuclear antibody[40]. Deutscher *et al*[41] noted 3 out of 50 WD children with elevated transaminases within 6 wk of DPA therapy who resolved subsequently following discontinuation. Trientine also present with similar adverse effects as DPA like nausea, vomiting, arthralgia, myalgia, leukopenia, elevation in anti-nuclear antibody (ANA), nephropathy but adverse effects requiring discontinuation of trientine is significantly lower compared to DPA[20].

In hepatic WD, paradoxical neurological worsening occurs commonly within 6 mo of therapy, in patients with an underlying overt or occult neuropsychiatric feature. Paradoxical neurological worsening occurs even when dosing and compliance is good [42]. It occurs due to the sudden release of Cu from the liver following chelation therapy causing oxidative brain injury. Overall incidence of paradoxical neurological worsening ranges from 7%-26%. Those with previous known neurological WD, the incidence of worsening is up to 75%[19,24,25]. Both DPA and TA have shown to cause neurological worsening. In series from Euro Wilson consortium, paradoxical neurological worsening occurred significantly more with TA compared to DPA[20]. Litwin *et al*[13] studied natural history of 143 WD (70 Neuro/Neurohepatic WD and 73 hepatic WD), of whom 23% neurological cohort and none of the hepatic cohort developed early neurological worsening on chelation. In this series, median time of onset of neurological worsening was 2.3 mo. Fifty-three percent were completely reversible and 13% were partially reversible on drug discontinuation with median time of reversibility of 9.2 mo[13]. Prior neurological involvement, lesions in brain stem or thalamus and concomitant anti-dopaminergic drugs had higher chances of neurological worsening. Treatment consists of drug discontinuation and addition of zinc for a transition period. Chelators can be restarted in lower doses with gradual increment once the symptoms improve[13].

Assessment of adequacy of chelation: Clinical parameters

Currently there is no fool-proof, gold standard yardstick to assess chelation adequacy. All have fallacies in assessment and hence multiple parameters are considered. Chelation adequacy can be assessed firstly by assessing compliance to drug intake. Compliance is assessed by having a pill count, self-reporting by patients themselves or by checking empty blister packs during follow up outpatient visits[43]. There are various scales being developed assessing medication adherence (MAQ: Medication adherence questionnaire, MARS: Medication adherence Rating scale) but none have been validated in children[44]. More objective way of assessing compliance is by measuring drug levels but it is not routinely available under clinical setting. Secondly, follow up of clinical parameters assess the adequacy of chelation like improvement in jaundice, ascites, encephalopathy which usually take 2-6 mo post therapy. Resolution of neurological symptoms may take longer than 2-3 years[12]. The resolution of Kayser-Fleischer ring on de-coppering therapy has considerable controversies to the

same. Studies have heterogeneity in their assessment and reports. It appears to be independent on type of presentation (neurologic *vs* hepatic), stage of disease (pre-symptomatic *vs* symptomatic) and choice of chelator and compliance. Initial reports showed, Kayser-Fleischer (KF) ring disappearance in 81% of the patients (completely in 41% and incompletely in 59%), more in pre-symptomatic stage (60%) than those in symptomatic phase with ongoing therapy (2%) over 22 years of follow-up on DPA (90%) and zinc or trientine (10%). Conversely one-third of asymptomatic patients the rings did not reabsorb even after therapy of > 10 years. In this study, the fading of KF rings seemed to be independent of the stage of the disease and effectiveness of the decopperizing treatment[45]. In a study by Fenu *et al*[46] where 66% were hepatic and 31% were neuro-hepatic (90% on DPA ± zinc therapy), partial or total KF ring resolution was observed in 28%, deterioration in 6% and static in the rest of the cohort over 1-3 years of therapy. Other smaller cohorts report reduction of KF ring in neuropsychiatric manifestation or disappearance over 10 years on maintenance zinc and molybdate therapy in pediatric hepatic WD[47,48]. KF rings may reappear with non-compliance, and occasionally even with successful maintenance therapy[49].

Liver status can be appropriately assessed by Pediatric end-stage liver disease or Child-Turcotte-Pugh score. Biochemical parameters like serum albumin, total bilirubin and prothrombin time normalizes by 6 mo but liver enzymes might take longer[12]. In the author's experience it takes 9-12 mo for complete normalization of Liver function tests in majority of the cases[50]. In patients who have additional neurological involvement, neurological response is monitored by indices such as Global assessment scale (GAS)[51]. Even with neurological WD with significant MRI changes, 50% show improvement with long term chelation[52].

Assessment of adequacy of chelation: Biochemical parameters

Presently the most widely acceptable way to assess adequacy of chelation is by 24-h urine copper and non-ceruloplasmin copper. Twenty-four hours urine copper (UCu) increases immediately following chelation and takes around 12-18 mo to reach a stable level[53]. European Association for the Study of the Liver (EASL) and American Association for the Study of Liver diseases (AALSD) recommends targeting 24-h urine copper between 200-500 mg/d for adequate chelation[12,15]. Values > 500 mg/d suggest under chelation as lot of unchelated copper is remaining in the body. Values < 200 mg/d may be either due to over chelation or poor compliance (Table 2). This can be differentiated by non-ceruloplasmin copper (NCC) levels calculated by the formula (serum copper (mg/L) - 0.3 x serum ceruloplasmin(mg/L)[54]. NCC has a few fallacies. Firstly, almost 20% of NCC are negative values, seen mostly when immunoassay method was used to measure ceruloplasmin as it measures both holoceruloplasmin and apoceruloplasmin. NCC calculation becomes inappropriate when inactive apoceruloplasmin is included. Secondly, there are variabilities in reference ranges in ceruloplasmin values between various laboratories across the world creating disparities in NCC cut-offs[55]. According to EASL guidelines, NCC > 15 mg/dL suggest poor compliance and < 5 mg/dL suggest over chelation. Additionally, 24-h urine copper after 48-h cessation of therapy has been recommended by EASL. Values > 100 mg/d is suggestive of under chelation or poor compliance while values < 100 mg/d suggest adequate treatment[15].

A novel and upcoming modality to assess chelation is the use of exchangeable copper. Exchangeable copper is the fraction of copper bound to albumin, peptide and amino acids which are easily chelated by chelating agents. It denotes a direct estimation of non-ceruloplasmin copper (NCC)[56]. On WD with chelation for long time, exchangeable copper values tend to reduce comparable to non-Wilson children. In a pilot study by the authors, the role of exchangeable copper was assessed in a cohort of 96 children with hepatic WD. Exchangeable copper was significantly higher in newly diagnosed WD compared to WD on chelation for more than 1 year (3 ± 7 μmol/L *vs* 0.9 ± 0.6 μmol/L, $P = 0.03$). Exchangeable copper values were lower in stable liver disease compared to unstable liver disease (0.86 ± 0.5mmol/L *vs* 1.3 ± 0.6 mmol/L, $P = 0.01$). Exchangeable copper values showed excellent correlation with non-ceruloplasmin copper ($r = 0.92$, $P < 0.001$). Predictive model incorporating exchangeable copper into standard monitoring tools improved the yield of disease control assessment by 21%[57].

Comparison of single vs dual chelation: Which is better in hepatic WD?

Strictly zinc is not considered as a systemic chelator. Oral zinc (Zn) induces metallothionine in enterocyte. Metallothionine is an endogenous chelator that has high affinity to copper. Hence induced metallothionine combines with luminal Cu, preventing its entry into circulation. This Cu is removed through feces when enterocyte is shed. Zn

Table 2 Twenty-four hours urine copper and non-ceruloplasmin copper in various stages of Wilson's disease treatment

Early stages of treatment (< 1 yr)	UCu > 500 μg/dNCC > 25 μg/dL
Good control (treatment > 1 yr)	UCu 200-500 μg/dNCC < 15 μg/dL
Poor compliance/uncontrolled disease	UCu > 500 μg/dNCC > 15 μg/dL
Inadequate dose	UCu < 200 μg/dNCC > 15 μg/dL
Over-treatment	UCu < 200 μg/dNCC < 5 μg/dL

UCu: Twenty-four hours urinary copper; NCC: Non ceruloplasmin copper.

also induces hepatic metallothionine[58]. Hence, Zn is used in pre-symptomatic WD, stable well chelated WD on maintenance therapy, severe neurological WD. It is also used as a last resort in those with DPA or trientine intolerance. In severe hepatic disease, many centers consider giving a trial of dual chelation DPA and zinc for rapid chelation and quick stabilization. In a study conducted by the authors, 65 children with > 9 mo chelation were followed up for long term outcome. Majority had advanced disease at presentation. 83% of children were treated with DPA monotherapy and 17% treated with DPA and zinc combination. Trientine was started in 4 children due to DPA toxicity. 77% of children responded to DPA monotherapy even when the disease is severe at presentation and 50% responded when DPA and zinc combination was started. The overall response to oral chelation is 71%[50]. Hence, DPA should be the first line of therapy for any hepatic WD and zinc is added in those who failed to show optimal response with DPA in desperate circumstances with the hope of rapid synergistic chelation and quicker liver recuperation[50]. Though there are no comparative trials of dual or single chelation therapy, there are limited case series that have used DPA or trientine with zinc for WD presenting with ascites, coagulopathy and encephalopathy[59-61]. Though the efficacy of dual therapy in these studies were 91%-100%, sample sizes were small. Systematic review of 17 studies that assessed the efficacy of dual therapy (DPA/ Trientine with zinc) showed pooled efficacy rate (60.4%, 95%CI: 55.8-65.0) compared to DPA (73.7%, 95%CI: 65.1-85.4) and trientine monotherapy (82.6%, 95%CI: 75.4-89.5). Adverse effects following monotherapy is also lesser with either DPA or trientine compared to combination therapy[62]. Another retrospective study assessed 30 of 313 patients on dual chelator therapy, showed long term discontinuation and non-adherence was higher as compared to monotherapy (P = 0.006). Combination therapy, may fare better in neurological WD compared to exclusive hepatic forms[63]. Compliance and adequate spacing with chelating agent need careful consideration in the treatment schedule. If consumed together, chelator can combine with zinc in the lumen and effective absorption of both the medication gets reduced. Animal studies have shown that hepatic zinc stores is also significantly reduced during decoppering[64]. Hence, when chelator is combined with zinc, a proportion of chelator is used up in removing the body zinc thereby compromising the efficacy.

Efficacy of ammonium tetra thiomolybdate

Ammonium tetra thiomolybdate is a strong decoppering agent used in limited trials. It prevents intestinal absorption of copper if given with meals but also reduces serum copper when given in between meals. Ammonium tetra thiomolybdate (ATM) is predominantly advised for neurological forms due to it low risk of neurological worsening[65]. In the comparative study of ATM with trientine in neurological WD, paradoxical neurological worsening is significantly lower with ATM (4%) compared to trientine (26.1%, P = 0.01)[66]. At larger doses, ATM can form toxic insoluble complex that gets deposited in liver causing hepatoxicity[67]. Hence the role of ATM in hepatic WD is precarious. Up to 10% of patients receiving ATM might develop bone marrow toxicity also[68]. Bis-choline tetra thiomolybdate (WTX101) is an investigational derivative of ATM being studied recently in neurological WD with better stability and lower toxicity[69]. Twenty-four weeks treatment of the drug caused improvement in 71% of neurological WD. Seven percent developed leukopenia and almost 39% developed elevated liver enzymes post therapy[69]. Robust experience in exclusive hepatic WD is not yet available.

CHELATION IN INDIAN CHILDHOOD CIRRHOSIS

Indian childhood cirrhosis is commonly seen in children between 6 mo and 5 years of age in Indian subcontinent with its peak incidence seen during 1970-1990[70]. Presently this entity seems to be waning in the Indian subcontinent. Predominant etiology advocated was excessive copper ingestion with use of copper utensils[71]. There was also a possibility of genetic predisposition affecting copper metabolism[70]. Clinical features consist of nonspecific symptoms to start with like fever, lethargy, easy fatiguability, palpable liver with leafy edges in stage I, splenomegaly and ascites in stage II and jaundice, coagulopathy and encephalopathy in stage III. Histopathological examination of liver shows diffuse hepatocyte necrosis, presence of Mallory bodies and granular orcein staining. Treatment monitoring is by liver function tests (LFT), serum copper and in many studies, by repeat hepatic copper and liver histology, while on treatment. Mortality is almost 60% in stage II but reaching almost 90% in stage III [72]. In the study by Bavdekar *et al*[73] 65 children with Indian childhood cirrhosis (ICC) on treatment with DPA were followed up for the mean duration of 3.5 years, showed response in 60% of the children in pre-icteric phase compared to only 6% response ($P < 0.01$) in icteric phase (Table 3). Another study in ICC children who received DPA or DPA with steroids showed 50% survival as compared to10% in placebo group ($P = 0.002$)[74].In a pediatric study, DPA therapy has showed better response compared to DPA with intravenous immunoglobulin ($P = 0.018$)[75]. Chelation may improve symptoms if given early as prognosis is poor in advanced disease despite treatment[75].

CHELATION IN NON-WILSONIAN COPPER RELATED DISORDERS

Non-Wilsonian copper related diseases termed by Baker *et al*[76] as copper associated childhood cirrhosis includes ICC from India and ICC-like illness from western countries. This ICC like illnesses is otherwise called idiopathic copper toxicosis. Type I copper associated childhood cirrhosis (CACC) resembles ICC, with an early onset of disease and related to increased copper intake. Type II CACC has onset later than 4 years of age and possibly has an autosomal recessive inheritance without an obvious increase in copper intake[77]. Although there are few case reports of ICC- like illnesses, meagre number of reports use chelation therapy probably due to its conflicting results. One child from Bangladeshi origin, presented with jaundice, anorexia, weight loss at 7 years, with normal serum ceruloplasmin, and elevated hepatic copper 2319 mg/g. Improvement in symptoms and decrease in liver copper (35 mg/g) was noted after 19 mo of DPA therapy (Table 3)[77]. In contrast, a 10 year old Italian child with ascites and hepatomegaly, normal ceruloplasmin levels and liver copper of 1970 mg/g did not show any improvement clinically and biochemically even after 2 years of DPA[78]. Largest cohort of endemic Tyrolean infantile cirrhosis studied by Muller *et al*[79] showed both genetics and copper contamination were responsible for the disease. However there is paucity of chelation therapy experience in this condition.

IRON CHELATION IN GESTATIONAL ALLOIMMUNE LIVER DISEASES

In Gestational alloimmune liver disease alloimmunization of fetal liver antigen occurs in maternal blood resulting in IgG fetal liver antibody causing complement activation in fetal liver and significant impairment in hepcidin production (Figure 2)[80]. This causes iron storage in various organs like liver, heart, gonads, pancreas *etc*. Gestational alloimmune liver disease (GALD) causes liver failure as a result of hemochromatosis in newborn period and has high mortality if not intervened earlier. The liver injury causes reduced production of hepcidin resulting in uncontrolled iron absorption through placenta. This excess iron might further aggravate liver injury and also result in extra-hepatic iron deposition[81,82]. There have been few studies of GALD being treated with iron chelators (intravenous deferoxamine) and antioxidants with no clear-cut benefit. In the series by Flynn *et al*[83] five infants with neonatal hemochromatosis received intravenous deferoxamine but only one survived without liver transplantation. In the study by Rodrigues *et al*[84] 10 infants received iron chelation but only one survived without transplantation. In another series by Sigurdsson *et al*[85] six infants with neonatal hemochromatosis received supportive measures whereas eight infants received combination of deferoxamine and antioxidants. Two out of six who

Table 3 Pediatric studies of chelation in liver diseases

Ref.	Disease	Drug	Follow up duration	Response	Adverse effects
Dhawan *et al* [60]	WD	DPA (*n* = 32)	Median:11.78 (1.45-34.2) yr	20/32 (62.5%)	Minor- 6.3%; Major- 21.9%
Wang *et al* [106]	WD	DPA/TA (*n* = 9)	Mean: 5.1 4.1 yr	All responded	Not mentioned
Das *et al*[50]	WD	DPA (*n* = 65), TA(*n* = 4)	Median: 3.6 (0.8-12) yr	DPA (42/65) 64.6%, TA (3/4) 75%	DPA 10.8%
Arnon *et al* [107]	WD	TA (*n* = 10)	Treatment duration: 18 mo. Follow up:12-60 mo	All responded	1/10 (10%) reported hepatotoxicity
Taylor *et al* [108]	WD	TA (*n* = 16)	6.4 (0.78-18.6) yr	14/16 (87.5%)	1 had allergic reaction
Santos Silva *et al*[59]	WDAll decompensated liver disease	DPA (*n* = 1)TA (*n* = 4)	18-60 mo	All responded one still had raised transaminase	3/4 (75%) on DPA developed cytopenia
Bavdekar *et al* [73]	ICC	DPA (*n* = 68)	3.5 (1-7) yr	29/68 (42.6%) alive after follow up	5 children had proteinuria
Tomar *et al* [75]	ICC	DPA (*n* = 60)	12 mo duration	13/17 (76.5%) of grade III survived	11.8% drug rash, 5.9% fever
Tanner *et al* [74]	ICC (15 children treated with DPA in both trials together)	DPA (*n* = 15)	6 yr	Trial I: 1/15 (6.7%) survived in 6 yr, Trial II: 5/10 (50%) survived in 6 yr	Not mentioned
Horselen *et al* [77]	Case report CACC (age 7 yr)	DPA	19 mo	Hepatic copper normalized	none
Maggiore *et al* [78]	Case report CACC (age 10 yr)	DPA	24 mo	No improvement	Not mentioned
Rodeck *et al* [109]	CACC (age 6 and 10 mo)	DPA	18 mo, other child deteriorated immediately following DPA initiation	One child improved and other developed acute liver failure requiring liver transplantation	None
Flynn *et al*[83] 2002	NH	DFO (*n* = 5) with antioxidant	Follow up at 48 mo	2/5 (40%) survived without transplantation	Not mentioned
Rodrigues *et al* [84] 2005	NH	DFO with antioxidant (*n* = 9)	Follow up 3-9.8 yr	1/9 (11.1%) survived without transplantation	Not mentioned
Sigurrdson *et al*[85] 1998	NH	DFO with antioxidant (*n* = 8)	Not mentioned	None survived without transplantation	Not mentioned
Masera *et al* [110] 2013	HJV hemochromatosis Case report (7/F)	DFX	12 mo of treatment	Iron indices improved on 12 mo treatment	Not mentioned

DPA: D-Penicillamine; TA: Trientine; WD: Wilson's disease; ICC: Indian childhood cirrhosis; NH: Neonatal Hemochromatosis; DFO: Deferoxamine; DFX: Deferasirox; CACC: Copper associated childhood cirrhosis.

received supportive measures survived compared to only one who received chelation. It is not clear if the small proportion of response to chelation is due to efficacy of the drug in already advanced disease or due to natural history. In the recent years, it now clear that intravenous immunoglobulin has a superior role than chelation therapy in GALD.

IRON CHELATION IN HEREDITARY HEMOCHROMATOSIS

Hemochromatosis is due to iron accumulation in various organs with secondary causes being commoner in children than hereditary hemochromatosis. Secondary causes of hemochromatosis are commonly related to repeated transfusions in hemolytic anemia especially thalassemia major. In normal individuals, increased plasma iron induces the genes like HFE, TFR2 and HJV. This causes release in hepcidin, binding with ferroportin in enterocytes and macrophages, reducing iron absorption. Hereditary hemochromatosis (HH), most commonly due to mutation in

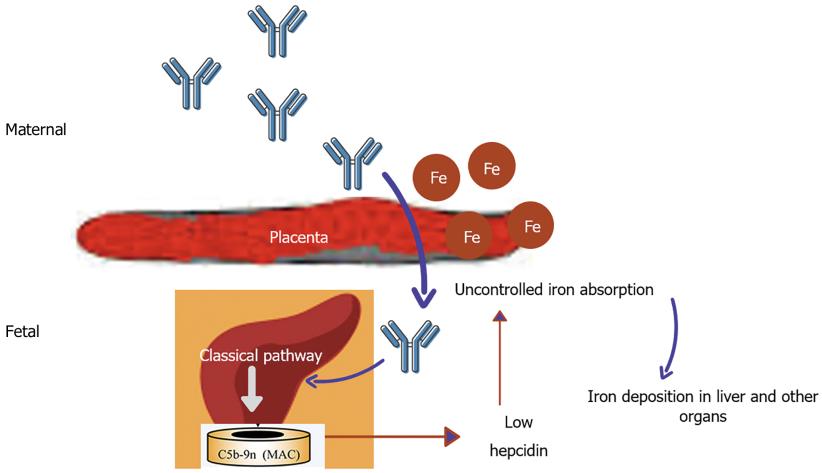

Figure 2 Pathogenesis of gestational alloimmune liver disease. Alloimmunization of fetal liver antigen by maternal blood produces IgG antibody passively transferred through the placenta to cause fetal liver injury by complement activation. Liver injury reduces the hepatic synthesis of hepcidin resulting in uncontrolled placental iron absorption. Excess iron is deposited in liver, pancreas, heart, gonads, *etc*.

HFE, cause impaired production of hepcidin making checkpoint for iron absorption defective[86]. Animal studies showed excessive fat intake causes impaired hepcidin production and increased transferrin receptor 1 and divalent metal transporter 1 Levels by altering mRNA expression. Hence, increased iron absorption and iron related liver injury may be responsible for development of non-alcoholic steatohepatitis[87]. Hereditary hemochromatosis (HH) is extremely rare in children. Excess iron in the serum causes liver cirrhosis, skin pigmentation, pancreatic insufficiency, cardiac dysfunction and hypothyroidism[88]. Iron chelation forms the mainstay of therapy in transfusion related siderosis in various hemolytic anemias in children. In a few studies, iron chelators have been implicated in treatment of HH also. Deferoxamine is parenteral iron chelator, given either as subcutaneous or intravenous infusion (20-50 mg/kg per day) over 8-24 h. Adverse effects seen are local reaction in injection site, hearing abnormalities, bone abnormalities *etc*. Deferasirox is an oral chelator with a similar efficacy as deferoxamine in removing hepatic iron but prone for its gastrointestinal side effects. Deferiprone, also an oral chelator is prone for gastrointestinal side effects and agranulocytosis and is highly effective in removing cardiac iron compared to other chelators (Table 4)[89]. Phatak *et al*[90] from Italy studied multiple doses of deferoxamine in HH, showed 10 mg/kg is the dose with optimal response and lower side effects. Nagler *et al*[91] analyzed 2 patients treated for 6 mo and 10 mo respectively who showed significant reduction in serum ferritin in the follow up. EASL and AASLD guidelines on HH recommend phlebotomy as the treatment of choice in HH[92,93]. Chelation may be considered in HH when phlebotomy is not tolerated due to severe congestive cardiac failure, anemia and in case of difficult venous access.

IRON CHELATION IN SECONDARY HEMOCHROMATOSIS

In children, secondary hemochromatosis is more common than HH and is usually caused by transfusion related iron overload seen in chronic hemolytic anemia especially beta thalassemia[94]. Each milliliter of packed RBC adds 1mg of iron to the body stores. Iron is usually bound to transferrin in plasma. However when the iron load increases, transferrin sites saturate and excess iron spills as labile plasma iron causing free radical injury to heart, liver and endocrine organs[95]. Multiple transfusion causes liver injury by various mechanisms such as siderosis causing hepatitis eventually progressing to fibrosis and cirrhosis. Hepatic foci of hemopoiesis and transfusion related hepatitis B and C infection are also seen[96].

Iron overload related liver injury can be assessed by various modalities. Serum ferritin is easily available and an inexpensive method to assess iron overload but its utility is limited in the presence of infection and inflammation. Liver iron concentration > 15 mg/g dry weight of liver is associated with significant mortality and morbidity[97]. The superconducting quantum interface device (SQUID) measures liver iron stores non-invasively but the SQUID scanners are not available in many centers

Table 4 Properties of iron-chelators

Properties	Deferoxamine (DFO)	Deferasirox (DFX)	Deferiprone (DFP)
Chelator: Iron ratio	1:1	2:1	3:1
Plasma $t_{1/2}$	30 min	12-16 h	2-3 h
Usual dose	20-50 mg/kg per day over 8-24 h	20-40 mg/kg per day once daily	75-100 mg/kg per day in 3 divided doses
Route of administration	Subcutaneous, intravenous	Oral	Oral
Clearance	Renal, hepatic	Hepatic	Renal
Efficacy in removing liver iron stores	Good	Good	Moderate
Efficacy in removing cardiac iron	Moderate	Moderate	Good
Advantages	Long safety data available, strongest chelator on molar basis	Oral once daily dose is sufficient	Oral, effective in removing cardiac iron
Adverse effects	Local reactions	Gastric intolerance	Nausea
	Sensorineural hearing loss	Rash	Vomiting
	Bone abnormalities	Diarrhea	Diarrhea
	Retinopathy	Elevation in creatinine	Arthralgia
	Pulmonary disease	Elevation in transaminases	Elevated liver enzymes
	Allergic reaction	Peptic ulcer	Agranulocytosis
	Bacterial infections (*e.g.*, *Listeria, Klebsiella*)	Renal dysfunction	
		Hepatic dysfunction	

worldwide[98]. Magnetic resonance imaging estimates liver iron by R2 and R2* techniques and it correlates well with liver iron concentration attained from biopsy. Magnetic resonance imaging (MRI) has now become the primary monitoring tool for both liver and cardiac iron[99].

Liver injury due to iron overload was common in children in pre-chelation era. Liver biopsies obtained in 80 children with beta thalassemia during splenectomy showed cirrhosis in 40% of children > 11 years with risk of cirrhosis increasing with age. 60% of the children showed hypoalbuminemia and 70% showed elevated transaminases[96]. Iron-chelators are well established treatment modality to prevent iron overload related liver injury. In a retrospective study by Maira *et al*[100] deferasirox for a duration of 4 ± 1.5 years showed significant improvement in liver stiffness measurement by transient elastography (7.4 ± 3.2 kPa *vs* 6.6 ± 3.2 kPa, $P = 0.017$) and liver iron concentration (LIC) (4.81 ± 3.82 mg/g *vs* 3.65 ± 3.45 mg/g, $P = 0.001$). Thus, iron chelation not only prevents progression of liver injury but also reverses inflammation and fibrosis. In the multicentric cross-sectional study from Italy, 924 beta-thalassemia patients were evaluated for iron overload assessment and management. The study showed serum ferritin had an excellent correlation with liver iron concentration. Deferasirox (38.3%) was most preferred chelator, especially in children because of its safety and easy administration[101]. Deferiprone was less commonly used when transaminases were elevated due to its concern of hepatic fibrosis[97]. Combination of two chelators were used whenever serum ferritin > 2500 ng/mL or MRI R2* values < 20 ms. Guidelines suggest that LIC assessment should be done at 1-2 yearly intervals [102]. Iron over load needs to be monitored and treated pre- and post-alloimmune hematopoietic stem cell transplantation (HSCT) for hemolytic anemia. Pre-transplant serum ferritin > 1000 ng/mL is associated with increased risk of post-transplant complications such as chronic liver disease, graft *vs* host disease (GVHD), sinusoidal obstruction syndrome and infection[103,104]. Hence it is mandatory to rapidly reduce ferritin levels before HSCT. Gruppo Italiano Trapianto di Midollo Osseo (GITMO) study group recommends switching to intravenous deferoxamine for rapid lowering of serum ferritin pre-transplant. From 6 mo post-transplant, iron overload is to be assessed by serum ferritin and MRI R2*. If LIC in MRI > 7 mg/g phlebotomy is preferred, but when LIC > 15 mg/g phlebotomy along with iron chelators are required to prevent complications[105].

CONCLUSION

Copper chelation by D-penicillamine and trientine forms the mainstay of treatment in childhood WD. Appropriate dosing, compliance to medications and scheduled monitoring with liver function tests, 24-h urine copper and non- ceruloplasmin copper are required for better control of the disease. D-penicillamine is a promising treatment for Indian childhood cirrhosis especially in early stages. The role in other non-Wilsonian copper diseases is doubtful. The use of iron chelator in Gestational alloimmune liver disease is waning due to its poor efficacy. Iron chelator may be considered as an alternative therapy in hereditary hemochromatosis when the primary treatment fails or not feasible but in case of secondary hemochromatosis chelation forms the main treatment.

REFERENCES

1 **Walshe JM**. Penicillamine, a new oral therapy for Wilson's disease. *Am J Med* 1956; **21**: 487-495 [PMID: 13362281 DOI: 10.1016/0002-9343(56)90066-3]

2 **Mettananda S**. Management of thalassaemia. *Sri Lanka J Child Heal* 2018; **47**: 159-165 [DOI: 10.4038/sljch.v47i2.8484]

3 **Aaseth J**, Skaug MA, Cao Y, Andersen O. Chelation in metal intoxication--Principles and paradigms. *J Trace Elem Med Biol* 2015; **31**: 260-266 [PMID: 25457281 DOI: 10.1016/j.jtemb.2014.10.001]

4 Al-Karadaghi S, Franco R, Hansson M, Shelnutt JA, Isaya G, Ferreira GC. Chelatases: distort to select? *Trends Biochem Sci.* 2006; 31(3):135-42 [DOI: 10.1016/j.tibs.2006.01.001.]

5 **Flora SJ**, Pachauri V. Chelation in metal intoxication. *Int J Environ Res Public Health* 2010; **7**: 2745-2788 [PMID: 20717537 DOI: 10.3390/ijerph7072745]

6 **de Bie P**, Muller P, Wijmenga C, Klomp LW. Molecular pathogenesis of Wilson and Menkes disease: correlation of mutations with molecular defects and disease phenotypes. *J Med Genet* 2007; **44**: 673-688 [PMID: 17717039 DOI: 10.1136/jmg.2007.052746]

7 **Lalioti V**, Sandoval I, Cassio D, Duclos-Vallée JC. Molecular pathology of Wilson's disease: a brief. *J Hepatol* 2010; **53**: 1151-1153 [PMID: 20832891 DOI: 10.1016/j.jhep.2010.07.008]

8 **Peisach J**, Blumberg WE. A mechanism for the action of penicillamine in the treatment of Wilson's disease. *Mol Pharmacol* 1969; **5**: 200-209 [PMID: 4306792]

9 **Kukovetz WR**, Beubler E, Kreuzig F, Moritz AJ, Nirnberger G, Werner-Breitenecker L. Bioavailability and pharmacokinetics of D-penicillamine. *J Rheumatol* 1983; **10**: 90-94 [PMID: 6842492]

10 **Netter P**, Bannwarth B, Péré P, Nicolas A. Clinical pharmacokinetics of D-penicillamine. *Clin Pharmacokinet* 1987; **13**: 317-333 [PMID: 3319347 DOI: 10.2165/00003088-198713050-00003]

11 **Langlois DK**, Lehner AF, Buchweitz JP, Ross DE, Johnson MB, Kruger JM, Bailie MB, Hauptman JG, Schall WD. Pharmacokinetics and relative bioavailability of D-penicillamine in fasted and nonfasted dogs. *J Vet Intern Med* 2013; **27**: 1071-1076 [PMID: 23875792 DOI: 10.1111/jvim.12147]

12 **Roberts EA**, Schilsky ML; American Association for Study of Liver Diseases (AASLD). Diagnosis and treatment of Wilson disease: an update. *Hepatology* 2008; **47**: 2089-2111 [PMID: 18506894 DOI: 10.1002/hep.22261]

13 **Litwin T**, Dzieżyc K, Karliński M, Chabik G, Czepiel W, Członkowska A. Early neurological worsening in patients with Wilson's disease. *J Neurol Sci* 2015; **355**: 162-167 [PMID: 26071888 DOI: 10.1016/j.jns.2015.06.010]

14 **Lu J**. Triethylenetetramine pharmacology and its clinical applications. *Mol Cancer Ther* 2010; **9**: 2458-2467 [PMID: 20660601 DOI: 10.1158/1535-7163.MCT-10-0523]

15 **European Association for Study of Liver**. EASL Clinical Practice Guidelines: Wilson's disease. *J Hepatol* 2012; **56**: 671-685 [PMID: 22340672 DOI: 10.1016/j.jhep.2011.11.007]

16 **Smirnova J**, Kabin E, Järving I, Bragina O, Tõugu V, Plitz T, Palumaa P. Copper(I)-binding properties of de-coppering drugs for the treatment of Wilson disease. α-Lipoic acid as a potential anti-copper agent. *Sci Rep* 2018; **8**: 1463 [PMID: 29362485 DOI: 10.1038/s41598-018-19873-2]

17 **Lau JY**, Lai CL, Wu PC, Pan HY, Lin HJ, Todd D. Wilson's disease: 35 years' experience. *Q J Med* 1990; **75**: 597-605 [PMID: 2217665]

18 **Svetel M**, Pekmezović T, Petrović I, Tomić A, Kresojević N, Jesić R, Kazić S, Raicević R, Stefanović D, Delibasić N, Zivanović D, Dordević M, Kostić VS. Long-term outcome in Serbian patients with Wilson disease. *Eur J Neurol* 2009; **16**: 852-857 [PMID: 19473354 DOI: 10.1111/j.1468-1331.2009.02607.x]

19 **Bruha R**, Marecek Z, Pospisilova L, Nevsimalova S, Vitek L, Martasek P, Nevoral J, Petrtyl J, Urbanek P, Jiraskova A, Ferenci P. Long-term follow-up of Wilson disease: natural history, treatment, mutations analysis and phenotypic correlation. *Liver Int* 2011; **31**: 83-91 [PMID: 20958917 DOI: 10.1111/j.1478-3231.2010.02354.x]

20 **Weiss KH**, Thurik F, Gotthardt DN, Schäfer M, Teufel U, Wiegand F, Merle U, Ferenci-Foerster D,

Maieron A, Stauber R, Zoller H, Schmidt HH, Reuner U, Hefter H, Trocello JM, Houwen RH, Ferenci P, Stremmel W; EUROWILSON Consortium. Efficacy and safety of oral chelators in treatment of patients with Wilson disease. *Clin Gastroenterol Hepatol* 2013; **11**: 1028-35.e1 [PMID: 23542331 DOI: 10.1016/j.cgh.2013.03.012]

21 **Walshe JM**. Treatment of Wilson's disease with trientine (triethylene tetramine) dihydrochloride. *Lancet* 1982; **1**: 643-647 [PMID: 6121964 DOI: 10.1016/s0140-6736(82)92201-2]

22 **Scheinberg IH**, Jaffe ME, Sternlieb I. The use of trientine in preventing the effects of interrupting penicillamine therapy in Wilson's disease. *N Engl J Med* 1987; **317**: 209-213 [PMID: 3600712 DOI: 10.1056/NEJM198707233170405]

23 **Hölscher S**, Leinweber B, Hefter H, Reuner U, Günther P, Weiss KH, Oertel WH, Möller JC. Evaluation of the symptomatic treatment of residual neurological symptoms in Wilson disease. *Eur Neurol* 2010; **64**: 83-87 [PMID: 20606453 DOI: 10.1159/000316066]

24 **Medici V**, Trevisan CP, D'Incà R, Barollo M, Zancan L, Fagiuoli S, Martines D, Irato P, Sturniolo GC. Diagnosis and management of Wilson's disease: results of a single center experience. *J Clin Gastroenterol* 2006; **40**: 936-941 [PMID: 17063115 DOI: 10.1097/01.mcg.0000225670.91722.59]

25 **Czlonkowska A**, Gajda J, Rodo M. Effects of long-term treatment in Wilson's disease with D-penicillamine and zinc sulphate. *J Neurol* 1996; **243**: 269-273 [PMID: 8936358 DOI: 10.1007/BF00868525]

26 LiverTox: Clinical and Research Information on Drug-Induced Liver Injury [Internet]. Bethesda (MD): National Institute of Diabetes and Digestive and Kidney Diseases; 2012 [PMID: 31643176]

27 **Hall CL**, Jawad S, Harrison PR, MacKenzie JC, Bacon PA, Klouda PT, MacIver AG. Natural course of penicillamine nephropathy: a long term study of 33 patients. *Br Med J (Clin Res Ed)* 1988; **296**: 1083-1086 [PMID: 3132218 DOI: 10.1136/bmj.296.6629.1083]

28 **Bienaimé F**, Clerbaux G, Plaisier E, Mougenot B, Ronco P, Rougier JP. D-Penicillamine-induced ANCA-associated crescentic glomerulonephritis in Wilson disease. *Am J Kidney Dis* 2007; **50**: 821-825 [PMID: 17954295 DOI: 10.1053/j.ajkd.2007.05.026]

29 **Derk CT**, Jimenez SA. Goodpasture-like syndrome induced by D-penicillamine in a patient with systemic sclerosis: report and review of the literature. *J Rheumatol* 2003; **30**: 1616-1620 [PMID: 12858467]

30 **Billingsley LM**, Stevens MB. The relationship between D-penicillamine--induced proteinuria and prior gold nephropathy. *Johns Hopkins Med J* 1981; **148**: 64-67 [PMID: 7206401]

31 **Steen VD**, Blair S, Medsger TA Jr. The toxicity of D-penicillamine in systemic sclerosis. *Ann Intern Med* 1986; **104**: 699-705 [PMID: 2938530 DOI: 10.7326/0003-4819-104-5-699]

32 Toxicity of longterm low dose D-penicillamine therapy in rheumatoid arthritis. Cooperative Systematic Studies of Rheumatic Disease Group. *J Rheumatol* 1987; **14**: 67-73 [PMID: 2952797]

33 **Kay AG**. Myelotoxicity of D-penicillamine. *Ann Rheum Dis* 1979; **38**: 232-236 [PMID: 485580 DOI: 10.1136/ard.38.3.232]

34 **Jaffe IA**. Adverse effects profile of sulfhydryl compounds in man. *Am J Med* 1986; **80**: 471-476 [PMID: 2937293 DOI: 10.1016/0002-9343(86)90722-9]

35 **Gupta P**, Choksi M, Goel A, Zachariah U, Sajith KG, Ramachandran J, Chandy G, Kurian G, Rebekah G, Eapen CE. Maintenance zinc therapy after initial penicillamine chelation to treat symptomatic hepatic Wilson's disease in resource constrained setting. *Indian J Gastroenterol* 2018; **37**: 31-38 [PMID: 29457214 DOI: 10.1007/s12664-018-0829-x]

36 **Pitman SK**, Huynh T, Bjarnason TA, An J, Malkhasyan KA. A case report and focused literature review of d-penicillamine and severe neutropenia: A serious toxicity from a seldom-used drug. *Clin Case Rep* 2019; **7**: 990-994 [PMID: 31110732 DOI: 10.1002/ccr3.2125]

37 **Petrides PE**, Gerhartz HH. D-penicillamine-induced agranulocytosis: hematological remission upon treatment with recombinant GM-CSF. *Z Rheumatol* 1991; **50**: 328-329 [PMID: 1776370]

38 **Ishak R**, Abbas O. Penicillamine revisited: historic overview and review of the clinical uses and cutaneous adverse effects. *Am J Clin Dermatol* 2013; **14**: 223-233 [PMID: 23605177 DOI: 10.1007/s40257-013-0022-z]

39 **Nishida H**, Sahashi K. [Penicillamine-induced myasthenia gravis]. *Ryoikibetsu Shokogun Shirizu* 2001; 351-353 [PMID: 11596408]

40 **Lee Y**, Lee ST, Cho H. D-penicillamine-induced ANA (+) ANCA (+) vasculitis in pediatric patients with Wilson's disease. *Clin Nephrol* 2016; **85**: 296-300 [PMID: 26784915 DOI: 10.5414/CN108763]

41 **Deutscher J**, Kiess W, Scheerschmidt G, Willgerodt H. Potential hepatotoxicity of penicillamine treatment in three patients with Wilson's disease. *J Pediatr Gastroenterol Nutr* 1999; **29**: 628 [PMID: 10554138 DOI: 10.1097/00005176-199911000-00031]

42 **Kalita J**, Kumar V, Ranjan A, Misra UK. Role of Oxidative Stress in the Worsening of Neurologic Wilson Disease Following Chelating Therapy. *Neuromolecular Med* 2015; **17**: 364-372 [PMID: 26224517 DOI: 10.1007/s12017-015-8364-8]

43 **Cramer JA**, Roy A, Burrell A, Fairchild CJ, Fuldeore MJ, Ollendorf DA, Wong PK. Medication compliance and persistence: terminology and definitions. *Value Health* 2008; **11**: 44-47 [PMID: 18237359 DOI: 10.1111/j.1524-4733.2007.00213.x]

44 **Maselbas W**, Członkowska A, Litwin T, Niewada M. Persistence with treatment for Wilson disease: a retrospective study. *BMC Neurol* 2019; **19**: 278 [PMID: 31718567 DOI: 10.1186/s12883-019-1502-4]

45 **Lössner A**, Lössner J, Bachmann H, Zotter J. The Kayser-Fleischer ring during long-term treatment in Wilson's disease (hepatolenticular degeneration). A follow-up study. *Graefes Arch Clin Exp*

Ophthalmol 1986; **224**: 152-155 [PMID: 3949188 DOI: 10.1007/BF02141489]
46 **Fenu M**, Liggi M, Demelia E, Sorbello O, Civolani A, Demelia L. Kayser-Fleischer ring in Wilson's disease: a cohort study. *Eur J Intern Med* 2012; **23**: e150-e156 [PMID: 22863441 DOI: 10.1016/j.ejim.2012.04.005]
47 **Esmaeli B**, Burnstine MA, Martonyi CL, Sugar A, Johnson V, Brewer GJ. Regression of Kayser-Fleischer rings during oral zinc therapy: correlation with systemic manifestations of Wilson's disease. *Cornea* 1996; **15**: 582-588 [PMID: 8899270]
48 **Marcellini M**, Di Ciommo V, Callea F, Devito R, Comparcola D, Sartorelli MR, Carelli G, Nobili V. Treatment of Wilson's disease with zinc from the time of diagnosis in pediatric patients: a single-hospital, 10-year follow-up study. *J Lab Clin Med* 2005; **145**: 139-143 [PMID: 15871305 DOI: 10.1016/j.lab.2005.01.007]
49 **Suvarna JC**. Kayser-Fleischer ring. *J Postgrad Med* 2008; **54**: 238-240 [PMID: 18626182 DOI: 10.4103/0022-3859.41816]
50 **Das MC**, Sen Sarma M, Srivastava A, Yachha SK, Poddar U. Effect of chelation therapy in pediatric Wilson's disease: Liver and endoscopic outcome. *J Hepatobiliary Pancreat Sci* 2021; **28**: 336-345 [PMID: 32745371 DOI: 10.1002/jhbp.812]
51 **Aggarwal A**, Aggarwal N, Nagral A, Jankharia G, Bhatt M. A novel Global Assessment Scale for Wilson's Disease (GAS for WD). *Mov Disord* 2009; **24**: 509-518 [PMID: 19115420 DOI: 10.1002/mds.22231]
52 **Prashanth LK**, Taly AB, Sinha S, Ravishankar S, Arunodaya GR, Vasudev MK, Swamy HS. Prognostic factors in patients presenting with severe neurological forms of Wilson's disease. *QJM* 2005; **98**: 557-563 [PMID: 16006499 DOI: 10.1093/qjmed/hci095]
53 **Walshe JM**. The pattern of urinary copper excretion and its response to treatment in patients with Wilson's disease. *QJM* 2011; **104**: 775-778 [PMID: 21622540 DOI: 10.1093/qjmed/hcr073]
54 **Twomey PJ**, Viljoen A, Reynolds TM, Wierzbicki AS. Non-ceruloplasmin-bound copper in routine clinical practice in different laboratories. *J Trace Elem Med Biol* 2008; **22**: 50-53 [PMID: 18319140 DOI: 10.1016/j.jtemb.2007.11.001]
55 **Duncan A**, Yacoubian C, Beetham R, Catchpole A, Bullock D. The role of calculated non-caeruloplasmin-bound copper in Wilson's disease. *Ann Clin Biochem* 2017; **54**: 649-654 [PMID: 27742851 DOI: 10.1177/0004563216676843]
56 **Schmitt F**, Podevin G, Poupon J, Roux J, Legras P, Trocello JM, Woimant F, Laprévote O, Nguyen TH, El Balkhi S. Evolution of exchangeable copper and relative exchangeable copper through the course of Wilson's disease in the Long Evans Cinnamon rat. *PLoS One* 2013; **8**: e82323 [PMID: 24358170 DOI: 10.1371/journal.pone.0082323]
57 UEG Week 2020 Poster Presentations. *United European Gastroenterol J* 2020; **8**: 144-887 [PMID: 33043826 DOI: 10.1177/2050640620927345]
58 **Cousins RJ**. Absorption, transport, and hepatic metabolism of copper and zinc: special reference to metallothionein and ceruloplasmin. *Physiol Rev* 1985; **65**: 238-309 [PMID: 3885271 DOI: 10.1152/physrev.1985.65.2.238]
59 **Santos Silva EE**, Sarles J, Buts JP, Sokal EM. Successful medical treatment of severely decompensated Wilson disease. *J Pediatr* 1996; **128**: 285-287 [PMID: 8636833 DOI: 10.1016/s0022-3476(96)70412-2]
60 **Dhawan A**, Taylor RM, Cheeseman P, De Silva P, Katsiyiannakis L, Mieli-Vergani G. Wilson's disease in children: 37-year experience and revised King's score for liver transplantation. *Liver Transpl* 2005; **11**: 441-448 [PMID: 15776453 DOI: 10.1002/Lt.20352]
61 **Askari FK**, Greenson J, Dick RD, Johnson VD, Brewer GJ. Treatment of Wilson's disease with zinc. XVIII. Initial treatment of the hepatic decompensation presentation with trientine and zinc. *J Lab Clin Med* 2003; **142**: 385-390 [PMID: 14713890 DOI: 10.1016/S0022-2143(03)00157-4]
62 **Chen JC**, Chuang CH, Wang JD, Wang CW. Combination Therapy Using Chelating Agent and Zinc for Wilson's Disease. *J Med Biol Eng* 2015; **35**: 697-708 [PMID: 26692828 DOI: 10.1007/s40846-015-0087-7]
63 **Weiss KH**, Gotthardt DN, Klemm D, Merle U, Ferenci-Foerster D, Schaefer M, Ferenci P, Stremmel W. Zinc monotherapy is not as effective as chelating agents in treatment of Wilson disease. *Gastroenterology* 2011; **140**: 1189-1198.e1 [PMID: 21185835 DOI: 10.1053/j.gastro.2010.12.034]
64 **Fieten H**, Dirksen K, van den Ingh TS, Winter EA, Watson AL, Leegwater PA, Rothuizen J. D-penicillamine treatment of copper-associated hepatitis in Labrador retrievers. *Vet J* 2013; **196**: 522-527 [PMID: 23375251 DOI: 10.1016/j.tvjl.2012.12.013]
65 **Brewer GJ**, Hedera P, Kluin KJ, Carlson M, Askari F, Dick RB, Sitterly J, Fink JK. Treatment of Wilson disease with ammonium tetrathiomolybdate: III. Initial therapy in a total of 55 neurologically affected patients and follow-up with zinc therapy. *Arch Neurol* 2003; **60**: 379-385 [PMID: 12633149 DOI: 10.1001/archneur.60.3.379]
66 **Brewer GJ**, Askari F, Lorincz MT, Carlson M, Schilsky M, Kluin KJ, Hedera P, Moretti P, Fink JK, Tankanow R, Dick RB, Sitterly J. Treatment of Wilson disease with ammonium tetrathiomolybdate: IV. Comparison of tetrathiomolybdate and trientine in a double-blind study of treatment of the neurologic presentation of Wilson disease. *Arch Neurol* 2006; **63**: 521-527 [PMID: 16606763 DOI: 10.1001/archneur.63.4.521]
67 **Medici V**, Trevisan CP, Bigotto MA, D'Incà R, Martines D, Dal Pont E, Sturniolo GC. Adverse reaction after tetrathiomolybdate treatment for Wilson's disease: a case report. *Mov Disord* 2006; **21**:

2030-2032 [PMID: 16991142 DOI: 10.1002/mds.21109]
68 **Karunajeewa H**, Wall A, Metz J, Grigg A. Cytopenias secondary to copper depletion complicating ammonium tetrathiomolybdate therapy for Wilson's disease. *Aust N Z J Med* 1998; **28**: 215-216 [PMID: 9612534 DOI: 10.1111/j.1445-5994.1998.tb02975.x]
69 **Weiss KH**, Askari FK, Czlonkowska A, Ferenci P, Bronstein JM, Bega D, Ala A, Nicholl D, Flint S, Olsson L, Plitz T, Bjartmar C, Schilsky ML. Bis-choline tetrathiomolybdate in patients with Wilson's disease: an open-label, multicentre, phase 2 study. *Lancet Gastroenterol Hepatol* 2017; **2**: 869-876 [PMID: 28988934 DOI: 10.1016/S2468-1253(17)30293-5]
70 **Pandit A**, Bhave S. Present interpretation of the role of copper in Indian childhood cirrhosis. *Am J Clin Nutr* 1996; **63**: 830S-835S [PMID: 8615370 DOI: 10.1093/ajcn/63.5.830]
71 **Chawla V**, Chandra RK, Verma IC, Ghai OP. An epidemiologic approach to Indian childhood cirrhosis. *Indian Pediatr* 1973; **10**: 73-79 [PMID: 4719650]
72 **Nayak NC**, Visalakshi S, Singh M, Chawla V, Chandra RK, Ramalingaswami V. Indian childhood cirrhosis--a re-evaluation of its pathomorphologic features and their significance in the light of clinical data and natural history of the disease. *Indian J Med Res* 1972; **60**: 246-259 [PMID: 5064312]
73 **Bavdekar AR**, Bhave SA, Pradhan AM, Pandit AN, Tanner MS. Long term survival in Indian childhood cirrhosis treated with D-penicillamine. *Arch Dis Child* 1996; **74**: 32-35 [PMID: 8660042 DOI: 10.1136/adc.74.1.32]
74 **Tanner MS**, Bhave SA, Pradhan AM, Pandit AN. Clinical trials of penicillamine in Indian childhood cirrhosis. *Arch Dis Child* 1987; **62**: 1118-1124 [PMID: 3318711 DOI: 10.1136/adc.62.11.1118]
75 **Tomar BS**, Saxena S, Prakash P, Tomar S, Verma C. D-penicillamine in the treatment of Indian childhood cirrhosis--a preliminary report. *Indian J Pediatr* 1983; **50**: 613-618 [PMID: 6680110 DOI: 10.1007/BF02957727]
76 **Baker A**, Gormally S, Saxena R, Baldwin D, Drumm B, Bonham J, Portmann B, Mowat AP. Copper-associated liver disease in childhood. *J Hepatol* 1995; **23**: 538-543 [PMID: 8583141 DOI: 10.1016/0168-8278(95)80059-x]
77 **Horslen SP**, Tanner MS, Lyon TD, Fell GS, Lowry MF. Copper associated childhood cirrhosis. *Gut* 1994; **35**: 1497-1500 [PMID: 7959213 DOI: 10.1136/gut.35.10.1497]
78 **Maggiore G**, De Giacomo C, Sessa F, Burgio GR. Idiopathic hepatic copper toxicosis in a child. *J Pediatr Gastroenterol Nutr* 1987; **6**: 980-983 [PMID: 3681585 DOI: 10.1097/00005176-198711000-00028]
79 **Muller T**, Feichtinger H, Berger H, Muller W. Endemic Tyrolean infantile cirrhosis: an ecogenetic disorder. *Lancet* 1996; **347**: 877-880 [PMID: 8622397 DOI: 10.1016/s0140-6736(96)91351-3]
80 **Kelly AL**, Lunt PW, Rodrigues F, Berry PJ, Flynn DM, McKiernan PJ, Kelly DA, Mieli-Vergani G, Cox TM. Classification and genetic features of neonatal haemochromatosis: a study of 27 affected pedigrees and molecular analysis of genes implicated in iron metabolism. *J Med Genet* 2001; **38**: 599-610 [PMID: 11546828 DOI: 10.1136/jmg.38.9.599]
81 **Feldman AG**, Whitington PF. Neonatal hemochromatosis. *J Clin Exp Hepatol* 2013; **3**: 313-320 [PMID: 25755519 DOI: 10.1016/j.jceh.2013.10.004]
82 **Lopriore E**, Mearin ML, Oepkes D, Devlieger R, Whitington PF. Neonatal hemochromatosis: management, outcome, and prevention. *Prenat Diagn* 2013; **33**: 1221-1225 [PMID: 24030714 DOI: 10.1002/pd.4232]
83 **Flynn DM**, Mohan N, McKiernan P, Beath S, Buckels J, Mayer D, Kelly DA. Progress in treatment and outcome for children with neonatal haemochromatosis. *Arch Dis Child Fetal Neonatal Ed* 2003; **88**: F124-F127 [PMID: 12598501 DOI: 10.1136/fn.88.2.f124]
84 **Rodrigues F**, Kallas M, Nash R, Cheeseman P, D'Antiga L, Rela M, Heaton ND, Mieli-Vergani G. Neonatal hemochromatosis--medical treatment vs. transplantation: the king's experience. *Liver Transpl* 2005; **11**: 1417-1424 [PMID: 16237701 DOI: 10.1002/Lt.20497]
85 **Sigurdsson L**, Reyes J, Kocoshis SA, Hansen TW, Rosh J, Knisely AS. Neonatal hemochromatosis: outcomes of pharmacologic and surgical therapies. *J Pediatr Gastroenterol Nutr* 1998; **26**: 85-89 [PMID: 9443126 DOI: 10.1097/00005176-199801000-00015]
86 **Pietrangelo A**. Hereditary hemochromatosis: pathogenesis, diagnosis, and treatment. *Gastroenterology* 2010; **139**: 393-408, 408.e1 [PMID: 20542038 DOI: 10.1053/j.gastro.2010.06.013]
87 **Higuchi T**, Moriyama M, Fukushima A, Matsumura H, Matsuoka S, Kanda T, Sugitani M, Tsunemi A, Ueno T, Fukuda N. Association of mRNA expression of iron metabolism-associated genes and progression of non-alcoholic steatohepatitis in rats. *Oncotarget* 2018; **9**: 26183-26194 [PMID: 29899851 DOI: 10.18632/oncotarget.25488]
88 **Salgia RJ**, Brown K. Diagnosis and management of hereditary hemochromatosis. *Clin Liver Dis* 2015; **19**: 187-198 [PMID: 25454304 DOI: 10.1016/j.cld.2014.09.011]
89 **Mobarra N**, Shanaki M, Ehteram H, Nasiri H, Sahmani M, Saeidi M, Goudarzi M, Pourkarim H, Azad M. A Review on Iron Chelators in Treatment of Iron Overload Syndromes. *Int J Hematol Oncol Stem Cell Res* 2016; **10**: 239-247 [PMID: 27928480]
90 **Phatak P**, Brissot P, Wurster M, Adams PC, Bonkovsky HL, Gross J, Malfertheiner P, McLaren GD, Niederau C, Piperno A, Powell LW, Russo MW, Stoelzel U, Stremmel W, Griffel L, Lynch N, Zhang Y, Pietrangelo A. A phase 1/2, dose-escalation trial of deferasirox for the treatment of iron overload in HFE-related hereditary hemochromatosis. *Hepatology* 2010; **52**: 1671-1779 [PMID:

20814896 DOI: 10.1002/hep.23879]
91 **Nagler M**, Gregor M, Wuillemin WA. Iron chelation with deferasirox in two patients with HFE hemochromatosis and chronic anemia. *Acta Haematol* 2011; **126**: 119-121 [PMID: 21659727 DOI: 10.1159/000328039]
92 **Bacon BR**, Adams PC, Kowdley KV, Powell LW, Tavill AS; American Association for the Study of Liver Diseases. Diagnosis and management of hemochromatosis: 2011 practice guideline by the American Association for the Study of Liver Diseases. *Hepatology* 2011; **54**: 328-343 [PMID: 21452290 DOI: 10.1002/hep.24330]
93 **European Association for the Study of the Liver**. EASL clinical practice guidelines for HFE hemochromatosis. *J Hepatol* 2010; **53**: 3-22 [PMID: 20471131 DOI: 10.1016/j.jhep.2010.03.001]
94 **Ware HM**, Kwiatkowski JL. Evaluation and treatment of transfusional iron overload in children. *Pediatr Clin North Am* 2013; **60**: 1393-1406 [PMID: 24237978 DOI: 10.1016/j.pcl.2013.09.003]
95 **Piga A**, Longo F, Duca L, Roggero S, Vinciguerra T, Calabrese R, Hershko C, Cappellini MD. High nontransferrin bound iron levels and heart disease in thalassemia major. *Am J Hematol* 2009; **84**: 29-33 [PMID: 19006228 DOI: 10.1002/ajh.21317]
96 **Jean G**, Terzoli S, Mauri R, Borghetti L, Di Palma A, Piga A, Magliano M, Melevendi M, Cattaneo M. Cirrhosis associated with multiple transfusions in thalassaemia. *Arch Dis Child* 1984; **59**: 67-70 [PMID: 6696498 DOI: 10.1136/adc.59.1.67]
97 **Olivieri NF**, Nathan DG, MacMillan JH, Wayne AS, Liu PP, McGee A, Martin M, Koren G, Cohen AR. Survival in medically treated patients with homozygous beta-thalassemia. *N Engl J Med* 1994; **331**: 574-578 [PMID: 8047081 DOI: 10.1056/NEJM199409013310903]
98 **Brittenham GM**, Farrell DE, Harris JW, Feldman ES, Danish EH, Muir WA, Tripp JH, Bellon EM. Magnetic-susceptibility measurement of human iron stores. *N Engl J Med* 1982; **307**: 1671-1675 [PMID: 7144866 DOI: 10.1056/NEJM198212303072703]
99 **Wood JC**, Enriquez C, Ghugre N, Tyzka JM, Carson S, Nelson MD, Coates TD. MRI R2 and R2* mapping accurately estimates hepatic iron concentration in transfusion-dependent thalassemia and sickle cell disease patients. *Blood* 2005; **106**: 1460-1465 [PMID: 15860670 DOI: 10.1182/blood-2004-10-3982]
100 **Maira D**, Cassinerio E, Marcon A, Mancarella M, Fraquelli M, Pedrotti P, Cappellini MD. Progression of liver fibrosis can be controlled by adequate chelation in transfusion-dependent thalassemia (TDT). *Ann Hematol* 2017; **96**: 1931-1936 [PMID: 28875336 DOI: 10.1007/s00277-017-3120-9]
101 **Piga A**, Longo F, Musallam KM, Cappellini MD, Forni GL, Quarta G, Chiavilli F, Commendatore F, Mulas S, Caruso V, Galanello R. Assessment and management of iron overload in β-thalassaemia major patients during the 21st century: a real-life experience from the Italian WEBTHAL project. *Br J Haematol* 2013; **161**: 872-883 [PMID: 23600689 DOI: 10.1111/bjh.12340]
102 **Angelucci E**, Barosi G, Camaschella C, Cappellini MD, Cazzola M, Galanello R, Marchetti M, Piga A, Tura S. Italian Society of Hematology practice guidelines for the management of iron overload in thalassemia major and related disorders. *Haematologica* 2008; **93**: 741-752 [PMID: 18413891 DOI: 10.3324/haematol.12413]
103 **Kamble RT**, Selby GB, Mims M, Kharfan-Dabaja MA, Ozer H, George JN. Iron overload manifesting as apparent exacerbation of hepatic graft-versus-host disease after allogeneic hematopoietic stem cell transplantation. *Biol Blood Marrow Transplant* 2006; **12**: 506-510 [PMID: 16635785 DOI: 10.1016/j.bbmt.2006.01.004]
104 **Majhail NS**, Lazarus HM, Burns LJ. Iron overload in hematopoietic cell transplantation. *Bone Marrow Transplant* 2008; **41**: 997-1003 [PMID: 18438425 DOI: 10.1038/bmt.2008.99]
105 **Sivgin S**, Eser B. The management of iron overload in allogeneic hematopoietic stem cell transplant (alloHSCT) recipients: where do we stand? *Ann Hematol* 2013; **92**: 577-586 [PMID: 23430087 DOI: 10.1007/s00277-013-1682-8]
106 **Wang LC**, Wang JD, Tsai CR, Cheng SB, Lin CC. Clinical features and therapeutic response in Taiwanese children with Wilson's disease: 12 years of experience in a single center. *Pediatr Neonatol* 2010; **51**: 124-129 [PMID: 20417464 DOI: 10.1016/S1875-9572(10)60022-8]
107 **Arnon R**, Calderon JF, Schilsky M, Emre S, Shneider BL. Wilson disease in children: serum aminotransferases and urinary copper on triethylene tetramine dihydrochloride (trientine) treatment. *J Pediatr Gastroenterol Nutr* 2007; **44**: 596-602 [PMID: 17460493 DOI: 10.1097/MPG.0b013e3180467715]
108 **Taylor RM**, Chen Y, Dhawan A; EuroWilson Consortium. Triethylene tetramine dihydrochloride (trientine) in children with Wilson disease: experience at King's College Hospital and review of the literature. *Eur J Pediatr* 2009; **168**: 1061-1068 [PMID: 19066958 DOI: 10.1007/s00431-008-0886-8]
109 **Rodeck B**, Kardoff R, Melter M. Treatment of copper associated liver disease in childhood. *Eur J Med Res* 1999; **4**: 253-256 [PMID: 10383883]
110 **Masera N**, Cattoni A, Decimi V, D'Apolito Valeria, Arosio C, Mariani R, Piperno A. Efficacy of deferasirox for the treatment of iron overload in a child affected by Juvenile Hemochromatosis. *Case Rep Clin Med* 2013; **2**: 126-128 [DOI: 10.4236/crcm.2013.22033]

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REVIEW

Hepatocellular carcinoma: Understanding molecular mechanisms for defining potential clinical modalities

Abhiram Natu, Anjali Singh, Sanjay Gupta

ORCID number: Abhiram Natu 0000-0002-6498-6134; Anjali Singh 0000-0002-0558-5664; Sanjay Gupta 0000-0002-1209-189X.

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Abhiram Natu, Anjali Singh, Sanjay Gupta, Epigenetics and Chromatin Biology Group, Gupta Laboratory, Cancer Research Institute, Advanced Centre for Treatment, Research and Education in Cancer, Tata Memorial Centre, Kharghar, Navi Mumbai 410210, Maharashtra, India

Abhiram Natu, Anjali Singh, Sanjay Gupta, Homi Bhabha National Institute, Training School Complex, Anushakti Nagar, Mumbai 400085, Maharashtra, India

Corresponding author: Sanjay Gupta, PhD, Senior Scientist, Epigenetics and Chromatin Biology Group, Gupta Laboratory, Cancer Research Institute, Advanced Centre for Treatment, Research and Education in Cancer, Tata Memorial Centre, Kharghar, Navi Mumbai 410210, Maharashtra, India. sgupta@actrec.gov.in

Abstract

Liver cancer is the sixth most commonly occurring cancer and costs millions of lives per year. The diagnosis of hepatocellular carcinoma (HCC) has relied on scanning techniques and serum-based markers such as α-fetoprotein. These measures have limitations due to their detection limits and asymptomatic conditions during the early stages, resulting in late-stage cancer diagnosis where targeted chemotherapy or systemic treatment with sorafenib is offered. However, the aid of conventional therapy for patients in the advanced stage of HCC has limited outcomes. Thus, it is essential to seek a new treatment strategy and improve the diagnostic techniques to manage the disease. Researchers have used the omics profile of HCC patients for sub-classification of tissues into different groups, which has helped us with prognosis. Despite these efforts, a promising target for treatment has not been identified. The hurdle in this situation is genetic and epigenetic variations in the tumor, leading to disparities in response to treatment. Understanding reversible epigenetic changes along with clinical traits help to define new markers for patient categorization and design personalized therapy. Many clinical trials of inhibitors of epigenetic modifiers (also known as epi-drugs) are in progress. Epi-drugs like azacytidine or belinostat are already approved for other cancer treatments. Furthermore, epigenetic changes have also been observed in drug-resistant HCC tumors. In such cases, combinatorial treatment of epi-drugs with systemic therapy or trans-arterial chemoembolization might re-sensitize resistant cells.

Key Words: Hepatocellular carcinoma; Diagnosis; Treatment; Epigenetics; Epi-drugs; Drug resistance

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Core Tip: This review article focuses on the limitations of diagnosis and treatment of hepatocellular carcinoma (HCC). Furthermore, the use of omics technology with clinical attributes for categorizing HCC patients in order that personalized treatment can be designed to prolong survival is discussed. Finally, the potential of epi-drugs in targeting epigenetic changes in the disease and resistance has been proposed.

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INTRODUCTION

Liver cancer ranks sixth in cancer incidence globally and accounts for 8.2% of total cancer deaths. The different categories of primary liver cancer are intrahepatic cholangiocarcinoma, hepatocellular carcinoma (HCC), fibrolamellar carcinoma, and hepatoblastoma. These categories have distinct changes in their molecular, histological, and pathological features. HCC alone accounts for 85%-90% of liver cancer cases[1]. Almost 2/3 of the population affected by HCC is found in east Asian and south-east Asian countries, making this disease endemic to the region[2]. Globally, 5-year median survival is below 20% for HCC[3]. Major risk factors for HCC include chronic infection with hepatitis B virus and hepatitis C virus, excessive consumption of alcohol, exposure to aflatoxin, physiological state such as non-alcoholic fatty liver disease, and diabetes[4]. According to the Barcelona Clinic Cancer Liver Classification (BCLC) algorithm, curative care for HCC involves tumor resection, ablation, and liver transplantation[5]. However, this mode of treatment is offered to patients diagnosed in an early stage of the disease. Current research suggests that only 20% of patients are diagnosed in the early stage[6]. The lacunae in diagnosis are the unavailability of promising liquid-based biomarkers and detection limits of scanning techniques. Palliative care involving chemo/radiation-based treatment is given to patients with intermediate and advanced stage disease. Following this, 70% of patients come back with a relapse of disease and suffer treatment side effects[7,8].

A new approach should be considered to identify diagnostic markers and achieve better therapy response to overcome disease management challenges. Recent advances in the omics field shed light on the pathogenesis and molecular classification of HCC [9-11]. The omics approach can help to investigate new markers to improve the therapeutic outcome. Liver carcinogenesis involves both genetic and epigenetic changes. It is impossible to target all genetic variations due to tumor heterogeneity, but gene signature can be manipulated as epigenetic changes are reversible[12]. Therefore, epi-drug-based treatment may act as an alternate treatment strategy instead of targeting a single protein or molecular pathway. Epi-drugs can be beneficial not only for the treatment of HCC but also for dealing with cancer resistance[13,14].

This article focuses on the existing approach for diagnosis and treatment in the management of HCC. We also review transcriptomic-based signatures of HCC for patient sub-categorization and their potential implications for diagnosis and therapy. Finally, we propose an epi-drug based treatment strategy based on the epigenetic landscape of HCC.

DIAGNOSIS OF LIVER CANCER

Five standard WHO-approved guidelines include the European Association for the Study of Liver Disease (EASL)[15], American Association for the Study of Liver Diseases (AASLD)[16], Asia-Pacific Association Study of the Liver[17], EASL-EORTC Clinical Practice Guidelines[18], and the updated AASLD guidelines are used for diagnosis of liver cancer. The diagnosis is primarily based on imaging techniques such

as ultrasound, computed tomography (CT) scan, and conventional magnetic resonance imaging (MRI)[19]. Invasive biopsies are not helpful for the diagnosis of liver tumors. The myriad risk factors involved in biopsy are the local spread of HCC along the needle track and different complications observed in individual patients[20]. The early-stage diagnosis of HCC continues to be crucial due to reduced sensitivity and specificity of the diagnostic methods, due to which an ample number of tumors are undetected. The complete list of diagnostic methods with detection limits is shown in Table 1. The various factors responsible for undetectable tumors involve a lack of specific markers and asymptomatic condition during the early stages of HCC[21]. Thus, the diagnosis of tumor occurs when it has spread and has reached an advanced stage.

The diagnostic marker used most frequently is serum α-fetoprotein (AFP)[22]. AFP level increases beyond 20 ng/mL in more than 70% of patients with HCC. However, AFP elevations are not explicitly associated with HCC as AFP levels from 10-500 ng/mL and even occasionally to 1000 ng/mL may be seen in patients with a high degree of necro-inflammatory activity such as chronic viral hepatitis[23]. Chan *et al*[24] in 2008 have shown that AFP could be better used as a prognostic marker to evaluate response to treatment and detection of recurrence instead of diagnosis[25]. Studies have shown that multiple combinations of markers provide more appropriate results in diagnosis than a single marker. A recent study investigated the use of HSP90α (heat shock protein 90) combined with AFP and thymidine kinase 1 to diagnose HCC with more efficiency[26]. A study from Beijing YouAn Hospital found that for early diagnosis of HBV-related HCC, a combination of AFP, GPC3, and GP73 had the highest diagnostic value[27]. Ghosh *et al*[28] have shown that the exosome encapsulated microRNAs could be used as a circulating diagnostic marker for HCC with low AFP levels.

Another marker, α-L-fucosidase (AFU), is expressed in liver cirrhosis patients[29]. However, limited research is available regarding the utility of AFU in the diagnosis of HCC. In the liver and gallbladder, cell membrane protein 5′-nucleotidase (5′-NT) is released into the blood during hepatic injury or obstruction[30]. It has been observed that 5′-NT levels also increase with age and during pregnancy[31]. Other markers such as AFP-L3, glypican-3, and des-γ-carboxy prothrombin also show inconsistent data due to low sensitivity and specificity. Hence, the discovery of putative liquid biomarkers is required, which can associate with tumor progression, recurrence, and effectiveness of therapeutic programs.

TREATMENT REGIME AND LIMITATIONS OF CHEMOTHERAPY IN LIVER CANCER

Treatment of HCC is decided based on different stages of tumor detection[32]. The BCLC algorithm is widely used for treatment as it considers tumor stage, liver function, performance status, and treatment impact (Figure 1). Early-stage cases are treated with surgery, ablation, or liver transplantation. The patients undergoing surgery showed 70% recurrence within five years[33]. The currently used methods for tumor ablation in HCC are percutaneous ethanol injection (PEI) and radiofrequency ablation (RFA). PEI consists of the direct injection of absolute ethanol into HCC nodules[34]. RFA is responsive in tumors > 4 cm in size. It involves necrosis of the tumor using a needle tip electrode that reaches temperatures up to 100°C[35]. Microwave ablation and irreversible electroporation have shown more promising results than tumor removal with PEI[36].

Patients with an intermediate stage having a tumor size greater than 5 cm or multinodular HCC with no vascular invasion are treated with trans-arterial chemoembolization (TACE). TACE is used to obstruct the nutrient supply to the tumor using the occlusion of arterial blood vessels[37]. Chemotherapeutic drugs such as doxorubicin or cisplatin are given during embolization, allowing prolonged exposure of the drug to tumor cells, resulting in tumor reduction. Yeo *et al*[38] showed that the overall response rate for doxorubicin-treated patients was 10.5%. Moreover, doxorubicin alone and combined with PIAF had no significant difference in response rate but showed treatment-associated toxicity in patients. Another study showed that combinatorial treatment of fluorouracil, leucovorin, and oxaliplatin failed to improve survival compared to doxorubicin[39]. In a multicohort study involving patients with unresectable tumors treated with TACE, overall survival (OS) was approximately 26-40 mo, with only 52% of patients achieving treatment benefits[40,41]. In some cases, selective internal radiation therapy is used in patients with intermediate-stage HCC.

Table 1 Utility and detection limits of existing diagnostic measures of hepatocellular carcinoma

Diagnostic methods	Definition/concept	Diagnostic limit/range	Ref.
Contrast-enhanced ultrasound	Inexpensive, non-invasive, first choice for screening HCC; Real time dynamic of blood supply.	Small HCC less than 1 cm	[101]
Multi phasic enhanced computed tomography	3 dimensional reconstructions, high sensitivity	1-2 cm HCC lesion	[102]
Magnetic resonance imaging	High resolution anatomic details, pre-contrast and multi-phasic enhanced 3D; Diffusion weighted imaging-functional imaging	2-3 cm HCC lesion	[103]
Positron emission tomography	Hepatocyte-specific PET tracer, 2-[18F] fluoro-2-deoxy-D-galactose, is used which accumulates in the liver compared with other tissues	Detection of small intrahepatic; HCC lesions	[104]
AFP	Elevated in HCC, non-specific	Range: > 500 ng/mL	[23]
α-L-fucosidase	Expressed in liver cirrhosis	Cut-off: 870 nmol/L	[105]
Des-γ-carboxy prothrombin	Sensitive; Not expressed in other liver disease	Cut-off: 40 mAU/mL	[105, 106]
HSP90α + AFP +TKI	Combination of markers have improved diagnostic value	HSP90- (76.65-144.00); AFP- (5.33-2000.00); TK1- (0.57-2.30)	[26]
AFP, GPC3, and GP73	Useful markers for early diagnosis and prognosis	Upregulated	[27, 107]
microRNA: miR-21, miR-199, and miR-122, miR-23a	Specific for diagnosis of HCC; Extremely sensitive	Cut-off value of ≥ 210	[108, 109]

HCC: Hepatocellular carcinoma; AFP: α-fetoprotein.

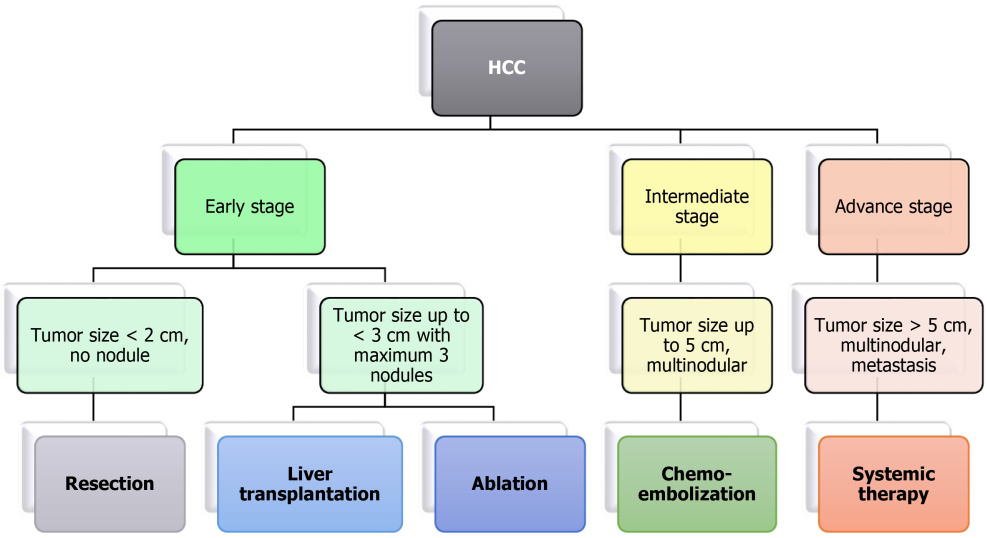

Figure 1 Treatment modalities for hepatocellular carcinoma based on tumor-node-metastasis staging. HCC: Hepatocellular carcinoma.

Intraarterial infusion of radioisotope labeled microspheres is carried out in this modality. Another radiation-based technique known as stereotactic body radiation is used for patients with > 3 cm of the tumor.

Systemic chemotherapy is given for advanced stages of HCC. NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines) have recommended sorafenib and lenvatinib as first-line systemic therapy for patients with unresectable HCC[42]. Brivanib, sunitinib, erlotinib, and regorafenib are other preferred drugs for late-stage HCC treatment. Kudo *et al*[43] observed that treatment with lenvatinib results in significantly higher OS than sorafenib and improvement in all secondary efficacy endpoints. This trial further results in FDA approval of lenvatinib as the first line of therapy for HCC[43]. Sorafenib and sunitinib are protein kinase inhibitors targeting VEGFR, PDGFR, and the Raf kinase pathway. However, a study suggested that sunitinib had an adverse effect in these patients and had no advantage over sorafenib [44]. Moreover, sorafenib has been extensively explored in the systemic treatment of

advanced stage HCC and combination with TACE, but it provided contradictory results[45,46]. Brivanib is an inhibitor of FGF1 and VEGFR2. Phase II clinical trials of brivanib showed the ineffectiveness of the drug compared to sorafenib for improving OS[47,48]. The EGFR inhibitor erlotinib or cetuximab was administered in phase II clinical trials of advanced stages of HCC. However, the trial results did not show the anti-tumor effect of cetuximab in HCC patients[49]. Interestingly, erlotinib showed a positive response in treatment by increasing OS to 13 mo and a response rate of 59% [50].

As discussed earlier, ablation treatment is possible in less than 40% of patients due to late diagnosis, and only 20% are treated with TACE. For the patients with advanced stages of HCC, treatment modalities are limited to systemic therapy, and response rates are also significantly less due to resistance towards available chemotherapy. Multimodal treatment involving more than one therapeutic drug has also failed in different combinations due to cytotoxicity and poor trial outcomes. Despite the significant research in targeted therapy of HCC management, a promising drug is yet to be identified. Thus, the hunt for combinatorial treatment with different therapeutic agents continues (Figure 2).

MOLECULAR LANDSCAPE OF LIVER TUMOR TISSUE FOR PATIENT STRATIFICATION AND IDENTIFICATION OF ALTERNATE TARGETS

Over the past years, HCC classification has mainly focused on histological analysis of tumor tissues. However, the molecular profile and clinical attributes have a significant impact on the prognosis of the disease, thereby redefining HCC into several subgroups. Boyault *et al*[51] published molecular classification systems for HCC composed of 6 groups. The groups were based on mutation profile, disease prognosis, and transcription landscape. The first group included patients with hepatitis B infection and low viral load, increased AFP levels, and high IGF2 expression, whereas the second group included patients with a high viral titer and associated microvascular invasion (MVI) and satellitosis. However, the difference in groups 3 and 4 was based on histological parameters. The third group consisted of poorly differentiated tumors with the worst prognosis; on the other hand, group 4 had well-differentiated tumors. Group 5 and 6 had a low proliferation rate and activated Wnt-signaling pathway. Moreover, pathways are differentially activated in different groups. Another group classified HCC into three groups based on histology and expression analysis of the tumor[52]. In this study, the first group showed the presence of satellitosis and MVI. Group 2 had high AFP expression, and the third group consisted of well-differentiated tumors with a low proliferation rate.

Tumor morphology-based classification has been proposed by Murakata *et al*[53]. The nodal status of the tumor was correlated with survival and recurrence of the disease. Moreover, the miRNA profile of HCC patients has been used to classify sorafenib responders[54]. *c-myc* signaling and EB-1 protein were functionally linked with HCC[55]. Similar findings were observed by Lee *et al*[56] in progenitor-like HCC, which correlated with poor prognosis. In another study, HCC progenitor-like signature consisting of CK-19, Ep-CAM, and CD133 was seen by Woo *et al*[57]. Morofuji *et al*[58] identified the gene signature of early recurrent HCC, including ERK1, PKG, Apaf1, and Bcl-X. Furthermore, ERK1 and Bcl-X were identified as genes associated with the poor prognosis of HCC[58]. However, these studies did not consider the survival status of an individual while proposing subtypes.

Jiang *et al*[59] showed that heterogeneity exists in proteomic profiling of paired early-stage HCC patients. The tumors were segregated into three subtypes: S-I, S-II, and S-III. S-I tumors had increased expression of liver-associated functional proteins. In contrast, S-II and S-III had a more proliferative nature due to overexpression of cell-cycle-related proteins. Furthermore, S-III were more aggressive and had a high expression of KRT19 and MMP9, associated with poor prognosis. Gao *et al*[60] sub-grouped 159 HBV infected patients based on survival, tumor thrombus, and multi-omics profile. These sub-groups were classified based on metabolic rewiring, alterations in the microenvironment, and cellular proliferation. Moreover, the study proposed two prognostic markers PYCR2 and ADH1A.

In the past decade, data generated under the TCGA consortium can be used to understand the gene expression profile of patients and obtain correlations with clinical attributes[9]. Machine learning algorithms are necessary to analyze such multivariate data. The molecular alterations obtained from the cancer genome atlas liver hepatocellular carcinoma (TCGA-LIHC) cohort (423 patients) can be explored to predict new

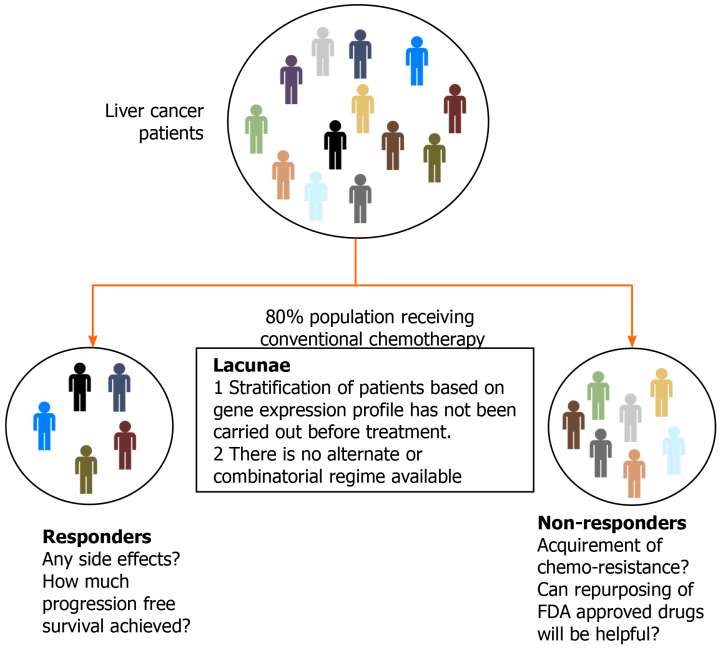

Figure 2 Challenges in the treatment of hepatocellular carcinoma.

targets and rationalize the combinatorial therapy. Transcriptome data generated from TCGA-LIHC identified over 13000 differentially expressed genes compared to cut-margin samples, and around 3330 genes correlated with poor survival (P value < 0.05). Furthermore, 1730 genes overlapped between the DE gene list and genes correlated with patient survival. The majority of overlapped genes showed more than 30% alteration compared to adjacent normal in this cohort and had a significant association with OS. Patients were categorized into different groups using clustering analysis of gene expression. It was observed that these genes belong to metabolism-related pathways and the cellular proliferation-related family (Figure 3). Deep learning computational framework on the TCGA-LIHC dataset suggested that aggressive subtype has TP53 inactivation with high expression of KRT10, EPCAM, and active AKT, WNT signaling[61]. Furthermore, drugs and small molecular compounds are available to target these genes. Schulze *et al*[62] reported that potential gene targets have FDA-approved drugs in 28% of liver tumors. Therefore, these genes can be used for prognosis of the disease, and targeting them may improve patient survival.

Gene expression analysis of liver cancer samples can also be utilized to identify new markers for diagnostic purposes. For example, SPP2 is downregulated at the transcript level in HCC. This gene is deregulated in multiple HCC cohorts. Moreover, a stage-wise decrease at the transcript level was observed in HCC TCGA data. Also, the downregulation of SPP2 leads to a significant decrease in patient survival (Figure 4). This observation indicates that SPP2 level is associated with normal liver function, and a change in levels can be a measure of liver carcinogenesis.

EPI-DRUG BASED TREATMENT FOR IMPROVEMENT OF THERAPEUTIC OUTCOME

The lack of success in disease management can be explained by the multifactorial nature of carcinogenesis involving multiple mutations and global level epigenome alterations[63-65]. Epigenetic changes being reversible can be useful to understand the relationship between tumor biology and help in redefining therapeutic response[12]. Epigenetics deals with changes in gene expression without change in the DNA sequences[66]. Despite all cells having the same DNA sequence, the epigenome decides cell fate regarding differentiation, cell proliferation, and cell death[67,68]. The widely studied epigenetic marks are DNA methylation, histone post-translational modifications, and non-coding RNAs. DNA methylation is the most characterized heritable epigenetic mark. This is where a methyl group is transferred onto the cytosine of the CpG di-nucleotide-rich region in DNA by DNMT enzymes[69]. DNA methylation plays a vital role in gene inactivation, genomic imprinting, attaining

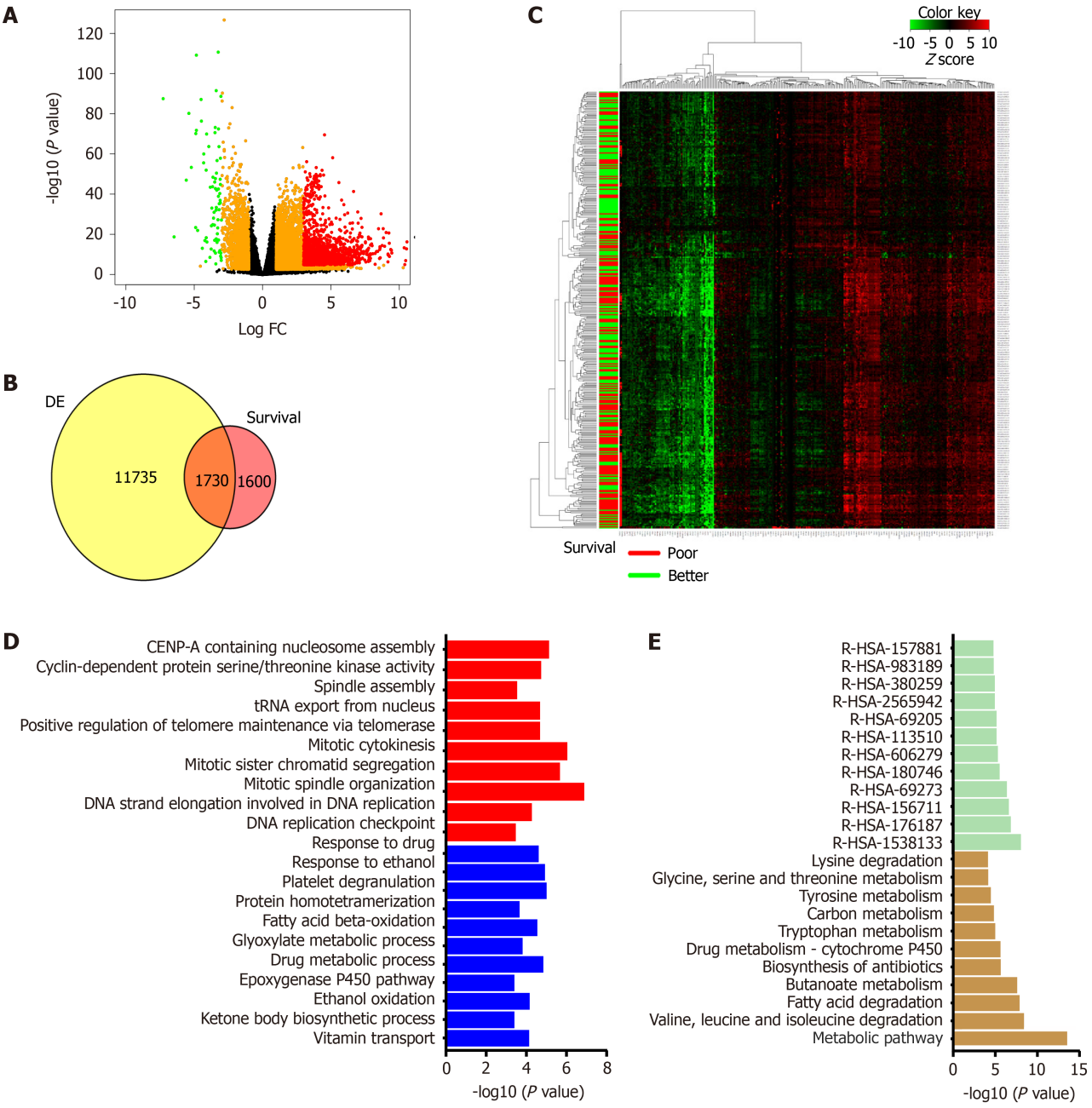

Figure 3 The cancer genome atlas liver hepatocellular carcinoma data analysis. A: Volcano plot representing differential gene expression between 373 tumor samples and 50 normal samples. Genes colored with red or green are most significantly altered; B: Venn diagram showing overlap between differentially expressed gene list and genes affecting survival of patients upon alteration (survival); C: Normalized expression of top 300 genes associated with overall survival represented using heatmap. Patients with overall survival below the median are marked with a red bar while those above the median are marked with a green bar; D: Altered biological process from overlap gene. Upregulated processes highlighted with red and downregulated processes are depicted as blue; E: Pathways analysis for overlap genes. Deregulated KEGG pathways shown by yellow bars and reactome pathways displayed using green bars. DE: Differentially expressed gene list.

tissue-specific gene expression, and X chromosome inactivation[69].

Similar to DNA modification, histone proteins also undergo post-translational modifications carried out by chromatin modifiers, namely writers, readers, and erasers [70]. The well-studied modifications include methylation, acetylation, phosphorylation, and ubiquitination. Histone methylation involves the addition of a methyl group at the lysine or arginine residue on the protruding histone tails. Histone methylation marks can result in repression of transcription or gene activation[71]. A typical example of gene suppression is trimethylation at H3K9, and H3K27 whereas methylation at H3K4, H3K36, and H3K79 enhance transcriptional activity[71]. Histone acetylation is the transfer of an acetyl group from acetyl CoA. This reaction leads to a change in electrostatic interaction between DNA and histones, resulting in the unwinding of chromatin and enhances gene transcription[72]. Histone phosphorylation has an essential role in DNA damage repair, gene transcription, and

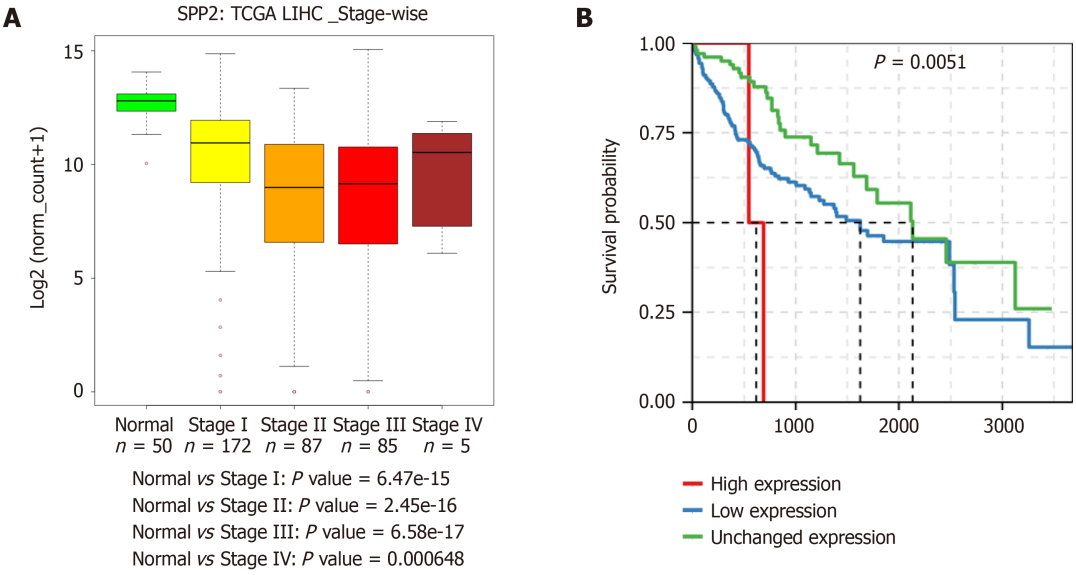

Figure 4 Expression of SPP2 in the cancer genome atlas liver cancer cohort. A: Stage-wise expression of SPP2; B: Patient survival associated with SPP2 expression. TCGA: The cancer genome atlas; LIHC: Liver hepatocellular carcinoma.

chromatin condensation during mitosis[72]. An illustration of chromatin-associated modifications and the role of epigenetic modifiers is shown in Figure 5. Non-coding RNAs are the transcribed intragenic regions of the DNA that are not translated into proteins. These entities govern gene silencing *via* RISC and RNA-induced transcriptional silencing complex formation[73].

Different research groups have extensively studied the epigenetic landscape of liver carcinogenesis. Moreover, in the past few years, researchers are investigating the epigenetic basis of chemoresistance in HCC. Lie *et al*[74] showed that lysine-specific demethylase 1 (LSD1) is upregulated in LGR5+ cells contributing to stemness and chemoresistance properties. Mechanistically, LSD1 removes the H3K4 methylation mark from the promoter of genes which inhibit Wnt-signaling. Thus, promoting pathway activation, which is essential for stemness and chemoresistance[74]. EpCAM+ liver cancer cells have high expression of chromodomain helicase DNA binding protein (CHD4), a DNA damage response protein. The abundance of CHD4 in liver cancer cells leads to epirubicin resistance[75]. Zinc-fingers and homeoboxes 2 (ZHX2) is one of the signature proteins which is downregulated in liver CSCs and is associated with tumor progression. It has been found that low expression of ZHX2 is correlated with epigenetic regulation of OCT4, SOX4, and NANOG by H3K36 methylation[76]. Oriana Lo Re *et al*[77] observed that low expression of MacroH2A1 leads to paracrine mediated chemoresistance and imparts CSCs properties to the tumor cells. Another study showed that the regulator of chromosome condensation 2 promotes metastasis and cisplatin resistance in HCC[78]. Ling *et al*[79] discovered that USP22 helps to attain chemoresistance by hypoxia-driven p53 mutant tumors. Hypoxia-induced expression of carbonyl reductase 1 leading to chemoresistance in HCC was observed by Tak *et al*[80]. H19 long non-coding (lnc)RNA has been shown to sensitize sorafenib or doxorubicin-resistant liver cancer cells[81]. The lncRNA CRNDE has been shown to interact with histone methyltransferase to enhance their effect on the inhibition of tumor suppressors and induce resistance in tumor cells[82].

Epigenetic alterations can be targeted by the class of small-molecule inhibitors that specifically inhibit or reverse the changes[83]. This class of inhibitors are referred to as epi-drugs. Different research groups have synthesized epi-drugs for all three prominent families of epigenetic modifiers- readers, writers, and erasers. Many epi-drugs have cleared pre-clinical trials, and initial phase trials have shown promising results. Few epi-drugs are clinically approved for the treatment of hematological malignancies. In some studies, treatment of solid tumors with an epi-drug helps in sensitizing tumor cells to chemotherapy[84,85]. These findings have promoted the research on inhibitors of HDAC, HAT, and DNMTs in combination with chemotherapeutic drugs. In HCC and gastric cancer, the inactive or suppressed state of tumor suppressor genes (TSGs) is mainly attributed to the overexpression of DNMTs and HDACs, leading to heterochromatinization. Reversion of the chromatin state using epi-drugs further leads to activation of TSGs and prevents tumor growth[86]. Ongoing

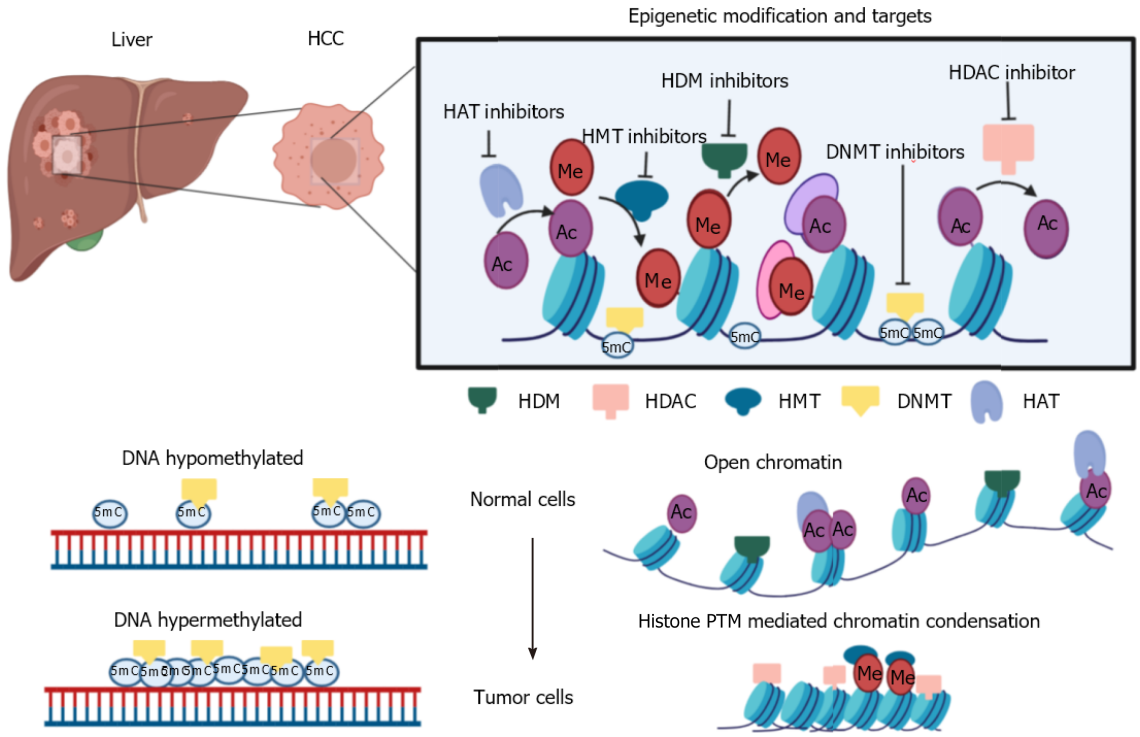

Figure 5 Schematic illustration of epigenetic modifications observed in hepatocellular carcinoma and chromatin modifiers targeted by epi-drugs. The figure represents general epigenetic alterations observed in hepatocellular carcinoma and different epigenetic modifiers that can be targeted *via* small molecule inhibitors. Moreover, DNA and chromatin mediated alterations observed in tumors are highlighted. Changes in DNA methylation and histone post-translational modifications levels inside normal cells lead to tumor formation. HCC: Hepatocellular carcinoma; HDM: Histone demethylase; HDAC: Histone deacetylase; HMT: Histone methyltransferase; DNMT: DNA methyltransferase; HAT: Histone acetyltransferase.

pre-clinical trials have been carried out with HDAC and DNMT inhibitors in combination or in comparison with each other to study the anti-tumor effects of the drugs. Guadecitabine (SGI-110), a DNMT inhibitor with sorafenib and oxaliplatin, is in phase II clinical trials for HCC (NCT01752933). Multicenter phase I/II clinical trials using belinostat (HDAC inhibitor) in patients with unresectable HCC showed a tumor stabilization effect[87]. One study showed that the combination of panobinostat and sorafenib significantly decreased tumor volume by inducing apoptosis in the tumor [88]. A group of researchers observed that the DNMT inhibitor 5'-aza-2' deoxycytidine and HDAC inhibitor SAHA down-regulated DNMT1, DNMT3a, DNMT3b, and HDAC1 and upregulated GSTP1 and SOCS1 gene expression, which further resulted in inhibition of cell viability and induced apoptosis[89]. A detailed list of potential epi-drugs is given in Table 2. These findings indicate the ability of epi-drugs, which can restructure the treatment strategy for HCC.

Future perspectives

The most effective way of controlling HCC is preventing the disease by spreading knowledge of etiological agents and hepatitis B vaccination. An increase in surveillance is one of the strategies to achieve better survival. This practice helps in the early diagnosis of HCC, monitors progression-free survival, and improves quality of life. Diagnosis of HCC at an early stage is crucial in order to start treatment at the right time and improve patient survival. Due to the reduced sensitivity of current diagnostic techniques, ultrasound scanning of high-risk individuals should be carried out every three months. Although ultrasound is cost-effective compared to MRI and CT scans, there is scope for developing more advanced MRI or CT versions to detect small lesions in the liver. Similarly, there is a need for an appropriate combination of liquid biomarkers used for the investigation of liver carcinogenesis. From a treatment perspective, upon early diagnosis, liver transplantation is preferred over surgical removal or ablation as it is has less than 15% chance of recurrence[90].

The primary cause of treatment failure in cancer is resistance to available chemotherapy, which results in relapse. From heterogeneous tumors, cells respond to treatment differently, and a rare small percentage of cells found in the quiescent G0 state of the cell cycle can escape treatment. These cells are inherently resistant to

Table 2 List of Food and Drug Administration approved/under trial epi-drugs

Drugs	Classification	Approved year	Indicated disease	Reference/ clinical trial number
Azacytidine	DNMT inhibitor	2004	MDS	NCT01186939
		2009	AML	NCT00887068
Decitabine	DNMT inhibitor	2006	MDS	NCT01751867
		2011	AML	NCT00260832
Vorinostat	HDAC inhibitor	2006	CTCL	NCT00773747
Romidepsin	HDAC inhibitor	2009	TCL	NCT02296398
Belinostat	HDAC inhibitor	2015	PTCL	NCT01839097
Panobinostat	HDAC inhibitor	2015	MM	NCT01023308
		2016	CML	NCT00451035
		2017	TCL	NCT00490776

MDS: Myelodysplastic syndrome; AML: Acute myeloid leukemia; CTCL: Cutaneous T cell lymphoma; TCL: T-cell lymphoma; PTCL: Peripheral T cell lymphoma; MM: Multiple myeloma; CML: Chronic myeloid leukemia; HDAC: Histone deacetylase; DNMT: DNA methyltransferase.

chemotherapy and involved in relapse. Studies have shown that tumor cells maintain the drug-tolerant state *via* chromatin-mediated changes after drug treatment[13]. The drug-tolerant persister (DTP) stage is reversible; however, prolonged exposure to chemotherapeutic drugs results in stable drug resistance properties[91-93]. DTP cells have non-random differential gene expressions, implicating chromatin-mediated changes leading to hetero-chromatinization of the transposable elements such as LINE1[94]. Recent findings suggest that ablation of the DTP cell population with FDA-approved epi-drugs impedes the development of resistance and relapse[13,94]. Hangauer *et al*[95] have shown DTP cells dependence on mesenchymal state and GPX4 (lipid hydroperoxide) for survival. Furthermore, inhibition of GPX4 triggers cell death of DTP cells *via* the ferroptosis pathway, indicating ferroptosis is required for the survival of DTP cells[95]. Thus, targeting inherently resistant residual cells could be helpful in reducing relapse in patients. However, more research on the identification and characterization of DTP cells is required to choose the appropriate drug combination for treatment purposes.

Targeted drug delivery is the critical factor in improving treatment outcomes and reducing the drug's side effects. Currently, researchers are investigating nanoparticle-mediated drug delivery. In addition, modified liposomal formulation showed a successful therapeutic response in HCC due to tumor-directed delivery and low drug load in the system[96]. Albumin is also a suitable drug-carrier molecule. An albumin-tagged drug has more potent effects compared to the drug alone[97]. Other materials such as dendrimers, micelles, polysaccharides, and silica are also used as carrier molecules[98-100]. Still, the hunt for an effective delivery system continues for targeted delivery.

CONCLUSION

Existing diagnostic methods are inadequate for the early detection of HCC. Similarly, implemented treatment modalities are unsuccessful in improving the survival of patients and result in cytotoxicity in normal cells. The use of credible biomarkers in the prognosis of HCC is essential to reduce mortality due to the disease. In the future, clinicians should focus on patient stratification based on molecular signatures and decide the treatment strategy to achieve maximum therapy outcome. The development of a combinatorial regime consisting of epi-drugs is urgently needed to treat the tumor mass.

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REFERENCES

1 **Sia D**, Villanueva A, Friedman SL, Llovet JM. Liver Cancer Cell of Origin, Molecular Class, and Effects on Patient Prognosis. *Gastroenterology* 2017; **152**: 745-761 [PMID: 28043904 DOI: 10.1053/j.gastro.2016.11.048]

2 **Bray F**, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* 2018; **68**: 394-424 [PMID: 30207593 DOI: 10.3322/caac.21492]

3 **Siegel RL**, Miller KD, Jemal A. Cancer statistics, 2018. *CA Cancer J Clin* 2018; **68**: 7-30 [PMID: 29313949 DOI: 10.3322/caac.21442]

4 **Villanueva A**. Hepatocellular Carcinoma. *N Engl J Med* 2019; **380**: 1450-1462 [PMID: 30970190 DOI: 10.1056/NEJMra1713263]

5 **Llovet JM**, Fuster J, Bruix J; Barcelona-Clínic Liver Cancer Group. The Barcelona approach: diagnosis, staging, and treatment of hepatocellular carcinoma. *Liver Transpl* 2004; **10**: S115-S120 [PMID: 14762851 DOI: 10.1002/Lt.20034]

6 **Farinati F**, Sergio A, Baldan A, Giacomin A, Di Nolfo MA, Del Poggio P, Benvegnu L, Rapaccini G, Zoli M, Borzio F, Giannini EG, Caturelli E, Trevisani F. Early and very early hepatocellular carcinoma: when and how much do staging and choice of treatment really matter? *BMC Cancer* 2009; **9**: 33 [PMID: 19171074 DOI: 10.1186/1471-2407-9-33]

7 **Bruix J**, Sala M, Llovet JM. Chemoembolization for hepatocellular carcinoma. *Gastroenterology* 2004; **127**: S179-S188 [PMID: 15508083 DOI: 10.1053/j.gastro.2004.09.032]

8 **Launay-Vacher V**, Deray G. Hypertension and proteinuria: a class-effect of antiangiogenic therapies. *Anticancer Drugs* 2009; **20**: 81-82 [PMID: 19343005 DOI: 10.1097/CAD.0b013e3283161012]

9 **Cancer Genome Atlas Research Network**. Cancer Genome Atlas Research Network. Comprehensive and Integrative Genomic Characterization of Hepatocellular Carcinoma. *Cell* 2017; **169**: 1327-1341.e23 [PMID: 28622513 DOI: 10.1016/j.cell.2017.05.046]

10 **Walther Z**, Jain D. Molecular pathology of hepatic neoplasms: classification and clinical significance. *Patholog Res Int* 2011; **2011**: 403929 [PMID: 21559202 DOI: 10.4061/2011/403929]

11 **Marquardt JU**, Galle PR, Teufel A. Molecular diagnosis and therapy of hepatocellular carcinoma (HCC): an emerging field for advanced technologies. *J Hepatol* 2012; **56**: 267-275 [PMID: 21782758 DOI: 10.1016/j.jhep.2011.07.007]

12 **Brien GL**, Valerio DG, Armstrong SA. Exploiting the Epigenome to Control Cancer-Promoting Gene-Expression Programs. *Cancer Cell* 2016; **29**: 464-476 [PMID: 27070701 DOI: 10.1016/j.ccell.2016.03.007]

13 **Sharma SV**, Lee DY, Li B, Quinlan MP, Takahashi F, Maheswaran S, McDermott U, Azizian N, Zou L, Fischbach MA, Wong KK, Brandstetter K, Wittner B, Ramaswamy S, Classon M, Settleman J. A chromatin-mediated reversible drug-tolerant state in cancer cell subpopulations. *Cell* 2010; **141**: 69-80 [PMID: 20371346 DOI: 10.1016/j.cell.2010.02.027]

14 **Dalvi MP**, Wang L, Zhong R, Kollipara RK, Park H, Bayo J, Yenerall P, Zhou Y, Timmons BC, Rodriguez-Canales J, Behrens C, Mino B, Villalobos P, Parra ER, Suraokar M, Pataer A, Swisher SG, Kalhor N, Bhanu NV, Garcia BA, Heymach JV, Coombes K, Xie Y, Girard L, Gazdar AF, Kittler R, Wistuba II, Minna JD, Martinez ED. Taxane-Platin-Resistant Lung Cancers Co-develop Hypersensitivity to JumonjiC Demethylase Inhibitors. *Cell Rep* 2017; **19**: 1669-1684 [PMID: 28538184 DOI: 10.1016/j.celrep.2017.04.077]

15 **European Association for the Study of the Liver**. EASL Clinical Practice Guidelines: Management of hepatocellular carcinoma. *J Hepatol* 2018; **69**: 182-236 [PMID: 29628281 DOI: 10.1016/j.jhep.2018.03.019]

16 **Merriman RB**, Tran TT. AASLD practice guidelines: The past, the present, and the future. *Hepatology* 2016; **63**: 31-34 [PMID: 26565582 DOI: 10.1002/hep.28345]

17 **Omata M**, Cheng AL, Kokudo N, Kudo M, Lee JM, Jia J, Tateishi R, Han KH, Chawla YK, Shiina S, Jafri W, Payawal DA, Ohki T, Ogasawara S, Chen PJ, Lesmana CRA, Lesmana LA, Gani RA, Obi S, Dokmeci AK, Sarin SK. Asia-Pacific clinical practice guidelines on the management of hepatocellular carcinoma: a 2017 update. *Hepatol Int* 2017; **11**: 317-370 [PMID: 28620797 DOI: 10.1007/s12072-017-9799-9]

18 **European Association for the Study of the Liver**; European Organisation for Research and Treatment of Cancer. EASL-EORTC clinical practice guidelines: management of hepatocellular carcinoma. *J Hepatol* 2012; **56**: 908-943 [PMID: 22424438 DOI: 10.1016/j.jhep.2011.12.001]

19 **Bargellini I**, Battaglia V, Bozzi E, Lauretti DL, Lorenzoni G, Bartolozzi C. Radiological diagnosis of hepatocellular carcinoma. *J Hepatocell Carcinoma* 2014; **1**: 137-148 [PMID: 27508183 DOI: 10.2147/JHC.S44379]

20 **Di Tommaso L**, Spadaccini M, Donadon M, Personeni N, Elamin A, Aghemo A, Lleo A. Role of liver biopsy in hepatocellular carcinoma. *World J Gastroenterol* 2019; **25**: 6041-6052 [PMID: 31686761 DOI: 10.3748/wjg.v25.i40.6041]

21 **Dimitroulis D**, Damaskos C, Valsami S, Davakis S, Garmpis N, Spartalis E, Athanasiou A, Moris

D, Sakellariou S, Kykalos S, Tsourouflis G, Garmpi A, Delladetsima I, Kontzoglou K, Kouraklis G. From diagnosis to treatment of hepatocellular carcinoma: An epidemic problem for both developed and developing world. *World J Gastroenterol* 2017; **23**: 5282-5294 [PMID: 28839428 DOI: 10.3748/wjg.v23.i29.5282]

22 **Galle PR**, Foerster F, Kudo M, Chan SL, Llovet JM, Qin S, Schelman WR, Chintharlapalli S, Abada PB, Sherman M, Zhu AX. Biology and significance of alpha-fetoprotein in hepatocellular carcinoma. *Liver Int* 2019; **39**: 2214-2229 [PMID: 31436873 DOI: 10.1111/liv.14223]

23 **Johnson PJ**. The role of serum alpha-fetoprotein estimation in the diagnosis and management of hepatocellular carcinoma. *Clin Liver Dis* 2001; **5**: 145-159 [PMID: 11218912 DOI: 10.1016/s1089-3261(05)70158-6]

24 **Chan SL**, Mo FK, Johnson PJ, Hui EP, Ma BB, Ho WM, Lam KC, Chan AT, Mok TS, Yeo W. New utility of an old marker: serial alpha-fetoprotein measurement in predicting radiologic response and survival of patients with hepatocellular carcinoma undergoing systemic chemotherapy. *J Clin Oncol* 2009; **27**: 446-452 [PMID: 19064965 DOI: 10.1200/JCO.2008.18.8151]

25 **Merani S**, Majno P, Kneteman NM, Berney T, Morel P, Mentha G, Toso C. The impact of waiting list alpha-fetoprotein changes on the outcome of liver transplant for hepatocellular carcinoma. *J Hepatol* 2011; **55**: 814-819 [PMID: 21334400 DOI: 10.1016/j.jhep.2010.12.040]

26 **Tang Y**, Li K, Cai Z, Xie Y, Tan X, Su C, Li J. HSP90α combined with AFP and TK1 improved the diagnostic value for hepatocellular carcinoma. *Biomark Med* 2020; **14**: 869-878 [PMID: 32490681 DOI: 10.2217/bmm-2019-0484]

27 **Wu M**, Liu Z, Li X, Zhang A, Li N. Dynamic Changes in Serum Markers and Their Utility in the Early Diagnosis of All Stages of Hepatitis B-Associated Hepatocellular Carcinoma. *Onco Targets Ther* 2020; **13**: 827-840 [PMID: 32095079 DOI: 10.2147/OTT.S229835]

28 **Ghosh S**, Bhowmik S, Majumdar S, Goswami A, Chakraborty J, Gupta S, Aggarwal S, Ray S, Chatterjee R, Bhattacharyya S, Dutta M, Datta S, Chowdhury A, Dhali GK, Banerjee S. The exosome encapsulated microRNAs as circulating diagnostic marker for hepatocellular carcinoma with low alpha-fetoprotein. *Int J Cancer* 2020; **147**: 2934-2947 [PMID: 32441313 DOI: 10.1002/ijc.33111]

29 **Fawzy Montaser M**, Amin Sakr M, Omar Khalifa M. Alpha-L-fucosidase as a tumour marker of hepatocellular carcinoma. *Arab J Gastroenterol* 2012; **13**: 9-13 [PMID: 22560818 DOI: 10.1016/j.ajg.2012.03.006]

30 **Hyder MA**, Hasan M, Mohieldein A. Comparative Study of 5'-Nucleotidase Test in Various Liver Diseases. *J Clin Diagn Res* 2016; **10**: BC01-BC03 [PMID: 27042442 DOI: 10.7860/JCDR/2016/12754.7163]

31 **Mikolasevic I**, Filipec-Kanizaj T, Jakopcic I, Majurec I, Brncic-Fischer A, Sobocan N, Hrstic I, Stimac T, Stimac D, Milic S. Liver Disease During Pregnancy: A Challenging Clinical Issue. *Med Sci Monit* 2018; **24**: 4080-4090 [PMID: 29905165 DOI: 10.12659/MSM.907723]

32 **Bruix J**, Sherman M; Practice Guidelines Committee, American Association for the Study of Liver Diseases. Management of hepatocellular carcinoma. *Hepatology* 2005; **42**: 1208-1236 [PMID: 16250051 DOI: 10.1002/hep.20933]

33 **Ishizawa T**, Hasegawa K, Aoki T, Takahashi M, Inoue Y, Sano K, Imamura H, Sugawara Y, Kokudo N, Makuuchi M. Neither multiple tumors nor portal hypertension are surgical contraindications for hepatocellular carcinoma. *Gastroenterology* 2008; **134**: 1908-1916 [PMID: 18549877 DOI: 10.1053/j.gastro.2008.02.091]

34 **Zuo CJ**, Wang PJ, Shao CW, Wang MJ, Tian JM, Xiao Y, Ren FY, Hao XY, Yuan M. CT-guided percutaneous ethanol injection with disposable curved needle for treatment of malignant liver neoplasms and their metastases in retroperitoneal lymph nodes. *World J Gastroenterol* 2004; **10**: 58-61 [PMID: 14695769 DOI: 10.3748/wjg.v10.i1.58]

35 **Sharma A**, Moore WH, Lanuti M, Shepard JA. How I do it: radiofrequency ablation and cryoablation of lung tumors. *J Thorac Imaging* 2011; **26**: 162-174 [PMID: 21508737 DOI: 10.1097/RTI.0b013e3182171b75]

36 **Yu H**, Burke CT. Comparison of percutaneous ablation technologies in the treatment of malignant liver tumors. *Semin Intervent Radiol* 2014; **31**: 129-137 [PMID: 25071303 DOI: 10.1055/s-0034-1373788]

37 **Lencioni R**, Petruzzi P, Crocetti L. Chemoembolization of hepatocellular carcinoma. *Semin Intervent Radiol* 2013; **30**: 3-11 [PMID: 24436512 DOI: 10.1055/s-0033-1333648]

38 **Yeo W**, Mok TS, Zee B, Leung TW, Lai PB, Lau WY, Koh J, Mo FK, Yu SC, Chan AT, Hui P, Ma B, Lam KC, Ho WM, Wong HT, Tang A, Johnson PJ. A randomized phase III study of doxorubicin *vs* cisplatin/interferon alpha-2b/doxorubicin/fluorouracil (PIAF) combination chemotherapy for unresectable hepatocellular carcinoma. *J Natl Cancer Inst* 2005; **97**: 1532-1538 [PMID: 16234567 DOI: 10.1093/jnci/dji315]

39 **Qin S**, Bai Y, Lim HY, Thongprasert S, Chao Y, Fan J, Yang TS, Bhudhisawasdi V, Kang WK, Zhou Y, Lee JH, Sun Y. Randomized, multicenter, open-label study of oxaliplatin plus fluorouracil/Leucovorin *vs* doxorubicin as palliative chemotherapy in patients with advanced hepatocellular carcinoma from Asia. *J Clin Oncol* 2013; **31**: 3501-3508 [PMID: 23980077 DOI: 10.1200/JCO.2012.44.5643]

40 **Lencioni R**, de Baere T, Soulen MC, Rilling WS, Geschwind JF. Lipiodol transarterial chemoembolization for hepatocellular carcinoma: A systematic review of efficacy and safety data. *Hepatology* 2016; **64**: 106-116 [PMID: 26765068 DOI: 10.1002/hep.28453]

41 **Kudo M**, Han G, Finn RS, Poon RT, Blanc JF, Yan L, Yang J, Lu L, Tak WY, Yu X, Lee JH, Lin SM, Wu C, Tanwandee T, Shao G, Walters IB, Dela Cruz C, Poulart V, Wang JH. Brivanib as adjuvant therapy to transarterial chemoembolization in patients with hepatocellular carcinoma: A randomized phase III trial. *Hepatology* 2014; **60**: 1697-1707 [PMID: 24996197 DOI: 10.1002/hep.27290]

42 **Benson AB**, D'Angelica MI, Abbott DE, Abrams TA, Alberts SR, Anaya DA, Anders R, Are C, Brown D, Chang DT, Cloyd J, Covey AM, Hawkins W, Iyer R, Jacob R, Karachristos A, Kelley RK, Kim R, Palta M, Park JO, Sahai V, Schefter T, Sicklick JK, Singh G, Sohal D, Stein S, Tian GG, Vauthey JN, Venook AP, Hammond LJ, Darlow SD. Guidelines Insights: Hepatobiliary Cancers, Version 2.2019. *J Natl Compr Canc Netw* 2019; **17**: 302-310 [PMID: 30959462 DOI: 10.6004/jnccn.2019.0019]

43 **Kudo M**, Finn RS, Qin S, Han KH, Ikeda K, Piscaglia F, Baron A, Park JW, Han G, Jassem J, Blanc JF, Vogel A, Komov D, Evans TRJ, Lopez C, Dutcus C, Guo M, Saito K, Kraljevic S, Tamai T, Ren M, Cheng AL. Lenvatinib *vs* sorafenib in first-line treatment of patients with unresectable hepatocellular carcinoma: a randomised phase 3 non-inferiority trial. *Lancet* 2018; **391**: 1163-1173 [PMID: 29433850 DOI: 10.1016/S0140-6736(18)30207-1]

44 **Cheng AL**, Kang YK, Lin DY, Park JW, Kudo M, Qin S, Chung HC, Song X, Xu J, Poggi G, Omata M, Pitman Lowenthal S, Lanzalone S, Yang L, Lechuga MJ, Raymond E. Sunitinib *vs* sorafenib in advanced hepatocellular cancer: results of a randomized phase III trial. *J Clin Oncol* 2013; **31**: 4067-4075 [PMID: 24081937 DOI: 10.1200/JCO.2012.45.8372]

45 **Park JW**, Kim YJ, Kim DY, Bae SH, Paik SW, Lee YJ, Kim HY, Lee HC, Han SY, Cheong JY, Kwon OS, Yeon JE, Kim BH, Hwang J. Sorafenib with or without concurrent transarterial chemoembolization in patients with advanced hepatocellular carcinoma: The phase III STAH trial. *J Hepatol* 2019; **70**: 684-691 [PMID: 30529387 DOI: 10.1016/j.jhep.2018.11.029]

46 **Zhao Y**, Wang WJ, Guan S, Li HL, Xu RC, Wu JB, Liu JS, Li HP, Bai W, Yin ZX, Fan DM, Zhang ZL, Han GH. Sorafenib combined with transarterial chemoembolization for the treatment of advanced hepatocellular carcinoma: a large-scale multicenter study of 222 patients. *Ann Oncol* 2013; **24**: 1786-1792 [PMID: 23508822 DOI: 10.1093/annonc/mdt072]

47 **Llovet JM**, Decaens T, Raoul JL, Boucher E, Kudo M, Chang C, Kang YK, Assenat E, Lim HY, Boige V, Mathurin P, Fartoux L, Lin DY, Bruix J, Poon RT, Sherman M, Blanc JF, Finn RS, Tak WY, Chao Y, Ezzeddine R, Liu D, Walters I, Park JW. Brivanib in patients with advanced hepatocellular carcinoma who were intolerant to sorafenib or for whom sorafenib failed: results from the randomized phase III BRISK-PS study. *J Clin Oncol* 2013; **31**: 3509-3516 [PMID: 23980090 DOI: 10.1200/JCO.2012.47.3009]

48 **Johnson PJ**, Qin S, Park JW, Poon RT, Raoul JL, Philip PA, Hsu CH, Hu TH, Heo J, Xu J, Lu L, Chao Y, Boucher E, Han KH, Paik SW, Robles-Aviña J, Kudo M, Yan L, Sobhonslidsuk A, Komov D, Decaens T, Tak WY, Jeng LB, Liu D, Ezzeddine R, Walters I, Cheng AL. Brivanib *vs* sorafenib as first-line therapy in patients with unresectable, advanced hepatocellular carcinoma: results from the randomized phase III BRISK-FL study. *J Clin Oncol* 2013; **31**: 3517-3524 [PMID: 23980084 DOI: 10.1200/JCO.2012.48.4410]

49 **Zhu AX**, Stuart K, Blaszkowsky LS, Muzikansky A, Reitberg DP, Clark JW, Enzinger PC, Bhargava P, Meyerhardt JA, Horgan K, Fuchs CS, Ryan DP. Phase 2 study of cetuximab in patients with advanced hepatocellular carcinoma. *Cancer* 2007; **110**: 581-589 [PMID: 17583545 DOI: 10.1002/cncr.22829]

50 **Philip PA**, Mahoney MR, Allmer C, Thomas J, Pitot HC, Kim G, Donehower RC, Fitch T, Picus J, Erlichman C. Phase II study of Erlotinib (OSI-774) in patients with advanced hepatocellular cancer. *J Clin Oncol* 2005; **23**: 6657-6663 [PMID: 16170173 DOI: 10.1200/JCO.2005.14.696]

51 **Boyault S**, Rickman DS, de Reyniès A, Balabaud C, Rebouissou S, Jeannot E, Hérault A, Saric J, Belghiti J, Franco D, Bioulac-Sage P, Laurent-Puig P, Zucman-Rossi J. Transcriptome classification of HCC is related to gene alterations and to new therapeutic targets. *Hepatology* 2007; **45**: 42-52 [PMID: 17187432 DOI: 10.1002/hep.21467]

52 **Hoshida Y**, Nijman SM, Kobayashi M, Chan JA, Brunet JP, Chiang DY, Villanueva A, Newell P, Ikeda K, Hashimoto M, Watanabe G, Gabriel S, Friedman SL, Kumada H, Llovet JM, Golub TR. Integrative transcriptome analysis reveals common molecular subclasses of human hepatocellular carcinoma. *Cancer Res* 2009; **69**: 7385-7392 [PMID: 19723656 DOI: 10.1158/0008-5472.CAN-09-1089]

53 **Murakata A**, Tanaka S, Mogushi K, Yasen M, Noguchi N, Irie T, Kudo A, Nakamura N, Tanaka H, Arii S. Gene expression signature of the gross morphology in hepatocellular carcinoma. *Ann Surg* 2011; **253**: 94-100 [PMID: 21233610 DOI: 10.1097/SLA.0b013e3181f9bc00]

54 **Nishida N**, Arizumi T, Hagiwara S, Ida H, Sakurai T, Kudo M. MicroRNAs for the Prediction of Early Response to Sorafenib Treatment in Human Hepatocellular Carcinoma. *Liver Cancer* 2017; **6**: 113-125 [PMID: 28275578 DOI: 10.1159/000449475]

55 **Orimo T**, Ojima H, Hiraoka N, Saito S, Kosuge T, Kakisaka T, Yokoo H, Nakanishi K, Kamiyama T, Todo S, Hirohashi S, Kondo T. Proteomic profiling reveals the prognostic value of adenomatous polyposis coli-end-binding protein 1 in hepatocellular carcinoma. *Hepatology* 2008; **48**: 1851-1863 [PMID: 18937283 DOI: 10.1002/hep.22552]

56 **Lee JS**, Heo J, Libbrecht L, Chu IS, Kaposi-Novak P, Calvisi DF, Mikaelyan A, Roberts LR, Demetris AJ, Sun Z, Nevens F, Roskams T, Thorgeirsson SS. A novel prognostic subtype of human hepatocellular carcinoma derived from hepatic progenitor cells. *Nat Med* 2006; **12**: 410-416 [PMID:

16532004 DOI: 10.1038/nm1377]
57 **Woo HG**, Lee JH, Yoon JH, Kim CY, Lee HS, Jang JJ, Yi NJ, Suh KS, Lee KU, Park ES, Thorgeirsson SS, Kim YJ. Identification of a cholangiocarcinoma-like gene expression trait in hepatocellular carcinoma. *Cancer Res* 2010; **70**: 3034-3041 [PMID: 20395200 DOI: 10.1158/0008-5472.CAN-09-2823]
58 **Morofuji N**, Ojima H, Hiraoka N, Okusaka T, Esaki M, Nara S, Shimada K, Kishi Y, Kondo T. Antibody-based proteomics to identify an apoptosis signature for early recurrence of hepatocellular carcinoma. *Clin Proteomics* 2016; **13**: 28 [PMID: 27799868 DOI: 10.1186/s12014-016-9130-0]
59 **Jiang Y**, Sun A, Zhao Y, Ying W, Sun H, Yang X, Xing B, Sun W, Ren L, Hu B, Li C, Zhang L, Qin G, Zhang M, Chen N, Huang Y, Zhou J, Liu M, Zhu X, Qiu Y, Sun Y, Huang C, Yan M, Wang M, Liu W, Tian F, Xu H, Wu Z, Shi T, Zhu W, Qin J, Xie L, Fan J, Qian X, He F; Chinese Human Proteome Project (CNHPP) Consortium. Proteomics identifies new therapeutic targets of early-stage hepatocellular carcinoma. *Nature* 2019; **567**: 257-261 [PMID: 30814741 DOI: 10.1038/s41586-019-0987-8]
60 **Gao Q**, Zhu H, Dong L, Shi W, Chen R, Song Z, Huang C, Li J, Dong X, Zhou Y, Liu Q, Ma L, Wang X, Zhou J, Liu Y, Boja E, Robles AI, Ma W, Wang P, Li Y, Ding L, Wen B, Zhang B, Rodriguez H, Gao D, Zhou H, Fan J. Integrated Proteogenomic Characterization of HBV-Related Hepatocellular Carcinoma. *Cell* 2019; **179**: 561-577.e22 [PMID: 31585088 DOI: 10.1016/j.cell.2019.08.052]
61 **Chaudhary K**, Poirion OB, Lu L, Garmire LX. Deep Learning-Based Multi-Omics Integration Robustly Predicts Survival in Liver Cancer. *Clin Cancer Res* 2018; **24**: 1248-1259 [PMID: 28982688 DOI: 10.1158/1078-0432.CCR-17-0853]
62 **Schulze K**, Imbeaud S, Letouzé E, Alexandrov LB, Calderaro J, Rebouissou S, Couchy G, Meiller C, Shinde J, Soysouvanh F, Calatayud AL, Pinyol R, Pelletier L, Balabaud C, Laurent A, Blanc JF, Mazzaferro V, Calvo F, Villanueva A, Nault JC, Bioulac-Sage P, Stratton MR, Llovet JM, Zucman-Rossi J. Exome sequencing of hepatocellular carcinomas identifies new mutational signatures and potential therapeutic targets. *Nat Genet* 2015; **47**: 505-511 [PMID: 25822088 DOI: 10.1038/ng.3252]
63 **Sharma S**, Kelly TK, Jones PA. Epigenetics in cancer. *Carcinogenesis* 2010; **31**: 27-36 [PMID: 19752007 DOI: 10.1093/carcin/bgp220]
64 **Herceg Z**, Hainaut P. Genetic and epigenetic alterations as biomarkers for cancer detection, diagnosis and prognosis. *Mol Oncol* 2007; **1**: 26-41 [PMID: 19383285 DOI: 10.1016/j.molonc.2007.01.004]
65 **Shah MA**, Denton EL, Arrowsmith CH, Lupien M, Schapira M. A global assessment of cancer genomic alterations in epigenetic mechanisms. *Epigenetics Chromatin* 2014; **7**: 29 [PMID: 25484917 DOI: 10.1186/1756-8935-7-29]
66 **Weinhold B**. Epigenetics: the science of change. *Environ Health Perspect* 2006; **114**: A160-A167 [PMID: 16507447 DOI: 10.1289/ehp.114-a160]
67 **Kanherkar RR**, Bhatia-Dey N, Csoka AB. Epigenetics across the human lifespan. *Front Cell Dev Biol* 2014; **2**: 49 [PMID: 25364756 DOI: 10.3389/fcell.2014.00049]
68 **Srinageshwar B**, Maiti P, Dunbar GL, Rossignol J. Role of Epigenetics in Stem Cell Proliferation and Differentiation: Implications for Treating Neurodegenerative Diseases. *Int J Mol Sci* 2016; **17** [PMID: 26848657 DOI: 10.3390/ijms17020199]
69 **Moore LD**, Le T, Fan G. DNA methylation and its basic function. *Neuropsychopharmacology* 2013; **38**: 23-38 [PMID: 22781841 DOI: 10.1038/npp.2012.112]
70 **Gillette TG**, Hill JA. Readers, writers, and erasers: chromatin as the whiteboard of heart disease. *Circ Res* 2015; **116**: 1245-1253 [PMID: 25814685 DOI: 10.1161/CIRCRESAHA.116.303630]
71 **Greer EL**, Shi Y. Histone methylation: a dynamic mark in health, disease and inheritance. *Nat Rev Genet* 2012; **13**: 343-357 [PMID: 22473383 DOI: 10.1038/nrg3173]
72 **Bannister AJ**, Kouzarides T. Regulation of chromatin by histone modifications. *Cell Res* 2011; **21**: 381-395 [PMID: 21321607 DOI: 10.1038/cr.2011.22]
73 **Shimada Y**, Mohn F, Bühler M. The RNA-induced transcriptional silencing complex targets chromatin exclusively *via* interacting with nascent transcripts. *Genes Dev* 2016; **30**: 2571-2580 [PMID: 27941123 DOI: 10.1101/gad.292599.116]
74 **Lei ZJ**, Wang J, Xiao HL, Guo Y, Wang T, Li Q, Liu L, Luo X, Fan LL, Lin L, Mao CY, Wang SN, Wei YL, Lan CH, Jiang J, Yang XJ, Liu PD, Chen DF, Wang B. Lysine-specific demethylase 1 promotes the stemness and chemoresistance of Lgr5+ liver cancer initiating cells by suppressing negative regulators of β-catenin signaling. *Oncogene* 2015; **34**: 3214 [PMID: 26062983 DOI: 10.1038/onc.2015.182]
75 **Nio K**, Yamashita T, Okada H, Kondo M, Hayashi T, Hara Y, Nomura Y, Zeng SS, Yoshida M, Sunagozaka H, Oishi N, Honda M, Kaneko S. Defeating EpCAM(+) liver cancer stem cells by targeting chromatin remodeling enzyme CHD4 in human hepatocellular carcinoma. *J Hepatol* 2015; **63**: 1164-1172 [PMID: 26095183 DOI: 10.1016/j.jhep.2015.06.009]
76 **Lin Q**, Wu Z, Yue X, Yu X, Wang Z, Song X, Xu L, He Y, Ge Y, Tan S, Wang T, Song H, Yuan D, Gong Y, Gao L, Liang X, Ma C. ZHX2 restricts hepatocellular carcinoma by suppressing stem cell-like traits through KDM2A-mediated H3K36 demethylation. *EBioMedicine* 2020; **53**: 102676 [PMID: 32114388 DOI: 10.1016/j.ebiom.2020.102676]
77 **Lo Re O**, Mazza T, Giallongo S, Sanna P, Rappa F, Vinh Luong T, Li Volti G, Drovakova A, Roskams T, Van Haele M, Tsochatzis E, Vinciguerra M. Loss of histone macroH2A1 in

hepatocellular carcinoma cells promotes paracrine-mediated chemoresistance and CD4$^+$CD25$^+$FoxP3$^+$ regulatory T cells activation. *Theranostics* 2020; **10**: 910-924 [PMID: 31903159 DOI: 10.7150/thno.35045]

78 **Chen Q**, Jiang P, Jia B, Liu Y, Zhang Z. RCC2 contributes to tumor invasion and chemoresistance to cisplatin in hepatocellular carcinoma. *Hum Cell* 2020; **33**: 709-720 [PMID: 32239438 DOI: 10.1007/s13577-020-00353-7]

79 **Ling S**, Shan Q, Zhan Q, Ye Q, Liu P, Xu S, He X, Ma J, Xiang J, Jiang G, Wen X, Feng Z, Wu Y, Feng T, Xu L, Chen K, Zhang X, Wei R, Zhang C, Cen B, Xie H, Song P, Liu J, Zheng S, Xu X. USP22 promotes hypoxia-induced hepatocellular carcinoma stemness by a HIF1α/USP22 positive feedback loop upon TP53 inactivation. *Gut* 2020; **69**: 1322-1334 [PMID: 31776228 DOI: 10.1136/gutjnl-2019-319616]

80 **Tak E**, Lee S, Lee J, Rashid MA, Kim YW, Park JH, Park WS, Shokat KM, Ha J, Kim SS. Human carbonyl reductase 1 upregulated by hypoxia renders resistance to apoptosis in hepatocellular carcinoma cells. *J Hepatol* 2011; **54**: 328-339 [PMID: 21056497 DOI: 10.1016/j.jhep.2010.06.045]

81 **Schultheiss CS**, Laggai S, Czepukojc B, Hussein UK, List M, Barghash A, Tierling S, Hosseini K, Golob-Schwarzl N, Pokorny J, Hachenthal N, Schulz M, Helms V, Walter J, Zimmer V, Lammert F, Bohle RM, Dandolo L, Haybaeck J, Kiemer AK, Kessler SM. The long non-coding RNA *H19* suppresses carcinogenesis and chemoresistance in hepatocellular carcinoma. *Cell Stress* 2017; **1**: 37-54 [PMID: 31225433 DOI: 10.15698/cst2017.10.105]

82 **Xie SC**, Zhang JQ, Jiang XL, Hua YY, Xie SW, Qin YA, Yang YJ. LncRNA CRNDE facilitates epigenetic suppression of CELF2 and LATS2 to promote proliferation, migration and chemoresistance in hepatocellular carcinoma. *Cell Death Dis* 2020; **11**: 676 [PMID: 32826865 DOI: 10.1038/s41419-020-02853-8]

83 **Berdasco M**, Esteller M. Clinical epigenetics: seizing opportunities for translation. *Nat Rev Genet* 2019; **20**: 109-127 [PMID: 30479381 DOI: 10.1038/s41576-018-0074-2]

84 **Italiano A**, Soria JC, Toulmonde M, Michot JM, Lucchesi C, Varga A, Coindre JM, Blakemore SJ, Clawson A, Suttle B, McDonald AA, Woodruff M, Ribich S, Hedrick E, Keilhack H, Thomson B, Owa T, Copeland RA, Ho PTC, Ribrag V. Tazemetostat, an EZH2 inhibitor, in relapsed or refractory B-cell non-Hodgkin lymphoma and advanced solid tumours: a first-in-human, open-label, phase 1 study. *Lancet Oncol* 2018; **19**: 649-659 [PMID: 29650362 DOI: 10.1016/S1470-2045(18)30145-1]

85 **Li J**, Hao D, Wang L, Wang H, Wang Y, Zhao Z, Li P, Deng C, Di LJ. Epigenetic targeting drugs potentiate chemotherapeutic effects in solid tumor therapy. *Sci Rep* 2017; **7**: 4035 [PMID: 28642588 DOI: 10.1038/s41598-017-04406-0]

86 **Amnekar RV**, Khan SA, Rashid M, Khade B, Thorat R, Gera P, Shrikhande SV, Smoot DT, Ashktorab H, Gupta S. Histone deacetylase inhibitor pre-treatment enhances the efficacy of DNA-interacting chemotherapeutic drugs in gastric cancer. *World J Gastroenterol* 2020; **26**: 598-613 [PMID: 32103870 DOI: 10.3748/wjg.v26.i6.598]

87 **Yeo W**, Chung HC, Chan SL, Wang LZ, Lim R, Picus J, Boyer M, Mo FK, Koh J, Rha SY, Hui EP, Jeung HC, Roh JK, Yu SC, To KF, Tao Q, Ma BB, Chan AW, Tong JH, Erlichman C, Chan AT, Goh BC. Epigenetic therapy using belinostat for patients with unresectable hepatocellular carcinoma: a multicenter phase I/II study with biomarker and pharmacokinetic analysis of tumors from patients in the Mayo Phase II Consortium and the Cancer Therapeutics Research Group. *J Clin Oncol* 2012; **30**: 3361-3367 [PMID: 22915658 DOI: 10.1200/JCO.2011.41.2395]

88 **Lachenmayer A**, Toffanin S, Cabellos L, Alsinet C, Hoshida Y, Villanueva A, Minguez B, Tsai HW, Ward SC, Thung S, Friedman SL, Llovet JM. Combination therapy for hepatocellular carcinoma: additive preclinical efficacy of the HDAC inhibitor panobinostat with sorafenib. *J Hepatol* 2012; **56**: 1343-1350 [PMID: 22322234 DOI: 10.1016/j.jhep.2012.01.009]

89 **Sanaei M**, Kavoosi F, Esmi Z. The Effect of 5-Aza-2'-Deoxycytidine in Combination to and in Comparison with Vorinostat on DNA Methyltransferases, Histone Deacetylase 1, Glutathione S-Transferase 1 and Suppressor of Cytokine Signaling 1 Genes Expression, Cell Growth Inhibition and Apoptotic Induction in Hepatocellular LCL-PI 11 Cell Line. *Int J Hematol Oncol Stem Cell Res* 2020; **14**: 45-55 [PMID: 32337014]

90 **Mehta N**, Heimbach J, Harnois DM, Sapisochin G, Dodge JL, Lee D, Burns JM, Sanchez W, Greig PD, Grant DR, Roberts JP, Yao FY. Validation of a Risk Estimation of Tumor Recurrence After Transplant (RETREAT) Score for Hepatocellular Carcinoma Recurrence After Liver Transplant. *JAMA Oncol* 2017; **3**: 493-500 [PMID: 27838698 DOI: 10.1001/jamaoncol.2016.5116]

91 **Ravindran Menon D**, Das S, Krepler C, Vultur A, Rinner B, Schauer S, Kashofer K, Wagner K, Zhang G, Bonyadi Rad E, Haass NK, Soyer HP, Gabrielli B, Somasundaram R, Hoefler G, Herlyn M, Schaider H. A stress-induced early innate response causes multidrug tolerance in melanoma. *Oncogene* 2015; **34**: 4448-4459 [PMID: 25417704 DOI: 10.1038/onc.2014.372]

92 **Hata AN**, Niederst MJ, Archibald HL, Gomez-Caraballo M, Siddiqui FM, Mulvey HE, Maruvka YE, Ji F, Bhang HE, Krishnamurthy Radhakrishna V, Siravegna G, Hu H, Raoof S, Lockerman E, Kalsy A, Lee D, Keating CL, Ruddy DA, Damon LJ, Crystal AS, Costa C, Piotrowska Z, Bardelli A, Iafrate AJ, Sadreyev RI, Stegmeier F, Getz G, Sequist LV, Faber AC, Engelman JA. Tumor cells can follow distinct evolutionary paths to become resistant to epidermal growth factor receptor inhibition. *Nat Med* 2016; **22**: 262-269 [PMID: 26828195 DOI: 10.1038/nm.4040]

93 **Ramirez M**, Rajaram S, Steininger RJ, Osipchuk D, Roth MA, Morinishi LS, Evans L, Ji W, Hsu CH, Thurley K, Wei S, Zhou A, Koduru PR, Posner BA, Wu LF, Altschuler SJ. Diverse drug-resistance mechanisms can emerge from drug-tolerant cancer persister cells. *Nat Commun* 2016; **7**:

10690 [PMID: 26891683 DOI: 10.1038/ncomms10690]
94 **Guler GD**, Tindell CA, Pitti R, Wilson C, Nichols K, KaiWai Cheung T, Kim HJ, Wongchenko M, Yan Y, Haley B, Cuellar T, Webster J, Alag N, Hegde G, Jackson E, Nance TL, Giresi PG, Chen KB, Liu J, Jhunjhunwala S, Settleman J, Stephan JP, Arnott D, Classon M. Repression of Stress-Induced LINE-1 Expression Protects Cancer Cell Subpopulations from Lethal Drug Exposure. *Cancer Cell* 2017; **32**: 221-237.e13 [PMID: 28781121 DOI: 10.1016/j.ccell.2017.07.002]
95 **Hangauer MJ**, Viswanathan VS, Ryan MJ, Bole D, Eaton JK, Matov A, Galeas J, Dhruv HD, Berens ME, Schreiber SL, McCormick F, McManus MT. Drug-tolerant persister cancer cells are vulnerable to GPX4 inhibition. *Nature* 2017; **551**: 247-250 [PMID: 29088702 DOI: 10.1038/nature24297]
96 **Shah SM**, Goel PN, Jain AS, Pathak PO, Padhye SG, Govindarajan S, Ghosh SS, Chaudhari PR, Gude RP, Gopal V, Nagarsenker MS. Liposomes for targeting hepatocellular carcinoma: use of conjugated arabinogalactan as targeting ligand. *Int J Pharm* 2014; **477**: 128-139 [PMID: 25311181 DOI: 10.1016/j.ijpharm.2014.10.014]
97 **Zhou Q**, Ching AK, Leung WK, Szeto CY, Ho SM, Chan PK, Yuan YF, Lai PB, Yeo W, Wong N. Novel therapeutic potential in targeting microtubules by nanoparticle albumin-bound paclitaxel in hepatocellular carcinoma. *Int J Oncol* 2011; **38**: 721-731 [PMID: 21240457 DOI: 10.3892/ijo.2011.902]
98 **Madaan K**, Kumar S, Poonia N, Lather V, Pandita D. Dendrimers in drug delivery and targeting: Drug-dendrimer interactions and toxicity issues. *J Pharm Bioallied Sci* 2014; **6**: 139-150 [PMID: 25035633 DOI: 10.4103/0975-7406.130965]
99 **Zhang N**, Wardwell PR, Bader RA. Polysaccharide-based micelles for drug delivery. *Pharmaceutics* 2013; **5**: 329-352 [PMID: 24300453 DOI: 10.3390/pharmaceutics5020329]
100 **Bharti C**, Nagaich U, Pal AK, Gulati N. Mesoporous silica nanoparticles in target drug delivery system: A review. *Int J Pharm Investig* 2015; **5**: 124-133 [PMID: 26258053 DOI: 10.4103/2230-973X.160844]
101 **Maida M**, Orlando E, Cammà C, Cabibbo G. Staging systems of hepatocellular carcinoma: a review of literature. *World J Gastroenterol* 2014; **20**: 4141-4150 [PMID: 24764652 DOI: 10.3748/wjg.v20.i15.4141]
102 **Bruix J**, Sherman M; American Association for the Study of Liver Diseases. Management of hepatocellular carcinoma: an update. *Hepatology* 2011; **53**: 1020-1022 [PMID: 21374666 DOI: 10.1002/hep.24199]
103 **Chou R**, Cuevas C, Fu R, Devine B, Wasson N, Ginsburg A, Zakher B, Pappas M, Graham E, Sullivan SD. Imaging Techniques for the Diagnosis of Hepatocellular Carcinoma: A Systematic Review and Meta-analysis. *Ann Intern Med* 2015; **162**: 697-711 [PMID: 25984845 DOI: 10.7326/M14-2509]
104 **Sørensen M**, Frisch K, Bender D, Keiding S. The potential use of 2-[^{18}F]fluoro-2-deoxy-D-galactose as a PET/CT tracer for detection of hepatocellular carcinoma. *Eur J Nucl Med Mol Imaging* 2011; **38**: 1723-1731 [PMID: 21553087 DOI: 10.1007/s00259-011-1831-z]
105 **Bialecki ES**, Di Bisceglie AM. Diagnosis of hepatocellular carcinoma. *HPB (Oxford)* 2005; **7**: 26-34 [PMID: 18333158 DOI: 10.1080/13651820410024049]
106 **Lok AS**, Sterling RK, Everhart JE, Wright EC, Hoefs JC, Di Bisceglie AM, Morgan TR, Kim HY, Lee WM, Bonkovsky HL, Dienstag JL; HALT-C Trial Group. Des-gamma-carboxy prothrombin and alpha-fetoprotein as biomarkers for the early detection of hepatocellular carcinoma. *Gastroenterology* 2010; **138**: 493-502 [PMID: 19852963 DOI: 10.1053/j.gastro.2009.10.031]
107 **El-Saadany S**, El-Demerdash T, Helmy A, Mayah WW, El-Sayed Hussein B, Hassanien M, Elmashad N, Fouad MA, Basha EA. Diagnostic Value of Glypican-3 for Hepatocellular Carcinomas Asian Pac J Cancer Prev 2018; 19: 811-817 [PMID: 29582639 DOI: 10.22034/APJCP.2018.19.3.811]
108 **Elhendawy M**, Abdul-Baki EA, Abd-Elsalam S, Hagras MM, Zidan AA, Abdel-Naby AY, Watny M, Elkabash IA, Salem ML, Elshanshoury M, Soliman S, Abdou S. MicroRNA signature in hepatocellular carcinoma patients: identification of potential markers. *Mol Biol Rep* 2020; **47**: 4945-4953 [PMID: 32430845 DOI: 10.1007/s11033-020-05521-4]
109 **Abdalla MA**, Haj-Ahmad Y. Promising Candidate Urinary MicroRNA Biomarkers for the Early Detection of Hepatocellular Carcinoma among High-Risk Hepatitis C Virus Egyptian Patients. *J Cancer* 2012; **3**: 19-31 [PMID: 22211142 DOI: 10.7150/jca.3.19]

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REVIEW

Heterogeneity of non-alcoholic fatty liver disease: Implications for clinical practice and research activity

Partha Pal, Rajan Palui, Sayantan Ray

ORCID number: Partha Pal 0000-0002-7090-9004; Rajan Palui 0000-0002-2429-3595; Sayantan Ray 0000-0002-6274-465X.

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Partha Pal, Department of Medical Gastroenterology, Asian Institute of Gastroenterology, Hyderabad 500082, India

Rajan Palui, Department of Endocrinology, The Mission Hospital, Durgapur 713212, West Bengal, India

Sayantan Ray, Department of Endocrinology, Jagannath Gupta Institute of Medical Sciences and Hospital, Kolkata 700137, West Bengal, India

Sayantan Ray, Diabetes and Endocrinology, Apollo Clinic, Ballygunge, Kolkata 700019, West Bengal, India

Corresponding author: Sayantan Ray, MBBS, MD, Department of Endocrinology, Jagannath Gupta Institute of Medical Sciences and Hospital, KP Mondal Road, Budge Budge, Kolkata 700137, West Bengal, India. sayantan.ray30@gmail.com

Abstract

Non-alcoholic fatty liver disease (NAFLD) is a heterogeneous condition with a wide spectrum of clinical presentations and natural history and disease severity. There is also substantial inter-individual variation and variable response to a different therapy. This heterogeneity of NAFLD is in turn influenced by various factors primarily demographic/dietary factors, metabolic status, gut microbiome, genetic predisposition together with epigenetic factors. The differential impact of these factors over a variable period of time influences the clinical phenotype and natural history. Failure to address heterogeneity partly explains the sub-optimal response to current and emerging therapies for fatty liver disease. Consequently, leading experts across the globe have recently suggested a change in nomenclature of NAFLD to metabolic-associated fatty liver disease (MAFLD) which can better reflect current knowledge of heterogeneity and does not exclude concomitant factors for fatty liver disease (*e.g.* alcohol, viral hepatitis, *etc.*). Precise identification of disease phenotypes is likely to facilitate clinical trial recruitment and expedite translational research for the development of novel and effective therapies for NAFLD/MAFLD.

Key Words: Non-alcoholic fatty liver disease; Metabolic-associated fatty liver disease; Heterogeneity; Phenotypes; nomenclature; Clinical trial; Effective therapies

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Core Tip: It is being increasingly recognized that non-alcoholic fatty liver disease (NAFLD) is a heterogenous condition with wide variability in clinical presentation and natural history. This heterogeneity is driven by genetic predisposition, metabolic factors, gut microbiota, diet and demographic factors. The suboptimal response to current pharmacotherapy in NAFLD highlights the failure to recognize this heterogeneity. Experts believe that updating NAFLD nomenclature is the first step towards this. Identification of disease subtypes can help development of preclinical model evaluating novel targets. This would in turn help clinical trial design by comparing and pooling results and thus improve disease outcomes.

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INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is increasing in both developed and developing countries, in parallel with the global obesity epidemic. Nevertheless, much is still unknown on the NAFLD phenotype. Moreover, since the term NAFLD was coined by Ludwig *et al*[1] in 1980, the nomenclature and diagnostic criteria have not been revisited. With a deeper understanding of the natural history of NAFLD, it has become gradually more obvious that this term is inherently complicated, chiefly due to the heterogeneity of NAFLD and principal driving factors between individuals. This heterogeneity in clinical presentation and the course of NAFLD is probably influenced by several factors which include age, gender, ethnicity, diet, alcohol consumption, genetic predisposition, microbiota, and metabolic milieu[2].The combined effect of the dynamic and complex systems-level interactions of these drivers is probably reflected in the phenotypic manifestations of NAFLD. Therefore, comprehensive phenotyping will translate into individual-level risk prediction and preventive strategies, and improvements in the design of clinical trials[2]. The heterogeneity of NAFLD and the presence of multiple pathophysiological pathways intrinsic to its progression suggest that the nomenclature should be revised and NAFLD may be classified in a way that takes into account the various underlying processes[3]. However, a change of name of any disease has considerable implications for both clinical practice as well as public health policy. Based on these evolving paradigms, this review will explore the factors contributing to NAFLD heterogeneity and its clinical and therapeutic implications. Besides, proposed changes in the current nomenclature and definition of NAFLD are discussed along with future perspectives.

HETEROGENEITY OF NAFLD: NEED FOR A NEW TERMINOLOGY

NAFLD represents an umbrella term with considerable heterogeneity among its subtypes. This is evidenced by variable disease severity and progression (disease phenotype) among patients with NAFLD[4]. The disease phenotype in NAFLD is in turn influenced by primary drivers of the disease and dynamic interaction between various disease modifiers (age, sex, ethnicity, co-existing disease, diet, alcohol consumption, smoking, hormonal status, genetic and epigenetic factors, gut microbiota, and metabolic risk factors)[2]. Although steatosis is highly prevalent, progression to steatohepatitis or other liver-related complications like cirrhosis and hepatocellular carcinoma (HCC) is highly unpredictable. The rate of fibrosis progression can also vary widely among patients. Moreover, there is growing evidence that HCC can develop in NAFLD without cirrhosis[5].

The suboptimal response rates of current investigational therapies (20%-40%) reflect a lack of consideration of heterogeneity of NAFLD[2,6]. Hence, a structured dissection of the key pathogenetic pathway and precise disease sub-typing based on genetic background, metabolic profile and anthropometric parameters shall help predict individualized risk and provide effective treatment[2]. The term NAFLD was coined in

1980 by Ludwig *et al*[1] and it was used to describe fatty liver disease without a history of significant alcohol intake. Although the prevalence of NAFLD has grown to epidemic proportions involving one-fourth of the population, the nomenclature and the diagnostic criteria have not been reevaluated[2]. The term NAFLD does not consider the heterogeneity of the disease and hence does not reflect current knowledge.

Based on recent epidemiological studies, it has been increasingly recognized that there is no cut-off for safe drinking in so-called NAFLD as there is frequent co-existence of at-risk drinking and dysmetabolism[7]. Moreover, accurate assessment of alcohol intake is often challenging especially in subpopulations like children and women due to cultural interdiction[8]. To further confuse the issue, there is evidence that an altered gut microbiome can lead to excess production of endogenous alcohol in non-drinkers[9]. Hence, the dichotomy between alcoholic liver disease and NAFLD should be abandoned. Until now, diagnosis of NAFLD was based on the exclusion of excess alcohol intake, concomitant viral hepatitis/other liver diseases, and secondary cause of fatty liver (*e.g.* drug-induced). With the increasing prevalence of NAFLD and the high prevalence of other liver diseases such as viral hepatitis particularly in countries like Middle East and north Africa, dual causes of liver disease should be considered[8]. The current definition of metabolic-associated fatty liver disease (MAFLD) does not require the exclusion of the above, considering the co-existence of different pathology for fatty liver disease (Figure 1). However, it requires the presence of overweight/obesity, type 2 diabetes mellitus (T2DM), or 2 metabolic risk factors. The term "non" in "nonalcoholic fatty liver disease" trivializes a disease that has major hepatic, cardiovascular (CV), and oncological sequelae[2,10]. Due to the "non"-rubric, it could be misinterpreted as something not serious and even encourage alcohol consumption. The term "alcohol" makes the nomenclature derogatory and thus stigmatizing the condition blaming the patient for their condition[2]. This has profound implications on recognition of the disease as a major public health problem and resource allocation by regulatory authorities to intercept this potentially deadly disease.

Due to the aforementioned reasons, the term MAFLD was proposed by Lonardo and Carulli 16 years back[11]. However, NAFLD nomenclature remained unchanged until now. For the same reasons, Polyzos and Mantzoros[12] have proposed the term dysmetabolism associated fatty liver disease (DAFLD). Recently two consensus guidelines have proposed a change in the nomenclature of NAFLD to MAFLD and have redefined the condition based on the presence of hepatic steatosis and metabolic risk factors[2,13] (Figure 2). The impact of such change was reflected in the identification of patients with hepatic steatosis with a higher risk of disease progression in a cross-sectional study of more than 13000 patients based on data from the third National Health and Nutrition Examination Surveys of the United States[14]. Another study from Hong Kong has shown that MAFLD definition reduces the incidence of fatty liver disease by 25% [more so in patients with low body mass index (BMI)], while the prevalence remains unchanged. Patients with a fatty liver disease not fulfilling the criteria of MAFLD were unlikely to have significant liver disease.

However, the future implications of change in the nomenclature are still unknown. Hence, Younossi *et al*[15], on behalf of the American Association for the Study of Liver Disease[15] have cautioned about the impact of premature change in terminology to MAFLD. While there are still existing challenges in widespread disease awareness, identification of treatment endpoints, and biomarkers for risk stratification, changing terminology may negatively impact the field[15]. Moreover, international consensus involving all scientific societies, regulatory bodies, pharmacological industry, and patient organizations is required before a change in terminology. No matter what is the terminology for fatty liver disease, it is clear that it is a heterogeneous disease with varying manifestations.

NAFLD AND CARDIOVASCULAR RISK

Patients with NAFLD are more likely to have morbidity and mortality from cardiovascular disease (CVD). Currently proposed term MAFLD is closely linked to DM, dyslipidemia, hypertension, systemic inflammation which are known to increase CVD risk. A higher risk of CVD and CVD associated events have been noted in epidemiological and observational studies in NAFLD[16,17]. NAFLD not only damages the coronary arteries (atherosclerosis and ischemic heart disease), but also the other cardiac structures like myocardium (heart failure), cardiac valves (aortic stenosis,

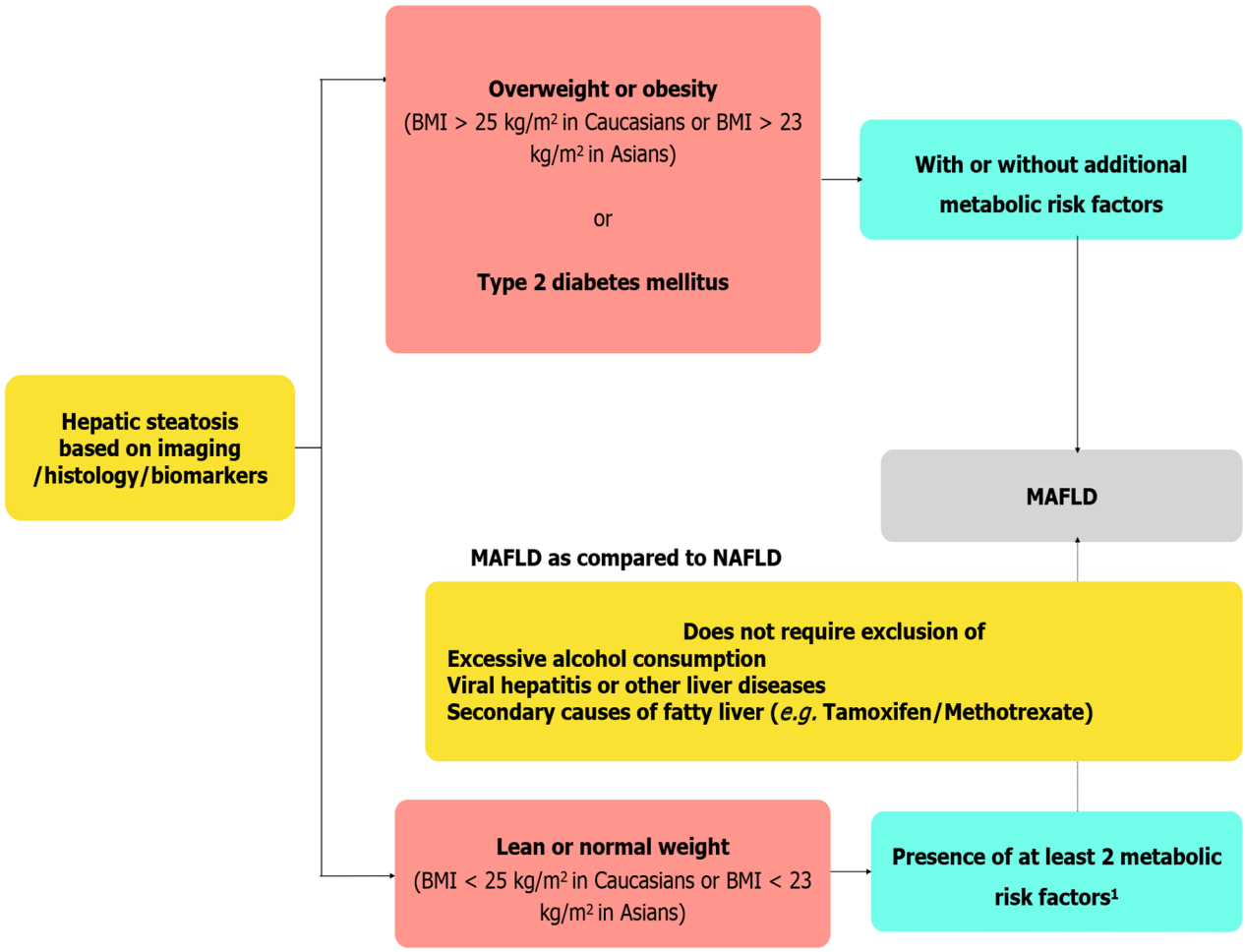

Figure 1 Proposed diagnostic criteria of metabolic associated fatty liver disease and key differences with non-alcoholic fatty liver disease definition. [1]Metabolic risk factors include (1) Waist circumference ≥ 102/88 cm in Caucasian men and women (≥ 90/80 cm for Asian men and women); (2) Blood pressure ≥ 130/85 mmHg or on drug treatment; (3) Triglyceride levels ≥ 150 mg/dL (≥ 1.70 mmol/L) or on drug treatment; (4) Plasma high density lipoprotein [HDL < 40 mg/dL (< 1.0 mmol/L) for men and < 50 mg/dL (< 1.3 mmol/L)] for women or on drug treatment; (5) Pre-diabetes [*i.e.*, fasting glucose levels 100 to 125 mg/dL (5.6 to 6.9 mmol/L), or 2-h post-load glucose levels 140 to 199 mg/dL (7.8 to 11.0 mmoL) or HbA1c 5.7% to 6.4% (39 to 47 mmol/moL)]; (6) Homeostasis model assessment of insulin resistance score ≥ 2.5; and (7) Plasma high-sensitivity C-reactive protein level > 2 mg/L. BMI: Body mass index; MAFLD: Metabolic-associated fatty liver disease; NAFLD: Non-alcoholic fatty liver disease.

mitral annular calcification), and conduction system (atrial fibrillation, conduction defects)[18]. CV disease in NAFLD can be subclinical (coronary and courted atherosclerosis) or clinical (myocardial infarction, stroke). Pathophysiological factors include dyslipidemia, oxidative stress, systemic inflammation, endothelial dysfunction, and a pro-thrombotic state leading to structural and functional cardiac changes including arterial stiffness, atherogenic plaque formation, and coronary calcification[19]. Among genetic factors related to NAFLD, MBOAT7 may promote venous thromboembolism whereas Transmembrane 6 superfamily 2 (TM6SF2) appears to be protective and PNPLA3 seems not to be associated with the risk of CVD. Other pathogenetic mechanisms of NAFLD such as environmental factors (diet, obesity, *etc.*), gut microbiota (through the gut liver axis and altered intestinal permeability), and epigenetic alterations also influence the CV risk[16].

Lifestyle modification and weight loss help in primary and secondary prevention of CVD in NAFLD. Aspirin and statins may be considered for primary and secondary prevention in individuals with NAFLD who are at high risk of CVD. Newer anti-diabetic medications such as SGLT2 inhibitors and GLP-1 receptor agonists are known to reduce CV events in T2DM and may be useful in this regard. Additional data are required on CV risk modification by farnesoid X receptor (FXR) agonists such as obeticholic acid. Future studies will likely address the predictive factors responsible for elevated CVD risk in NAFLD as there is a lack of targeted pharmacological therapy. Hence, CV endpoints should be included in clinical trials in NAFLD/MAFLD [16,19].

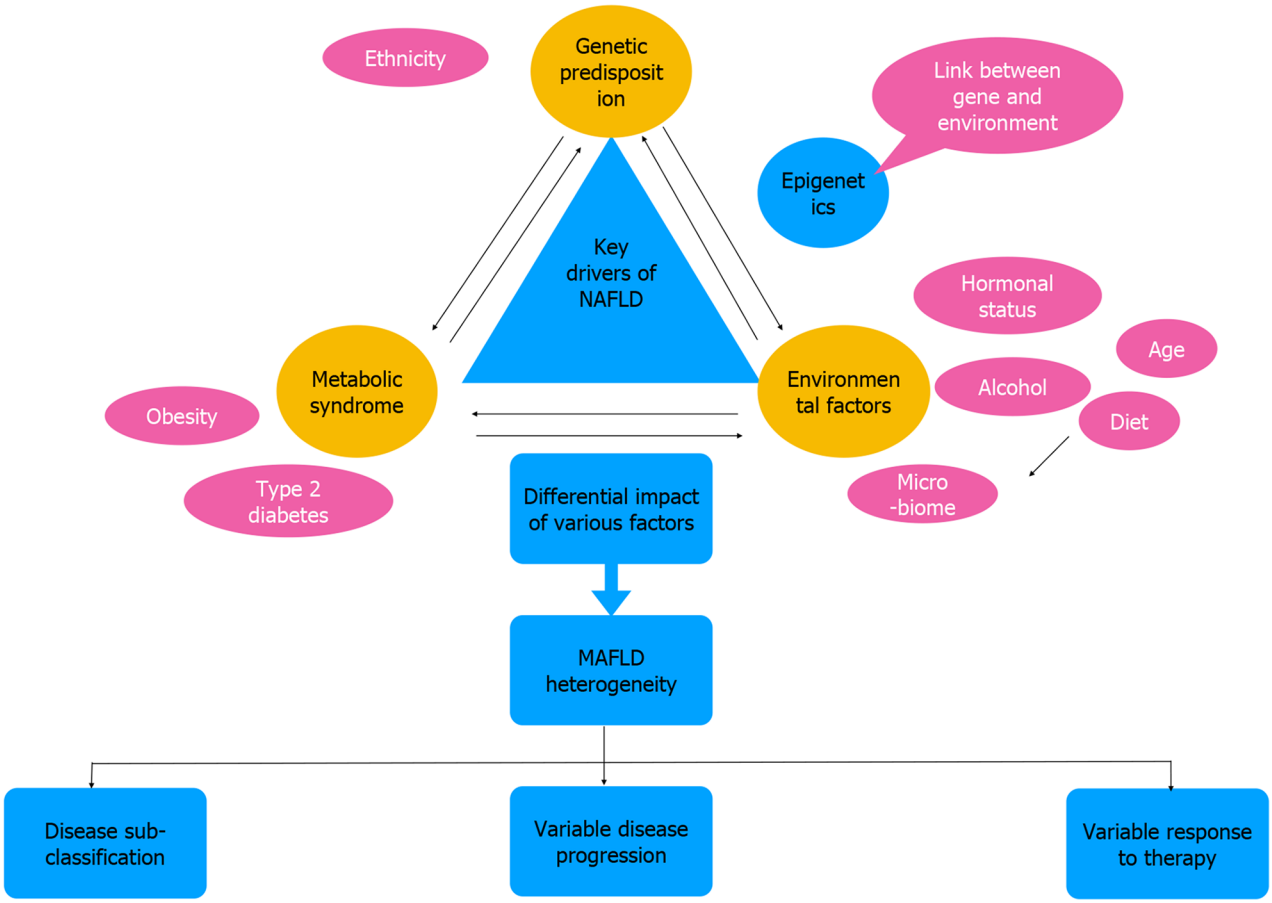

Figure 2 Key drivers of metabolic-associated fatty liver disease, resulting in disease heterogeneity and its clinical implications. Genetic predisposition, metabolic health, and environmental factors influence molecular and phenotypical heterogeneity of metabolic-associated fatty liver disease leading to various disease subtypes, variable disease progression, and response to therapy. MAFLD: Metabolic-associated fatty liver disease; NAFLD: Non-alcoholic fatty liver disease.

FACTORS FOR HETEROGENEITY

Age

The prevalence, risk of hepatic/extra-hepatic complications, and all-cause mortality of NAFLD increase with age. This is due to multiple factors like reduction in hepatic blood flow/volume, decrease in bile acid synthesis, altered cholesterol metabolism, increase in oxidative respiration due to decrease in mitochondria numbers, cellular aging, increased exposure to disease drivers over a prolonged period, and progressive increase in insulin resistance (IR) due to change in body composition (sarcopenia, abdominal and visceral adiposity with ectopic fat deposition)[20-23].

Gender and menopause effect

The prevalence of NAFLD and degree of hepatic fibrosis are lower in pre-menopausal women compared to men and postmenopausal women with better overall survival rates in the former[24]. Changes in body fat distribution (abdominal obesity after menopause), differences in metabolic risk factors, sexual dimorphism of key metabolic pathways (lipid metabolism, insulin signaling, and inflammation), and differences in hepatic gene expression of various metabolic pathways (*e.g.* FXR, liver X receptor) are likely mechanisms for the difference[25-27]. The prevalence of NAFLD and fibrosis risk is lower in postmenopausal women on hormone replacement therapy (HRT) compared to those who are not on HRT[28]. The extent of hepatic fibrosis increases with the prolonged duration of estrogen deficiency in postmenopausal women[29]. Hence, risk stratification in NAFLD should be based on gender and menopausal status.

Ethnicity

The prevalence of NAFLD and risk of nonalcoholic steatohepatitis (NASH) are seen in

decreasing order of frequency in Hispanics, non-Hispanic whites, and African Americans[30]. It is important to note that the risk of fibrosis did not vary based on ethnicity. The plausible explanations for such racial disparity are differences in genetic predisposition, metabolic traits (IR and body fat distribution), environmental factors (dietary habits like increased carbohydrate consumption, physical inactivity, and cultural factors). For example, the frequency of risk alleles of Patatin-like phospholipase domain-containing protein 3 (*PNPLA3*) gene in Hispanics, non-Hispanic whites, and African-Americans are 49%, 23%, and 17% respectively[31]. Importantly, Asian individuals tend to accumulate liver fat at lower BMI, have a higher degree of inflammation, and have a possibly higher risk of fibrosis compared to other ethnicities[32, 33]. PNPLA3 rs738409 risk allele frequency is more common in East Asians compared to Caucasians[34].

Diet and gut microbiota

It is well known that a Western diet with high fat and fruit content leads to a higher incidence of NAFLD. On the other hand, the adoption of the Mediterranean diet is associated with decreased liver fat content and CV risk[35]. Gut microbial composition changes rapidly according to changing dietary patterns. The effect of diet in fatty liver disease is difficult to differentiate from those due to diet-induced change in gut microbial composition[36]. Gut microbiome composition can identify individuals with a higher risk of NAFLD progression[37]. The gut microbiome and its metabolites influence bile acid metabolism, which in turn influences lipid, choline, and glucose metabolism. Alteration in gut microbial composition and intestinal permeability in NAFLD leads to the circulation of bacterial metabolites such as lipopolysaccharide which is in turn sensed by hepatic Toll-like receptors which induce activation of hepatic pro-inflammatory cells and stellate cells leading to inflammation and fibrosis progression[38,39]. Apart from dietary factors, genetic makeup and ethnicity influence gut microbiome composition[40,41].

Metabolic health

Obese *vs* lean NASH: Although intra-hepatic fat content is closely influenced by obesity, 45% of the obese are said to be metabolically healthy as they don't have any components of metabolic syndrome (MetS)[42]. It is not clear whether these individuals have a lower risk of CV complications compared to normal-weight, metabolically healthy individuals[43]. On the other hand, 30% of normal-weight individuals have MetS and higher cardiometabolic risk. This is because the distribution and nature of fat are more important than the amount of fat in predicting metabolic risk[2]. Visceral fat is associated with higher metabolic risk compared to peripheral and subcutaneous fat. Fat distribution is influenced by ethnicity (higher visceral adiposity in Asians) and genetic makeup[44]. 5%-45% of NAFLD (20% among Europeans) are also lean NAFLD as defined by the presence of hepatic steatosis with normal BMI in the absence of significant alcohol intake[45]. Lean NAFLD has distinct genetic predisposition, metabolic and microbial profiles. Increased prevalence of TM6SF2 risk allele, increased bile acids/Farnesoid receptor activity due to intact metabolic adaptation, and gut microbial profile which facilitates liver fat generation have been seen in lean NAFLD. Individuals with lean NALFD have a better metabolic profile compared to their obese counterparts[46]. The data on the natural history of disease progression in lean NAFLD have shown variable outcomes. Distinct pathways of liver fat accumulation are being recognized. In type 1/metabolic NAFLD, calorie excess due to dietary intake and physical inactivity leads to increased hepatic fatty acid supply by peripheral lipolysis and hepatic lipogenesis[4]. This is associated with IR and other components of MetS thus leading to increased cardiometabolic risk. The accumulated liver fat is composed of monounsaturated triacylglycerols and free fatty acids enriched with ceramides. In type 2/PLNPLA3 NAFLD (with rs738409 risk allele), there is increased intra-hepatic lipogenesis and impaired lipolysis leading to steatosis[47]. The fat composition is predominantly polyunsaturated triacylglycerols. This is not associated with IR and adverse cardiometabolic outcomes although the risk of NASH and HCC is increased. Increasingly various metabolomic signatures leading to hepatic steatosis are being recognized based on RNA-sequencing analysis study [48]. Identification of the key pathway for hepatic steatosis by genetic and molecular profiling may thus help in predicting the risk of progression, cardio-metabolic, and treatment outcomes.

Genetics and epigenetics

Among the multiple variant genes associated with NAFLD identified on genome-wide

association studies, few common variants (PNPLA3, TM6SF2, GCKR, MBOAT7, HSD17B13) are worth mentioning which have divergent metabolic effects[49]. PNPLA3 and TM6SF2 variants increase the risk of NAFLD and advanced fibrosis[50,51]. PLPLA3, TM6SF2, and GCKR variants are associated with T2DM[52]. MBOAT7 and HSD17B13 variants do not affect serum lipid or glucose levels and do not increase cardiometabolic risk[53,54]. These variants explain only a minority of NAFLD. That is why it is important to consider the effect of other variants, gene-environment interactions (described with the *PNPLA3* gene), and epigenetics. Epigenetic alterations of key regulators of metabolic, inflammatory, and fibrotic pathways represent a bridge between variant genes and the environment in NAFLD. Micro-RNAs such as miRNA-122, miRNA-192, and miRNA-34a are unregulated in NAFLD[55]. miRNA-34A also correlates with disease activity. The role of long non-coding RNAs (lncRNAs) in NAFLD is limited requiring further elucidation[56]. Reversible alteration of methylation signatures of key regulatory pathways is seen in NAFLD which reverses following weight reduction surgery[57]. Methylation signatures can help identify patients with advanced fibrosis [*e.g.* hyper-methylation of peroxisome proliferator-activated receptor gamma (PPARγ)][58]. Epigenetic alterations can alter the expression of PNPLA3 explaining the gene-environment link[59]. There is increasing evidence that maternal high fat diet leads to epigenetic alterations in fetal liver and increasing the possibility of NAFLD in adolescence in the offspring[60,61]. Higher maternal BMI is associated with hypermethylation of the PPARγ coactivator 1(*PGC1*) gene which regulates energy metabolism in the newborn[62].

Familial risk

Twin studies, prospective and retrospective family studies have shown heritable factors in hepatic steatosis and fibrosis. In a prospective study, the risk of advanced fibrosis in first-degree relatives of patients with NAFLD-cirrhosis was 18% which is significantly higher than the general population risk[63,64]. Hence family history also should be considered while doing risk stratification of NAFLD patients.

Alcohol intake

The effect of alcohol use in fatty liver disease has a dose-dependent response which synergistically increases in the presence of metabolic risk factors[65]. This is contrary to the earlier belief that alcohol consumption has a "J" shaped effect on fatty liver disease progression with a beneficial effect on light to moderate use and deleterious effect on excessive use[66]. Hence, it is being increasingly revealed that there is no safe cutoff of alcohol consumption in fatty liver disease.

CLINICAL IMPLICATIONS OF NAFLD HETEROGENEITY

NAFLD sub-classification

The heterogeneity in NAFLD due to its multifactorial etiology, pathophysiological diversity, genetic polymorphisms, and on the other side, the ultimate unifying fate of steatosis and its progression, made NAFLD more like an umbrella disease with multiple subtypes. The proposed change of nomenclature as MAFLD, will not truly represent the full spectrum of the disease pathophysiology and thus this over-generalized new nomenclature has been criticized. Singh *et al*[3] had proposed the 'MEGA-D' classification representing the 'Mega-diversity' of the NAFLD. They had proposed five sub-types of the disease, each representing a major pathophysiological hypothesis behind each subtype. The subtypes are as follows: M-Metabolic syndrome, E-Environmental stressor, G-Genetic Factor, A-Bile Acid dysregulation, and D-Gut dysbiosis related NAFLD. Moreover, it is also suggested to consider fatty liver disease as an umbrella term to include the whole spectrum of cryptogenic to classic to alcohol-associated fatty liver disease. Till any consensus-driven widely accepted terminology and sub-classification of NAFLD comes into place, it is prudent to consider fatty liver disease as common outcome pathology with different etiological triggers.

Alteration of lipid metabolism is one of the major pathophysiological factors behind the development and progression of NAFLD. Lipidomics based sub-classification of patients with NAFLD had been proposed which depends upon the signature patterns of alteration in the fatty acid homeostasis pathway[67]. 'M-subtype' is characterized by increased hepatic fatty acid uptake and reduced hepatic glutathione and S-adenosine methionine (SAM) content. On the other hand, the 'non-M subtype' occurs due to increased de novo hepatic lipogenesis and is characterized by normal hepatic SAM levels. Gut microbiota composition-based sub-classification of NAFLD had also been

proposed. However, till now no studies had been able to reveal any signature gut microbiota profile suitable for phenotypical classification of NAFLD patients.

Automated algorithm-driven cluster sub-classification, based on demographic factors (age, gender, ethnicity), clinical and laboratory findings[68], had been evaluated in a cohort of 13290 NAFLD patients in the United States. The whole cohort had been divided into 5 subtypes and evaluated for disease outcomes including survival rates. In subtype 1, there were mostly female Hispanics with mild metabolic comorbidities with minimal fibrosis, but on the other hand subtype 2 had mostly patients with MetS with signs of developing liver dysfunction. Subtype 3 was a mostly young and healthy population with mild disease and minimal abnormalities. Subtype 4 patients were predominantly elderly male Caucasians who had more severe disease at baseline with features of fibrosis and also showed features of progression to cirrhosis stage. Subtype 5 patients were the oldest with more severe cirrhosis and associated with significant co-morbidities. Among the disease outcome, subtype 5 was at the highest risk mortality and subtype 4 had the highest risk of cirrhosis and HCC. Although this type of cluster-based subtyping of the disease needs to be validated clinically it can help to identify relevant disease subtypes in future studies.

In a gene expression study by Hoang *et al*[48], the disease progression score of individual genes had been evaluated and it showed a strong correlation with histological manifestations of disease severity. In this study, the authors proposed NAS (gene-level NAFLD activity score) and gene-level fibrosis stage (gFib) scores. These score-based subtypes of NAFLD not only can assess the risk of disease progression but also can predict the response to therapy. This molecular-based cluster classification either can be the forerunner of different clinical subtypes of NAFLD or can represent different phases of a dynamic spectrum of the disease.

Though genetic, clinical cluster, and pathophysiological based sub-classification of NAFLD had been proposed as discussed above, none of them are universally accepted. Moreover, detailed literature is mainly limited to disease phenotypes depending upon demographic factors, obesity, and clinical outcomes.

Inter-individual variation

Demography (Asian *vs* Western countries): The prevalence of NAFLD is now showing an increasing trend in Asian countries. A meta-analysis done in 2016[69] showed a higher prevalence in Asia (27.4%) than North America (24%) or European Union (23.7%). In a recent meta-analysis[70], the prevalence in Asia was found to have increased further (29.62%) and a secular trend of the rising prevalence in the last few decades had been reported. The increase in prevalence in Asia is likely due to an increase in obesity, sedentary lifestyle, changing westernized eating habits, and various socio-economic factors[71]. The prevalence in the rural area was significantly lower than in the urban areas, suggesting the detrimental effect of urbanization on obesity and the consequent NAFLD[72]. In both Asian and western countries, the prevalence increases with age. Prevalence is higher in males as well as among elderly women indicating protective effects of estrogen in females in the reproductive age group. Apart from the increased prevalence of metabolically unhealthy obesity and excessive visceral obesity, alteration of gut microbiota and bile acid profiles has also been postulated as possible contributing factors behind the development of steatosis [40]. Among the genetic factors, PNPLA3 polymorphism (rs738409) had been strongly associated with hepatic steatosis in both western and eastern studies[31]. However, a higher prevalence of PNPLA3 risk allele had been reported in Asia than in African or European countries[73,74]. Genetic polymorphisms of other genes like *TM6SF2*, *AGTR1*, *HSD17B13*, and *GCKR* genes had also been linked with increased susceptibility of NAFLD in Asian subjects[54,75-77]. Sarcopenia and hypovitaminosis D also was associated with NAFLD development[78,79]. One of the major differences in Asian countries from their western counterpart is the increased prevalence of lean NAFLD (discussed later) in the former. Though the overall prevalence of NAFLD is almost similar in eastern and western countries, however, the rate of complications is still lesser in Asian countries. In a retrospective study from Japan with a median follow-up of 5.8 years, only 0.25% of patients developed HCC with an annual incidence of 0.043%[80]. In contrast to western countries, NAFLD still contributes only to a minor proportion of liver-related complications requiring liver transplantation in Asia. In a Japanese nationwide survey, only 2.1% of patients with cirrhosis had NASH and almost two-thirds of the patients had viral hepatitis[81]. The indolent course of NAFLD in Asian countries is likely due to relatively short disease duration in the majority of the patients in this part of the world. As there is a considerable lag in economic growth and consequent obesity epidemic in Asian countries, the rise in NAFLD and its complications are likely to follow the western trend in the coming

years. Moreover, the relatively higher chance of co-existence of viral hepatitis and NAFLD in Asian countries increases the risk of hepatic complications further[82].

Ethnicity: Irrespective of ethnic variability, a trend of overall increased prevalence of NAFLD had been seen globally. In the world, Middle East had the highest prevalence of NAFLD, and in Africa; it is the lowest[69]. Studies from the United States reported that Hispanics had shown the highest risk of NAFLD and on the other hand, the risk is much less in the Alaskan Native. Among Asian ethnicity, the prevalence is highest among Indonesian and lowest in Japanese[70]. Interestingly, people of South Asian origin who are living in the United Kingdom, also showed higher risk[83]. In a recent meta-analysis, which evaluated ethnic heterogeneity of NAFLD in the United States, both higher overall prevalence of NAFLD and risk of progression to NASH had been reported in Hispanics and the risks were lowest among Blacks[30]. Although there was no significant difference in patients with fibrosis among different ethnicities. The reasons behind the ethnic variation are multifactorial. A significantly high risk of NAFLD among American Japanese than the native Japanese suggests the impact of socio-economic development and differences in lifestyles in the pathogenesis[70]. Specific western dietary patterns in different ethnicities, like consumption of red meat and hydrogenated fat, had also been associated with an increased risk of fibrosis[84]. Intake of saturated fatty acids increases and on the other hand, consumption of omega 3 fatty acid-rich food reduces the risk of steatosis. Genetic factors can explain the heterogeneity of NAFLD across different ethnicities. Among genetic variants of the *PNPLA3* gene, rs738409 increases the risk of NAFLD in Hispanics and Southeast Asians[85]. On the other hand, the increased prevalence of protective polymorphism of the same *PNPLA3* gene (rs6006460) can explain the reduced risk of NAFLD among African Americans[31]. The rs738409 variant had been also associated with an increased risk of progression to NASH and hepatic fibrosis[86,87]. However, in a study from Malaysia, though the frequency of *PNPLA3* risk allele was higher among Chinese individuals but the prevalence of NAFLD was much less in them in comparison to Malay and Indian participants[87]. This paradox can be explained by the involvement of multiple candidate genes in disease pathophysiology among different ethnicities. With the advent of Genome Wise Association studies, the role of predisposing polymorphisms of other candidate genes like *TM6SF2* and *GCKR* gene had been explored further. The rs58542926 variants of the *TM6SF2* gene were significantly associated with intra-hepatic fat (triglyceride) accumulation in White and African-American but not among Hispanic individuals[88]. Different polymorphisms in the *AGTR1* gene were protective among Indians but not in Chinese and Malay subjects [75]. Recently, polygenic gene scores had been developed to evaluate the cumulative effects of multiple candidate genes in the development and progression of NAFLD [89]. Further studies are needed in the future to explore the complex interaction of different genetic polymorphisms which can explain disease heterogeneity across different ethnic populations.

Age (Children and adolescents): With the increasing prevalence of pediatric obesity, the prevalence of NAFLD in children and adolescents is ever rising. The pooled prevalence of pediatric NAFLD in general population and obesity clinic were 7.6% (95%CI: 5.5%-10.3%) and 34.2% (95%CI: 27.8%-41.2%) respectively[90]. The factors which can influence the intrauterine metabolic milieu of the developing fetus, like maternal obesity and diabetes, had been postulated to increase the future risk of NAFLD[91,92]. Increased consumption of fructose-rich beverages, processed food, saturated fat along with decreased intake of dietary fibers (westernized dietary habits) had been strongly associated with the development of NAFLD in children[93]. On the other hand, breastfeeding was protective against the development of NAFLD[94]. The genes which had been shown to increase the risk of pediatric NAFLD are similar to the adults. Genetic variants of*PNPLA3* (rs738409), *TM6SF2* (rs58542926), and *GCKR* gene had been shown to increase the susceptibility of development of NAFLD in pediatric patients[31,88]. Though histological diagnosis of NAFLD remains ideal, diagnosis by imaging (ultrasound/MRI) is the most practical one in the pediatric population. As the prevalence of obesity in children is ever-increasing, the chance of co-existence of other secondary causes of hepatic steatosis should also be carefully evaluated before confirming the diagnosis of NAFLD. Histological pattern in pediatric NAFLD (peri-portal distribution-Type 2 NASH) differs from that of their adult counter-part (peri-central distribution-Type 1 NASH)[95]. Both fibrosis and steatosis are mainly present in the periportal region in type 2 NASH and are seen more in younger children. Moreover, the classical 'ballooning' change is also seen less frequently in children. On the other hand, type 1 NASH of the adult pattern can be seen in the older adolescent

age group[96]. There is a paucity of longitudinal studies evaluating the natural history of pediatric NAFLD. Around 10%-25% of patients had advanced fibrosis and almost half of the patients had NASH at the time of diagnosis[97]. Though the incidence of HCC in the pediatric age group is extremely rare, a large number of pediatric patients with NAFLD are at increased risk of developing HCC in early adulthood. Weight loss and lifestyle changes were effective in the reversal of steatosis in pediatric patients[98].

BMI (lean/non-obese NAFLD): Lean and non-obese NAFLD is defined as NAFLD in a person with BMI < 25 kg/m^2 (< 23 for Asian subjects) and < 30 kg/m^2 (< 25 for Asian subjects) respectively. In a meta-analysis that included 93 studies from 24 countries, the prevalence of lean and non-obese NAFLD in the general population was reported as 5.1% and 12.1% respectively[99]. Globally, the prevalence of non-obese NAFLD among the whole NAFLD group was 40% and in countries like India, it is as high as 47%, indicating that a large proportion of fatty liver disease is now developing in the non-obese population. Though non-obese NAFLD initially was more common in Asian countries, now almost similar prevalence of NAFLD is being reported from the western part of the world (United States 43.2%). Globally the prevalence of lean/non-obese NAFLD is showing an increasing trend over the last 3 decades[100]. Though Shi *et al*[101] had reported a lower prevalence of hypertension, hyperuricemia, and fasting blood glucose in lean/non-obese NAFLD patients compared to obese NAFLD, these lean patients are not necessarily metabolically healthy. Rather lean NAFLD patients are more likely to have visceral obesity, metabolic syndrome, dyslipidemia, hypertension, and DM as co-morbidities than the lean controls[101]. The pathophysiological basis of the development of NAFLD in lean/non-obese individuals is complex and multi-factorial. Increased prevalence of the PNPLA3 G allele had been found in lean NAFLD patients[102]. Other genetic factors like TM6SF2 (T)[46], cholesteryl ester transfer protein, and interferon lambda 3 (IFNL3)/IFNL4(C) had also been found to increase the risk of lean/non-obese NAFLD[103,104]. On the other hand, possible roles of distinct gut microbiota, bile acid profile[46,105], increased lysine, tyrosine, lysophosphatidylcholines, and phosphatidylcholines, had also been implicated in the development of NAFLD among lean individuals[106]. The progression of NAFLD in the lean population can be conceptualized as a state of gradual attenuation of metabolic adaptation. Pathophysiologically, this can be divided into 3 stages- stage of susceptibility, stage of adaptation, and stage of failure[107]. Studies evaluating the true natural history of lean NAFLD are sparse in the literature. In the largest meta-analysis Ye *et al*[99] reported that among lean/non-obese NAFLD patients, NASH and fibrosis (> stage 2) were present in 39% and 29% of patients respectively, which was lesser than the prevalence among obese NAFLD population. However, liver-related mortality was reported as almost twice in lean/non-obese NAFLD patients than in the obese NAFLD group. In another study with a mean longitudinal follow-up of almost 20 years, lean NAFLD patients did not show any significantly increased risk of overall mortality but the risk of progression to severe hepatic diseases was significantly higher (HR 2.69) than the obese NAFLD population [108]. Like obese NAFLD, lifestyle modification in the form of dietary modifications and increased physical activity remains the main therapeutic approach in lean NAFLD patients[109].

Variable natural history

Classic and dynamic model: Previously, the natural history of NAFLD had been conceptualized as a disease spectrum that follows a linear model of disease progression. This classic model hypothesized that there is a gradual progression of the disease from NAFL to NASH to cirrhosis and HCC. However, this progressive worsening of the disease does not occur in all of the patients of NAFLD and significant heterogeneity in the natural history of NAFLD had been observed. Recent literature had identified that not all the patients with NAFLD follow this 'classic linear model' of natural history. A study by Pais *et al*[110], which systemically evaluated serial liver biopsy in NAFLD patients, had shown that 60% of NAFL patients had progressed to NASH and around 25% of patients of NAFL had directly progressed to the fibrotic stage. Various factors like DM, obesity, old age, and a higher degree of baseline abnormality were identified as possible risk factors for disease progression. In another longitudinal follow-up study by McPherson *et al*[111], no significant difference in the rate of fibrosis progression between NAFL and NASH patients was found. In an excellent systematic review by Singh *et al*[112], serial liver biopsy data of 411 biopsy-proven NAFLD from 11 cohort studies were analyzed. They had also re-emphasized that both NAFL and NASH can progress to the fibrotic stage. However, it takes much

longer (14 years) time to progress one fibrosis stage in NAFL than in NASH (7 years). The annual fibrosis progression rate was slower in NAFL (0.07 stage) than in NASH (0.14 stage). Moreover, NAFL and NASH had a comparable rate of CV mortality (OR 0.9) though all-cause and liver-related mortality are higher in NASH[113]. To summarize, NAFL can progress both to the NASH and fibrosis stage directly and on the other hand, NASH can also regress to NAFL or progress to the fibrotic stage. Thus, in the 'dynamic model' of NAFLD, it has been conceptualized that in early NAFLD, there is dynamic cycling between NAFL and NASH[114] (Figure 3).

Slow and rapid progressor: In the same meta-analysis discussed above, Singh *et al* [112] also had identified significant heterogeneity among disease progression in NAFLD. They reported 2 subtypes of NAFLD patients according to fibrosis progression rate- rapid and slow progressor. The rapid progressors were around 20% of the NAFLD group who progressed rapidly from baseline (stage 0 fibrosis) to advanced (stage 3 or 4 fibrosis). On the other hand, the majority of NAFLD patients are slow progressors who only progressed 1 or 2 stage fibrosis in a similar time frame. Older age, low ASL: Alanine aminotransferase (ALT) ratio, co-morbidities like diabetes mellitus or hypertension, and genetic polymorphisms are probable risk factors for rapid progressors[103,115] (Figure 3).

HCC: With the progressive increase in the prevalence of NAFLD worldwide, the risk of HCC and liver-related mortality are likely to rise as a consequence. Viral hepatitis-related HCC usually occurs in the background of the advanced stage of cirrhosis. Though classically HCC usually occurs in the advanced stage of cirrhosis in the NAFLD spectrum, this is not true for all the cases of NAFLD-related HCC[116]. Rather one of the most common causes of chronic liver disease-related HCC without evidence of cirrhosis is NAFLD[5]. Leung *et al*[117] had reported 15% percent of NAFLD-related HCC as non-cirrhotic and they usually had larger hepatic tumor diameter at diagnosis. In a retrospective analysis, Mohamad *et al*[118] also reported that HCC in NAFLD patients without cirrhosis are likely to present in the older age group with a larger tumor size with a high recurrence rate in comparison to those with cirrhosis (Figure 3).

THERAPEUTIC AND RESEARCH IMPLICATIONS

NAFLD progression and prognostication

Many factors may influence the progression of NAFLD to the more advanced stage but are not routinely or easily assessed in day-to-day practice (*e.g.*, genotype, gut microbiome, mitochondrial function, immunological response)[119]. Consequently, we need to consider the natural history studies to help provide clinical, biochemical, and histological variables that can be utilized to decipher which patients will develop severe disease with worse outcomes. With regard to clinical features, a paired biopsy study by McPherson *et al*[111] underscores the impact of IR with 80% of patients with NAFL and progression of fibrosis developing diabetes by the time of follow-up biopsy compared with 25% of nonprogressors. Other studies have also shown that weight gain and worsening IR are associated with fibrosis progression in NAFLD[110]. Data for biochemical predictors are somewhat deficient. However, a study found that in patients with biopsy-proven NASH and compensated cirrhosis; lower levels of serum cholesterol, ALT, and platelets are independently associated with hepatic complications and higher aspartate aminotransferase (AST)/ALT ratio with overall mortality [120]. In NAFLD, baseline histology can provide a good prognostic value. According to a systemic review and meta-analysis of paired-biopsy studies, a third of individuals with NAFLD will have progression of fibrosis with a mean progression rate of 0.14 stages *per* annum for NASH, corresponding to one stage of fibrosis progression over a median of 7.1 years[112]. Nevertheless, many epidemiological studies have de-emphasized the presence of NASH and confirmed the presence and degree of fibrosis as the most important histologic predictor of liver-related morbidity and mortality [121,122].

It is now widely accepted that the severity of fibrosis is the only significant predictor of outcomes in NAFLD. The histological differentiation between NAFL and NASH is unlikely to predict fibrosis progression and carries very little prognostic value. Thus, it is better to consider the diagnosis of patients with advanced fibrosis (F3 and F4) because this stage is a predictor for hepatic and extrahepatic morbidity and mortality [123]. This strategy identifies those with liver disease sufficient to call for specific interventions to prevent complications of cirrhosis and the development of HCC. People with NAFL or NASH with early F0–F2 don't need to be considered as having

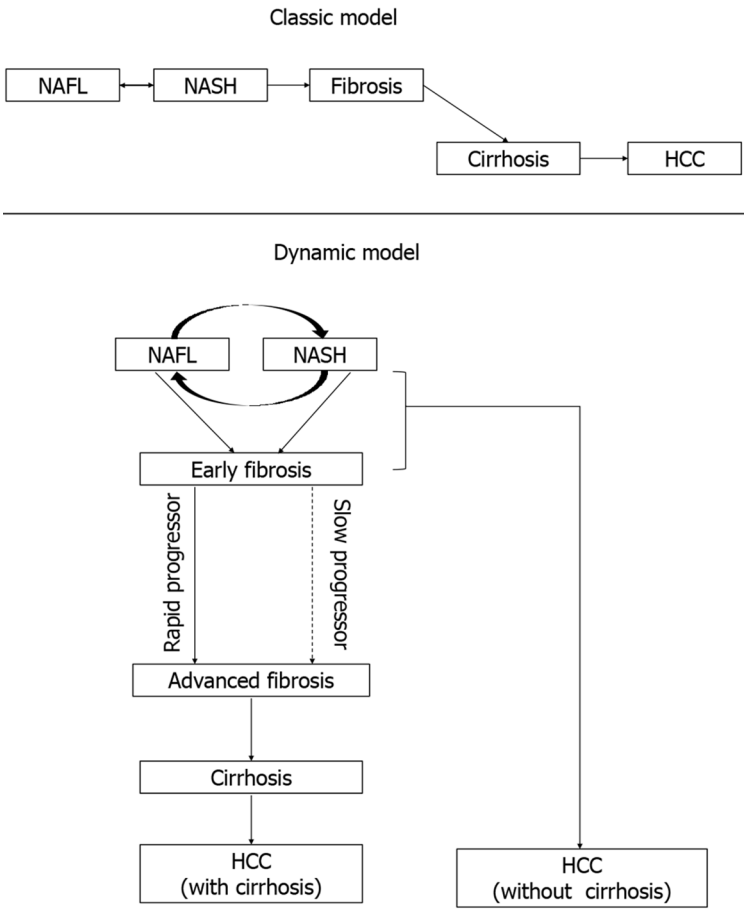

Figure 3 Natural history of non-alcoholic fatty liver disease (classic and dynamic model). HCC: Hepatocellular carcinoma; NAFL: Nonalcoholic fatty liver; NASH: Nonalcoholic steatohepatitis.

liver disease necessitating intervention owing to the low risk of liver-related complications. In these persons, metabolic risk factors like diabetes should be addressed to optimize CV outcomes, with likely benefits on liver disease[123]. As progressive fibrosis indicates a poor prognosis with unfavorable CV and adverse hepatic outcomes, the approach should now focus on the risk stratification of patients and identify those needing liver-specific intervention.

Non-invasive tests of hepatic fibrosis

As the severity of fibrosis is the major driver for the long-term prognosis of NAFLD patients, it is, therefore, critical to identify patients at higher risk of advanced fibrosis to optimize their management[124]. Although required to detect patients with NASH and early fibrosis, liver biopsy is an invasive procedure. Patient acceptability is low, and it is not desirable to perform liver biopsy repetitively to assess disease progression and response to treatment. Moreover, as only a small proportion of the patients would develop liver-related complications, performing non-invasive tests (NITs) as the primary assessment is preferable[125]. This section focuses on the confounding factors that can affect the performance and accuracy of NITs of liver fibrosis in patients with NAFLD.

Impact of confounding factors

Non-invasive fibrosis scores are usually used to detect or exclude advanced fibrosis in individuals with NAFLD. A few studies purposely looked at reasons for imprecise prediction by these scores. In a multicentric European study in subjects with biopsy-proven NAFLD, the AST-to-ALT ratio, NAFLD fibrosis score (NFS) and Fibrosis-4 (FIB-4) index performed poorly for the detection of significant fibrosis in persons aged 35 years or below[126]. The specificity of the FIB-4 index and NFS reduced to unacceptable levels in those aged 65 years and older in the same study. This reason is that age is a component of both the fibrosis scores. The performance of NITs and the used transient elastography (TE) liver stiffness cutoffs in different ethnic populations

and special subpopulations such as individuals with diabetes and obesity also need to be taken into account. For example, depending on the ethnicity, the diagnostic accuracy of the NITs may be altered. Compared to Western populations, South Asians develop more metabolic complications at lower body mass indices. The accuracy of the NFS, AST-to-platelet ratio index, FIB-4, AST/ALT ratio, and BARD score is found to be lower in the South Asian population in comparison with the Caucasian population [127]. In addition, the NFS has a lower sensitivity in individuals of South Asian descent, as the majority had a lower BMI and were younger than Caucasian counterparts with a comparable disease stage, and therefore had a lower score[125]. Serum markers of liver fibrosis and possible confounding factors are summarized in Table 1.

With regards to imaging modalities that estimate liver stiffness as a potential surrogate of hepatic fibrosis, vibration-controlled transient elastography (VCTE) has been widely validated against liver histology[128] and shows correlation with clinical outcomes in longitudinal studies[129]. However, there are a number of factors to be considered while using this modality. Pathologies that increase liver stiffness can lead to a false-positive diagnosis of advanced fibrosis. Besides, high BMI and severe hepatic steatosis have been reported to increase the false positive rate of VCTE[130]. A recent study suggests that when using the XL probe in obese patients, steatosis does not augment liver stiffness independent of fibrosis[128]. Magnetic resonance elastography (MRE) can surmount many of these barriers, except for iron overload and acute inflammation; nonetheless, restricted availability at most centers and cost are the limiting factors. MRE has higher applicability and accuracy than VCTE when compared head-to-head[131].

While it is expected that blood-based parameters or imaging modalities will replace liver biopsy for the diagnosis in people who would benefit from treatment, equally it indicates that validation of any future marker should be done in more specifically defined cohorts. A recent International Consensus Panel suggested that the factors that shape the NAFLD heterogeneity should be taken into account when devising risk-stratification scores and algorithms[2]. Caution should be exercised by clinicians during the interpretation of test results when the tests are applied in patients with potential confounding factors.

Considerations for best practice

Early detection of advanced fibrosis is essential in the efforts to halt the NASH progression. Therefore, screening is vital to ensure that patients, mainly those with advanced F3–F4, are identified and linked to care before they develop end-stage liver disease. With the development of reliable NITs to identify patients with advanced fibrosis, there is now potential to put management strategies earlier in place[132]. Clinicians need to be more proactive in detecting patients with advanced fibrosis due to NASH. Figure 4 shows a diagnostic algorithm that targets screening of patients with characteristics of MetS who are at risk of progressive fibrosis. This is in accordance with guideline recommendations to screen this high-risk group[133]. This pathway includes sequential use of NITs (preferably a serum biomarker and an imaging technique) and can decrease secondary and tertiary referral rates and achieve larger cost savings.

In the Asia–Pacific region, quite a few studies have assessed the cross-sectional accuracy of non-invasive surrogates of liver biopsy among NAFLD patients[134,135]. It has been suggested that the serum tests and physical tools when used in combinations can yield more reliable data than that provided by either method alone[136]. Nevertheless, concerns are there regarding the definition of threshold values in Asian patients and Asia-Pacific Working Party stated that "at the present time, the clinical use of such tools to avoid liver biopsy remains undefined"[137].

Newsome *et al*[138] recently published the FibroScan-AST (FAST) score for the non-invasive identification of patients with significant fibrosis (≥ F2) and a NAFLD activity score (NAS) of ≥ 4 to detect those at increased risk of disease progression. This could reduce unnecessary liver biopsies in patients unlikely to have significant disease. The incorporation of VCTE values in the score enhanced the diagnostic performance. This prospective study was validated in multiple global cohorts from North America, Europe, and Asia. Discrimination was considerably higher for the FAST score when compared with FIB-4 and NFS. Now, further research on the performance of the FAST score is required to transition the use of such predictive models to clinical practice. The diagnostic accuracy of the sequential combination of FIB-4 and VCTE had been evaluated recently in an individual participant data meta-analysis that included 5735 patients. Depending upon the different cut-offs used, this combined algorithm can diagnose cirrhosis with a specificity of 95%-98%, obviating the need for liver biopsy

Table 1 Non-invasive tests of hepatic fibrosis and potential confounding factors

Biomarker panel	Parameters	Validation	Prognostic ability	Confounding factors/limitations
APRI	AST, platelet	Good	Fair	Large number of individuals fall in the indeterminate range
Fibrosis-4 index	Age, AST, ALT, platelet	Very good	Very good	Poor performance in patients aged ≤ 35 yr Low specificity in patients aged ≥ 65 yr Less sensitive in South Asian Population
NAFLD fibrosis score	Age, BMI, IFG or diabetes, AST, ALT, platelet, albumin	Very good	Good	Different cutoff values needed for younger or older participants Albumin may decrease in chronic illnesses, malnutrition, nephrotic syndrome and protein-losing enteropathy Less sensitive in South Asian Population
Enhanced liver fibrosis panel	PIIINP, HA, TIMP1	Good	Very good	PIIINP is increased in other fibrotic diseases or bone fracture TIMP1 is increased in cancer and inflammation Not as widely available as non-patented scores and more expensive
FibroMeter NAFLD	Age, weight, prothrombin index, ALT, AST, ferritin, fasting glucose	Fair	NA	Prothrombin index affected by anti-coagulants Ferritin is an acute phase protein Glucose is affected by anti-diabetic treatment More validation needed
NIS4	miR-34a-5p, α2-M, YKL-40, and glycated hemoglobin	Fair	NA	Not as widely available as non-patented scores and more expensive More validation is needed

ALT: Alanine aminotransferase; APRI: AST-to platelet ratio index; AST: Aspartate aminotransferase; BMI: Body mass index; HA: Hyaluronic acid; IFG: Impaired fasting glucose; α2-M: α2 macroglobulin; NA: Not applicable; NAFLD: Non-alcoholic fatty liver disease; PIIINP: Procollagen type III N-terminal peptide; PTI: Prothrombin index; TIMP-1: Tissue inhibitor of matrix metalloproteinase 1.

[139].

Identification of novel therapeutic targets

As the burden of NAFLD has become increasingly evident, so also have hurdles to developing effective therapeutic points of action. The development of progressive steatohepatitis is connected to excess metabolic substrate delivery to the liver that, in turn, induces cell stress, which can activate inflammatory and apoptotic signaling. Eventually, inflammation triggers a fibrogenic response that can lead to cirrhosis in the end[140]. This simplified model facilitates the evaluation of precise mechanisms underlying each of these factors and targeting them for treatment. Table 2 summarizes proposed 'druggable' pathophysiologic targets in NAFLD[141-153].

Quite a few of the recently carried out phase 2 and 3 studies failed to reproduce the encouraging antifibrotic or NASH-resolving effects observed in animal models. Reasons for this discrepancy between preclinical models and clinical settings are likely diverse. Most importantly, no model can ever assess compounds in the actual physiological settings of heterogeneous human populations. This aspect may become further relevant if mechanisms are not entirely translatable between two different species[154]. Additionally, none of the available NASH models used for preclinical trials adequately represents all the human disease aspects from the macroscopic to the molecular level. Moreover, only a few models reflect linked extrahepatic diseases (such as atherosclerosis, obesity, or IR). Finally, a higher heterogeneity in humans in relation to genetics, the gut microbiota, gender, and existing comorbidities leads to even more complications. It is, therefore, critical to recognize the drawbacks of preclinical models to improve clinical trial outcomes in drug development.

There is significant interindividual variability in the NAFLD susceptibility and for progression to liver-related complications[49]. It is becoming more and more apparent that there is substantial heterogeneity in the molecular and cellular processes

Table 2 Liver-targeted therapies in development for the treatment of nonalcoholic fatty liver disease

Treatment targets	Mechanism of action	Agent (oral/injectable)	Current status
Metabolism	FXR agonism	Obeticholic acid	Interim analysis of a phase 3 RCT (REGENERATE) showed significant histological improvement[141]
		Tropifexor (LJN452)	A phase 2 study recently completed (NCT02855164)
		Cilofexor	A phase 2 study in patients with NASH showed a decrease in hepatic fat[142]
	PPAR agonism	Elafibranor	Interim analysis a phase 3 trial (RESOLVE-IT) failed to show any treatment effect
		Lanifibranor (IVA337)	A phase 2 study in patients with T2DM and NAFLD is actively recruiting (NCT03459079)
		Saroglitazar	A phase 2 RCT (EVIDENCES IV) in participants with NAFLD/NASH has shown significant improvement in ALT, LFC, and IR[143]
	Acetyl-CoA Carboxylase inhibition	PF-05221304	Improved liver chemistry and liver fat in an RCT[144]
	GLP-1 agonism	Liraglutide	Only data from small studies have been published and the relative contribution of weight loss and improvement in glycemic control to the observed benefits in NASH are yet to be determined[145-147]
		Semaglutide	In a phase 2 trial, the primary endpoint (resolution of NASH with no worsening in fibrosis), was met[148]
	FGF21 agonism	Pegbelfermin (BMS-986036)	A series of phase 2b trials of pegbelfermin are underway
	MCP2 antagonism	MSDC-0602 K	The EMMINENCE phase 2b trial didn't meet the primary end point[149]
	THRβ agonism	Resmetirom (MGL-3196)	A phase 3 study is actively recruiting (NCT03900429)
Cell stress and apoptosis	Antioxidant	Vitamin E	Resolution of NASH in some studies, but not all; no impact on fibrosis[150]
	Pan-caspase inhibition	Emricasan	Phase 2b clinical trials for NASH failed to meet their primary efficacy end points[151]
	ASK1 inhibition	Selonsertib	Phase 3 STELLAR trials discontinued due to lack of efficacy
Inflammation	CCR2/CCR5 inhibition	Cenicriviroc	Phase 3 trial AURORA terminated due to lack of efficacy
	Inflammasome inhibition	SGM-1019	A phase 2 study is terminated due to a safety event (NCT03676231)
Fibrosis	LOXL2 inhibition	Simtuzumab	No benefit on histological analysis or on clinical outcomes[152]
Gut–liver signaling axis	FGF19 agonism	Aldafermin (NGM282)	In a phase 2 trial of patients with NASH, aldafermin reduced liver fat and produced a trend toward fibrosis improvement[153]

ACC: Acetyl-CoA carboxylase; ALT: Alanine aminotransferase; ASK1: Apoptosis signal-regulating kinase; CCR: C–C motif chemokine receptor; FGF: Fibroblast growth factor; FXR: Farnesoid X receptor; GLP1: Glucagon-like peptide 1; IR: Insulin resistance; LFC: Liver fat content; LOXL2: Lysyl oxidase homolog 2; NAFLD: Nonalcoholic fatty liver disease; NASH: Nonalcoholic steatohepatitis; PPAR: Peroxisome proliferator-activated receptor; THRβ: Thyroid hormone receptor β.

propelling the disease from one patient to the next. This understanding raises the possibility of matching specific therapeutic strategies to the particular disease drivers in a given patient. The development of such personalized approaches and the detection of subpopulations with distinctive disease drivers will need a combination of phenotypic, genetic, and molecular data[140]. Furthermore, genetic insights present a powerful approach to deduce and prioritize candidate drugs. Such selection can avoid numerous drawbacks while defining likely benefits[155]. However, drug discovery based on genetics is still in its infancy, and this area will present its challenges. NAFLD is associated with several metabolic disturbances. As many circadian clock-controlled genes are fundamental in the metabolic processes of the body, it is not unexpected that some of these genes can be potential therapeutic targets[156]. Thus, by considering the circadian cycling of their targets, new drugs for NAFLD can be administered in a way that optimizes the benefits and minimizes the side effects.

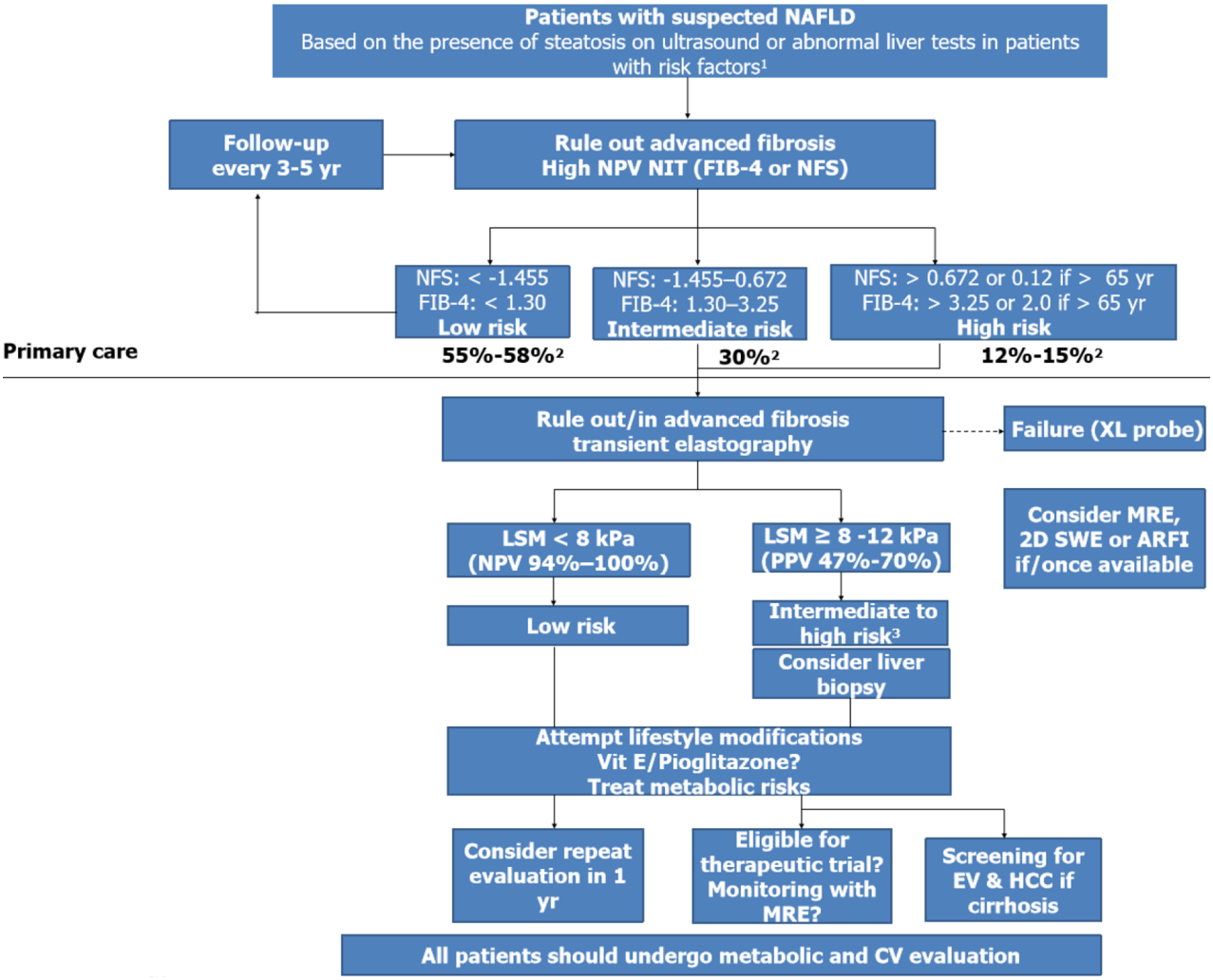

Figure 4 A suggested algorithm for the use of non-invasive tests for risk stratification of patients with suspected non-alcoholic fatty liver disease in clinical practice. [1]Obesity, type 2 diabetes, or metabolic syndrome; [2]Estimated prevalence for low, intermediate, and high risks groups; [3]Patented serum biomarkers (FibroTest, Fibrometer, or ELF) could be considered in patients with intermediate-risk. ARFI: Acoustic radiation force imaging; LSM: Liver stiffness measurement; MRE: Magnetic resonance elastography; NPV: Negative predictive value; PPV: Positive predictive value; SWE: Shear wave elastography.

Impact on clinical trials and endpoints

Given the rising disease burden associated with NAFLD, the development of outcome measures to assess the at-risk population and validate clinically relevant study endpoints is vital. Nevertheless, the natural history of NAFLD is highly variable, often nonlinear in progression. In addition, NAFLD itself is a heterogeneous disease that is shaped by the dynamic interaction between genetic predisposition, environmental factors, and several modifiable risk factors[157]. This pathogenetic background provides numerous potential targets for therapeutic intervention, however, this same complexity limits defining clear, measurable, and objective clinical endpoints[158]. Considering these factors, surrogate endpoints, which can be used to predict outcomes on clinically relevant endpoints, are expected to be beneficial in most patients. Furthermore, NAFLD is a slowly progressive disease, with a gap of many years between onset and development of "hard" clinical outcomes, such as liver-related and all-cause mortality. As stated earlier, the fibrosis stage is the most important predictor of liver-related outcomes. Unfortunately, the progression of fibrosis itself is also slow, with a median of 7.1 years in subjects with NASH[112]. Thus, selecting meaningful clinical endpoints has been a major challenge in drug development and validation. At present, before enrolling patients into NASH clinical trials, identifying which patients with NAFLD have NASH, particularly those with advanced fibrosis, is one of the major stumbling blocks. Once these at-risk patients have been selected, monitoring for fibrosis regression in individuals with advanced fibrosis appears to be the optimal endpoint in clinical trials and should supplant NASH-based endpoints[158]. Surrogate measures of liver-related outcomes also seem reliable. Although important, to assess for all-cause mortality (primarily CV death) and liver-related mortality will require

longer-term follow-up.

Liver biopsy is essentially prone to sampling error and interobserver variability; its invasive nature also makes it a barrier for large clinical trials. Given these limitations, the development of accurate, robust, and reproducible noninvasive surrogate endpoints which may ultimately replace biopsy in trials are eagerly sought in NAFLD research[159]. Algorithms such as NFS and FIB-4 may be useful tools for prescreening, in order to enrich the patient group with an appropriate spectrum of NASH and fibrosis for enrollment. Noninvasive imaging methods such as VCTE and MRE are likely to play a future role but presently lack the ability to differentiate between closely related fibrosis stages[160].

To summarize, a combination of the slow nature of disease progression in NAFLD, heterogeneity of therapeutic targets, and inherent limitations of serial liver biopsy to evaluate effects of intervention have considerably hampered clinical trial design as well as the development of new and effective therapies[158]. Thus, the standard trial design that does not consider the disease heterogeneity may not be the best approach for learning this complex disease. Future clinical trials need to target patients with specific characteristics (gender, hormonal status, genetic susceptibility, metabolic and microbiota signatures, and the presence or absence of comorbidities) once the connections between these characteristics and the therapeutic targets are clearly understood[2].

FUTURE PERSPECTIVES

With increasing recognition of heterogeneous molecular and genetic drivers of NAFLD, there is a possibility of precision medicine based on the identification of specific drivers of the disease. An integrated model of NAFLD development based on genetic, molecular, histology, "omics" based data (transcriptome, metabolite, proteome, microbiome), and disease phenotype to identify disease subpopulations is required for such personalized approaches[140]. Critical data on molecular heterogeneity and its relation to clinical outcomes of NAFLD to going to explore new horizons in the management of this global pandemic[161]. A better understanding of bidirectional and dynamic disease progression and regression (*e.g.* fibrosis), the influence of behavioral factors, and establishing a correlation with end-organ damage is warranted. Prospective follow-up data on the evolution of pediatric NAFLD into adulthood shall shed light on pediatric disease evolution[162]. Identification and validation of non-invasive methods of disease assessment and biomarkers will accelerate the development of pharmacotherapy and testing of combination therapies. Seamless phase II-IV trial designs, virtual placebo cohort analysis, master clinical trials testing multiple agents and multiple disease types, use of effectiveness trials in real-world settings, and patient-reported outcomes would revolutionize clinical trials for NAFLD. Precise terminology, characterization of disease heterogeneity (both molecular and clinical), novel translational models to identify new therapeutic target, and thus better designed clinical trials would help reduce the burden of the disease[2].

CONCLUSION

The impact of the upsurge in NAFLD patients and a rising proportion with advanced disease will be reflected in higher rates of hepatic and extrahepatic morbidity and mortality, which will continue to burden the health care system heavily. On the other hand, a lack of enough consideration of heterogeneity in risk profiles and responsiveness to treatment posing impediments that hampers progress to effective treatments. It is anticipated that a more robust understanding of pathophysiology will result in better characterization and subphenotyping of the disease and its drivers. In turn, this understanding of disease variability may help the introduction of appropriate noninvasive biomarkers for each subtype, thus promoting more individualized interventions. In this regard, any discussions on the update of nomenclature or more appropriate terminology are in the right direction. However, the proposed redefining of the disease should increase the prioritization of research activity on NAFLD to fill current knowledge gaps and find new tools to overcome the challenges. It appears to be important to place NAFLD/MAFLD/DAFLD under the same umbrella with significant comorbidities and approach NAFLD/MAFLD/DAFLD holistically rather than facing NAFLD as a separate entity. Future studies are likely to provide us the necessary prerequisites for designing more appropriate clinical trials to

identify finely tailored diagnostic and treatment strategies for our patients.

REFERENCES

1 **Ludwig J**, Viggiano TR, McGill DB, Oh BJ. Nonalcoholic steatohepatitis: Mayo Clinic experiences with a hitherto unnamed disease. *Mayo Clin Proc* 1980; **55**: 434-438 [PMID: 7382552]

2 **Eslam M**, Sanyal AJ, George J; International Consensus Panel. MAFLD: A Consensus-Driven Proposed Nomenclature for Metabolic Associated Fatty Liver Disease. *Gastroenterology* 2020; **158**: 1999-2014.e1 [PMID: 32044314 DOI: 10.1053/j.gastro.2019.11.312]

3 **Singh SP**, Anirvan P, Reddy KR, Conjeevaram HS, Marchesini G, Rinella ME, Madan K, Petroni ML, Al-Mahtab M, Caldwell SH, Aithal GP, Hamid SS, Farrell GC, Satapathy SK, Duseja A, Acharya SK, Dassanayake AS, Goh KL. Non-alcoholic fatty liver disease: Not time for an obituary just yet! *J Hepatol* 2021; **74**: 972-974 [PMID: 33340575 DOI: 10.1016/j.jhep.2020.10.015]

4 **Yki-Järvinen H**, Luukkonen PK. Heterogeneity of non-alcoholic fatty liver disease. *Liver Int* 2015; **35**: 2498-2500 [PMID: 26386359 DOI: 10.1111/liv.12970]

5 **Mittal S**, El-Serag HB, Sada YH, Kanwal F, Duan Z, Temple S, May SB, Kramer JR, Richardson PA, Davila JA. Hepatocellular Carcinoma in the Absence of Cirrhosis in United States Veterans is Associated With Nonalcoholic Fatty Liver Disease. *Clin Gastroenterol Hepatol* 2016; **14**: 124-31.e1 [PMID: 26196445 DOI: 10.1016/j.cgh.2015.07.019]

6 **Friedman SL**, Neuschwander-Tetri BA, Rinella M, Sanyal AJ. Mechanisms of NAFLD development and therapeutic strategies. *Nat Med* 2018; **24**: 908-922 [PMID: 29967350 DOI: 10.1038/s41591-018-0104-9]

7 **Åberg F**, Helenius-Hietala J, Puukka P, Färkkilä M, Jula A. Interaction between alcohol consumption and metabolic syndrome in predicting severe liver disease in the general population. *Hepatology* 2018; **67**: 2141-2149 [PMID: 29164643 DOI: 10.1002/hep.29631]

8 **Shiha G**, Alswat K, Al Khatry M, Sharara AI, Örmeci N, Waked I, Benazzouz M, Al-Ali F, Hamed AE, Hamoudi W, Attia D, Derbala M, Sharaf-Eldin M, Al-Busafi SA, Zaky S, Bamakhrama K, Ibrahim N, Ajlouni Y, Sabbah M, Salama M, Anushiravani A, Afredj N, Barakat S, Hashim A, Fouad Y, Soliman R. Nomenclature and definition of metabolic-associated fatty liver disease: a consensus from the Middle East and north Africa. *Lancet Gastroenterol Hepatol* 2021; **6**: 57-64 [PMID: 33181119 DOI: 10.1016/S2468-1253(20)30213-2]

9 **Yuan J**, Chen C, Cui J, Lu J, Yan C, Wei X, Zhao X, Li N, Li S, Xue G, Cheng W, Li B, Li H, Lin W, Tian C, Zhao J, Han J, An D, Zhang Q, Wei H, Zheng M, Ma X, Li W, Chen X, Zhang Z, Zeng H, Ying S, Wu J, Yang R, Liu D. Fatty Liver Disease Caused by High-Alcohol-Producing Klebsiella pneumoniae. *Cell Metab* 2019; **30**: 675-688.e7 [PMID: 31543403 DOI: 10.1016/j.cmet.2019.08.018]

10 **Jepsen P**, Vilstrup H, Mellemkjaer L, Thulstrup AM, Olsen JH, Baron JA, Sørensen HT. Prognosis of patients with a diagnosis of fatty liver--a registry-based cohort study. *Hepatogastroenterology* 2003; **50**: 2101-2104 [PMID: 14696473]

11 **Loria P**, Lonardo A, Carulli N. Should nonalcoholic fatty liver disease be renamed? *Dig Dis* 2005; **23**: 72-82 [PMID: 15920328 DOI: 10.1159/000084728]

12 **Polyzos SA**, Mantzoros CS. Making progress in nonalcoholic fatty liver disease (NAFLD) as we are transitioning from the era of NAFLD to dys-metabolism associated fatty liver disease (DAFLD). *Metabolism* 2020; **111S**: 154318 [PMID: 32707055 DOI: 10.1016/j.metabol.2020.154318]

13 **Eslam M**, Newsome PN, Sarin SK, Anstee QM, Targher G, Romero-Gomez M, Zelber-Sagi S, Wai-Sun Wong V, Dufour JF, Schattenberg JM, Kawaguchi T, Arrese M, Valenti L, Shiha G, Tiribelli C, Yki-Järvinen H, Fan JG, Grønbæk H, Yilmaz Y, Cortez-Pinto H, Oliveira CP, Bedossa P, Adams LA, Zheng MH, Fouad Y, Chan WK, Mendez-Sanchez N, Ahn SH, Castera L, Bugianesi E, Ratziu V, George J. A new definition for metabolic dysfunction-associated fatty liver disease: An international expert consensus statement. *J Hepatol* 2020; **73**: 202-209 [PMID: 32278004 DOI: 10.1016/j.jhep.2020.03.039]

14 **Lin S**, Huang J, Wang M, Kumar R, Liu Y, Liu S, Wu Y, Wang X, Zhu Y. Comparison of MAFLD and NAFLD diagnostic criteria in real world. *Liver Int* 2020; **40**: 2082-2089 [PMID: 32478487 DOI: 10.1111/liv.14548]

15 **Younossi ZM**, Rinella ME, Sanyal AJ, Harrison SA, Brunt EM, Goodman Z, Cohen DE, Loomba R. From NAFLD to MAFLD: Implications of a Premature Change in Terminology. *Hepatology* 2021; **73**: 1194-1198 [PMID: 32544255 DOI: 10.1002/hep.31420]

16 **Dongiovanni P**, Paolini E, Corsini A, Sirtori CR, Ruscica M. Nonalcoholic fatty liver disease or metabolic dysfunction-associated fatty liver disease diagnoses and cardiovascular diseases: From epidemiology to drug approaches. *Eur J Clin Invest* 2021; **51**: e13519 [PMID: 33583033 DOI: 10.1111/eci.13519]

17 **Cai J**, Zhang XJ, Ji YX, Zhang P, She ZG, Li H. Nonalcoholic Fatty Liver Disease Pandemic Fuels the Upsurge in Cardiovascular Diseases. *Circ Res* 2020; **126**: 679-704 [PMID: 32105577 DOI: 10.1161/CIRCRESAHA.119.316337]

18 **Targher G**, Corey KE, Byrne CD. NAFLD, and cardiovascular and cardiac diseases: Factors influencing risk, prediction and treatment. *Diabetes Metab* 2021; **47**: 101215 [PMID: 33296704 DOI: 10.1016/j.diabet.2020.101215]

19 **Shroff H**, VanWagner LB. Cardiovascular Disease in Nonalcoholic Steatohepatitis: Screening and

Management. *Curr Hepatol Rep* 2020; **19**: 315-326 [PMID: 33585157 DOI: 10.1007/s11901-020-00530-0]
20 **Frith J**, Day CP, Henderson E, Burt AD, Newton JL. Non-alcoholic fatty liver disease in older people. *Gerontology* 2009; **55**: 607-613 [PMID: 19690397 DOI: 10.1159/000235677]
21 **Frith J**, Jones D, Newton JL. Chronic liver disease in an ageing population. *Age Ageing* 2009; **38**: 11-18 [PMID: 19029099 DOI: 10.1093/ageing/afn242]
22 **Donati B**, Valenti L. Telomeres, NAFLD and Chronic Liver Disease. *Int J Mol Sci* 2016; **17**: 383 [PMID: 26999107 DOI: 10.3390/ijms17030383]
23 **Kuk JL**, Saunders TJ, Davidson LE, Ross R. Age-related changes in total and regional fat distribution. *Ageing Res Rev* 2009; **8**: 339-348 [PMID: 19576300 DOI: 10.1016/j.arr.2009.06.001]
24 **Lonardo A**, Nascimbeni F, Ballestri S, Fairweather D, Win S, Than TA, Abdelmalek MF, Suzuki A. Sex Differences in Nonalcoholic Fatty Liver Disease: State of the Art and Identification of Research Gaps. *Hepatology* 2019; **70**: 1457-1469 [PMID: 30924946 DOI: 10.1002/hep.30626]
25 **Lovejoy JC**, Champagne CM, de Jonge L, Xie H, Smith SR. Increased visceral fat and decreased energy expenditure during the menopausal transition. *Int J Obes (Lond)* 2008; **32**: 949-958 [PMID: 18332882 DOI: 10.1038/ijo.2008.25]
26 **Kurt Z**, Barrere-Cain R, LaGuardia J, Mehrabian M, Pan C, Hui ST, Norheim F, Zhou Z, Hasin Y, Lusis AJ, Yang X. Tissue-specific pathways and networks underlying sexual dimorphism in non-alcoholic fatty liver disease. *Biol Sex Differ* 2018; **9**: 46 [PMID: 30343673 DOI: 10.1186/s13293-018-0205-7]
27 **Cvitanović Tomaš T**, Urlep Ž, Moškon M, Mraz M, Rozman D. *LiverSex* Computational Model: Sexual Aspects in Hepatic Metabolism and Abnormalities. *Front Physiol* 2018; **9**: 360 [PMID: 29706895 DOI: 10.3389/fphys.2018.00360]
28 **Yang JD**, Abdelmalek MF, Pang H, Guy CD, Smith AD, Diehl AM, Suzuki A. Gender and menopause impact severity of fibrosis among patients with nonalcoholic steatohepatitis. *Hepatology* 2014; **59**: 1406-1414 [PMID: 24123276 DOI: 10.1002/hep.26761]
29 **Klair JS**, Yang JD, Abdelmalek MF, Guy CD, Gill RM, Yates K, Unalp-Arida A, Lavine JE, Clark JM, Diehl AM, Suzuki A; Nonalcoholic Steatohepatitis Clinical Research Network. A longer duration of estrogen deficiency increases fibrosis risk among postmenopausal women with nonalcoholic fatty liver disease. *Hepatology* 2016; **64**: 85-91 [PMID: 26919573 DOI: 10.1002/hep.28514]
30 **Rich NE**, Oji S, Mufti AR, Browning JD, Parikh ND, Odewole M, Mayo H, Singal AG. Racial and Ethnic Disparities in Nonalcoholic Fatty Liver Disease Prevalence, Severity, and Outcomes in the United States: A Systematic Review and Meta-analysis. *Clin Gastroenterol Hepatol* 2018; **16**: 198-210.e2 [PMID: 28970148 DOI: 10.1016/j.cgh.2017.09.041]
31 **Romeo S**, Kozlitina J, Xing C, Pertsemlidis A, Cox D, Pennacchio LA, Boerwinkle E, Cohen JC, Hobbs HH. Genetic variation in PNPLA3 confers susceptibility to nonalcoholic fatty liver disease. *Nat Genet* 2008; **40**: 1461-1465 [PMID: 18820647 DOI: 10.1038/ng.257]
32 **Mohanty SR**, Troy TN, Huo D, O'Brien BL, Jensen DM, Hart J. Influence of ethnicity on histological differences in non-alcoholic fatty liver disease. *J Hepatol* 2009; **50**: 797-804 [PMID: 19231016 DOI: 10.1016/j.jhep.2008.11.017]
33 **Tabibian JH**, Lazo M, Durazo FA, Yeh HC, Tong MJ, Clark JM. Nonalcoholic fatty liver disease across ethno-racial groups: do Asian-American adults represent a new at-risk population? *J Gastroenterol Hepatol* 2011; **26**: 501-509 [PMID: 21332546 DOI: 10.1111/j.1440-1746.2010.06443.x]
34 **Shen J**, Wong GL, Chan HL, Chan HY, Yeung DK, Chan RS, Chim AM, Chan AW, Choi PC, Woo J, Chu WC, Wong VW. PNPLA3 gene polymorphism accounts for fatty liver in community subjects without metabolic syndrome. *Aliment Pharmacol Ther* 2014; **39**: 532-539 [PMID: 24417250 DOI: 10.1111/apt.12609]
35 **Trovato FM**, Catalano D, Martines GF, Pace P, Trovato GM. Mediterranean diet and non-alcoholic fatty liver disease: the need of extended and comprehensive interventions. *Clin Nutr* 2015; **34**: 86-88 [PMID: 24529325 DOI: 10.1016/j.clnu.2014.01.018]
36 **David LA**, Maurice CF, Carmody RN, Gootenberg DB, Button JE, Wolfe BE, Ling AV, Devlin AS, Varma Y, Fischbach MA, Biddinger SB, Dutton RJ, Turnbaugh PJ. Diet rapidly and reproducibly alters the human gut microbiome. *Nature* 2014; **505**: 559-563 [PMID: 24336217 DOI: 10.1038/nature12820]
37 **Loomba R**, Seguritan V, Li W, Long T, Klitgord N, Bhatt A, Dulai PS, Caussy C, Bettencourt R, Highlander SK, Jones MB, Sirlin CB, Schnabl B, Brinkac L, Schork N, Chen CH, Brenner DA, Biggs W, Yooseph S, Venter JC, Nelson KE. Gut Microbiome-Based Metagenomic Signature for Non-invasive Detection of Advanced Fibrosis in Human Nonalcoholic Fatty Liver Disease. *Cell Metab* 2017; **25**: 1054-1062.e5 [PMID: 28467925 DOI: 10.1016/j.cmet.2017.04.001]
38 **Ilan Y**. Leaky gut and the liver: a role for bacterial translocation in nonalcoholic steatohepatitis. *World J Gastroenterol* 2012; **18**: 2609-2618 [PMID: 22690069 DOI: 10.3748/wjg.v18.i21.2609]
39 **Jialal I**, Kaur H, Devaraj S. Toll-like receptor status in obesity and metabolic syndrome: a translational perspective. *J Clin Endocrinol Metab* 2014; **99**: 39-48 [PMID: 24187406 DOI: 10.1210/jc.2013-3092]
40 **Hall AB**, Tolonen AC, Xavier RJ. Human genetic variation and the gut microbiome in disease. *Nat Rev Genet* 2017; **18**: 690-699 [PMID: 28824167 DOI: 10.1038/nrg.2017.63]
41 **Brooks AW**, Priya S, Blekhman R, Bordenstein SR. Gut microbiota diversity across ethnicities in

the United States. *PLoS Biol* 2018; **16**: e2006842 [PMID: 30513082 DOI: 10.1371/journal.pbio.2006842]

42 **Loos RJF**, Kilpeläinen TO. Genes that make you fat, but keep you healthy. *J Intern Med* 2018; **284**: 450-463 [PMID: 30144199 DOI: 10.1111/joim.12827]

43 **Eckel N**, Li Y, Kuxhaus O, Stefan N, Hu FB, Schulze MB. Transition from metabolic healthy to unhealthy phenotypes and association with cardiovascular disease risk across BMI categories in 90 257 women (the Nurses' Health Study): 30 year follow-up from a prospective cohort study. *Lancet Diabetes Endocrinol* 2018; **6**: 714-724 [PMID: 29859908 DOI: 10.1016/S2213-8587(18)30137-2]

44 **Agbim U**, Carr RM, Pickett-Blakely O, Dagogo-Jack S. Ethnic Disparities in Adiposity: Focus on Non-alcoholic Fatty Liver Disease, Visceral, and Generalized Obesity. *Curr Obes Rep* 2019; **8**: 243-254 [PMID: 31144261 DOI: 10.1007/s13679-019-00349-x]

45 **Ding C**, Chan Z, Magkos F. Lean, but not healthy: the 'metabolically obese, normal-weight' phenotype. *Curr Opin Clin Nutr Metab Care* 2016; **19**: 408-417 [PMID: 27552473 DOI: 10.1097/MCO.0000000000000317]

46 **Chen F**, Esmaili S, Rogers GB, Bugianesi E, Petta S, Marchesini G, Bayoumi A, Metwally M, Azardaryany MK, Coulter S, Choo JM, Younes R, Rosso C, Liddle C, Adams LA, Craxì A, George J, Eslam M. Lean NAFLD: A Distinct Entity Shaped by Differential Metabolic Adaptation. *Hepatology* 2020; **71**: 1213-1227 [PMID: 31442319 DOI: 10.1002/hep.30908]

47 **Park JH**, Cho B, Kwon H, Prilutsky D, Yun JM, Choi HC, Hwang KB, Lee IH, Kim JI, Kong SW. I148M variant in PNPLA3 reduces central adiposity and metabolic disease risks while increasing nonalcoholic fatty liver disease. *Liver Int* 2015; **35**: 2537-2546 [PMID: 26148225 DOI: 10.1111/liv.12909]

48 **Hoang SA**, Oseini A, Feaver RE, Cole BK, Asgharpour A, Vincent R, Siddiqui M, Lawson MJ, Day NC, Taylor JM, Wamhoff BR, Mirshahi F, Contos MJ, Idowu M, Sanyal AJ. Gene Expression Predicts Histological Severity and Reveals Distinct Molecular Profiles of Nonalcoholic Fatty Liver Disease. *Sci Rep* 2019; **9**: 12541 [PMID: 31467298 DOI: 10.1038/s41598-019-48746-5]

49 **Eslam M**, Valenti L, Romeo S. Genetics and epigenetics of NAFLD and NASH: Clinical impact. *J Hepatol* 2018; **68**: 268-279 [PMID: 29122391 DOI: 10.1016/j.jhep.2017.09.003]

50 **Liu YL**, Reeves HL, Burt AD, Tiniakos D, McPherson S, Leathart JB, Allison ME, Alexander GJ, Piguet AC, Anty R, Donaldson P, Aithal GP, Francque S, Van Gaal L, Clement K, Ratziu V, Dufour JF, Day CP, Daly AK, Anstee QM. TM6SF2 rs58542926 influences hepatic fibrosis progression in patients with non-alcoholic fatty liver disease. *Nat Commun* 2014; **5**: 4309 [PMID: 24978903 DOI: 10.1038/ncomms5309]

51 **Diogo D**, Tian C, Franklin CS, Alanne-Kinnunen M, March M, Spencer CCA, Vangjeli C, Weale ME, Mattsson H, Kilpeläinen E, Sleiman PMA, Reilly DF, McElwee J, Maranville JC, Chatterjee AK, Bhandari A, Nguyen KH, Estrada K, Reeve MP, Hutz J, Bing N, John S, MacArthur DG, Salomaa V, Ripatti S, Hakonarson H, Daly MJ, Palotie A, Hinds DA, Donnelly P, Fox CS, Day-Williams AG, Plenge RM, Runz H. Phenome-wide association studies across large population cohorts support drug target validation. *Nat Commun* 2018; **9**: 4285 [PMID: 30327483 DOI: 10.1038/s41467-018-06540-3]

52 **Mahajan A**, Wessel J, Willems SM, Zhao W, Robertson NR, Chu AY, Gan W, Kitajima H, Taliun D, Rayner NW, Guo X, Lu Y, Li M, Jensen RA, Hu Y, Huo S, Lohman KK, Zhang W, Cook JP, Prins BP, Flannick J, Grarup N, Trubetskoy VV, Kravic J, Kim YJ, Rybin DV, Yaghootkar H, Müller-Nurasyid M, Meidtner K, Li-Gao R, Varga TV, Marten J, Li J, Smith AV, An P, Ligthart S, Gustafsson S, Malerba G, Demirkan A, Tajes JF, Steinthorsdottir V, Wuttke M, Lecoeur C, Preuss M, Bielak LF, Graff M, Highland HM, Justice AE, Liu DJ, Marouli E, Peloso GM, Warren HR; ExomeBP Consortium; MAGIC Consortium; GIANT Consortium, Afaq S, Afzal S, Ahlqvist E, Almgren P, Amin N, Bang LB, Bertoni AG, Bombieri C, Bork-Jensen J, Brandslund I, Brody JA, Burtt NP, Canouil M, Chen YI, Cho YS, Christensen C, Eastwood SV, Eckardt KU, Fischer K, Gambaro G, Giedraitis V, Grove ML, de Haan HG, Hackinger S, Hai Y, Han S, Tybjærg-Hansen A, Hivert MF, Isomaa B, Jäger S, Jørgensen ME, Jørgensen T, Käräjämäki A, Kim BJ, Kim SS, Koistinen HA, Kovacs P, Kriebel J, Kronenberg F, Läll K, Lange LA, Lee JJ, Lehne B, Li H, Lin KH, Linneberg A, Liu CT, Liu J, Loh M, Mägi R, Mamakou V, McKean-Cowdin R, Nadkarni G, Neville M, Nielsen SF, Ntalla I, Peyser PA, Rathmann W, Rice K, Rich SS, Rode L, Rolandsson O, Schönherr S, Selvin E, Small KS, Stančáková A, Surendran P, Taylor KD, Teslovich TM, Thorand B, Thorleifsson G, Tin A, Tönjes A, Varbo A, Witte DR, Wood AR, Yajnik P, Yao J, Yengo L, Young R, Amouyel P, Boeing H, Boerwinkle E, Bottinger EP, Chowdhury R, Collins FS, Dedoussis G, Dehghan A, Deloukas P, Ferrario MM, Ferrières J, Florez JC, Frossard P, Gudnason V, Harris TB, Heckbert SR, Howson JMM, Ingelsson M, Kathiresan S, Kee F, Kuusisto J, Langenberg C, Launer LJ, Lindgren CM, Männistö S, Meitinger T, Melander O, Mohlke KL, Moitry M, Morris AD, Murray AD, de Mutsert R, Orho-Melander M, Owen KR, Perola M, Peters A, Province MA, Rasheed A, Ridker PM, Rivadineira F, Rosendaal FR, Rosengren AH, Salomaa V, Sheu WH, Sladek R, Smith BH, Strauch K, Uitterlinden AG, Varma R, Willer CJ, Blüher M, Butterworth AS, Chambers JC, Chasman DI, Danesh J, van Duijn C, Dupuis J, Franco OH, Franks PW, Froguel P, Grallert H, Groop L, Han BG, Hansen T, Hattersley AT, Hayward C, Ingelsson E, Kardia SLR, Karpe F, Kooner JS, Köttgen A, Kuulasmaa K, Laakso M, Lin X, Lind L, Liu Y, Loos RJF, Marchini J, Metspalu A, Mook-Kanamori D, Nordestgaard BG, Palmer CNA, Pankow JS, Pedersen O, Psaty BM, Rauramaa R, Sattar N, Schulze MB, Soranzo N, Spector TD, Stefansson K, Stumvoll M, Thorsteinsdottir U, Tuomi T, Tuomilehto J, Wareham NJ, Wilson JG, Zeggini E, Scott RA,

Barroso I, Frayling TM, Goodarzi MO, Meigs JB, Boehnke M, Saleheen D, Morris AP, Rotter JI, McCarthy MI. Refining the accuracy of validated target identification through coding variant fine-mapping in type 2 diabetes. *Nat Genet* 2018; **50**: 559-571 [PMID: 29632382 DOI: 10.1038/s41588-018-0084-1]

53 **Simons N**, Isaacs A, Koek GH, Kuč S, Schaper NC, Brouwers MCGJ. PNPLA3, TM6SF2, and MBOAT7 Genotypes and Coronary Artery Disease. *Gastroenterology* 2017; **152**: 912-913 [PMID: 28157516 DOI: 10.1053/j.gastro.2016.12.020]

54 **Abul-Husn NS**, Cheng X, Li AH, Xin Y, Schurmann C, Stevis P, Liu Y, Kozlitina J, Stender S, Wood GC, Stepanchick AN, Still MD, McCarthy S, O'Dushlaine C, Packer JS, Balasubramanian S, Gosalia N, Esopi D, Kim SY, Mukherjee S, Lopez AE, Fuller ED, Penn J, Chu X, Luo JZ, Mirshahi UL, Carey DJ, Still CD, Feldman MD, Small A, Damrauer SM, Rader DJ, Zambrowicz B, Olson W, Murphy AJ, Borecki IB, Shuldiner AR, Reid JG, Overton JD, Yancopoulos GD, Hobbs HH, Cohen JC, Gottesman O, Teslovich TM, Baras A, Mirshahi T, Gromada J, Dewey FE. A Protein-Truncating HSD17B13 Variant and Protection from Chronic Liver Disease. *N Engl J Med* 2018; **378**: 1096-1106 [PMID: 29562163 DOI: 10.1056/NEJMoa1712191]

55 **Pirola CJ**, Fernández Gianotti T, Castaño GO, Mallardi P, San Martino J, Mora Gonzalez Lopez Ledesma M, Flichman D, Mirshahi F, Sanyal AJ, Sookoian S. Circulating microRNA signature in non-alcoholic fatty liver disease: from serum non-coding RNAs to liver histology and disease pathogenesis. *Gut* 2015; **64**: 800-812 [PMID: 24973316 DOI: 10.1136/gutjnl-2014-306996]

56 **Sookoian S**, Rohr C, Salatino A, Dopazo H, Fernandez Gianotti T, Castaño GO, Pirola CJ. Genetic variation in long noncoding RNAs and the risk of nonalcoholic fatty liver disease. *Oncotarget* 2017; **8**: 22917-22926 [PMID: 28206970 DOI: 10.18632/oncotarget.15286]

57 **Ahrens M**, Ammerpohl O, von Schönfels W, Kolarova J, Bens S, Itzel T, Teufel A, Herrmann A, Brosch M, Hinrichsen H, Erhart W, Egberts J, Sipos B, Schreiber S, Häsler R, Stickel F, Becker T, Krawczak M, Röcken C, Siebert R, Schafmayer C, Hampe J. DNA methylation analysis in nonalcoholic fatty liver disease suggests distinct disease-specific and remodeling signatures after bariatric surgery. *Cell Metab* 2013; **18**: 296-302 [PMID: 23931760 DOI: 10.1016/j.cmet.2013.07.004]

58 **Hardy T**, Zeybel M, Day CP, Dipper C, Masson S, McPherson S, Henderson E, Tiniakos D, White S, French J, Mann DA, Anstee QM, Mann J. Plasma DNA methylation: a potential biomarker for stratification of liver fibrosis in non-alcoholic fatty liver disease. *Gut* 2017; **66**: 1321-1328 [PMID: 27002005 DOI: 10.1136/gutjnl-2016-311526]

59 **Kitamoto T**, Kitamoto A, Ogawa Y, Honda Y, Imajo K, Saito S, Yoneda M, Nakamura T, Nakajima A, Hotta K. Targeted-bisulfite sequence analysis of the methylation of CpG islands in genes encoding PNPLA3, SAMM50, and PARVB of patients with non-alcoholic fatty liver disease. *J Hepatol* 2015; **63**: 494-502 [PMID: 25776890 DOI: 10.1016/j.jhep.2015.02.049]

60 **Baker PR 2nd**, Friedman JE. Mitochondrial role in the neonatal predisposition to developing nonalcoholic fatty liver disease. *J Clin Invest* 2018; **128**: 3692-3703 [PMID: 30168806 DOI: 10.1172/JCI120846]

61 **Suter MA**, Ma J, Vuguin PM, Hartil K, Fiallo A, Harris RA, Charron MJ, Aagaard KM. In utero exposure to a maternal high-fat diet alters the epigenetic histone code in a murine model. *Am J Obstet Gynecol* 2014; **210**: 463.e1-463.e11 [PMID: 24793723 DOI: 10.1016/j.ajog.2014.01.045]

62 **Gemma C**, Sookoian S, Alvariñas J, García SI, Quintana L, Kanevsky D, González CD, Pirola CJ. Maternal pregestational BMI is associated with methylation of the PPARGC1A promoter in newborns. *Obesity (Silver Spring)* 2009; **17**: 1032-1039 [PMID: 19148128 DOI: 10.1038/oby.2008.605]

63 **Struben VM**, Hespenheide EE, Caldwell SH. Nonalcoholic steatohepatitis and cryptogenic cirrhosis within kindreds. *Am J Med* 2000; **108**: 9-13 [PMID: 11059435 DOI: 10.1016/s0002-9343(99)00315-0]

64 **Caussy C**, Soni M, Cui J, Bettencourt R, Schork N, Chen CH, Ikhwan MA, Bassirian S, Cepin S, Gonzalez MP, Mendler M, Kono Y, Vodkin I, Mekeel K, Haldorson J, Hemming A, Andrews B, Salotti J, Richards L, Brenner DA, Sirlin CB, Loomba R; Familial NAFLD Cirrhosis Research Consortium. Nonalcoholic fatty liver disease with cirrhosis increases familial risk for advanced fibrosis. *J Clin Invest* 2017; **127**: 2697-2704 [PMID: 28628033 DOI: 10.1172/JCI93465]

65 **Boyle M**, Masson S, Anstee QM. The bidirectional impacts of alcohol consumption and the metabolic syndrome: Cofactors for progressive fatty liver disease. *J Hepatol* 2018; **68**: 251-267 [PMID: 29113910 DOI: 10.1016/j.jhep.2017.11.006]

66 **Rehm J**, Taylor B, Mohapatra S, Irving H, Baliunas D, Patra J, Roerecke M. Alcohol as a risk factor for liver cirrhosis: a systematic review and meta-analysis. *Drug Alcohol Rev* 2010; **29**: 437-445 [PMID: 20636661 DOI: 10.1111/j.1465-3362.2009.00153.x]

67 **Mato JM**, Alonso C, Noureddin M, Lu SC. Biomarkers and subtypes of deranged lipid metabolism in non-alcoholic fatty liver disease. *World J Gastroenterol* 2019; **25**: 3009-3020 [PMID: 31293337 DOI: 10.3748/wjg.v25.i24.3009]

68 **Vandromme M**, Jun T, Perumalswami P, Dudley JT, Branch A, Li L. Automated phenotyping of patients with non-alcoholic fatty liver disease reveals clinically relevant disease subtypes. *Pac Symp Biocomput* 2020; **25**: 91-102 [PMID: 31797589]

69 **Younossi ZM**, Koenig AB, Abdelatif D, Fazel Y, Henry L, Wymer M. Global epidemiology of nonalcoholic fatty liver disease-Meta-analytic assessment of prevalence, incidence, and outcomes. *Hepatology* 2016; **64**: 73-84 [PMID: 26707365 DOI: 10.1002/hep.28431]

70 **Li J**, Zou B, Yeo YH, Feng Y, Xie X, Lee DH, Fujii H, Wu Y, Kam LY, Ji F, Li X, Chien N, Wei M, Ogawa E, Zhao C, Wu X, Stave CD, Henry L, Barnett S, Takahashi H, Furusyo N, Eguchi Y, Hsu YC, Lee TY, Ren W, Qin C, Jun DW, Toyoda H, Wong VW, Cheung R, Zhu Q, Nguyen MH. Prevalence, incidence, and outcome of non-alcoholic fatty liver disease in Asia, 1999-2019: a systematic review and meta-analysis. *Lancet Gastroenterol Hepatol* 2019; **4**: 389-398 [PMID: 30902670 DOI: 10.1016/S2468-1253(19)30039-1]

71 **Fan JG**, Kim SU, Wong VW. New trends on obesity and NAFLD in Asia. *J Hepatol* 2017; **67**: 862-873 [PMID: 28642059 DOI: 10.1016/j.jhep.2017.06.003]

72 **Mohan V**, Farooq S, Deepa M, Ravikumar R, Pitchumoni CS. Prevalence of non-alcoholic fatty liver disease in urban south Indians in relation to different grades of glucose intolerance and metabolic syndrome. *Diabetes Res Clin Pract* 2009; **84**: 84-91 [PMID: 19168251 DOI: 10.1016/j.diabres.2008.11.039]

73 **Lin YC**, Chang PF, Hu FC, Yang WS, Chang MH, Ni YH. A common variant in the PNPLA3 gene is a risk factor for non-alcoholic fatty liver disease in obese Taiwanese children. *J Pediatr* 2011; **158**: 740-744 [PMID: 21168155 DOI: 10.1016/j.jpeds.2010.11.016]

74 **Bhatt SP**, Nigam P, Misra A, Guleria R, Pandey RM, Pasha MA. Genetic variation in the patatin-like phospholipase domain-containing protein-3 (PNPLA-3) gene in Asian Indians with nonalcoholic fatty liver disease. *Metab Syndr Relat Disord* 2013; **11**: 329-335 [PMID: 23734760 DOI: 10.1089/met.2012.0064]

75 **Zain SM**, Mohamed Z, Mahadeva S, Rampal S, Basu RC, Cheah PL, Salim A, Mohamed R. Susceptibility and gene interaction study of the angiotensin II type 1 receptor (AGTR1) gene polymorphisms with non-alcoholic fatty liver disease in a multi-ethnic population. *PLoS One* 2013; **8**: e58538 [PMID: 23484035 DOI: 10.1371/journal.pone.0058538]

76 **Yang Z**, Wen J, Tao X, Lu B, Du Y, Wang M, Wang X, Zhang W, Gong W, Ling C, Wu S, Hu R. Genetic variation in the GCKR gene is associated with non-alcoholic fatty liver disease in Chinese people. *Mol Biol Rep* 2011; **38**: 1145-1150 [PMID: 20625834 DOI: 10.1007/s11033-010-0212-1]

77 **Cai W**, Weng DH, Yan P, Lin YT, Dong ZH, Mailamuguli, Yao H. Genetic polymorphisms associated with nonalcoholic fatty liver disease in Uyghur population: a case-control study and meta-analysis. *Lipids Health Dis* 2019; **18**: 14 [PMID: 30646922 DOI: 10.1186/s12944-018-0877-3]

78 **Zhai HL**, Wang NJ, Han B, Li Q, Chen Y, Zhu CF, Chen YC, Xia FZ, Cang Z, Zhu CX, Lu M, Lu YL. Low vitamin D levels and non-alcoholic fatty liver disease, evidence for their independent association in men in East China: a cross-sectional study (Survey on Prevalence in East China for Metabolic Diseases and Risk Factors (SPECT-China)). *Br J Nutr* 2016; **115**: 1352-1359 [PMID: 26888280 DOI: 10.1017/S0007114516000386]

79 **Lim S**, Kim JH, Yoon JW, Kang SM, Choi SH, Park YJ, Kim KW, Lim JY, Park KS, Jang HC. Sarcopenic obesity: prevalence and association with metabolic syndrome in the Korean Longitudinal Study on Health and Aging (KLoSHA). *Diabetes Care* 2010; **33**: 1652-1654 [PMID: 20460442 DOI: 10.2337/dc10-0107]

80 **Kawamura Y**, Arase Y, Ikeda K, Seko Y, Imai N, Hosaka T, Kobayashi M, Saitoh S, Sezaki H, Akuta N, Suzuki F, Suzuki Y, Ohmoto Y, Amakawa K, Tsuji H, Kumada H. Large-scale long-term follow-up study of Japanese patients with non-alcoholic Fatty liver disease for the onset of hepatocellular carcinoma. *Am J Gastroenterol* 2012; **107**: 253-261 [PMID: 22008893 DOI: 10.1038/ajg.2011.327]

81 **Michitaka K**, Nishiguchi S, Aoyagi Y, Hiasa Y, Tokumoto Y, Onji M; Japan Etiology of Liver Cirrhosis Study Group. Etiology of liver cirrhosis in Japan: a nationwide survey. *J Gastroenterol* 2010; **45**: 86-94 [PMID: 19789837 DOI: 10.1007/s00535-009-0128-5]

82 **Wong SW**, Chan WK. Epidemiology of non-alcoholic fatty liver disease in Asia. *Indian J Gastroenterol* 2020; **39**: 1-8 [PMID: 32152903 DOI: 10.1007/s12664-020-01018-x]

83 **Alazawi W**, Mathur R, Abeysekera K, Hull S, Boomla K, Robson J, Foster GR. Ethnicity and the diagnosis gap in liver disease: a population-based study. *Br J Gen Pract* 2014; **64**: e694-e702 [PMID: 25348993 DOI: 10.3399/bjgp14X682273]

84 **Soleimani D**, Ranjbar G, Rezvani R, Goshayeshi L, Razmpour F, Nematy M. Dietary patterns in relation to hepatic fibrosis among patients with nonalcoholic fatty liver disease. *Diabetes Metab Syndr Obes* 2019; **12**: 315-324 [PMID: 30881075 DOI: 10.2147/DMSO.S198744]

85 **Huang Y**, Cohen JC, Hobbs HH. Expression and characterization of a PNPLA3 protein isoform (I148M) associated with nonalcoholic fatty liver disease. *J Biol Chem* 2011; **286**: 37085-37093 [PMID: 21878620 DOI: 10.1074/jbc.M111.290114]

86 **Kawaguchi T**, Sumida Y, Umemura A, Matsuo K, Takahashi M, Takamura T, Yasui K, Saibara T, Hashimoto E, Kawanaka M, Watanabe S, Kawata S, Imai Y, Kokubo M, Shima T, Park H, Tanaka H, Tajima K, Yamada R, Matsuda F, Okanoue T; Japan Study Group of Nonalcoholic Fatty Liver Disease. Genetic polymorphisms of the human PNPLA3 gene are strongly associated with severity of non-alcoholic fatty liver disease in Japanese. *PLoS One* 2012; **7**: e38322 [PMID: 22719876 DOI: 10.1371/journal.pone.0038322]

87 **Zain SM**, Mohamed R, Mahadeva S, Cheah PL, Rampal S, Basu RC, Mohamed Z. A multi-ethnic study of a PNPLA3 gene variant and its association with disease severity in non-alcoholic fatty liver disease. *Hum Genet* 2012; **131**: 1145-1152 [PMID: 22258181 DOI: 10.1007/s00439-012-1141-y]

88 **Kozlitina J**, Smagris E, Stender S, Nordestgaard BG, Zhou HH, Tybjærg-Hansen A, Vogt TF, Hobbs HH, Cohen JC. Exome-wide association study identifies a TM6SF2 variant that confers susceptibility to nonalcoholic fatty liver disease. *Nat Genet* 2014; **46**: 352-356 [PMID: 24531328

DOI: 10.1038/ng.2901]
89 **Goffredo M**, Caprio S, Feldstein AE, D'Adamo E, Shaw MM, Pierpont B, Savoye M, Zhao H, Bale AE, Santoro N. Role of TM6SF2 rs58542926 in the pathogenesis of nonalcoholic pediatric fatty liver disease: A multiethnic study. *Hepatology* 2016; **63**: 117-125 [PMID: 26457389 DOI: 10.1002/hep.28283]
90 **Anderson EL**, Howe LD, Jones HE, Higgins JP, Lawlor DA, Fraser A. The Prevalence of Non-Alcoholic Fatty Liver Disease in Children and Adolescents: A Systematic Review and Meta-Analysis. *PLoS One* 2015; **10**: e0140908 [PMID: 26512983 DOI: 10.1371/journal.pone.0140908]
91 **Brumbaugh DE**, Tearse P, Cree-Green M, Fenton LZ, Brown M, Scherzinger A, Reynolds R, Alston M, Hoffman C, Pan Z, Friedman JE, Barbour LA. Intrahepatic fat is increased in the neonatal offspring of obese women with gestational diabetes. *J Pediatr* 2013; **162**: 930-6.e1 [PMID: 23260099 DOI: 10.1016/j.jpeds.2012.11.017]
92 **Modi N**, Murgasova D, Ruager-Martin R, Thomas EL, Hyde MJ, Gale C, Santhakumaran S, Doré CJ, Alavi A, Bell JD. The influence of maternal body mass index on infant adiposity and hepatic lipid content. *Pediatr Res* 2011; **70**: 287-291 [PMID: 21629154 DOI: 10.1203/PDR.0b013e318225f9b1]
93 **Oddy WH**, Herbison CE, Jacoby P, Ambrosini GL, O'Sullivan TA, Ayonrinde OT, Olynyk JK, Black LJ, Beilin LJ, Mori TA, Hands BP, Adams LA. The Western dietary pattern is prospectively associated with nonalcoholic fatty liver disease in adolescence. *Am J Gastroenterol* 2013; **108**: 778-785 [PMID: 23545714 DOI: 10.1038/ajg.2013.95]
94 **Ayonrinde OT**, Oddy WH, Adams LA, Mori TA, Beilin LJ, de Klerk N, Olynyk JK. Infant nutrition and maternal obesity influence the risk of non-alcoholic fatty liver disease in adolescents. *J Hepatol* 2017; **67**: 568-576 [PMID: 28619255 DOI: 10.1016/j.jhep.2017.03.029]
95 **Molleston JP**, Schwimmer JB, Yates KP, Murray KF, Cummings OW, Lavine JE, Brunt EM, Scheimann AO, Unalp-Arida A; NASH Clinical Research Network. Histological abnormalities in children with nonalcoholic fatty liver disease and normal or mildly elevated alanine aminotransferase levels.*J Pediatr* 2014; **164**: 707-713. e3 [PMID: 24360992 DOI: 10.1016/j.jpeds.2013.10.071]
96 **Schwimmer JB**, Behling C, Newbury R, Deutsch R, Nievergelt C, Schork NJ, Lavine JE. Histopathology of pediatric nonalcoholic fatty liver disease. *Hepatology* 2005; **42**: 641-649 [PMID: 16116629 DOI: 10.1002/hep.20842]
97 **Goyal NP**, Schwimmer JB. The Progression and Natural History of Pediatric Nonalcoholic Fatty Liver Disease. *Clin Liver Dis* 2016; **20**: 325-338 [PMID: 27063272 DOI: 10.1016/j.cld.2015.10.003]
98 **Nobili V**, Marcellini M, Devito R, Ciampalini P, Piemonte F, Comparcola D, Sartorelli MR, Angulo P. NAFLD in children: a prospective clinical-pathological study and effect of lifestyle advice. *Hepatology* 2006; **44**: 458-465 [PMID: 16871574 DOI: 10.1002/hep.21262]
99 **Ye Q**, Zou B, Yeo YH, Li J, Huang DQ, Wu Y, Yang H, Liu C, Kam LY, Tan XXE, Chien N, Trinh S, Henry L, Stave CD, Hosaka T, Cheung RC, Nguyen MH. Global prevalence, incidence, and outcomes of non-obese or lean non-alcoholic fatty liver disease: a systematic review and meta-analysis. *Lancet Gastroenterol Hepatol* 2020; **5**: 739-752 [PMID: 32413340 DOI: 10.1016/S2468-1253(20)30077-7]
100 **Lu FB**, Zheng KI, Rios RS, Targher G, Byrne CD, Zheng MH. Global epidemiology of lean non-alcoholic fatty liver disease: A systematic review and meta-analysis. *J Gastroenterol Hepatol* 2020; **35**: 2041-2050 [PMID: 32573017 DOI: 10.1111/jgh.15156]
101 **Shi Y**, Wang Q, Sun Y, Zhao X, Kong Y, Ou X, Jia J, Wu S, You H. The Prevalence of Lean/Nonobese Nonalcoholic Fatty Liver Disease: A Systematic Review and Meta-Analysis. *J Clin Gastroenterol* 2020; **54**: 378-387 [PMID: 31651571 DOI: 10.1097/MCG.0000000000001270]
102 **Honda Y**, Yoneda M, Kessoku T, Ogawa Y, Tomeno W, Imajo K, Mawatari H, Fujita K, Hyogo H, Ueno T, Chayama K, Saito S, Nakajima A, Hotta K. Characteristics of non-obese non-alcoholic fatty liver disease: Effect of genetic and environmental factors. *Hepatol Res* 2016; **46**: 1011-1018 [PMID: 26763865 DOI: 10.1111/hepr.12648]
103 **Adams LA**, Marsh JA, Ayonrinde OT, Olynyk JK, Ang WQ, Beilin LJ, Mori T, Palmer LJ, Oddy WW, Lye SJ, Pennell CE. Cholesteryl ester transfer protein gene polymorphisms increase the risk of fatty liver in females independent of adiposity. *J Gastroenterol Hepatol* 2012; **27**: 1520-1527 [PMID: 22414273 DOI: 10.1111/j.1440-1746.2012.07120.x]
104 **Eslam M**, Hashem AM, Leung R, Romero-Gomez M, Berg T, Dore GJ, Chan HL, Irving WL, Sheridan D, Abate ML, Adams LA, Mangia A, Weltman M, Bugianesi E, Spengler U, Shaker O, Fischer J, Mollison L, Cheng W, Powell E, Nattermann J, Riordan S, McLeod D, Armstrong NJ, Douglas MW, Liddle C, Booth DR, George J, Ahlenstiel G; International Hepatitis C Genetics Consortium (IHCGC). Interferon-λ rs12979860 genotype and liver fibrosis in viral and non-viral chronic liver disease. *Nat Commun* 2015; **6**: 6422 [PMID: 25740255 DOI: 10.1038/ncomms7422]
105 **Yun Y**, Kim HN, Lee EJ, Ryu S, Chang Y, Shin H, Kim HL, Kim TH, Yoo K, Kim HY. Fecal and blood microbiota profiles and presence of nonalcoholic fatty liver disease in obese vs lean subjects. *PLoS One* 2019; **14**: e0213692 [DOI: 10.1371/journal.pone.0213692]
106 **Feldman A**, Eder SK, Felder TK, Kedenko L, Paulweber B, Stadlmayr A, Huber-Schönauer U, Niederseer D, Stickel F, Auer S, Haschke-Becher E, Patsch W, Datz C, Aigner E. Clinical and Metabolic Characterization of Lean Caucasian Subjects With Non-alcoholic Fatty Liver. *Am J Gastroenterol* 2017; **112**: 102-110 [PMID: 27527746 DOI: 10.1038/ajg.2016.318]
107 **Eslam M**, Chen F, George J. NAFLD in Lean Asians. *Clin Liver Dis (Hoboken)* 2020; **16**: 240-243 [PMID: 33489095 DOI: 10.1002/cld.930]

108 **Hagström H**, Nasr P, Ekstedt M, Hammar U, Stål P, Hultcrantz R, Kechagias S. Risk for development of severe liver disease in lean patients with nonalcoholic fatty liver disease: A long-term follow-up study. *Hepatol Commun* 2018; **2**: 48-57 [PMID: 29404512 DOI: 10.1002/hep4.1124]

109 **Wang AY**, Dhaliwal J, Mouzaki M. Lean non-alcoholic fatty liver disease. *Clin Nutr* 2019; **38**: 975-981 [PMID: 30466956 DOI: 10.1016/j.clnu.2018.08.008]

110 **Pais R**, Charlotte F, Fedchuk L, Bedossa P, Lebray P, Poynard T, Ratziu V; LIDO Study Group. A systematic review of follow-up biopsies reveals disease progression in patients with non-alcoholic fatty liver. *J Hepatol* 2013; **59**: 550-556 [PMID: 23665288 DOI: 10.1016/j.jhep.2013.04.027]

111 **McPherson S**, Hardy T, Henderson E, Burt AD, Day CP, Anstee QM. Evidence of NAFLD progression from steatosis to fibrosing-steatohepatitis using paired biopsies: implications for prognosis and clinical management. *J Hepatol* 2015; **62**: 1148-1155 [PMID: 25477264 DOI: 10.1016/j.jhep.2014.11.034]

112 **Singh S**, Allen AM, Wang Z, Prokop LJ, Murad MH, Loomba R. Fibrosis progression in nonalcoholic fatty liver vs nonalcoholic steatohepatitis: a systematic review and meta-analysis of paired-biopsy studies. *Clin Gastroenterol Hepatol* 2015; **13**: 643-54.e1 [PMID: 24768810 DOI: 10.1016/j.cgh.2014.04.014]

113 **Musso G**, Gambino R, Cassader M, Pagano G. Meta-analysis: natural history of non-alcoholic fatty liver disease (NAFLD) and diagnostic accuracy of non-invasive tests for liver disease severity. *Ann Med* 2011; **43**: 617-649 [PMID: 21039302 DOI: 10.3109/07853890.2010.518623]

114 **De A**, Duseja A. Natural History of Simple Steatosis or Nonalcoholic Fatty Liver. *J Clin Exp Hepatol* 2020; **10**: 255-262 [PMID: 32405182 DOI: 10.1016/j.jceh.2019.09.005]

115 **Kechagias S**, Nasr P, Blomdahl J, Ekstedt M. Established and emerging factors affecting the progression of nonalcoholic fatty liver disease. *Metabolism* 2020; **111S**: 154183 [PMID: 32061907 DOI: 10.1016/j.metabol.2020.154183]

116 **Anastasopoulos NT**, Lianos GD, Tatsi V, Karampa A, Goussia A, Glantzounis GK. Clinical heterogeneity in patients with non-alcoholic fatty liver disease-associated hepatocellular carcinoma. *Expert Rev Gastroenterol Hepatol* 2020; **14**: 1025-1033 [PMID: 32746645 DOI: 10.1080/17474124.2020.1802244]

117 **Leung C**, Yeoh SW, Patrick D, Ket S, Marion K, Gow P, Angus PW. Characteristics of hepatocellular carcinoma in cirrhotic and non-cirrhotic non-alcoholic fatty liver disease. *World J Gastroenterol* 2015; **21**: 1189-1196 [PMID: 25632192 DOI: 10.3748/wjg.v21.i4.1189]

118 **Mohamad B**, Shah V, Onyshchenko M, Elshamy M, Aucejo F, Lopez R, Hanouneh IA, Alhaddad R, Alkhouri N. Characterization of hepatocellular carcinoma (HCC) in non-alcoholic fatty liver disease (NAFLD) patients without cirrhosis. *Hepatol Int* 2016; **10**: 632-639 [PMID: 26558795 DOI: 10.1007/s12072-015-9679-0]

119 **Marjot T**, Moolla A, Cobbold JF, Hodson L, Tomlinson JW. Nonalcoholic Fatty Liver Disease in Adults: Current Concepts in Etiology, Outcomes, and Management. *Endocr Rev* 2020; **41** [PMID: 31629366 DOI: 10.1210/endrev/bnz009]

120 **Bhala N**, Angulo P, van der Poorten D, Lee E, Hui JM, Saracco G, Adams LA, Charatcharoenwitthaya P, Topping JH, Bugianesi E, Day CP, George J. The natural history of nonalcoholic fatty liver disease with advanced fibrosis or cirrhosis: an international collaborative study. *Hepatology* 2011; **54**: 1208-1216 [PMID: 21688282 DOI: 10.1002/hep.24491]

121 **Ekstedt M**, Hagström H, Nasr P, Fredrikson M, Stål P, Kechagias S, Hultcrantz R. Fibrosis stage is the strongest predictor for disease-specific mortality in NAFLD after up to 33 years of follow-up. *Hepatology* 2015; **61**: 1547-1554 [PMID: 25125077 DOI: 10.1002/hep.27368]

122 **Hagström H**, Nasr P, Ekstedt M, Hammar U, Stål P, Hultcrantz R, Kechagias S. Fibrosis stage but not NASH predicts mortality and time to development of severe liver disease in biopsy-proven NAFLD. *J Hepatol* 2017; **67**: 1265-1273 [PMID: 28803953 DOI: 10.1016/j.jhep.2017.07.027]

123 **Rowe IA**, Parker R. The diagnosis of nonalcoholic fatty liver disease should carry important prognostic information. *Nat Rev Gastroenterol Hepatol* 2019; **16**: 449-450 [PMID: 31197261 DOI: 10.1038/s41575-019-0168-0]

124 **Vilar-Gomez E**, Chalasani N. Non-invasive assessment of non-alcoholic fatty liver disease: Clinical prediction rules and blood-based biomarkers. *J Hepatol* 2018; **68**: 305-315 [PMID: 29154965 DOI: 10.1016/j.jhep.2017.11.013]

125 **Wai JW**, Fu C, Wong VW. Confounding factors of non-invasive tests for nonalcoholic fatty liver disease. *J Gastroenterol* 2020; **55**: 731-741 [PMID: 32451628 DOI: 10.1007/s00535-020-01686-8]

126 **McPherson S**, Hardy T, Dufour JF, Petta S, Romero-Gomez M, Allison M, Oliveira CP, Francque S, Van Gaal L, Schattenberg JM, Tiniakos D, Burt A, Bugianesi E, Ratziu V, Day CP, Anstee QM. Age as a Confounding Factor for the Accurate Non-Invasive Diagnosis of Advanced NAFLD Fibrosis. *Am J Gastroenterol* 2017; **112**: 740-751 [PMID: 27725647 DOI: 10.1038/ajg.2016.453]

127 **De Silva S**, Li W, Kemos P, Brindley JH, Mecci J, Samsuddin S, Chin-Aleong J, Feakins RM, Foster GR, Syn WK, Alazawi W. Non-invasive markers of liver fibrosis in fatty liver disease are unreliable in people of South Asian descent. *Frontline Gastroenterol* 2018; **9**: 115-121 [PMID: 29588839 DOI: 10.1136/flgastro-2017-100865]

128 **Xia B**, Wang F, Friedrich-Rust M, Zhou F, Zhu J, Yang H, Ruan W, Zeng Z. Feasibility and Efficacy of Transient Elastography using the XL probe to diagnose liver fibrosis and cirrhosis: A meta-analysis. *Medicine (Baltimore)* 2018; **97**: e11816 [PMID: 30278481 DOI: 10.1097/MD.0000000000011816]

129 **Shili-Masmoudi S**, Wong GL, Hiriart JB, Liu K, Chermak F, Shu SS, Foucher J, Tse YK, Bernard

PH, Yip TC, Merrouche W, Chan HL, Wong VW, de Lédinghen V. Liver stiffness measurement predicts long-term survival and complications in non-alcoholic fatty liver disease. *Liver Int* 2020; **40**: 581-589 [PMID: 31749300 DOI: 10.1111/liv.14301]

130 **Petta S**, Maida M, Macaluso FS, Di Marco V, Cammà C, Cabibi D, Craxì A. The severity of steatosis influences liver stiffness measurement in patients with nonalcoholic fatty liver disease. *Hepatology* 2015; **62**: 1101-1110 [PMID: 25991038 DOI: 10.1002/hep.27844]

131 **Park CC**, Nguyen P, Hernandez C, Bettencourt R, Ramirez K, Fortney L, Hooker J, Sy E, Savides MT, Alquiraish MH, Valasek MA, Rizo E, Richards L, Brenner D, Sirlin CB, Loomba R. Magnetic Resonance Elastography vs Transient Elastography in Detection of Fibrosis and Noninvasive Measurement of Steatosis in Patients With Biopsy-Proven Nonalcoholic Fatty Liver Disease. *Gastroenterology* 2017; **152**: 598-607.e2 [PMID: 27911262 DOI: 10.1053/j.gastro.2016.10.026]

132 **Augustin S**, Ahmed A, Alkhouri N, Canbay A, Corless L, Nakajima A, Okanoue T, Petta S, Ratziu V, Tsochatzis EA, Wong VW, Romero-Gómez M. Identification of Patients with Advanced Fibrosis Due to Nonalcoholic Fatty Liver Disease: Considerations for Best Practice. *J Gastrointestin Liver Dis* 2020; **29**: 235-245 [PMID: 32530991 DOI: 10.15403/jgld-775]

133 Association for the Study of the Liver (EASL).; European Association for the Study of Diabetes (EASD); European Association for the Study of Obesity (EASO). EASL-EASD-EASO Clinical Practice Guidelines for the management of non-alcoholic fatty liver disease. *J Hepatol* 2016; **64**: 1388-1402 [DOI: 10.1159/000443344]

134 **Sumida Y**, Yoneda M, Hyogo H, Itoh Y, Ono M, Fujii H, Eguchi Y, Suzuki Y, Aoki N, Kanemasa K, Fujita K, Chayama K, Saibara T, Kawada N, Fujimoto K, Kohgo Y, Yoshikawa T, Okanoue T; Japan Study Group of Nonalcoholic Fatty Liver Disease (JSG-NAFLD). Validation of the FIB4 index in a Japanese nonalcoholic fatty liver disease population. *BMC Gastroenterol* 2012; **12**: 2 [PMID: 22221544 DOI: 10.1186/1471-230X-12-2]

135 **Yu SJ**, Kim DH, Lee JH, Chung GE, Yim JY, Park MJ, Kim YJ, Yoon JH, Jang JJ, Lee HS. Validation of P2/MS and other noninvasive fibrosis scoring systems in the Korean population with nonalcoholic fatty liver disease. *Korean J Gastroenterol* 2011; **57**: 19-27 [PMID: 21258197 DOI: 10.4166/kjg.2011.57.1.19]

136 **Wong GL**, Chan HL. Two are better than one: noninvasive assessment of liver fibrosis in nonalcoholic fatty liver disease. *Hepatol Int* 2015; **9**: 481-483 [PMID: 25788206 DOI: 10.1007/s12072-015-9625-1]

137 **Wong VW**, Chan WK, Chitturi S, Chawla Y, Dan YY, Duseja A, Fan J, Goh KL, Hamaguchi M, Hashimoto E, Kim SU, Lesmana LA, Lin YC, Liu CJ, Ni YH, Sollano J, Wong SK, Wong GL, Chan HL, Farrell G. Asia-Pacific Working Party on Non-alcoholic Fatty Liver Disease guidelines 2017-Part 1: Definition, risk factors and assessment. *J Gastroenterol Hepatol* 2018; **33**: 70-85 [PMID: 28670712 DOI: 10.1111/jgh.13857]

138 **Newsome PN**, Sasso M, Deeks JJ, Paredes A, Boursier J, Chan WK, Yilmaz Y, Czernichow S, Zheng MH, Wong VW, Allison M, Tsochatzis E, Anstee QM, Sheridan DA, Eddowes PJ, Guha IN, Cobbold JF, Paradis V, Bedossa P, Miette V, Fournier-Poizat C, Sandrin L, Harrison SA. FibroScan-AST (FAST) score for the non-invasive identification of patients with non-alcoholic steatohepatitis with significant activity and fibrosis: a prospective derivation and global validation study. *Lancet Gastroenterol Hepatol* 2020; **5**: 362-373 [PMID: 32027858 DOI: 10.1016/S2468-1253(19)30383-8]

139 **Mózes FE**, Lee JA, Selvaraj EA, Jayaswal ANA, Trauner M, Boursier J, Fournier C, Staufer K, Stauber RE, Bugianesi E, Younes R, Gaia S, Lupșor-Platon M, Petta S, Shima T, Okanoue T, Mahadeva S, Chan WK, Eddowes PJ, Hirschfield GM, Newsome PN, Wong VW, de Ledinghen V, Fan J, Shen F, Cobbold JF, Sumida Y, Okajima A, Schattenberg JM, Labenz C, Kim W, Lee MS, Wiegand J, Karlas T, Yılmaz Y, Aithal GP, Palaniyappan N, Cassinotto C, Aggarwal S, Garg H, Ooi GJ, Nakajima A, Yoneda M, Ziol M, Barget N, Geier A, Tuthill T, Brosnan MJ, Anstee QM, Neubauer S, Harrison SA, Bossuyt PM, Pavlides M; LITMUS Investigators. Diagnostic accuracy of non-invasive tests for advanced fibrosis in patients with NAFLD: an individual patient data meta-analysis. *Gut* 2021 [PMID: 34001645 DOI: 10.1136/gutjnl-2021-324243]

140 **Sanyal AJ**. Past, present and future perspectives in nonalcoholic fatty liver disease. *Nat Rev Gastroenterol Hepatol* 2019; **16**: 377-386 [PMID: 31024089 DOI: 10.1038/s41575-019-0144-8]

141 **Younossi ZM**, Ratziu V, Loomba R, Rinella M, Anstee QM, Goodman Z, Bedossa P, Geier A, Beckebaum S, Newsome PN, Sheridan D, Sheikh MY, Trotter J, Knapple W, Lawitz E, Abdelmalek MF, Kowdley KV, Montano-Loza AJ, Boursier J, Mathurin P, Bugianesi E, Mazzella G, Olveira A, Cortez-Pinto H, Graupera I, Orr D, Gluud LL, Dufour JF, Shapiro D, Campagna J, Zaru L, MacConell L, Shringarpure R, Harrison S, Sanyal AJ; REGENERATE Study Investigators. Obeticholic acid for the treatment of non-alcoholic steatohepatitis: interim analysis from a multicentre, randomised, placebo-controlled phase 3 trial. *Lancet* 2019; **394**: 2184-2196 [PMID: 31813633 DOI: 10.1016/S0140-6736(19)33041-7]

142 **Patel K**, Harrison SA, Elkhashab M, Trotter JF, Herring R, Rojter SE, Kayali Z, Wong VW, Greenbloom S, Jayakumar S, Shiffman ML, Freilich B, Lawitz EJ, Gane EJ, Harting E, Xu J, Billin AN, Chung C, Djedjos CS, Subramanian GM, Myers RP, Middleton MS, Rinella M, Noureddin M. Cilofexor, a Nonsteroidal FXR Agonist, in Patients With Noncirrhotic NASH: A Phase 2 Randomized Controlled Trial. *Hepatology* 2020; **72**: 58-71 [PMID: 32115759 DOI: 10.1002/hep.31205]

143 **Gawrieh S**, Noureddin M, Loo N, Mohseni R, Awasty V, Cusi K, Kowdley KV, Lai M, Schiff E, Parmar D, Patel P, Chalasani N. Saroglitazar, a PPAR-α/γ Agonist, for Treatment of NAFLD: A

Randomized Controlled Double-Blind Phase 2 Trial. *Hepatology* 2021 [DOI: 10.1002/hep.31843]

144 **Amin N**, Carvajal-Gonzalez S, Aggarwal N, Tuthill T, Inglot M, Bergman A, Esler W. Abstract 31: PF-05221304 (PF'1304), a liver-targeted acetyl-CoA carboxylase inhibitor (ACCI), in adults with nonalcoholic fatty liver disease (NAFLD) demonstrates robust reductions in liver fat and ALT: phase 2a, dose-ranging study. *Hepatology* 2019; **70**: 21A-22A [DOI: 10.1016/s0168-8278(20)31392-1]

145 **Armstrong MJ**, Gaunt P, Aithal GP, Barton D, Hull D, Parker R, Hazlehurst JM, Guo K; LEAN trial team, Abouda G, Aldersley MA, Stocken D, Gough SC, Tomlinson JW, Brown RM, Hübscher SG, Newsome PN. Liraglutide safety and efficacy in patients with non-alcoholic steatohepatitis (LEAN): a multicentre, double-blind, randomised, placebo-controlled phase 2 study. *Lancet* 2016; **387**: 679-690 [PMID: 26608256 DOI: 10.1016/S0140-6736(15)00803-X]

146 **Petit JM**, Cercueil JP, Loffroy R, Denimal D, Bouillet B, Fourmont C, Chevallier O, Duvillard L, Vergès B. Effect of Liraglutide Therapy on Liver Fat Content in Patients With Inadequately Controlled Type 2 Diabetes: The Lira-NAFLD Study. *J Clin Endocrinol Metab* 2017; **102**: 407-415 [PMID: 27732328 DOI: 10.1210/jc.2016-2775]

147 **Eguchi Y**, Kitajima Y, Hyogo H, Takahashi H, Kojima M, Ono M, Araki N, Tanaka K, Yamaguchi M, Matsuda Y, Ide Y, Otsuka T, Ozaki I, Ono N, Eguchi T, Anzai K; Japan Study Group for NAFLD (JSG-NAFLD). Pilot study of liraglutide effects in non-alcoholic steatohepatitis and non-alcoholic fatty liver disease with glucose intolerance in Japanese patients (LEAN-J). *Hepatol Res* 2015; **45**: 269-278 [PMID: 24796231 DOI: 10.1111/hepr.12351]

148 **Newsome PN**, Buchholtz K, Cusi K, Linder M, Okanoue T, Ratziu V, Sanyal AJ, Sejling AS, Harrison SA; NN9931-4296 Investigators. A Placebo-Controlled Trial of Subcutaneous Semaglutide in Nonalcoholic Steatohepatitis. *N Engl J Med* 2021; **384**: 1113-1124 [PMID: 33185364 DOI: 10.1056/NEJMoa2028395]

149 **Harrison SA**, Alkhouri N, Davison BA, Sanyal A, Edwards C, Colca JR, Lee BH, Loomba R, Cusi K, Kolterman O, Cotter G, Dittrich HC. Insulin sensitizer MSDC-0602K in non-alcoholic steatohepatitis: A randomized, double-blind, placebo-controlled phase IIb study. *J Hepatol* 2020; **72**: 613-626 [PMID: 31697972 DOI: 10.1016/j.jhep.2019.10.023]

150 **Sanyal AJ**, Chalasani N, Kowdley KV, McCullough A, Diehl AM, Bass NM, Neuschwander-Tetri BA, Lavine JE, Tonascia J, Unalp A, Van Natta M, Clark J, Brunt EM, Kleiner DE, Hoofnagle JH, Robuck PR; NASH CRN. Pioglitazone, vitamin E, or placebo for nonalcoholic steatohepatitis. *N Engl J Med* 2010; **362**: 1675-1685 [PMID: 20427778 DOI: 10.1056/NEJMoa0907929]

151 **Harrison SA**, Goodman Z, Jabbar A, Vemulapalli R, Younes ZH, Freilich B, Sheikh MY, Schattenberg JM, Kayali Z, Zivony A, Sheikh A, Garcia-Samaniego J, Satapathy SK, Therapondos G, Mena E, Schuppan D, Robinson J, Chan JL, Hagerty DT, Sanyal AJ. A randomized, placebo-controlled trial of emricasan in patients with NASH and F1-F3 fibrosis. *J Hepatol* 2020; **72**: 816-827 [PMID: 31887369 DOI: 10.1016/j.jhep.2019.11.024]

152 **Harrison SA**, Abdelmalek MF, Caldwell S, Shiffman ML, Diehl AM, Ghalib R, Lawitz EJ, Rockey DC, Schall RA, Jia C, McColgan BJ, McHutchison JG, Subramanian GM, Myers RP, Younossi Z, Ratziu V, Muir AJ, Afdhal NH, Goodman Z, Bosch J, Sanyal AJ; GS-US-321-0105 and GS-US-321-0106 Investigators. Simtuzumab Is Ineffective for Patients With Bridging Fibrosis or Compensated Cirrhosis Caused by Nonalcoholic Steatohepatitis. *Gastroenterology* 2018; **155**: 1140-1153 [PMID: 29990488 DOI: 10.1053/j.gastro.2018.07.006]

153 **Harrison SA**, Neff G, Guy CD, Bashir MR, Paredes AH, Frias JP, Younes Z, Trotter JF, Gunn NT, Moussa SE, Kohli A, Nelson K, Gottwald M, Chang WCG, Yan AZ, DePaoli AM, Ling L, Lieu HD. Efficacy and Safety of Aldafermin, an Engineered FGF19 Analog, in a Randomized, Double-Blind, Placebo-Controlled Trial of Patients With Nonalcoholic Steatohepatitis. *Gastroenterology* 2021; **160**: 219-231.e1 [PMID: 32781086 DOI: 10.1053/j.gastro.2020.08.004]

154 **Reimer KC**, Wree A, Roderburg C, Tacke F. New drugs for NAFLD: lessons from basic models to the clinic. *Hepatol Int* 2020; **14**: 8-23 [PMID: 31802390 DOI: 10.1007/s12072-019-10001-4]

155 **Eslam M**, George J. Genetic Insights for Drug Development in NAFLD. *Trends Pharmacol Sci* 2019; **40**: 506-516 [PMID: 31160124 DOI: 10.1016/j.tips.2019.05.002]

156 **Saran AR**, Dave S, Zarrinpar A. Circadian Rhythms in the Pathogenesis and Treatment of Fatty Liver Disease.*Gastroenterology* 2020; **158**: 1948-1966. e1 [PMID: 32061597 DOI: 10.1053/j.gastro.2020.01.050]

157 **Hardy T**, Oakley F, Anstee QM, Day CP. Nonalcoholic Fatty Liver Disease: Pathogenesis and Disease Spectrum. *Annu Rev Pathol* 2016; **11**: 451-496 [PMID: 26980160 DOI: 10.1146/annurev-pathol-012615-044224]

158 **Hannah WN Jr**, Torres DM, Harrison SA. Nonalcoholic Steatohepatitis and Endpoints in Clinical Trials. *Gastroenterol Hepatol (N Y)* 2016; **12**: 756-763 [PMID: 28035202]

159 **Thiagarajan P**, Aithal GP. Drug Development for Nonalcoholic Fatty Liver Disease: Landscape and Challenges. *J Clin Exp Hepatol* 2019; **9**: 515-521 [PMID: 31516268 DOI: 10.1016/j.jceh.2019.03.002]

160 **Bawden SJ**, Scott RA, Aithal GP. Current and Future Magnetic Resonance Technologies for Assessing Liver Disease in Clinical and Experimental Medicine. *Dig Dis* 2017; **35**: 314-322 [PMID: 28467990 DOI: 10.1159/000456582]

161 **Cerami E**, Gao J, Dogrusoz U, Gross BE, Sumer SO, Aksoy BA, Jacobsen A, Byrne CJ, Heuer ML, Larsson E, Antipin Y, Reva B, Goldberg AP, Sander C, Schultz N. The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data. *Cancer Discov* 2012; **2**: 401-404 [PMID: 22588877 DOI: 10.1158/2159-8290.CD-12-0095]

162 **Africa JA**, Behling CA, Brunt EM, Zhang N, Luo Y, Wells A, Hou J, Belt PH, Kohil R, Lavine JE, Molleston JP, Newton KP, Whitington PF, Schwimmer JB; Nonalcoholic Steatohepatitis Clinical Research Network. In Children With Nonalcoholic Fatty Liver Disease, Zone 1 Steatosis Is Associated With Advanced Fibrosis. *Clin Gastroenterol Hepatol* 2018; **16**: 438-446.e1 [PMID: 28286193 DOI: 10.1016/j.cgh.2017.02.030]

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REVIEW

Newly discovered endocrine functions of the liver

Jane Rhyu, Run Yu

ORCID number: Jane Rhyu 0000-0003-0407-4907; Run Yu 0000-0003-2901-5102.

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Jane Rhyu, Run Yu, Division of Endocrinology, Diabetes, and Metabolism, UCLA David Geffen School of Medicine, Los Angeles, CA 90095, United States

Corresponding author: Run Yu, MD, PhD, Professor, Division of Endocrinology, Diabetes, and Metabolism, UCLA David Geffen School of Medicine, 200 Medical Plaza Driveway #530, Los Angeles, CA 90095, United States. runyu@mednet.ucla.edu

Abstract

The liver, the largest solid visceral organ of the body, has numerous endocrine functions, such as direct hormone and hepatokine production, hormone metabolism, synthesis of binding proteins, and processing and redistribution of metabolic fuels. In the last 10 years, many new endocrine functions of the liver have been discovered. Advances in the classical endocrine functions include delineation of mechanisms of liver production of endocrine hormones [including 25-hydroxyvitamin D, insulin-like growth factor 1 (IGF-1), and angiotensinogen], hepatic metabolism of hormones (including thyroid hormones, glucagon-like peptide-1, and steroid hormones), and actions of specific binding proteins to glucocorticoids, sex steroids, and thyroid hormones. These studies have furthered insight into cirrhosis-associated endocrinopathies, such as hypogonadism, osteoporosis, IGF-1 deficiency, vitamin D deficiency, alterations in glucose and lipid homeostasis, and controversially relative adrenal insufficiency. Several novel endocrine functions of the liver have also been unraveled, elucidating the liver's key negative feedback regulatory role in the pancreatic α cell-liver axis, which regulates pancreatic α cell mass, glucagon secretion, and circulating amino acid levels. Betatrophin and other hepatokines, such as fetuin-A and fibroblast growth factor 21, have also been discovered to play important endocrine roles in modulating insulin sensitivity, lipid metabolism, and body weight. It is expected that more endocrine functions of the liver will be revealed in the near future.

Key Words: Liver; Endocrine function; Hormone; Amino acids; Hepatokine; Fibroblast growth factor 21

Core Tip: The liver has many newly discovered endocrine functions, most of which are in regulating metabolism, underscoring the functioning of the liver as a major metabolic organ. Convincing evidence has shown that the liver regulates endocrine

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functions in mineral and fuel metabolism, especially in the metabolism of glucose and lipids *via* hepatokines and amino acids *via* negative feedback on pancreatic α cells. As research into the endocrine function of the liver is a rapidly evolving field, controversial findings often exist; caution needs to be taken when interpreting novel findings to avoid over-simplification of complex metabolic processes and premature allocation of research resources.

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INTRODUCTION

The liver is a dynamic endocrine organ and mediates critical metabolic pathways *via* roles in direct hormone and hepatokine production, hormone metabolism, synthesis of binding proteins, detoxification, and processing and redistribution of metabolic fuels [1-4]. It participates in multiple signaling pathways with other endocrine organs, including the pituitary, pancreas, gut, thyroid, adrenal glands, and bone, with hormones in turn modulating the liver's metabolic and synthetic functions[1,5]. Diseases that affect the liver lead to a variety of endocrine manifestations, including hypogonadism, osteoporosis, effects on glucose metabolism and growth hormone (GH), and controversial effects on cortisol[1,5].

The liver, with its vascularity, is well-positioned to provide and receive endocrine signals, including those from pancreatic and gut hormones[6]. It also receives exposure to antigen-rich blood systemically and from the gastrointestinal system as a lymphoid organ[7] and serves as a principal organ in drug metabolism and clearance[8]. Despite only representing 2.5% of the body weight, the liver receives up to 25% of the total cardiac output at rest[9]. It also receives a unique double afferent blood flow from the hepatic artery and partially deoxygenated portal vein, with around 75% of the blood flow from the latter[9]. The portal vein, in turn, receives blood from the stomach, small and large intestines, pancreas, spleen, and gallbladder[9], with direct physiological implications on the regulation of metabolism by endocrine liver functions[6]. Great progress has been made in the understanding of the endocrine functions of the liver in the last 10 years.

ADVANCES IN CLASSIC ENDOCRINE FUNCTIONS OF THE LIVER

We will first briefly summarize the advances in the understanding of the liver classic endocrine functions (Table 1).

Direct hormone production

The liver directly synthesizes multiple hormones, including 25-hydroxyvitamin D, insulin-like growth factor 1 (IGF-1), and angiotensinogen. Given roles in direct hormone production, the liver also has permissive roles of normal hormone function, in particular with effects on bone health, the GH-IGF-1 axis, and renin-angiotensin-aldosterone (RAA) pathway.

Vitamin D: The liver is the primary site of 25-hydroxylation of vitamin D to 25-hydroxyvitamin D (calcidiol), the main storage form of vitamin D[10]. Vitamin D is a secosteroid hormone well known for its role in calcium and bone homeostasis, with pleiotropic effects on cellular proliferation, differentiation, and immunomodulation [11-13]. 25-hydroxyvitamin D (calcidiol) then undergoes 1-alpha-hydroxylation in the kidney to the activated form 1,25-dihydroxyvitamin D (calcitriol)[10], which provides the active hormonal effects of vitamin D. The hydroxylation of vitamin D to produce calcidiol is mainly carried out in the liver by multiple cytochrome P450 mixed-function oxidases (CYPs) located in the mitochondria, endoplasmic reticulum (ER), and microsomes, though studies also show presence of these CYPs in extrahepatic tissues [10,11].

Table 1 Classic endocrine functions of the liver

Hormone	Liver function	Target organ	Action on target organ	Alteration in liver diseases
25-hydroxyvitamin D	Direct production	Gut	Prohormone of calcitriol which stimulates gut calcium absorption	Decreased production resulting in low bone density
Insulin-like growth factor 1	Direct production	Ubiquitous	Promoting growth and differentiation and regulating nutrients metabolism	Decreased production resulting in dysmetabolism
Angiotensinogen	Direct production	Cardiovascular system	Precursor of angiotensin II which regulates aldosterone level. Both regulate vascular tone, sodium retention, and cardiac remodeling	Near-normal function
Thyroid hormone	Activation through T4 to T3 conversion; inactivation through degradation; TBG production	Ubiquitous	Increasing metabolism and energy expenditure	Low T3 syndrome
Glucagon-like peptide 1 (GLP-1)	Metabolism of GLP-1 *via* dipeptidyl peptidase-4(DPPIV)	Pancreas, gut, and brain	Stimulating insulin production, decreasing gut motility, and suppressing appetite	Increased DPPIV expression resulting in higher risk of diabetes
Sex hormones	Hormone metabolism and SHBG production	Ubiquitous	Numerous (details beyond this review)	Hypogonadism
Glucocorticoids	Hormone metabolism and CBG production	Ubiquitous	Numerous (details beyond this review)	Relative adrenal insufficiency
Mineralocorticoids	Hormone metabolism	Cardiovascular system	Maintaining electrolyte balance and blood pressure	Largely intact

TBG: Thyroxine binding globulin; CBG: Cortisol binding globulin; SHBG: Sex hormone binding globulin.

IGF-1: The liver is the primary source of IGF-1, a 70-amino acid polypeptide hormone with endocrine, paracrine, and autocrine effects[14]. IGF-1 affects almost every tissue and organ[15], and its receptors are ubiquitously expressed[16]. Besides mediating the actions of GH, more recently, non-growth-related actions of IGF-1 are found. IGF-1 binds to the insulin receptor and the hybrid IGF-1/insulin receptors, with implications on the metabolic effects of IGF-1[14]. IGF-1, GH, and insulin are hypothesized to constitute a regulated axis to inform cells about nutritional status, helping direct cells grow and differentiate *vs* induce a state of quiescence, senescence or apoptosis[14]. The IGF-1 receptor also participates in a crosstalk with the thyrotropin receptor by forming heterodimers[17], with implications on cellular growth and pathological implications in Graves' eye disease.

Angiotensinogen: The liver is the primary source of angiotensinogen, which is involved in the RAA system[18]. The RAA system is vital for maintaining blood pressure homeostasis, *via* effects on sodium balance, intra- and extra-vascular volume, and systemic vascular tone[19]. Angiotensinogen, an alpha-globulin, is the only known substrate for renin and the main precursor molecule for angiotensin II (AngII), the major biologically active peptide in the RAA pathway[19]. Despite local tissue production of AngII, liver angiotensinogen is the primary source of renal AngII[18]. Hepatocytes tonically secrete angiotensinogen and primarily determine plasma angiotensinogen levels, with small increases in angiotensinogen levels increasing blood pressure and AngII levels[20].

Hormone metabolism

The liver is involved in the metabolism of multiple endocrine hormones, including thyroid hormones, glucagon-like peptide-1, and steroid hormones, with roles in both activation and inactivation of the hormones.

Thyroid hormone: Hepatic metabolism has roles in both activation and inactivation of thyroid hormones. The biologic activity of thyroid hormone is mainly mediated through the active thyroid hormone T3. The thyroid only secretes 20% of the daily T3 requirement, with the remainder 80% converted from T4 by peripheral selenium-containing deiodinase enzymes (DIO), of which three primary deiodinases (type 1, 2, and 3) have been identified[21]. The liver expresses DIO1, along with the kidney and thyroid, which converts T4 to T3, though with less kinetic efficiency compared to

DIO2, which is expressed by brown adipose tissue and the pituitary. Subsequently, the thyroid hormone is metabolized by conjugation with sulfate or glucuronic acid, which occurs prominently in the liver[22].

Glucagon-like peptide 1: With the discovery of glucagon-like peptide 1 (GLP-1), increasing research has been studying the gut-pancreas-liver axis, and the liver has been shown to play a key role in the hormone's metabolism[23]. GLP-1 is an incretin hormone produced by the intestinal L-cells in response to ingestion of nutrients, including carbohydrates, fatty acids, and fiber[24]. It stimulates insulin secretion in a glucose-dependent manner, with associated inhibition of hepatic gluconeogenesis, and promotes insulin gene transcription and growth and proliferation of islet cells[24]. GLP-1 is inactivated by dipeptidyl peptidase-4 (DPPIV), also known as CD26, a ubiquitous membrane-associated peptidase[25]. DPPIV has pleiotropic effects and widespread tissue distribution in all organs, with expression in capillary endothelial cells and high expression in the liver[25].

Steroid hormone metabolism: The liver participates in most steps of steroid hormone regulation, starting from being the primary site of cholesterol biosynthesis[26,27]. At the liver, steroid hormones undergo phase I metabolism by cytochrome P450 enzymes (CYPs), *via* multiple pathways including hydroxylation or reduction, and phase II metabolism, also *via* various processes including glucuronidation, sulfation, or methylation[27], ultimately leading to excretion of their conjugates in urine or bile.

Steroid hormone metabolism: Sex hormones: The liver is the main site for metabolic conversion of estrogens, progesterone, and androgens to their metabolites *via* CYPs, which are abundantly expressed in the liver[28]. In particular, as part of the first phase of metabolism, estrogens undergo hydroxylation by numerous CYPs, including 2-hydroxylation to 2-hydroxyestradiol and 4-hydroxylation to 4-hydroxestradiol, which represent 80% and 20% of biotransformation of estradiol in the liver, respectively. 2-hydroxylation is mainly catalyzed by CYP1A2 and CYP3A4, which are expressed in the liver, and CYP1A1 in extrahepatic tissues[28]. 4-hydroxestradiol, unlike 2-hydroxestradiol, is associated with free radical generation and cellular damage, with associated increased risk of carcinogenesis in the breast and endometrium. Subsequent phase II metabolism of sex hormones, *via* O-methylation by catechol O-methyltransferase (COMT), glucuronidation, or sulfation, occurs at high levels at the liver, with subsequent elimination in the urine or stool[28-30].

Steroid hormone metabolism: Glucocorticoids and mineralocorticoids: The liver is also the primary site of glucocorticoid and mineralocorticoid metabolism[27]. Cortisol is converted to and from its inactive metabolite cortisone by two isozymes of 11-beta hydroxysteroid dehydrogenase (11-beta-HSD)[31]. 11-beta-HSD type 1 (11-beta-HSD1) is widely distributed, though most abundantly located in the liver and adipose tissue, and is responsible for converting cortisone back to cortisol[31], with *in vitro* activity being greater in omental than subcutaneous adipose tissue[32]. In healthy individuals, local splanchnic cortisol production, including from the liver, can equal or even exceed that produced by extra-splanchnic tissues, including the adrenal gland[32]. In obese, non-diabetic individuals, the liver has been shown to account for virtually all splanchnic cortisol production[32]. Though primarily secreted from the adrenal glands under the regulation of the RAA axis, animal studies suggest possibility of local hepatic aldosterone production during liver injury, which may contribute to fibrogenesis[33]. Glucocorticoids and mineralocorticoids, like other steroid hormones, undergo phase I and phase II metabolism in the liver, with excretion of their conjugates in urine or bile[27].

Binding protein production

Lipophilic hormones, including steroid hormones, are not water soluble and need to be carried in the blood stream by binding proteins[2,34]. The liver is the primary source of binding proteins for many hormones. The liver produces specific binding proteins to multiple lipophilic hormones, including glucocorticoids, mineralocorticoids, sex steroids, thyroid hormones (T3 and T4), and vitamin D metabolites[2,34]. Binding globulins for these lipophilic hormones include cortisol binding globulin (CBG, which binds cortisol, aldosterone, and progesterone), sex hormone binding globulin (SHBG, which binds estradiol, testosterone, and other sex hormones), thyroxine binding globulin (TBG, which binds T3 and T4), and vitamin D binding globulin (DBG, which binds vitamin D metabolites)[2,34]. Binding proteins that are produced by the liver also include transthyretin (which binds thyroid hormone and

retinol), IGF-1 binding proteins (IGFBP, which binds IGF, including IGF-1), and non-specific binding proteins including albumin and lipoproteins. Binding proteins serve as a circulating reservoir for hormones, potentially regulating tissue distribution and target destination in a manner that can be highly selective and targeted[2,35]. Binding protein expression and production, which occur primarily at the liver, is complex and under the regulation and influence of multiple factors[2]. Most binding protein expression increase in response to estrogens, including physiologically with pregnancy or with oral contraceptives[2,34]. Hepatic failure and protein-losing nephropathies lead to decrease of binding proteins in general[2,34].

Endocrine dysregulation in liver disease

The liver mediates the effects of numerous hormonal pathways, whether directly or indirectly; thus, not surprisingly, derangements affecting the liver lead to disruptions of various hormonal pathways. Patients with cirrhosis are characterized by various endocrinopathies, including relative increase in estrogen compared to androgens, hypogonadism, osteoporosis, IGF-1 deficiency, vitamin D deficiency, alterations in glucose and lipid homeostasis, and perhaps more controversially a relative adrenal insufficiency.

Sex hormones: Cirrhosis is characterized by symptoms of estrogen-androgen imbalance, with relatively higher estradiol and lower testosterone concentrations[36]. The etiology of estrogen-testosterone imbalance is at least in part due to conversion of androgens to estrogens in cirrhosis, which in large part occurs peripherally[36]. The pathophysiology of hypogonadism is complex, including potential contribution from hypothalamic-pituitary suppression from a relatively increased estrogen circulation. SHBG is elevated in compensated cirrhotic patients, with subsequent decreases with decompensated cirrhosis, leading to concern for potential underestimation of hypogonadism in cirrhosis[34].

Cortisol: Patients with cirrhosis have relatively lower cortisol levels, also in the setting of lower production of cortisol binding globulin[37]. Some studies suggest the presence of a relative adrenal insufficiency in cirrhosis, also termed critical illness-associated corticosteroid insufficiency[38]. These studies suggest a potential hepatoadrenal syndrome in advanced liver disease, with associated inadequate cortisol production during stress response[38]. The decrease in cortisol binding globulin makes the diagnosis more difficult, though some studies suggest that free cortisol levels are decreased in relative adrenal insufficiency[37]. Hepatoadrenal syndrome and associated low free cortisol are attributed to decreased formation of HDL precursors and formation of proinflammatory cytokines and endotoxins[38].

RAA system: In liver disease, the systemic RAA pathway is upregulated due to systemic and splanchnic arterial vasodilation and associated hypoperfusion of the renal system[39]. Notably, the cirrhotic liver is able to produce angiotensinogen to near-normal plasma levels until the end stages[40].

DPPIV and GLP-1: DPPIV may play a role in linking type 2 diabetes with chronic liver disease. Type 2 diabetes has been associated with a greater than 2-fold increased risk of liver disease[41], and *in vitro* studies have suggested that elevated glucose can induce DPPIV expression in liver cells[42]. The increased DPPIV activity, which degrades the incretin hormone GLP-1, may contribute towards development of IGT, insulin resistance, lipogenesis, and hepatic injury in liver disease[25,43]. Serum DPPIV levels are notably increased in cirrhosis[25], and increased DPPIV expression in the liver has been observed in hepatitis C, NAFLD, experimental liver regeneration, and cirrhosis[25,43]. Cirrhotic nodules show diffuse and uniform staining of DPPIV, with loss of usual zonal expression of DPPIV[43], and degree of hepatic expression of DPPIV has also been shown to correlate with NAFLD grading[25]. Increased DPPIV expression has also been seen in various malignant tumors, including hepatocellular carcinoma, with DPPIV noted to promote resistance to anticancer agents[25].

Thyroid hormone: Given the liver's role in thyroid hormone metabolism, including local conversion of T4 to T3 by DIO1[21], patients with cirrhosis may present with abnormalities in thyroid hormone levels[44]. Though a variety of patterns are seen, the most common pattern is a low total T3 (TT3), low free T3 (FT3), elevated reverse T3 (rT3), low total T4 (TT4), variable literature on elevated *vs* low free T4 (FT4) levels, and possible elevations in TSH[44,45]. The low total hormone levels are attributable to low TBG[44]. The pattern is consistent with low T3 syndrome, which occurs in systemic illnesses, and represents non-thyroidal illness syndrome, previously known as

euthyroid sick syndrome[44].

IGF-1: Systemic IGF-1 deficiency in cirrhosis has been associated with an altered metabolic profile, including diabetes, deregulated lipid profile, and cardiovascular disease[14]. Lack of liver-derived IGF-1, in particular, has been associated with resultant insulin insensitivity in the liver, skeletal muscle, and adipose tissue, and corresponding hyperinsulinemia[46]. In NAFLD, the severity of steatosis has been correlated with a decrease in IGF-1 levels, with statistically significant differences in IGF-1 levels between mild-moderate *vs* severe steatosis[14,47].

Bone health and vitamin D: Chronic liver disease, including cirrhosis regardless of etiology, is associated with osteomalacia, osteopenia, and osteoporosis, and up to 40% of patients with chronic liver disease may develop an osteoporotic fracture[48]. The etiology of hepatic osteodystrophy is not well understood, though potential contributing factors include hypogonadism, and decreased hepatic production of IGF-1 and fibronectin[48]. There is a shift in cytokine production with changes in the receptor activator of nuclear factor kappa-B ligand (RANKL)/osteoprotegerin (OPG) system and an up-regulation of IL-6, which stimulates osteoclasts[48]. Decreased vitamin D synthesis, which is more marked in severely compromised liver function or in cholestatic liver disease, can further contribute to increased osteoporotic risk[49]. History of steroid treatment in chronic liver disease may be a risk factor for osteoporosis as well[48,49]. Different etiologies of liver disease may differ in their pathogenesis of osteoporosis, and in particular, diseases such as hemochromatosis and Wilson's may also directly impact bone health[48].

NOVEL ENDOCRINE FUNCTIONS OF THE LIVER

Besides the advances in the understanding of classic endocrine functions of the liver, novel liver endocrine functions have been unraveled in the last several years (Table 2), including endocrine regulation of pancreatic α cells, adipose tissue, and insulin sensitivity.

Feedback regulation of pancreatic α cells and glucagon

A major novel endocrine function of the liver is its critical role in a pancreatic α cell-liver axis that regulates pancreatic α cell proliferation and circulating glucagon and amino acid levels[50,51]. The pancreatic α cells, unlike the insulin-secreting β cells, have been considered a mysterious cell type until recently[52,53]. The α cells appear first during embryogenesis[54]. The main known function of the α cells is to produce and secrete the hormone glucagon[55]. Glucagon raises circulating glucose levels directly by stimulating gluconeogenesis and glycogenolysis, and indirectly by inhibiting insulin secretion[55,56].

Recently, a new α cell-liver axis has been discovered, endowing the liver with new endocrine functions[50,51]. The first clue of the α cell-liver axis came from glucagon receptor (GCGR) knockout mice[57,58]. The GCGR knockout mice harbor diffusely enlarged pancreas and exhibit extremely high glucagon levels[57-59]. Histologically, the pancreas of GCGR knockout mice contain numerous islets at various sizes, which are composed of mostly α cells as demonstrated by immunochemistry[57-59]. Normally the number of islets is quite small, and the islets are mostly composed of β cells. Mahvash disease, a human autosomal recessive hereditary disease discovered by our group, is caused by biallelic inactivating GCGR mutations, and its universal features are also α cell hyperplasia and hyperglucagonemia[60-62]. GCGR inactivation in zebra fish and non-human primates also result in α cell hyperplasia and hyperglucagonemia[63-66]. Thus, preservation of glucagon function is conserved throughout evolution.

Although a physiological compensation of hyperglucagonemia in animals and humans with inactive GCGR is quite intuitive, the specific mechanism of the compensation was initially not clear[67]. The liver-specific GCGR knockout mice interestingly have similar α cell hyperplasia and hyperglucagonemia, as those in global GCGR knockout mice[57,58,68], suggesting that the liver is the only target organ of glucagon that sends feedback signals to α cells, and that loss of the usual negative feedback mechanism stimulates α cell hyperplasia and glucagon secretion. This theory is also supported by the liver-specific stimulatory G protein α subunit (Gsα) knockout mice, which also exhibit α cell hyperplasia and hyperglucagonemia[69]. As glucagon antagonists were a promising anti-diabetes medication, both academia and pharmaco-

Table 2 Novel endocrine functions of the liver

Liver hormone	Target organ	Action on target organ	Alteration in liver diseases
Amino acids	Pancreatic α cells	Stimulate cell proliferation and glucagon secretion	Not studied yet
Betatrophin	Pancreatic β cells (?)	Stimulate cell proliferation (?)	Increased in cirrhosis
Fetuin	Skeletal muscle; Adipose tissue	Decrease insulin sensitivity; Reduce adiponectin expression	Elevated in nonalcoholic fatty liver disease
FGF21	Adipose tissue; Brain	Increase insulin sensitivity; Reduce food intake	Elevated in nonalcoholic fatty liver disease
Activin E	Adipose tissue	Increase fat oxidation	Increased in nonalcoholic fatty liver disease
Tsukushi	Adipose tissue	Increase thermogenesis	Increased in nonalcoholic fatty liver disease
GPNMB	Adipose tissue	Increase lipogenesis	Increased in nonalcoholic fatty liver disease

FGF21: Fibroblast growth factor 21; GPNMB: Glycoprotein nonmetastatic melanoma protein B.

logical companies became interested in the α cell-liver axis due to potential applications in diabetes drug development[70,71]. Some of the key original large-scale experiments leading to the discovery of the role of amino acids in regulating α cells were performed by pharmaceutical companies[72-74].

The liver may regulate α cells *via* neural or humoral mechanisms[67,68]. Islet transplantation experiments demonstrate that the liver uses a humoral mechanism [68]. Wild-type islets transplanted into the kidney of GCGR knockout mice undergo α cell hyperplasia, while GCGR knockout islets transplanted into wild-type kidney undergo reduced α cell proliferation. Thus, it is assumed that the liver sends a humoral factor (hormone) to stimulate pancreatic α cells, a phenomenon that is pronounced in diseases where the usual negative feedback mechanism is affected.

Initially, it was hoped that a single liver hormone would be isolated from differential liver gene expression patterns of wild-type and GCGR knockout mice[67]. Several groups, including ours, performed liver mRNA arrays of GCGR knockout mice and in wild-type mice treated with inhibitory GCGR antibodies, using wild-type mice as control[67,68,72]. Not surprisingly, many genes are overexpressed (potential stimulatory hormones) or underexpressed (potential inhibitory hormones) in the GCGR knockout liver[67,68,72]. Genes involved in gluconeogenesis are downregulated in the GCGR knockout liver[67,68,72]. On the other hand, genes involved in amino acid synthesis (*e.g.*, asparagine synthetase, Asns) are upregulated, and genes involved in amino acid catabolism (*e.g.*, glutaminase 2, Gls2) are downregulated[67,68,72]. Genes regulating lipid metabolism are also differentially expressed[67,68,72]. Most of the genes with significant differential expression were not bona fide hormone candidates because they were not secreted proteins[67,68,72]. InhbA and DefB1 were the only 2 overexpressed secreted proteins by both the GCGR knockout liver and wild-type liver treated with inhibitory GCGR antibodies; however, these two proteins were are also upregulated by glucagon in primary hepatocytes and thus unlikely the pursued liver hormone[67,68,75].

Another possibility was that the liver hormone may not be a direct gene product such as a protein or polypeptide; rather, the hormone may be a small molecule or metabolite[67]. Metabolomes of the GCGR knockout and wild-type mice were compared[72]. Many differences exist but most notable differences were in glucose, amino acid, nucleotide, and bile acid levels[72]. The GCGR knockout mice have lower glucose levels (70% of wild-type value) and higher levels of most amino acids (up to 15-fold for alanine, glutamine, glycine, lysine, and threonine) and 2 bile acids (cholic acid and glycocholic acid, both about 200-fold) [72]. In humans with Mahvash disease, glucose levels are generally normal, but the levels of amino acids, especially alanine and glutamine, are clearly elevated[62,76-78].

Pinpointing the identity of the novel liver hormone requires tremendous amount of work. Parabiosis of GCGR knockout and wild-type mice was considered, but no such models were published[67]. A more practical *in vitro* islet culture assay was adopted by most groups to screen for the liver hormone that stimulates α cell hyperplasia and hyperglucagonemia[73-75]. With the islet culture assay, it is shown that a < 10 kDa

fraction of serum from GCGR knockout mice sufficiently stimulates α cell proliferation [75]. This fraction contains small proteins or peptides, lipids, amino acids, and metabolites[75]. We have discussed earlier that most proteins or peptides are unlikely the liver hormone. Eliminating lipids from the fraction does not change the activity of the fraction in stimulating α cell proliferation[75]. Finally, as amino acids levels are much higher in GCGR knockout serum, cocktails that mimic the amino acids levels in GCGR knockout mice serum have been tested for their ability to stimulate α cell proliferation, and indeed they do[73-75].

Individual amino acids were further tested to see if a particular amino acid is sufficient to stimulate α cell proliferation[73-75,79]. So far, the data on individual amino acids are still somewhat controversial. Most individual amino acid do not stimulate α cell proliferation or glucagon secretion[73-75,79]. Glutamine alone stimulated α cell proliferation in 2 studies, but it did not stimulate glucagon secretion in another, which is intriguing as α cell hyperplasia and hyperglucagonemia coexist in all models of GCGR inhibition[74,75,79]. Alanine alone stimulated α cell proliferation in one study, but not in another, albeit acutely stimulating glucagon release[75,79]. Experimental conditions may explain some of the different results. It is also possible that α cell proliferation and acute glucagon release may be separate processes.

The α cell receptor for amino acids is under active research. In GCCR knockout mice and in wild-type type mice treated with inhibitory GCGR antibodies, the most upregulated α cell gene is the amino acid transporter Slc38a5 (20-80-fold increase)[74, 75]. Slc38a5 preferentially transports glutamine and several other amino acids, which is concordant with the stimulatory effect of glutamine on α cell proliferation[74,75]. Slc38a5 knockout mice treated with inhibitory glucagon antibodies and Slc38a5 and GCGR double knockout mice exhibited less prominent α cell hyperplasia (approximately 50% less) but similar hyperglucagonemia[74]; this data suggested that Slc38a5 is at least partially responsible for amino acid-stimulated α cell hyperplasia and that α cell hyperplasia and hyperglucagonemia may be regulated separately. Slc38a5, however, is not expressed in human α cells[74]. Another amino acid transporter Slc38a4 is enriched in human α cells when mice with human islet implants are treated with inhibitory GCGR antibodies[80]. In humans with Mahvash disease, Slc38a4 is expressed in the α cells[80], supporting a role of the amino acid transporter in mediating amino acid-stimulated α cell hyperplasia in humans as well. The mTOR pathway in α cells is activated by amino acids as well, contributing to α cell hyperplasia[73-75].

As a result of these studies, the α cell-liver axis has largely been clarified (Figure 1). The α cells secrete glucagon, which signals the liver to increase hepatic amino acid breakdown and reduce amino acid synthesis, consequently leading to desirable amino acid levels in the circulation. After glucagon signaling is inhibited, the liver decreases amino acid breakdown and increases amino acid synthesis, thus raising circulating amino acid levels. The amino acid levels, in turn, act on the α cell amino acid transporters to stimulate α cell proliferation. The evolutionarily conserved α cell-liver axis suggests that glucagon's primary role may be regulating amino acid levels.

Betatrophin

Betatrophin (also known as angiopoietin-like protein 8, ANGPTL8) is a 22-kD protein produced and secreted by the liver and adipose tissue[81,82]. Several years ago, betatrophin was touted as the long sought-after liver hormone that stimulates pancreatic β cell proliferation and insulin production in conditions with insulin resistance[83,84]. An insulin resistance mouse model based on insulin receptor antagonist (S961) infusion exhibits remarkable hyperinsulinemia and beta cell hyperproliferation[83]. As S961 does not directly stimulate β cell proliferation, it was hypothesized that a humoral factor mediates the stimulation of β cell proliferation in this mouse model[83]. Screening of liver genes that were differentially expressed as a result of S961 infusion suggested that betatrophin, a secreted protein that is upregulated by S961 infusion, could be the humoral factor[83]. Betatrophin expression correlated well with β cell proliferation rates. The original report found that liver overexpression of betatrophin stimulated β cell proliferation[83].

The potential of betatrophin as the Holy Grail for diabetes treatment attracted much attention, but later experiments strongly argue against this function of betatrophin[85-87]. Betatrophin knockout mice exhibited normal glucose metabolism and similar hyperinsulinemia and β cell hyperproliferation in response to S961 infusion[85,86]. Detailed analysis of pancreas morphometry by several laboratories definitively showed that betatrophin overexpression does not stimulate β cell proliferation[88]. The only exception was that direct delivery of betatrophin to pancreas does stimulate β cell proliferation in rats[89]. In some mouse models of diabetes, betatrophin lowered

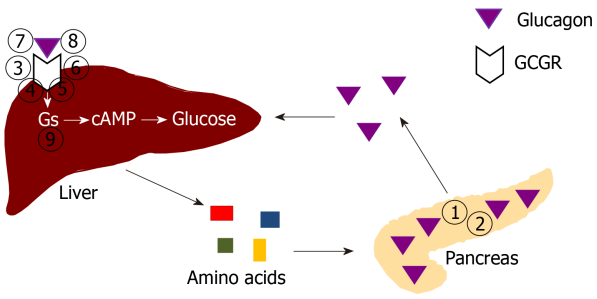

Figure 1 Schematic drawing of regulation of pancreatic α cell number and glucagon secretion by amino acid levels controlled by the liver. The numbers indicate specific ways to disrupt glucagon signaling. (1) Glucagon deletion; (2) Prohormone convertase 2 deletion (with no mature glucagon secretion); (3) Glucagon receptor (GCGR) global deletion; (4) GCGR liver-specific deletion; (5) GCGR inactivating mutation; (6) GCGR antisense RNA; (7) GCGR antagonists; (8) GCGR antibodies; and (9) Gsα liver-specific deletion. See text for details. Citation: Yu R, Zheng Y, Lucas MB, Tong YG. Elusive liver factor that causes pancreatic α cell hyperplasia: A review of literature. *World J Gastrointest Pathophysiol* 2015; 6(4): 131-139. Copyright ©The Author(s) 2015. Published by Baishideng Publishing Group Inc[67]. GCGR: Glucagon receptor.

glucose levels without effects on β cell proliferation[90]. Overall, betatrophin, despite the name, does not appear to stimulate β cell proliferation.

Betatrophin, however, could be a circulating marker of insulin resistance[82]. Early studies of betatrophin levels in various forms of human insulin resistance were quite conflictory, partly due to the differences in measurement methods[82]. Later studies using more standardized methods for measuring betatrophin were summarized by several meta-analyses on the correlation of circulating betatrophin levels and type 2 diabetes, gestational diabetes, polycystic ovary syndrome (PCOS), and obesity — all conditions with insulin resistance[91-95]. Xu *et al*[91] analyzed 25 such studies and showed a positive and significant correlation between circulating betatrophin levels and insulin resistance. Yue *et al*[92] analyzed 11 studies on betatrophin in type 2 diabetes and found that betatrophin is significantly elevated in type 2 diabetes. Kong *et al*[93] analyzed 8 studies on betatrophin in gestational diabetes and concluded that betatrophin is significantly elevated in gestational diabetes. Varikasuvu *et al*[94] analyzed 11 studies on betatrophin in PCOS and concluded that betatrophin is significantly elevated in PCOS. Similarly, Ye *et al*[95] analyzed 6 studies on betatrophin in obesity and concluded that betatrophin is significantly elevated in obesity. Thus, overall, circulating betatrophin is likely a marker of insulin resistance in humans. The high betatrophin liver expression in mice treated with S961, in retrospect, could simply be a sign of insulin resistance caused by S961[83]. It is, however, not clear how insulin resistance upregulates betatrophin. In humans, hyperinsulinemia, often associated with insulin resistance, and metformin, an insulin sensitizer, both decrease betatrophin levels, suggesting that insulin resistance per se upregulates betatrophin levels[96]. Betatrophin overexpression could further worsen hepatocyte sensitivity to insulin, the significance of which needs to be further explored[97].

Betatrophin also has a role in lipids regulation[98]. Betatrophin knockout mice exhibit much reduced triglyceride levels due to reduction in liver VLDL secretion[86]; betatrophin also forms a complex with ANGPTL3, which inhibits lipoprotein lipase (LPL) activity[86]. The increased production of VLDL and decreased LPL activity both contribute to hypertriglyceridemia. Betatrophin overexpression doubles triglyceride levels in mice[86]. In humans, circulating betatrophin levels are positively correlated with triglyceride levels in the general population[99]. In people with dyslipidemia, however, betatrophin levels were lower than in controls[100]. Betatrophin may potentially be a target in dyslipidemia treatment[101].

Hepatokines

Hepatokines are metabolism-regulating proteins produced and secreted by the liver [102,103]. Several hepatokines have been reported and studied. Five of the most studied hepatokines are discussed in this review: Fetuin-A, fibroblast growth factor 21 (FGF21), activin E, Tsukushi, and glycoprotein nonmetastatic melanoma protein B (GPNMB).

Fetuin-A: Fetuin-A, also known as α2-Heremans-Schmid glycoprotein in humans, is one of the first discovered hepatokines[104]. A 52-kD glycoprotein, fetuin-A has diverse metabolic functions[104]. Under physiological conditions, fetuin-A mostly functions as a carrier protein and regulates osteogenesis and inhibits extra-skeletal

calcification[105]. Fetuin-A's role in regulating insulin sensitivity has also been studied in detail[106,107]. Fetuin-A knockout mice exhibit higher insulin sensitivity and have less tendency to develop obesity[106]. At the molecular level, fetuin-A inhibits insulin receptor phosphorylation in myocytes and adipocytes and adiponectin expression in adipocytes[107]. Fetuin-A levels are elevated in patients with insulin resistance or type 2 diabetes, likely mediated by high free fatty acid levels, and high fetuin-A levels are a risk factor for type 2 diabetes[108,109]. The thiazolidinedione-type diabetes medication pioglitazone directly inhibits hepatic production of fetuin-A, partly contributing to its action in improving insulin sensitivity[110].

FGF21: FGF21 is a hepatokine that was first discovered in 2000, but its metabolic regulation functions were not characterized until recently[111,112]. Although FGF21 is also expressed in adipose tissue and the pancreas, circulating FGF21 is predominantly derived from the liver[113]. Hepatic FGF21 expression is regulated by a number of physiological conditions and factors[114]. Prolonged starvation (> 7 d) and overnutrition both upregulate FGF21 expression[115,116]. Glucagon and the thyroid hormone triiodothyronine (T3) both stimulate FGF21 expression, while insulin may inhibit FGF21 expression in liver[117,118]. High-carbohydrate, high-fat diet, and low protein diets stimulate FGF21 expression as well[119,120]. The microRNAs miR-577 and miR-212 target FGF21 mRNA for degradation, thus suppressing FGF21 expression[121, 122]. FGF21 is also upregulated by ER stress[123]. At the molecular level, at least some of the above actions are mediated by the nuclear hormone receptor peroxisome proliferation-activated receptor α (PPARα), which binds to regions of the FGF21 promoter and simulates FGF21 expression[124-126].

The human pre-FGF21 (precursor of mature FGF21) includes a 28-amino-acid signaling peptide and a 181-amino-acid FGF21 proper as the circulating form[127]. FGF21 signals through its transmembrane tyrosine kinase receptors, FGFR1c and FGFR3c, and its transmembrane co-receptor, Klotho-β (KLB)[128]. FGF21 downstream signaling is tissue-specific but generally leads to metabolic benefits such as increased insulin sensitivity and weight loss[129]. In the adipose tissue, FGF21 stimulates the Ras/Raf/MAPK pathway, with phosphorylation of ERK1 and ERK2, and the mTOR pathway, contributing to higher insulin sensitivity[130-132]. Other FGF21 metabolic benefits such as weight loss is mediated by non-adipose tissue such as the brain[133]. FGF21 has been a major interest of metabolic drug development. As the native FGF21 is not stable in the usual formulation, re-engineered FGF21 analogues and PEGylated FGF21 have been developed to be more stable[134]. Activating monoclonal antibodies targeting FGFR1–β-klotho have also been developed[135]. Preclinical and clinical studies have demonstrated clear metabolic benefits of the FGF21 analogs and activating antibodies, such as appetite suppression, weight loss, improved glycemia, and favorable lipid profile[134,135].

Activin E: Activin E belongs to the family of transforming growth factor-β (TGF-β) proteins[136]. Activin E is a secreted homodimer of inhibin-βE, which is mainly expressed in the liver[137]. Each mature inhibin-βE monomer has 113 amino acids [137]. In both mice and humans, inhibin-βE is upregulated by obesity and insulin resistance[138]. In mice, hepatic overexpression of inhibin-βE prevents excess weight gain and improves insulin sensitivity by promoting energy expenditure *via* increased fat oxidation[139,140]. Inhibin-βE ablation in mice gives conflictory results[138,139]. In one study using the transcriptional activator-like effector nucleases (TALENs) to remove liver specific inhibin-βE expression, inhibin-βE-deficient mice exhibited normal weight but had impaired thermogenesis during cold exposure[139]. In another study, however, use of small interfering RNA (siRNA) to silence Inhibin-βE expression in the liver reduced weight gain in obese mice[138]. Thus, the roles of Activin E in metabolic regulation are still controversial.

Tsukushi: Tsukushi belongs to the family of small leucine-rich proteoglycan (SLRP) extracellular matrix proteins[141]. The secreted human Tsukushi protein has 337 amino acids. Besides its role in regulating embryonic development, Tsukushi is found to be a hepatokine, potentially regulating adipose tissue, weight, and energy expenditure[142]. In both mice and humans, Tsukushi is upregulated by thyroid hormone[142,143]; in mice, Tsukushi is induced by obesity and cold exposure[142]. Tsukushi deficiency in mice protects them from diet-induced obesity by increasing adipose tissue thermogenesis and energy expenditure[142]. Using mice from a different genetic background, another group could not reproduce the metabolic benefits of Tsukushi deficiency[144]. Furthermore, studies have also failed to show deleterious metabolic effects from Tsukushi overexpression[144]. The roles of

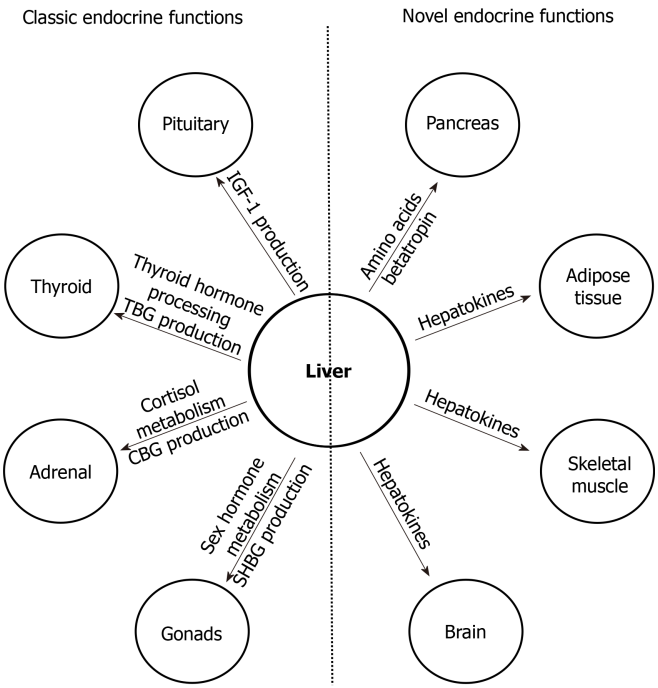

Figure 2 Major classic and novel endocrine functions of the liver. Left, major classic endocrine functions of the liver; right, novel endocrine functions of the liver. See text for details. IGF-1: Insulin-like growth factor 1; TBG: Thyroxine binding globulin; CBG: Cortisol binding globulin; SHBG: Sex hormone binding globulin.

Tsukushi in metabolic regulation thus also remain controversial.

GPNMB: GPNMB is a transmembrane glycoprotein expressed in the liver and other organs[145]. The cleaved extracellular domain of GPNMB (a glycosylated 480-amino-acid protein) is a hepatokine targeting adipose tissue[146,147]. In 2 obese mouse models, GPNMB expression was upregulated in the liver and secreted GPNMB levels were higher as well. Secreted GPNMB stimulates lipogenesis *in vitro* and *in vivo*[147]. A neutralizing antibody targeting GPNMB reduces obesity and improves insulin sensitivity[147]. In both mice and humans, GPNMB levels are positively correlated with obesity and insulin resistance[147]. GPNMB is thus a promising therapeutic target for treatments of obesity and diabetes.

CONCLUSION

The liver has numerous endocrine functions such as direct hormone and hepatokine production, hormone metabolism, synthesis of binding proteins, and processing and redistribution of metabolic fuels. In the last 10 years, many new endocrine functions of the liver have been discovered (Figure 2). Several novel endocrine functions of the liver have been unraveled. The liver plays a key negative feedback regulatory role in the pancreatic α cell-liver axis which regulates pancreatic α cell mass, glucagon secretion, and circulating amino acid levels. Betatrophin and other hepatokines such as fetuin-A and FGF21 play important endocrine roles in modulating insulin sensitivity, lipid metabolism, and body fat weight. It is expected that more endocrine functions of the liver will be discovered in the near future. As endocrine function of the liver is a rapidly evolving field, controversial findings often exist; caution needs to be taken when interpreting novel findings to avoid over-simplification of complex metabolic processes and premature allocation of research resources.

REFERENCES

1 **Watt MJ**, Miotto PM, De Nardo W, Montgomery MK. The Liver as an Endocrine Organ-Linking NAFLD and Insulin Resistance. *Endocr Rev* 2019; **40**: 1367-1393 [PMID: 31098621 DOI: 10.1210/er.2019-00034]

2 **Bikle DD**. The Free Hormone Hypothesis: When, Why, and How to Measure the Free Hormone Levels to Assess Vitamin D, Thyroid, Sex Hormone, and Cortisol Status. *JBMR Plus* 2021; **5**: e10418 [PMID: 33553985 DOI: 10.1002/jbm4.10418]
3 **Grant DM**. Detoxification pathways in the liver. *J Inherit Metab Dis* 1991; **14**: 421-430 [PMID: 1749210 DOI: 10.1007/BF01797915]
4 **Rui L**. Energy metabolism in the liver. *Compr Physiol* 2014; **4**: 177-197 [PMID: 24692138 DOI: 10.1002/cphy.c130024]
5 **Malespin M**, Nassri A. Endocrine Diseases and the Liver: An Update. *Clin Liver Dis* 2019; **23**: 233-246 [PMID: 30947874 DOI: 10.1016/j.cld.2018.12.006]
6 **Svegliati-Baroni G**, Patrício B, Lioci G, Macedo MP, Gastaldelli A. Gut-Pancreas-Liver Axis as a Target for Treatment of NAFLD/NASH. *Int J Mol Sci* 2020; **21** [PMID: 32823659 DOI: 10.3390/ijms21165820]
7 **Crispe IN**. The liver as a lymphoid organ. *Annu Rev Immunol* 2009; **27**: 147-163 [PMID: 19302037 DOI: 10.1146/annurev.immunol.021908.132629]
8 **Corsini A**, Bortolini M. Drug-induced liver injury: the role of drug metabolism and transport. *J Clin Pharmacol* 2013; **53**: 463-474 [PMID: 23436293 DOI: 10.1002/jcph.23]
9 **Eipel C**, Abshagen K, Vollmar B. Regulation of hepatic blood flow: the hepatic arterial buffer response revisited. *World J Gastroenterol* 2010; **16**: 6046-6057 [PMID: 21182219 DOI: 10.3748/wjg.v16.i48.6046]
10 **Bikle DD**. Vitamin D metabolism, mechanism of action, and clinical applications. *Chem Biol* 2014; **21**: 319-329 [PMID: 24529992 DOI: 10.1016/j.chembiol.2013.12.016]
11 **Elangovan H**, Chahal S, Gunton JE. Vitamin D in liver disease: Current evidence and potential directions. *Biochim Biophys Acta Mol Basis Dis* 2017; **1863**: 907-916 [PMID: 28064017 DOI: 10.1016/j.bbadis.2017.01.001]
12 **Christakos S**, Dhawan P, Verstuyf A, Verlinden L, Carmeliet G. Vitamin D: Metabolism, Molecular Mechanism of Action, and Pleiotropic Effects. *Physiol Rev* 2016; **96**: 365-408 [PMID: 26681795 DOI: 10.1152/physrev.00014.2015]
13 **Konstantakis C**, Tselekouni P, Kalafateli M, Triantos C. Vitamin D deficiency in patients with liver cirrhosis. *Ann Gastroenterol* 2016; **29**: 297-306 [PMID: 27366029 DOI: 10.20524/aog.2016.0037]
14 **Kineman RD**, Del Rio-Moreno M, Sarmento-Cabral A. 40 YEARS of IGF1: Understanding the tissue-specific roles of IGF1/IGF1R in regulating metabolism using the Cre/LoxP system. *J Mol Endocrinol* 2018; **61**: T187-T198 [PMID: 29743295 DOI: 10.1530/JME-18-0076]
15 **Lara-Diaz VJ**, Castilla-Cortazar I, Martín-Estal I, García-Magariño M, Aguirre GA, Puche JE, de la Garza RG, Morales LA, Muñoz U. IGF-1 modulates gene expression of proteins involved in inflammation, cytoskeleton, and liver architecture. *J Physiol Biochem* 2017; **73**: 245-258 [PMID: 28124277 DOI: 10.1007/s13105-016-0545-x]
16 **Riedemann J**, Macaulay VM. IGF1R signalling and its inhibition. *Endocr Relat Cancer* 2006; **13** Suppl 1: S33-S43 [PMID: 17259557 DOI: 10.1677/erc.1.01280]
17 **Krieger CC**, Place RF, Bevilacqua C, Marcus-Samuels B, Abel BS, Skarulis MC, Kahaly GJ, Neumann S, Gershengorn MC. TSH/IGF-1 Receptor Cross Talk in Graves' Ophthalmopathy Pathogenesis. *J Clin Endocrinol Metab* 2016; **101**: 2340-2347 [PMID: 27043163 DOI: 10.1210/jc.2016-1315]
18 **Matsusaka T**, Niimura F, Shimizu A, Pastan I, Saito A, Kobori H, Nishiyama A, Ichikawa I. Liver angiotensinogen is the primary source of renal angiotensin II. *J Am Soc Nephrol* 2012; **23**: 1181-1189 [PMID: 22518004 DOI: 10.1681/ASN.2011121159]
19 **Patel S**, Rauf A, Khan H, Abu-Izneid T. Renin-angiotensin-aldosterone (RAAS): The ubiquitous system for homeostasis and pathologies. *Biomed Pharmacother* 2017; **94**: 317-325 [PMID: 28772209 DOI: 10.1016/j.biopha.2017.07.091]
20 **Caulfield M**, Lavender P, Newell-Price J, Kamdar S, Farrall M, Clark AJ. Angiotensinogen in human essential hypertension. *Hypertension* 1996; **28**: 1123-1125 [PMID: 8952609 DOI: 10.1161/01.hyp.28.6.1123]
21 **Luongo C**, Dentice M, Salvatore D. Deiodinases and their intricate role in thyroid hormone homeostasis. *Nat Rev Endocrinol* 2019; **15**: 479-488 [PMID: 31160732 DOI: 10.1038/s41574-019-0218-2]
22 **Findlay KA**, Kaptein E, Visser TJ, Burchell B. Characterization of the uridine diphosphate-glucuronosyltransferase-catalyzing thyroid hormone glucuronidation in man. *J Clin Endocrinol Metab* 2000; **85**: 2879-2883 [PMID: 10946897 DOI: 10.1210/jcem.85.8.6715]
23 **Jin T**, Weng J. Hepatic functions of GLP-1 and its based drugs: current disputes and perspectives. *Am J Physiol Endocrinol Metab* 2016; **311**: E620-E627 [PMID: 27507553 DOI: 10.1152/ajpendo.00069.2016]
24 **MacDonald PE**, El-Kholy W, Riedel MJ, Salapatek AM, Light PE, Wheeler MB. The multiple actions of GLP-1 on the process of glucose-stimulated insulin secretion. *Diabetes* 2002; **51** Suppl 3: S434-S442 [PMID: 12475787 DOI: 10.2337/diabetes.51.2007.s434]
25 **Itou M**, Kawaguchi T, Taniguchi E, Sata M. Dipeptidyl peptidase-4: a key player in chronic liver disease. *World J Gastroenterol* 2013; **19**: 2298-2306 [PMID: 23613622 DOI: 10.3748/wjg.v19.i15.2298]
26 **Trapani L**, Segatto M, Pallottini V. Regulation and deregulation of cholesterol homeostasis: The liver as a metabolic "power station". *World J Hepatol* 2012; **4**: 184-190 [PMID: 22761969 DOI: 10.4254/wjh.v4.i6.184]

27 **Schiffer L**, Barnard L, Baranowski ES, Gilligan LC, Taylor AE, Arlt W, Shackleton CHL, Storbeck KH. Human steroid biosynthesis, metabolism and excretion are differentially reflected by serum and urine steroid metabolomes: A comprehensive review. *J Steroid Biochem Mol Biol* 2019; **194**: 105439 [PMID: 31362062 DOI: 10.1016/j.jsbmb.2019.105439]

28 **Tsuchiya Y**, Nakajima M, Yokoi T. Cytochrome P450-mediated metabolism of estrogens and its regulation in human. *Cancer Lett* 2005; **227**: 115-124 [PMID: 16112414 DOI: 10.1016/j.canlet.2004.10.007]

29 **Raftogianis R**, Creveling C, Weinshilboum R, Weisz J. Estrogen metabolism by conjugation. *J Natl Cancer Inst Monogr* 2000; 113-124 [PMID: 10963623 DOI: 10.1093/oxfordjournals.jncimonographs.a024234]

30 **Wilson VS**, LeBlanc GA. The contribution of hepatic inactivation of testosterone to the lowering of serum testosterone levels by ketoconazole. *Toxicol Sci* 2000; **54**: 128-137 [PMID: 10746940 DOI: 10.1093/toxsci/54.1.128]

31 **Quinkler M**, Stewart PM. Hypertension and the cortisol-cortisone shuttle. *J Clin Endocrinol Metab* 2003; **88**: 2384-2392 [PMID: 12788832 DOI: 10.1210/jc.2003-030138]

32 **Basu R**, Basu A, Grudzien M, Jung P, Jacobson P, Johnson M, Singh R, Sarr M, Rizza RA. Liver is the site of splanchnic cortisol production in obese nondiabetic humans. *Diabetes* 2009; **58**: 39-45 [PMID: 18852327 DOI: 10.2337/db08-1079]

33 **Fujisawa G**, Muto S, Okada K, Kusano E, Ishibashi S. Mineralocorticoid receptor antagonist spironolactone prevents pig serum-induced hepatic fibrosis in rats. *Transl Res* 2006; **148**: 149-156 [PMID: 16938653 DOI: 10.1016/j.trsl.2006.03.007]

34 **Hammond GL**. Plasma steroid-binding proteins: primary gatekeepers of steroid hormone action. *J Endocrinol* 2016; **230**: R13-R25 [PMID: 27113851 DOI: 10.1530/JOE-16-0070]

35 **Hammond GL**. Potential functions of plasma steroid-binding proteins. *Trends Endocrinol Metab* 1995; **6**: 298-304 [PMID: 18406714 DOI: 10.1016/1043-2760(95)00162-x]

36 **Sinclair M**, Grossmann M, Gow PJ, Angus PW. Testosterone in men with advanced liver disease: abnormalities and implications. *J Gastroenterol Hepatol* 2015; **30**: 244-251 [PMID: 25087838 DOI: 10.1111/jgh.12695]

37 **Fede G**, Spadaro L, Tomaselli T, Privitera G, Scicali R, Vasianopoulou P, Thalassinos E, Martin N, Thomas M, Purrello F, Burroughs AK. Comparison of total cortisol, free cortisol, and surrogate markers of free cortisol in diagnosis of adrenal insufficiency in patients with stable cirrhosis. *Clin Gastroenterol Hepatol* 2014; **12**: 504-12.e8; quiz e23 [PMID: 23978347 DOI: 10.1016/j.cgh.2013.08.028]

38 **Rakici H**. Adrenal Insufficiency in Cirrhosis Patients: Evaluation of 108 Case Series. *Euroasian J Hepatogastroenterol* 2017; **7**: 150-153 [PMID: 29201798 DOI: 10.5005/jp-journals-10018-1237]

39 **Di Pascoli M**, La Mura V. Renin-angiotensin-aldosterone system in cirrhosis: There's room to try! *Dig Liver Dis* 2019; **51**: 297-298 [PMID: 30220630 DOI: 10.1016/j.dld.2018.07.038]

40 **Sansoè G**, Aragno M, Wong F. Pathways of hepatic and renal damage through non-classical activation of the renin-angiotensin system in chronic liver disease. *Liver Int* 2020; **40**: 18-31 [PMID: 31580514 DOI: 10.1111/liv.14272]

41 **Jarvis H**, Craig D, Barker R, Spiers G, Stow D, Anstee QM, Hanratty B. Metabolic risk factors and incident advanced liver disease in non-alcoholic fatty liver disease (NAFLD): A systematic review and meta-analysis of population-based observational studies. *PLoS Med* 2020; **17**: e1003100 [PMID: 32353039 DOI: 10.1371/journal.pmed.1003100]

42 **Miyazaki M**, Kato M, Tanaka K, Tanaka M, Kohjima M, Nakamura K, Enjoji M, Nakamuta M, Kotoh K, Takayanagi R. Increased hepatic expression of dipeptidyl peptidase-4 in non-alcoholic fatty liver disease and its association with insulin resistance and glucose metabolism. *Mol Med Rep* 2012; **5**: 729-733 [PMID: 22179204 DOI: 10.3892/mmr.2011.707]

43 **Matsumoto Y**, Bishop GA, McCaughan GW. Altered zonal expression of the CD26 antigen (dipeptidyl peptidase IV) in human cirrhotic liver. *Hepatology* 1992; **15**: 1048-1053 [PMID: 1350563 DOI: 10.1002/hep.1840150613]

44 **Moura Neto A**, Zantut-Wittmann DE. Abnormalities of Thyroid Hormone Metabolism during Systemic Illness: The Low T3 Syndrome in Different Clinical Settings. *Int J Endocrinol* 2016; **2016**: 2157583 [PMID: 27803712 DOI: 10.1155/2016/2157583]

45 **Green JR**. Thyroid function in chronic liver disease. *Z Gastroenterol* 1979; **17**: 447-451 [PMID: 112792]

46 **Ohlsson C**, Mohan S, Sjögren K, Tivesten A, Isgaard J, Isaksson O, Jansson JO, Svensson J. The role of liver-derived insulin-like growth factor-I. *Endocr Rev* 2009; **30**: 494-535 [PMID: 19589948 DOI: 10.1210/er.2009-0010]

47 **Mallea-Gil MS**, Ballarino MC, Spiraquis A, Iriarte M, Kura M, Gimenez S, Oneto A, Guitelman M, Machado R, Miguel CM. IGF-1 Levels in different stages of liver steatosis and its association with metabolic syndrome. *Acta Gastroenterol Latinoam* 2012; **42**: 20-26 [PMID: 22616493]

48 **Handzlik-Orlik G**, Holecki M, Wilczyński K, Duława J. Osteoporosis in liver disease: pathogenesis and management. *Ther Adv Endocrinol Metab* 2016; **7**: 128-135 [PMID: 27293541 DOI: 10.1177/2042018816641351]

49 **Nair S**. Vitamin d deficiency and liver disease. *Gastroenterol Hepatol (N Y)* 2010; **6**: 491-493 [PMID: 20978551]

50 **Wewer Albrechtsen NJ**, Pedersen J, Galsgaard KD, Winther-Sørensen M, Suppli MP, Janah L, Gromada J, Vilstrup H, Knop FK, Holst JJ. The Liver-α-Cell Axis and Type 2 Diabetes. *Endocr Rev*

2019; **40**: 1353-1366 [PMID: 30920583 DOI: 10.1210/er.2018-00251]
51 **Dean ED**. A Primary Role for α-Cells as Amino Acid Sensors. *Diabetes* 2020; **69**: 542-549 [PMID: 31653720 DOI: 10.2337/dbi19-0021]
52 **Gromada J**, Franklin I, Wollheim CB. Alpha-cells of the endocrine pancreas: 35 years of research but the enigma remains. *Endocr Rev* 2007; **28**: 84-116 [PMID: 17261637 DOI: 10.1210/er.2006-0007]
53 **Habener JF**, Stanojevic V. Alpha cells come of age. *Trends Endocrinol Metab* 2013; **24**: 153-163 [PMID: 23260869 DOI: 10.1016/j.tem.2012.10.009]
54 **Habener JF**, Stanojevic V. α-cell role in β-cell generation and regeneration. *Islets* 2012; **4**: 188-198 [PMID: 22847495 DOI: 10.4161/isl.20500]
55 **Quesada I**, Tudurí E, Ripoll C, Nadal A. Physiology of the pancreatic alpha-cell and glucagon secretion: role in glucose homeostasis and diabetes. *J Endocrinol* 2008; **199**: 5-19 [PMID: 18669612 DOI: 10.1677/JOE-08-0290]
56 **Habegger KM**, Heppner KM, Geary N, Bartness TJ, DiMarchi R, Tschöp MH. The metabolic actions of glucagon revisited. *Nat Rev Endocrinol* 2010; **6**: 689-697 [PMID: 20957001 DOI: 10.1038/nrendo.2010.187]
57 **Parker JC**, Andrews KM, Allen MR, Stock JL, McNeish JD. Glycemic control in mice with targeted disruption of the glucagon receptor gene. *Biochem Biophys Res Commun* 2002; **290**: 839-843 [PMID: 11785978 DOI: 10.1006/bbrc.2001.6265]
58 **Gelling RW**, Du XQ, Dichmann DS, Romer J, Huang H, Cui L, Obici S, Tang B, Holst JJ, Fledelius C, Johansen PB, Rossetti L, Jelicks LA, Serup P, Nishimura E, Charron MJ. Lower blood glucose, hyperglucagonemia, and pancreatic alpha cell hyperplasia in glucagon receptor knockout mice. *Proc Natl Acad Sci U S A* 2003; **100**: 1438-1443 [PMID: 12552113 DOI: 10.1073/pnas.0237106100]
59 **Yu R**, Dhall D, Nissen NN, Zhou C, Ren SG. Pancreatic neuroendocrine tumors in glucagon receptor-deficient mice. *PLoS One* 2011; **6**: e23397 [PMID: 21853126 DOI: 10.1371/journal.pone.0023397]
60 **Yu R**, Nissen NN, Dhall D, Heaney AP. Nesidioblastosis and hyperplasia of alpha cells, microglucagonoma, and nonfunctioning islet cell tumor of the pancreas: review of the literature. *Pancreas* 2008; **36**: 428-431 [PMID: 18437091 DOI: 10.1097/MPA.0b013e31815ceb23]
61 **Zhou C**, Dhall D, Nissen NN, Chen CR, Yu R. Homozygous P86S mutation of the human glucagon receptor is associated with hyperglucagonemia, alpha cell hyperplasia, and islet cell tumor. *Pancreas* 2009; **38**: 941-946 [PMID: 19657311 DOI: 10.1097/MPA.0b013e3181b2bb03]
62 **Yu R**. Mahvash Disease: 10 Years After Discovery. *Pancreas* 2018; **47**: 511-515 [PMID: 29702528 DOI: 10.1097/MPA.0000000000001044]
63 **Yu R**. Pancreatic α-cell hyperplasia: facts and myths. *J Clin Endocrinol Metab* 2014; **99**: 748-756 [PMID: 24285676 DOI: 10.1210/jc.2013-2952]
64 **Li M**, Dean ED, Zhao L, Nicholson WE, Powers AC, Chen W. Glucagon receptor inactivation leads to α-cell hyperplasia in zebrafish. *J Endocrinol* 2015; **227**: 93-103 [PMID: 26446275 DOI: 10.1530/JOE-15-0284]
65 **Bai X**, Jia J, Kang Q, Fu Y, Zhou Y, Zhong Y, Zhang C, Li M. Integrated Metabolomics and Lipidomics Analysis Reveal Remodeling of Lipid Metabolism and Amino Acid Metabolism in Glucagon Receptor-Deficient Zebrafish. *Front Cell Dev Biol* 2020; **8**: 605979 [PMID: 33520988 DOI: 10.3389/fcell.2020.605979]
66 **Yan H**, Gu W, Yang J, Bi V, Shen Y, Lee E, Winters KA, Komorowski R, Zhang C, Patel JJ, Caughey D, Elliott GS, Lau YY, Wang J, Li YS, Boone T, Lindberg RA, Hu S, Véniant MM. Fully human monoclonal antibodies antagonizing the glucagon receptor improve glucose homeostasis in mice and monkeys. *J Pharmacol Exp Ther* 2009; **329**: 102-111 [PMID: 19129372 DOI: 10.1124/jpet.108.147009]
67 **Yu R**, Zheng Y, Lucas MB, Tong YG. Elusive liver factor that causes pancreatic α cell hyperplasia: A review of literature. *World J Gastrointest Pathophysiol* 2015; **6**: 131-139 [PMID: 26600971 DOI: 10.4291/wjgp.v6.i4.131]
68 **Longuet C**, Robledo AM, Dean ED, Dai C, Ali S, McGuinness I, de Chavez V, Vuguin PM, Charron MJ, Powers AC, Drucker DJ. Liver-specific disruption of the murine glucagon receptor produces α-cell hyperplasia: evidence for a circulating α-cell growth factor. *Diabetes* 2013; **62**: 1196-1205 [PMID: 23160527 DOI: 10.2337/db11-1605]
69 **Chen M**, Gavrilova O, Zhao WQ, Nguyen A, Lorenzo J, Shen L, Nackers L, Pack S, Jou W, Weinstein LS. Increased glucose tolerance and reduced adiposity in the absence of fasting hypoglycemia in mice with liver-specific Gs alpha deficiency. *J Clin Invest* 2005; **115**: 3217-3227 [PMID: 16239968 DOI: 10.1172/JCI24196]
70 **Shen DM**, Lin S, Parmee ER. A survey of small molecule glucagon receptor antagonists from recent patents (2006 - 2010). *Expert Opin Ther Pat* 2011; **21**: 1211-1240 [PMID: 21635155 DOI: 10.1517/13543776.2011.587001]
71 **Scheen AJ**, Paquot N, Lefèbvre PJ. Investigational glucagon receptor antagonists in Phase I and II clinical trials for diabetes. *Expert Opin Investig Drugs* 2017; **26**: 1373-1389 [PMID: 29052441 DOI: 10.1080/13543784.2017.1395020]
72 **Yang J**, MacDougall ML, McDowell MT, Xi L, Wei R, Zavadoski WJ, Molloy MP, Baker JD, Kuhn M, Cabrera O, Treadway JL. Polyomic profiling reveals significant hepatic metabolic alterations in glucagon-receptor (GCGR) knockout mice: implications on anti-glucagon therapies for diabetes. *BMC Genomics* 2011; **12**: 281 [PMID: 21631939 DOI: 10.1186/1471-2164-12-281]

73 **Solloway MJ**, Madjidi A, Gu C, Eastham-Anderson J, Clarke HJ, Kljavin N, Zavala-Solorio J, Kates L, Friedman B, Brauer M, Wang J, Fiehn O, Kolumam G, Stern H, Lowe JB, Peterson AS, Allan BB. Glucagon Couples Hepatic Amino Acid Catabolism to mTOR-Dependent Regulation of α-Cell Mass. *Cell Rep* 2015; **12**: 495-510 [PMID: 26166562 DOI: 10.1016/j.celrep.2015.06.034]

74 **Kim J**, Okamoto H, Huang Z, Anguiano G, Chen S, Liu Q, Cavino K, Xin Y, Na E, Hamid R, Lee J, Zambrowicz B, Unger R, Murphy AJ, Xu Y, Yancopoulos GD, Li WH, Gromada J. Amino Acid Transporter Slc38a5 Controls Glucagon Receptor Inhibition-Induced Pancreatic α Cell Hyperplasia in Mice. *Cell Metab* 2017; **25**: 1348-1361.e8 [PMID: 28591637 DOI: 10.1016/j.cmet.2017.05.006]

75 **Dean ED**, Li M, Prasad N, Wisniewski SN, Von Deylen A, Spaeth J, Maddison L, Botros A, Sedgeman LR, Bozadjieva N, Ilkayeva O, Coldren A, Poffenberger G, Shostak A, Semich MC, Aamodt KI, Phillips N, Yan H, Bernal-Mizrachi E, Corbin JD, Vickers KC, Levy SE, Dai C, Newgard C, Gu W, Stein R, Chen W, Powers AC. Interrupted Glucagon Signaling Reveals Hepatic α Cell Axis and Role for L-Glutamine in α Cell Proliferation. *Cell Metab* 2017; **25**: 1362-1373.e5 [PMID: 28591638 DOI: 10.1016/j.cmet.2017.05.011]

76 **Larger E**, Wewer Albrechtsen NJ, Hansen LH, Gelling RW, Capeau J, Deacon CF, Madsen OD, Yakushiji F, De Meyts P, Holst JJ, Nishimura E. Pancreatic α-cell hyperplasia and hyperglucagonemia due to a glucagon receptor splice mutation. *Endocrinol Diabetes Metab Case Rep* 2016; **2016** [PMID: 27933176 DOI: 10.1530/EDM-16-0081]

77 **Li H**, Zhao L, Singh R, Ham JN, Fadoju DO, Bean LJH, Zhang Y, Xu Y, Xu HE, Gambello MJ. The first pediatric case of glucagon receptor defect due to biallelic mutations in *GCGR* is identified by newborn screening of elevated arginine. *Mol Genet Metab Rep* 2018; **17**: 46-52 [PMID: 30294546 DOI: 10.1016/j.ymgmr.2018.09.006]

78 **Gild ML**, Tsang V, Samra J, Clifton-Bligh RJ, Tacon L, Gill AJ. Hypercalcemia in Glucagon Cell Hyperplasia and Neoplasia (Mahvash Syndrome): A New Association. *J Clin Endocrinol Metab* 2018; **103**: 3119-3123 [PMID: 30032256 DOI: 10.1210/jc.2018-01074]

79 **Galsgaard KD**, Jepsen SL, Kjeldsen SAS, Pedersen J, Wewer Albrechtsen NJ, Holst JJ. Alanine, arginine, cysteine, and proline, but not glutamine, are substrates for, and acute mediators of, the liver-α-cell axis in female mice. *Am J Physiol Endocrinol Metab* 2020; **318**: E920-E929 [PMID: 32255678 DOI: 10.1152/ajpendo.00459.2019]

80 **Kim J**, Dominguez Gutierrez G, Xin Y, Cavino K, Sung B, Sipos B, Kloeppel G, Gromada J, Okamoto H. Increased SLC38A4 Amino Acid Transporter Expression in Human Pancreatic α-Cells After Glucagon Receptor Inhibition. *Endocrinology* 2019; **160**: 979-988 [PMID: 30938753 DOI: 10.1210/en.2019-00022]

81 **Kaestner KH**. Betatrophin--promises fading and lessons learned. *Cell Metab* 2014; **20**: 932-933 [PMID: 25470542 DOI: 10.1016/j.cmet.2014.11.016]

82 **Abu-Farha M**, Abubaker J, Tuomilehto J. ANGPTL8 (betatrophin) role in diabetes and metabolic diseases. *Diabetes Metab Res Rev* 2017; **33** [PMID: 28722798 DOI: 10.1002/dmrr.2919]

83 **Yi P**, Park JS, Melton DA. Betatrophin: a hormone that controls pancreatic β cell proliferation. *Cell* 2013; **153**: 747-758 [PMID: 23623304 DOI: 10.1016/j.cell.2013.04.008]

84 **Seymour PA**, Serup P. Bulking up on beta cells. *N Engl J Med* 2013; **369**: 777-779 [PMID: 23964941 DOI: 10.1056/NEJMcibr1307038]

85 **Gusarova V**, Alexa CA, Na E, Stevis PE, Xin Y, Bonner-Weir S, Cohen JC, Hobbs HH, Murphy AJ, Yancopoulos GD, Gromada J. ANGPTL8/betatrophin does not control pancreatic beta cell expansion. *Cell* 2014; **159**: 691-696 [PMID: 25417115 DOI: 10.1016/j.cell.2014.09.027]

86 **Wang Y**, Quagliarini F, Gusarova V, Gromada J, Valenzuela DM, Cohen JC, Hobbs HH. Mice lacking ANGPTL8 (Betatrophin) manifest disrupted triglyceride metabolism without impaired glucose homeostasis. *Proc Natl Acad Sci U S A* 2013; **110**: 16109-16114 [PMID: 24043787 DOI: 10.1073/pnas.1315292110]

87 **Cox AR**, Lam CJ, Bonnyman CW, Chavez J, Rios JS, Kushner JA. Angiopoietin-like protein 8 (ANGPTL8)/betatrophin overexpression does not increase beta cell proliferation in mice. *Diabetologia* 2015; **58**: 1523-1531 [PMID: 25917759 DOI: 10.1007/s00125-015-3590-z]

88 **Cox AR**, Barrandon O, Cai EP, Rios JS, Chavez J, Bonnyman CW, Lam CJ, Yi P, Melton DA, Kushner JA. Resolving Discrepant Findings on ANGPTL8 in β-Cell Proliferation: A Collaborative Approach to Resolving the Betatrophin Controversy. *PLoS One* 2016; **11**: e0159276 [PMID: 27410263 DOI: 10.1371/journal.pone.0159276]

89 **Chen J**, Chen S, Huang P, Meng XL, Clayton S, Shen JS, Grayburn PA. In vivo targeted delivery of ANGPTL8 gene for beta cell regeneration in rats. *Diabetologia* 2015; **58**: 1036-1044 [PMID: 25720603 DOI: 10.1007/s00125-015-3521-z]

90 **Liu S**, Smith EM, King TH, Glenn L, Trevino M, Park SH, Machida Y, Villaflor C, Grzesik W, Morris MA, Imai Y, Nadler JL. Host Factors Alter Effects of Angiopoietin-Like Protein 8 on Glucose Homeostasis in Diabetic Mice. *J Diabetes Mellitus* 2016; **6**: 277-290 [PMID: 31741751 DOI: 10.4236/jdm.2016.64029]

91 **Xu J**, Lin Y, Zhou H, Zhao L, Xiang G. The Correlation Between Circulating Betatrophin and Insulin Resistance in General Population: A Meta-Analysis. *Horm Metab Res* 2017; **49**: 760-771 [PMID: 28931172 DOI: 10.1055/s-0043-108911]

92 **Yue S**, Wu J, Zhang J, Liu L, Chen L. The Relationship between Betatrophin Levels in Blood and T2DM: A Systematic Review and Meta-Analysis. *Dis Markers* 2016; **2016**: 9391837 [PMID: 27242389 DOI: 10.1155/2016/9391837]

93 **Kong FJ**, Ma LL, Li G, Chen YX, Zhou JQ. Circulating Betatrophin Levels and Gestational

Diabetes Mellitus: A Systematic Review and Meta-Analysis. *PLoS One* 2017; **12**: e0169941 [PMID: 28081192 DOI: 10.1371/journal.pone.0169941]

94 **Varikasuvu SR**, Panga JR, Satyanarayana MV. Circulating Angiopoietin-like 8 protein (ANGPTL8/Betatrophin) in patients with polycystic ovary syndrome: a systematic review and multi effect size meta-analysis. *Gynecol Endocrinol* 2019; **35**: 190-197 [PMID: 30614305 DOI: 10.1080/09513590.2018.1536736]

95 **Ye J**, Qin Y, Wang D, Yang L, Yuan G. The Relationship between Circulating ANGPTL8/Betatrophin Concentrations and Adult Obesity: A Meta-Analysis. *Dis Markers* 2019; **2019**: 5096860 [PMID: 31772689 DOI: 10.1155/2019/5096860]

96 **Wang H**, Du L, Wu T, Yang G, Hu W, Wang H, Yang M, Liu D, Gu HF, Zhu Z, Zheng H, Li L. Circulating betatrophin is associated with insulin resistance in humans: cross-sectional and interventional studies *in vivo* and *in vitro*. *Oncotarget* 2017; **8**: 96604-96614 [PMID: 29228555 DOI: 10.18632/oncotarget.21852]

97 **Hao Q**, Zheng A, Zhang H, Cao H. Down-regulation of betatrophin enhances insulin sensitivity in type 2 diabetes mellitus through activation of the GSK-3β/PGC-1α signaling pathway. *J Endocrinol Invest* 2021; **44**: 1857-1868 [PMID: 33464548 DOI: 10.1007/s40618-020-01493-1]

98 **Zhang R**. Lipasin, a novel nutritionally-regulated liver-enriched factor that regulates serum triglyceride levels. *Biochem Biophys Res Commun* 2012; **424**: 786-792 [PMID: 22809513 DOI: 10.1016/j.bbrc.2012.07.038]

99 **Gao T**, Jin K, Chen P, Jin H, Yang L, Xie X, Yang M, Hu C, Yu X. Circulating Betatrophin Correlates with Triglycerides and Postprandial Glucose among Different Glucose Tolerance Statuses--A Case-Control Study. *PLoS One* 2015; **10**: e0133640 [PMID: 26247824 DOI: 10.1371/journal.pone.0133640]

100 **Gómez-Ambrosi J**, Pascual-Corrales E, Catalán V, Rodríguez A, Ramírez B, Romero S, Vila N, Ibáñez P, Margall MA, Silva C, Gil MJ, Salvador J, Frühbeck G. Altered Concentrations in Dyslipidemia Evidence a Role for ANGPTL8/Betatrophin in Lipid Metabolism in Humans. *J Clin Endocrinol Metab* 2016; **101**: 3803-3811 [PMID: 27472196 DOI: 10.1210/jc.2016-2084]

101 **Fu Z**, Abou-Samra AB, Zhang R. A lipasin/Angptl8 monoclonal antibody lowers mouse serum triglycerides involving increased postprandial activity of the cardiac lipoprotein lipase. *Sci Rep* 2015; **5**: 18502 [PMID: 26687026 DOI: 10.1038/srep18502]

102 **Jung TW**, Yoo HJ, Choi KM. Implication of hepatokines in metabolic disorders and cardiovascular diseases. *BBA Clin* 2016; **5**: 108-113 [PMID: 27051596 DOI: 10.1016/j.bbacli.2016.03.002]

103 **Jensen-Cody SO**, Potthoff MJ. Hepatokines and metabolism: Deciphering communication from the liver. *Mol Metab* 2021; **44**: 101138 [PMID: 33285302 DOI: 10.1016/j.molmet.2020.101138]

104 **Bourebaba L**, Marycz K. Pathophysiological Implication of Fetuin-A Glycoprotein in the Development of Metabolic Disorders: A Concise Review. *J Clin Med* 2019; **8** [PMID: 31766373 DOI: 10.3390/jcm8122033]

105 **Jahnen-Dechent W**, Heiss A, Schäfer C, Ketteler M. Fetuin-A regulation of calcified matrix metabolism. *Circ Res* 2011; **108**: 1494-1509 [PMID: 21659653 DOI: 10.1161/CIRCRESAHA.110.234260]

106 **Mathews ST**, Rakhade S, Zhou X, Parker GC, Coscina DV, Grunberger G. Fetuin-null mice are protected against obesity and insulin resistance associated with aging. *Biochem Biophys Res Commun* 2006; **350**: 437-443 [PMID: 17011519 DOI: 10.1016/j.bbrc.2006.09.071]

107 **Ren G**, Kim T, Papizan JB, Okerberg CK, Kothari VM, Zaid H, Bilan PJ, Araya-Ramirez F, Littlefield LA, Bowers RL, Mahurin AJ, Nickles MM, Ludvigsen R, He X, Grandjean PW, Mathews ST. Phosphorylation status of fetuin-A is critical for inhibition of insulin action and is correlated with obesity and insulin resistance. *Am J Physiol Endocrinol Metab* 2019; **317**: E250-E260 [PMID: 31084489 DOI: 10.1152/ajpendo.00089.2018]

108 **Ramírez-Vélez R**, García-Hermoso A, Hackney AC, Izquierdo M. Effects of exercise training on Fetuin-a in obese, type 2 diabetes and cardiovascular disease in adults and elderly: a systematic review and Meta-analysis. *Lipids Health Dis* 2019; **18**: 23 [PMID: 30670052 DOI: 10.1186/s12944-019-0962-2]

109 **Pan X**, Wen SW, Bestman PL, Kaminga AC, Acheampong K, Liu A. Fetuin-A in Metabolic syndrome: A systematic review and meta-analysis. *PLoS One* 2020; **15**: e0229776 [PMID: 32134969 DOI: 10.1371/journal.pone.0229776]

110 **Ochi A**, Mori K, Emoto M, Nakatani S, Morioka T, Motoyama K, Fukumoto S, Imanishi Y, Koyama H, Ishimura E, Inaba M. Direct inhibitory effects of pioglitazone on hepatic fetuin-A expression. *PLoS One* 2014; **9**: e88704 [PMID: 24551137 DOI: 10.1371/journal.pone.0088704]

111 **Fisher FM**, Maratos-Flier E. Understanding the Physiology of FGF21. *Annu Rev Physiol* 2016; **78**: 223-241 [PMID: 26654352 DOI: 10.1146/annurev-physiol-021115-105339]

112 **Tezze C**, Romanello V, Sandri M. FGF21 as Modulator of Metabolism in Health and Disease. *Front Physiol* 2019; **10**: 419 [PMID: 31057418 DOI: 10.3389/fphys.2019.00419]

113 **Markan KR**, Naber MC, Ameka MK, Anderegg MD, Mangelsdorf DJ, Kliewer SA, Mohammadi M, Potthoff MJ. Circulating FGF21 is liver derived and enhances glucose uptake during refeeding and overfeeding. *Diabetes* 2014; **63**: 4057-4063 [PMID: 25008183 DOI: 10.2337/db14-0595]

114 **Erickson A**, Moreau R. The regulation of FGF21 gene expression by metabolic factors and nutrients. *Horm Mol Biol Clin Investig* 2016; **30** [PMID: 27285327 DOI: 10.1515/hmbci-2016-0016]

115 **Fazeli PK**, Lun M, Kim SM, Bredella MA, Wright S, Zhang Y, Lee H, Catana C, Klibanski A, Patwari P, Steinhauser ML. FGF21 and the late adaptive response to starvation in humans. *J Clin*

Invest 2015; **125**: 4601-4611 [PMID: 26529252 DOI: 10.1172/JCI83349]

116 **Zhang X**, Yeung DC, Karpisek M, Stejskal D, Zhou ZG, Liu F, Wong RL, Chow WS, Tso AW, Lam KS, Xu A. Serum FGF21 Levels are increased in obesity and are independently associated with the metabolic syndrome in humans. *Diabetes* 2008; **57**: 1246-1253 [PMID: 18252893 DOI: 10.2337/db07-1476]

117 **Habegger KM**, Stemmer K, Cheng C, Müller TD, Heppner KM, Ottaway N, Holland J, Hembree JL, Smiley D, Gelfanov V, Krishna R, Arafat AM, Konkar A, Belli S, Kapps M, Woods SC, Hofmann SM, D'Alessio D, Pfluger PT, Perez-Tilve D, Seeley RJ, Konishi M, Itoh N, Kharitonenkov A, Spranger J, DiMarchi RD, Tschöp MH. Fibroblast growth factor 21 mediates specific glucagon actions. *Diabetes* 2013; **62**: 1453-1463 [PMID: 23305646 DOI: 10.2337/db12-1116]

118 **Adams AC**, Astapova I, Fisher FM, Badman MK, Kurgansky KE, Flier JS, Hollenberg AN, Maratos-Flier E. Thyroid hormone regulates hepatic expression of fibroblast growth factor 21 in a PPARalpha-dependent manner. *J Biol Chem* 2010; **285**: 14078-14082 [PMID: 20236931 DOI: 10.1074/jbc.C110.107375]

119 **Uebanso T**, Taketani Y, Fukaya M, Sato K, Takei Y, Sato T, Sawada N, Amo K, Harada N, Arai H, Yamamoto H, Takeda E. Hypocaloric high-protein diet improves fatty liver and hypertriglyceridemia in sucrose-fed obese rats *via* two pathways. *Am J Physiol Endocrinol Metab* 2009; **297**: E76-E84 [PMID: 19435858 DOI: 10.1152/ajpendo.00014.2009]

120 **Martínez-Garza Ú**, Torres-Oteros D, Yarritu-Gallego A, Marrero PF, Haro D, Relat J. Fibroblast Growth Factor 21 and the Adaptive Response to Nutritional Challenges. *Int J Mol Sci* 2019; **20** [PMID: 31546675 DOI: 10.3390/ijms20194692]

121 **Chen XY**, Li GM, Dong Q, Peng H. MiR-577 inhibits pancreatic β-cell function and survival by targeting fibroblast growth factor 21 (FGF-21) in pediatric diabetes. *Genet Mol Res* 2015; **14**: 15462-15470 [PMID: 26634512 DOI: 10.4238/2015.November.30.24]

122 **Xiao J**, Bei Y, Liu J, Dimitrova-Shumkovska J, Kuang D, Zhou Q, Li J, Yang Y, Xiang Y, Wang F, Yang C, Yang W. miR-212 downregulation contributes to the protective effect of exercise against non-alcoholic fatty liver *via* targeting FGF-21. *J Cell Mol Med* 2016; **20**: 204-216 [PMID: 26648452 DOI: 10.1111/jcmm.12733]

123 **Schaap FG**, Kremer AE, Lamers WH, Jansen PL, Gaemers IC. Fibroblast growth factor 21 is induced by endoplasmic reticulum stress. *Biochimie* 2013; **95**: 692-699 [PMID: 23123503 DOI: 10.1016/j.biochi.2012.10.019]

124 **Gälman C**, Lundåsen T, Kharitonenkov A, Bina HA, Eriksson M, Hafström I, Dahlin M, Amark P, Angelin B, Rudling M. The circulating metabolic regulator FGF21 is induced by prolonged fasting and PPARalpha activation in man. *Cell Metab* 2008; **8**: 169-174 [PMID: 18680716 DOI: 10.1016/j.cmet.2008.06.014]

125 **Vernia S**, Cavanagh-Kyros J, Garcia-Haro L, Sabio G, Barrett T, Jung DY, Kim JK, Xu J, Shulha HP, Garber M, Gao G, Davis RJ. The PPARα-FGF21 hormone axis contributes to metabolic regulation by the hepatic JNK signaling pathway. *Cell Metab* 2014; **20**: 512-525 [PMID: 25043817 DOI: 10.1016/j.cmet.2014.06.010]

126 **Videla LA**, Fernández V, Vargas R, Cornejo P, Tapia G, Varela N, Valenzuela R, Arenas A, Fernández J, Hernández-Rodas MC, Riquelme B. Upregulation of rat liver PPARα-FGF21 signaling by a docosahexaenoic acid and thyroid hormone combined protocol. *Biofactors* 2016; **42**: 638-646 [PMID: 27248050 DOI: 10.1002/biof.1300]

127 **Nishimura T**, Nakatake Y, Konishi M, Itoh N. Identification of a novel FGF, FGF-21, preferentially expressed in the liver. *Biochim Biophys Acta* 2000; **1492**: 203-206 [PMID: 10858549 DOI: 10.1016/s0167-4781(00)00067-1]

128 **Kilkenny DM**, Rocheleau JV. The FGF21 Receptor Signaling Complex: Klothoβ, FGFR1c, and Other Regulatory Interactions. *Vitam Horm* 2016; **101**: 17-58 [PMID: 27125737 DOI: 10.1016/bs.vh.2016.02.008]

129 **Staiger H**, Keuper M, Berti L, Hrabe de Angelis M, Häring HU. Fibroblast Growth Factor 21-Metabolic Role in Mice and Men. *Endocr Rev* 2017; **38**: 468-488 [PMID: 28938407 DOI: 10.1210/er.2017-00016]

130 **Li H**, Wu G, Fang Q, Zhang M, Hui X, Sheng B, Wu L, Bao Y, Li P, Xu A, Jia W. Fibroblast growth factor 21 increases insulin sensitivity through specific expansion of subcutaneous fat. *Nat Commun* 2018; **9**: 272 [PMID: 29348470 DOI: 10.1038/s41467-017-02677-9]

131 **Ge X**, Chen C, Hui X, Wang Y, Lam KS, Xu A. Fibroblast growth factor 21 induces glucose transporter-1 expression through activation of the serum response factor/Ets-like protein-1 in adipocytes. *J Biol Chem* 2011; **286**: 34533-34541 [PMID: 21846717 DOI: 10.1074/jbc.M111.248591]

132 **Minard AY**, Tan SX, Yang P, Fazakerley DJ, Domanova W, Parker BL, Humphrey SJ, Jothi R, Stöckli J, James DE. mTORC1 Is a Major Regulatory Node in the FGF21 Signaling Network in Adipocytes. *Cell Rep* 2016; **17**: 29-36 [PMID: 27681418 DOI: 10.1016/j.celrep.2016.08.086]

133 **Sarruf DA**, Thaler JP, Morton GJ, German J, Fischer JD, Ogimoto K, Schwartz MW. Fibroblast growth factor 21 action in the brain increases energy expenditure and insulin sensitivity in obese rats. *Diabetes* 2010; **59**: 1817-1824 [PMID: 20357365 DOI: 10.2337/db09-1878]

134 **Geng L**, Lam KSL, Xu A. The therapeutic potential of FGF21 in metabolic diseases: from bench to clinic. *Nat Rev Endocrinol* 2020; **16**: 654-667 [PMID: 32764725 DOI: 10.1038/s41574-020-0386-0]

135 **Baruch A**, Wong C, Chinn LW, Vaze A, Sonoda J, Gelzleichter T, Chen S, Lewin-Koh N, Morrow

L, Dheerendra S, Boismenu R, Gutierrez J, Wakshull E, Wilson ME, Arora PS. Antibody-mediated activation of the FGFR1/Klothoβ complex corrects metabolic dysfunction and alters food preference in obese humans. *Proc Natl Acad Sci U S A* 2020; **117**: 28992-29000 [PMID: 33139537 DOI: 10.1073/pnas.2012073117]

136 **Fang J**, Wang SQ, Smiley E, Bonadio J. Genes coding for mouse activin beta C and beta E are closely linked and exhibit a liver-specific expression pattern in adult tissues. *Biochem Biophys Res Commun* 1997; **231**: 655-661 [PMID: 9070865 DOI: 10.1006/bbrc.1997.6162]

137 **Namwanje M**, Brown CW. Activins and Inhibins: Roles in Development, Physiology, and Disease. *Cold Spring Harb Perspect Biol* 2016; **8** [PMID: 27328872 DOI: 10.1101/cshperspect.a021881]

138 **Sugiyama M**, Kikuchi A, Misu H, Igawa H, Ashihara M, Kushima Y, Honda K, Suzuki Y, Kawabe Y, Kaneko S, Takamura T. Inhibin βE (INHBE) is a possible insulin resistance-associated hepatokine identified by comprehensive gene expression analysis in human liver biopsy samples. *PLoS One* 2018; **13**: e0194798 [PMID: 29596463 DOI: 10.1371/journal.pone.0194798]

139 **Hashimoto O**, Funaba M, Sekiyama K, Doi S, Shindo D, Satoh R, Itoi H, Oiwa H, Morita M, Suzuki C, Sugiyama M, Yamakawa N, Takada H, Matsumura S, Inoue K, Oyadomari S, Sugino H, Kurisaki A. Activin E Controls Energy Homeostasis in Both Brown and White Adipose Tissues as a Hepatokine. *Cell Rep* 2018; **25**: 1193-1203 [PMID: 30380411 DOI: 10.1016/j.celrep.2018.10.008]

140 **Sekiyama K**, Ushiro Y, Kurisaki A, Funaba M, Hashimoto O. Activin E enhances insulin sensitivity and thermogenesis by activating brown/beige adipocytes. *J Vet Med Sci* 2019; **81**: 646-652 [PMID: 30880304 DOI: 10.1292/jvms.19-0036]

141 **Dellett M**, Hu W, Papadaki V, Ohnuma S. Small leucine rich proteoglycan family regulates multiple signalling pathways in neural development and maintenance. *Dev Growth Differ* 2012; **54**: 327-340 [PMID: 22524604 DOI: 10.1111/j.1440-169X.2012.01339.x]

142 **Wang Q**, Sharma VP, Shen H, Xiao Y, Zhu Q, Xiong X, Guo L, Jiang L, Ohta K, Li S, Shi H, Rui L, Lin JD. The hepatokine Tsukushi gates energy expenditure *via* brown fat sympathetic innervation. *Nat Metab* 2019; **1**: 251-260 [PMID: 31535079 DOI: 10.1038/s42255-018-0020-9]

143 **Liu D**, Zhang P, Wei X, Deng Y, Liu W, Guo D, Liu J, Xu B, Huang C, Huang J, Lin J, Liu S, Xue Y, Zhang H. Elevated Serum Tsukushi Levels in Patients With Hyperthyroidism. *Front Endocrinol (Lausanne)* 2020; **11**: 580097 [PMID: 33117292 DOI: 10.3389/fendo.2020.580097]

144 **Mouchiroud M**, Camiré É, Aldow M, Caron A, Jubinville É, Turcotte L, Kaci I, Beaulieu MJ, Roy C, Labbé SM, Varin TV, Gélinas Y, Lamothe J, Trottier J, Mitchell PL, Guénard F, Festuccia WT, Joubert P, Rose CF, Karvellas CJ, Barbier O, Morissette MC, Marette A, Laplante M. The Hepatokine TSK does not affect brown fat thermogenic capacity, body weight gain, and glucose homeostasis. *Mol Metab* 2019; **30**: 184-191 [PMID: 31767170 DOI: 10.1016/j.molmet.2019.09.014]

145 **van der Lienden MJC**, Gaspar P, Boot R, Aerts JMFG, van Eijk M. Glycoprotein Non-Metastatic Protein B: An Emerging Biomarker for Lysosomal Dysfunction in Macrophages. *Int J Mol Sci* 2018; **20** [PMID: 30586924 DOI: 10.3390/ijms20010066]

146 **Kuang H**, Lin JD. GPNMB: expanding the code for liver-fat communication. *Nat Metab* 2019; **1**: 507-508 [PMID: 32694853 DOI: 10.1038/s42255-019-0069-0]

147 **Gong XM**, Li YF, Luo J, Wang JQ, Wei J, Xiao T, Xie C, Hong J, Ning G, Shi XJ, Li BL, Qi W, Song BL. Gpnmb secreted from liver promotes lipogenesis in white adipose tissue and aggravates obesity and insulin resistance. *Nat Metab* 2019; **1**: 570-583 [PMID: 32694855 DOI: 10.1038/s42255-019-0065-4]

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MINIREVIEWS

Current strategies to induce liver remnant hypertrophy before major liver resection

Celeste Del Basso, Martin Gaillard, Panagiotis Lainas, Stella Zervaki, Gabriel Perlemuter, Pierre Chagué, Laurence Rocher, Cosmin Sebastian Voican, Ibrahim Dagher, Hadrien Tranchart

ORCID number: Celeste Del Basso 0000-0003-3606-9512; Martin Gaillard 0000-0003-1539-2118; Panagiotis Lainas 0000-0002-2438-8519; Stella Zervaki 0000-0001-6289-6437; Gabriel Perlemuter 0000-0001-9985-8725; Pierre Chagué 0000-0003-3992-537X; Laurence Rocher 0000-0002-3775-6886; Cosmin Sebastian Voican 0000-0002-7161-9631; Ibrahim Dagher 0000-0002-3989-7148; Hadrien Tranchart 0000-0003-2173-2828.

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Celeste Del Basso, Martin Gaillard, Panagiotis Lainas, Stella Zervaki, Ibrahim Dagher, Hadrien Tranchart, Department of Minimally Invasive Digestive Surgery, Antoine Béclère Hospital, Clamart 92140, France

Gabriel Perlemuter, Cosmin Sebastian Voican, Department of Hepato-Gastroenterology and Nutrition, Antoine Béclère Hospital, Clamart 92140, France

Pierre Chagué, Laurence Rocher, Department of Radiology, Antoine Béclère Hospital, Clamart 92140, France

Corresponding author: Hadrien Tranchart, MD, PhD, Associate Professor, Department of Minimally Invasive Digestive Surgery, Antoine Béclère Hospital, 157 rue de la Porte de Trivaux, Clamart 92140, France. hadrien.tranchart@aphp.fr

Abstract

Hepatic resection is the gold standard for patients affected by primary or metastatic liver tumors but is hampered by the risk of post-hepatectomy liver failure. Despite recent impro-vements, liver surgery still requires excellent clinical judgement in selecting patients for surgery and, above all, efficient pre-operative strategies to provide adequate future liver remnant. The aim of this article is to review the literature on the rational, the preliminary assessment, the advantages as well as the limits of each existing technique for preparing the liver for major hepatectomy.

Key Words: Liver regeneration; Major hepatectomy; Liver insufficiency; Future liver remnant; Portal vein embolization

Core Tip: Hepatic resection is the gold standard for patients affected by liver tumors but is hampered by the risk of post-hepatectomy liver failure. We herein review the literature on the rational, the preliminary assessment, the advantages as well as the limits of each existing technique for preparing the liver for major hepatectomy.

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INTRODUCTION

Hepatic resection is the gold standard for patients affected by primary or metastatic liver tumors but is hampered by the risk of post hepatectomy liver failure (PHLF). Indeed, PHLF is considered the most frightening complication of liver surgery, representing a major source of severe morbidity and mortality[1]. Despite recent improvements, liver surgery still requires excellent clinical judgement in selecting patients for surgery and, above all, efficient pre-operative tools to provide an adequate future liver remnant (FLR).

The liver has a unique capacity of preserving its volume due to regeneration. The atrophy-hypertrophy phenomenon is a prime example of the liver's pathophysiologic (atrophy) and restorative (hypertrophy) response to injury[2]. It occurs whenever there is impairment of bile or blood flow: the liver reacts with atrophy of the region concerned and with compensatory hypertrophy of the less or not impaired regions, resulting in characteristic gross deformity of the organ and, in some instances, in rotation of the liver around a virtual hilar axis[3]. The mechanisms that induce cellular division are complex and based on different inflammatory cytokines. The Hepatocyte Growth Factor (HGF) seems to be the main mitogenic factor and its role has been established in liver regeneration[4].

The first case of *in vivo* human hepatic regeneration was described by Pack *et al*[5] in 1962. Starting from animal models in the first half of the 20th century, it was recognized that liver regeneration could also be induced by portal vein ligation (PVL)[6]. In 1986, the first cases of percutaneous transhepatic portal vein embolization (PVE) were performed before liver resection in the setting of hepatocellular carcinoma[7], and a few years later Makuuchi *et al*[8] reported the utility of PVE in promoting FLR hypertrophy prior to hepatic resection in patients with hilar cholangiocarcinoma. Since those initial reports, preoperative PVE has been established as the standard procedure for obtaining FRL hypertrophy, increasing the eligibility of patients for major hepatectomy as well as improving postoperative outcomes and safety. However, concerns regarding the insufficient increase of FLR and/or concomitant tumoral progression after PVE have led to the development of recent alternative techniques to push further the limits of liver surgery.

The aim of this article is to review the techniques available for preparing the liver for major hepatectomy, and to depict their advantages and limitations.

LIVER REGENERATION

The liver's unique capacity for regeneration was first recorded in the legend of Prometheus in Greek mythology and it represents the basis of the treatment of many liver diseases. Regeneration of the liver is a pathophysiological process, embracing both hypertrophy (increase in cell size or protein content in the prereplicative phase) and hyperplasia (increase in cell numbers). Both events can take place independently [9]. The mechanisms of liver regeneration have mainly been studied after extensive hepatectomy. The players of regeneration following the different techniques exposed in this article are thought to be similar to those after hepatectomy, but the precise mechanism remains unknown. Basically, the regeneration process is a cytokine- and growth-factor-mediated pathway. The main cytokine-mediated pathways include members of the innate immune system, tumor necrosis factor (TNF)α and interleukin (IL)-6, and growth-factor-mediated pathways are regulated by HGF and transforming growth factor (TGF)α[10]. It is a multi-step process, starting from the "priming" of hepatocytes, the moment they acquire replicative capacity, followed by the proliferative step in which an adequate cell mass is attained, and a termination stage in which liver cell proliferation is ended once the necessary functional mass has been reached[11]. Proliferation of hepatocytes advances from periportal to pericentral areas of the lobule, as a wave of mitoses[12]. Proliferation of biliary epithelial cells occurs a

little later than hepatocytes. The particularity of liver regeneration is that replacement of the lost hepatic mass is not mediated by selected stem cells proliferation but it entirely depends on mature adult hepatocytes and other hepatic cell types. Concerning the time interval, as far as we know, normal liver weight is reestablished within 8-15 d in humans[13].

POST-HEPATECTOMY LIVER FAILURE

Although morbidity and mortality after liver surgery have improved over the past 10 years, PHLF is still reported in up to 8%, ranging from 1.2% to 32%, and depends on the patient's condition and functional reserve of the liver before resection[1]. Different definitions of PHLF are available. In 2011, the International Study Group of Liver Surgery (ISGLS) defined PHLF as "a post-operatively acquired deterioration in the ability of the liver to maintain its synthetic, excretory, and detoxifying functions, which are characterized by an increased International Normalized Ratio (INR) and concomitant hyperbilirubinemia on or after postoperative day 5"[14]. It is worth pointing out that severe PHLF is associated with a mortality rate of 54%.

A related syndrome that results in a transient but sometimes fatal form of liver failure has been described following liver transplantation (LT) but also after extensive liver resection. This is the so-called Small For Size Syndrome (SFSS). In 2005, Dahm *et al*[15] defined SFSS as a graft to recipient weight ratio < 0.8% alongside two of the following for three consecutive days; bilirubin > 100 mmol/L, INR > 2 and encephalopathy grade 3 or 4. In this definition, SFSS is a clinical syndrome characterized by post-operative liver dysfunction, prolonged cholestasis and coagulopathy, portal hypertension and ascites. It can lead to a higher rate of hemorrhage, sepsis and gastrointestinal bleeding[16]. The key point of SFSS is the presence of portal hypertension and intra-hepatic portal congestion as the underlying cause of liver failure[17].

PREDICTION OF PHLF RISK

Despite improvements in surgical and postoperative management, parameters determining the degree of possible hepatectomy remain largely uncertain. Different patient related and surgical factors have to be considered to decrease PHLF incidence. Surgical factors include the extent of resection and volume of FLR, duration of intraoperative liver ischemia during portal pedicle clamping, duration of surgery and the need for blood transfusion. The risk of PHLF is highly influenced by the quality of underlying liver parenchyma. The type of underlying liver parenchyma is frequently assessed by preoperative liver biopsy, but noninvasive methods, such as liver stiffness, are now available. For example, liver stiffness measurement by transient elastography (Fibroscan) predicts persistent hepatic decompensation in patients undergoing resection for hepatocellular carcinoma[18].

It is generally thought that the minimal functional liver mass needed for adequate postoperative liver function is estimated to be 20%-25% in patients with normal liver parenchyma, whereas those with chemotherapy-induced liver injury require a FLR volume of approximately 30%, while those with cirrhosis at least a 40% minimal functional liver mass[19]. Therefore, standardized FLR volume can be easily evaluated by a tridimensional computed tomography (CT) reconstruction method, as FLR/estimated total liver volume[20]. Estimated total liver volume is generally calculated using a formula based on body surface area[21].

In addition to volume, estimation of FLR function is an important factor. Typical biochemical parameters, such as liver function tests, albumin, and clotting factors must be evaluated. The old but effective Child-Turcotte-Pugh score, which was introduced in 1964, still represents a simple system for grading liver function[22]. The model for end-stage liver disease score, which is mainly used in liver transplantation, can also predict the survival rate of cirrhotic patients to better select ideal candidates for surgery[23]. A recent study also showed that mean serum level of hyaluronic acid can be a useful tool, especially when liver biopsy is not feasible[24].

Dynamic tests of liver function can also be used. The most well-known is indocyanine green (ICG) clearance. ICG is a water soluble, inert, fluorescent tricarbocyanine dye with protein binding close to 95% (mainly, alpha1- and beta-lipoproteins and albumin), a hepatic extraction rate above 70%, and is almost completely excreted in its unchanged form by the liver. ICG elimination can be

expressed as ICG plasma disappearance rate (ICGPDR) or retention rate at 15 min (ICGR15), reflecting liver function. Use of the ICG test for patient selection has been shown to decrease postoperative mortality[25].

In recent years, there have been several attempts to assess hepatobiliary magnetic resonance imaging (MRI) as a tool to predict liver dysfunction. Since it was first described in 1991 by Weinmann *et al*[26], MRI has been showed to provide both global and segmental liver function information, and postoperative remnant liver function thanks to the measurement of liver signal intensity in the hepatobiliary phase.

Liver function evaluation by nuclear medicine techniques is also more and more used. Dynamic 99mTc-mebrofenin hepatobiliary scintigraphy has been used to provide quantitative information on total and regional liver function. The hepatic uptake of 99mTc-mebrofenin is similar to the uptake of organic anions such as bilirubin[27]. This technique efficiently estimates the risk of postoperative liver failure especially in patients with uncertain quality of liver parenchyma[28]. The 99m Tc-GSA is another recently proposed agent that is not affected by hyperbilirubinemia and can be used for liver function assessment in cholestatic patients[29]. Finally, the LiMAx test allows real-time *in vivo* determination of liver Cytochrome P450 1A2 (CYP1A2) activity. The CYP1A2 is not influenced by cholestasis or drugs and is ubiquitous in liver parenchyma. Intravenous administration of 13C methacetin, a substance exclusively metabolized by CYP1A2, with continuous real-time breath analysis represents the basis of the LiMAx test[30].

PORTAL VEIN EMBOLIZATION

Since the first report in 1986, PVE has progressively become the gold standard for inducing liver hypertrophy with satisfying safety and efficacy[31]. Initially described by laparotomy, the portal system access is now obtained by percutaneous puncture of the portal vein. According to the operator's preference, an ipsilateral or contralateral approach can be chosen, in reference to the segment bearing the tumor. The ipsilateral approach has the main advantage of protecting the FLR from injury[2] whereas the contralateral approach facilitates embolization[32]. Irrespective of the approach chosen, PVE is performed in a retrograde manner (Figure 1). Many embolic materials have been used for PVE without significant differences in terms of hypertrophy. Embolic materials include fibrin glue, N-butyl-2-cyanoacrylate and ethiodized oil, gelatin sponge and thrombin, coils, microparticles [*e.g.*, polyvinyl alcohol (PVA) particles or tris-acryl gelatin microspheres] and absolute alcohol[33]. A non-absorbable material is generally used. However, interesting results were reported with the use of an absorbable powder material (Gelfoam® powder, Pfizer, New York, USA) that lasts approximately 2 wk, leading to temporary PVE. In an animal model, this method showed efficient and stable liver regeneration[34]. These results were confirmed in a limited preliminary series in clinical practice[35] and a prospective study is undergoing (EMBORES study, NCT02945059). One of the advantages of temporary PVE is that it can theoretically be repeated several times to boost more liver hypertrophy, as has been suggested in an animal model[36].

PVE is successfully performed in more than 90% of cases[37]. A computed tomography scan with volumetric evaluation is generally performed between 4 and 8 wk after embolization. PVE induces a FLR hypertrophy than can reach 40%[37], with a low 2% morbidity rate and no mortality in the vast majority of studies[37-39]. PVE is considered an efficient method, allowing successful hepatectomy in more than 70% of cases[37,38,40].

Contraindications to PVE are extensive portal thrombus and important portal hypertension[41]. Another potential limit of PVE is the risk of tumor growth during the 4 to 8 wk separating PVE and liver surgery. In addition, several authors have suggested that PVE itself could promote tumor growth within the embolized liver[42-45]. Among others, these reasons have led to the development of alternative strategies.

PORTAL VEIN LIGATION (PVL) AND TWO-STAGE HEPATECTOMY

As it requires a surgical procedure with portal pedicles dissection, PVL is nowadays mainly indicated in the setting of two-stage hepatectomy (TSH) for the treatment of bilobar liver disease[46,47]. In the TSH strategy, the first surgical step includes tumoral clearance of the FLR (usually by parenchymal spearing resections or locoregional treatment like radiofrequency ablation) and concomitant PVL that allows FLR growth.

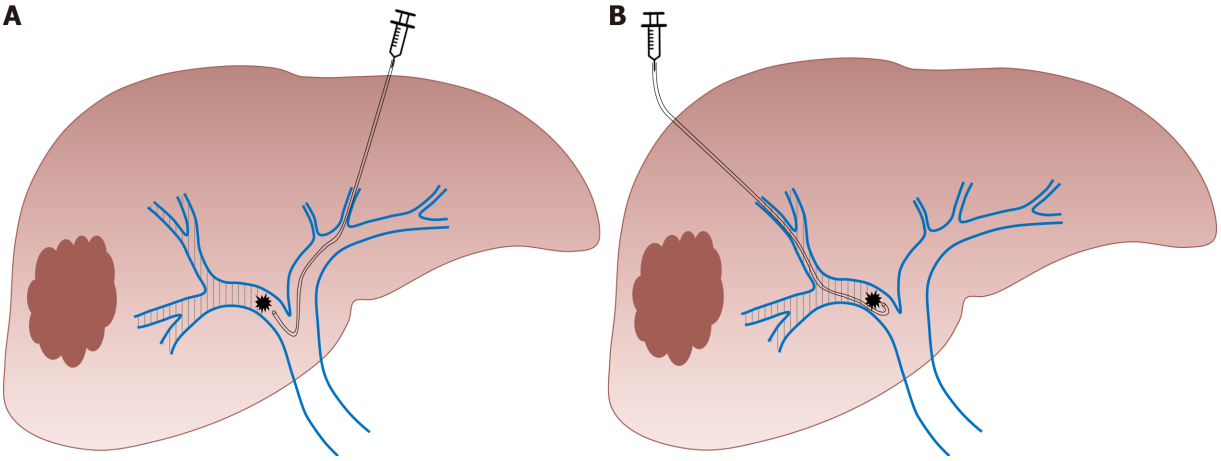

Figure 1 Right portal vein embolization using. A: Contralateral; B: Ipsilateral approach.

In the second step, after liver regeneration (approximately 4 to 8 wk later), major liver resection is performed (usually right or right extended hepatectomy) (Figure 2). Similarly, PVL can be performed for the management of patients presenting synchronous colorectal metastases or neuroendocrine tumors[47]. The first surgical step associates colorectal resection with PVL, followed by major liver surgery in the second procedure. However, many centers have adopted PVE (performed by the percutaneous approach after FLR clearance or colorectal resection) for two-step procedures, avoiding portal pedicle dissection and facilitating the second procedure [48].

It was initially suggested that PVE resulted in superior FLR growth compared to PVL[49] as in theory PVE allows distal portal obstruction which decreases the possibility of intrahepatic collateral development. Several studies demonstrated that the results are globally similar[50,51]. In fact, the debate concerning the efficiency of PVL compared to PVE is no longer relevant. PVL requires a surgical procedure and can appear as an alternative to PVE only when a two-step surgery is planned. In other cases, percutaneous PVE is clearly a simpler and better tolerated approach.

ASSOCIATING LIVER PARTITION AND PORTAL VEIN LIGATION FOR STAGED HEPATECTOMY

The aim of this alternative strategy, described by Schnizbauer *et al*[52] in 2012, is to induce rapid and massive liver hypertrophy, to allow liver surgery in a short period of time in patients with initially very limited FRL volume. The first step of the associating liver partition and portal vein ligation for staged hepatectomy (ALPPS) procedure consists of performing PVL and an *in situ* splitting of the liver parenchyma, leaving the hepatic artery, bile duct, and hepatic vein intact until the subsequent operation. This first surgical step can be associated with tumoral clearance of the FRL. During the second operation (that can be performed one to two weeks later) the remaining hepatic artery, bile duct, and hepatic vein are divided and the liver specimen is extracted (Figure 3).

The first report demonstrated a morbidity rate of 44% and a mortality rate of 12% [52], and triggered an intense debate on the safety of this procedure, limiting its promotion worldwide. The morbi-mortality rate decreased with experience but remains high, with approximately 40% of major postoperative complications and 9% of mortality[53]. Nevertheless, the ALPPS technique induces more than 65% of FLR growth in approximately 7 days[52-55] and the second procedure is feasible in more than 90% of cases[56]. The main advantage of the ALPPS procedure is the rapid increase in FLR volume in a short interval and therefore a shorter interval between the two stages. Although the volumetric results of this technique are impressive, several authors suggested that FLR volume hypertrophy is not correlated to functional improvement[57,58] which could partly explain the high morbidity of the procedure. Besides, concerns have been raised by some authors regarding potentially poorer oncological results comparing to the classical TSH[59]. The results of a meta-analysis comparing ALPPS to TSH showed that the extent of FLR increase was not different

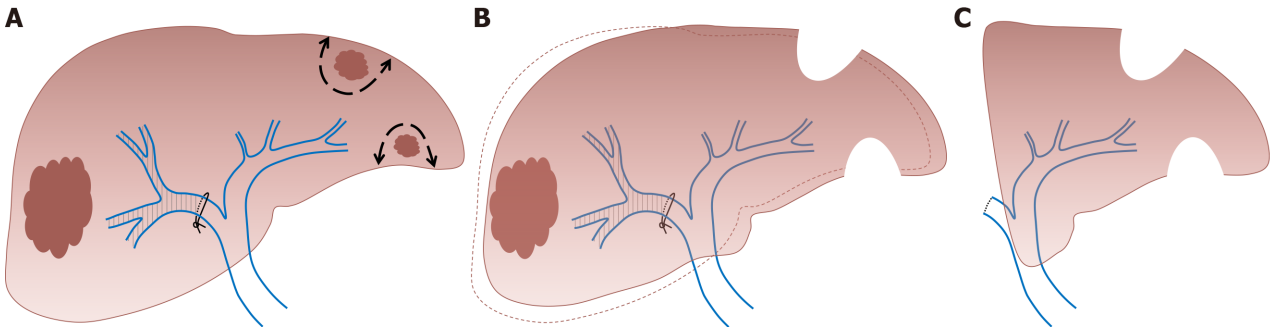

Figure 2 Two-stage hepatectomy procedure starts with tumoral clearance of the future liver remnant. A: Concomitant right portal vein ligation; B: Allowing left liver growth; C: Ends with right hepatectomy.

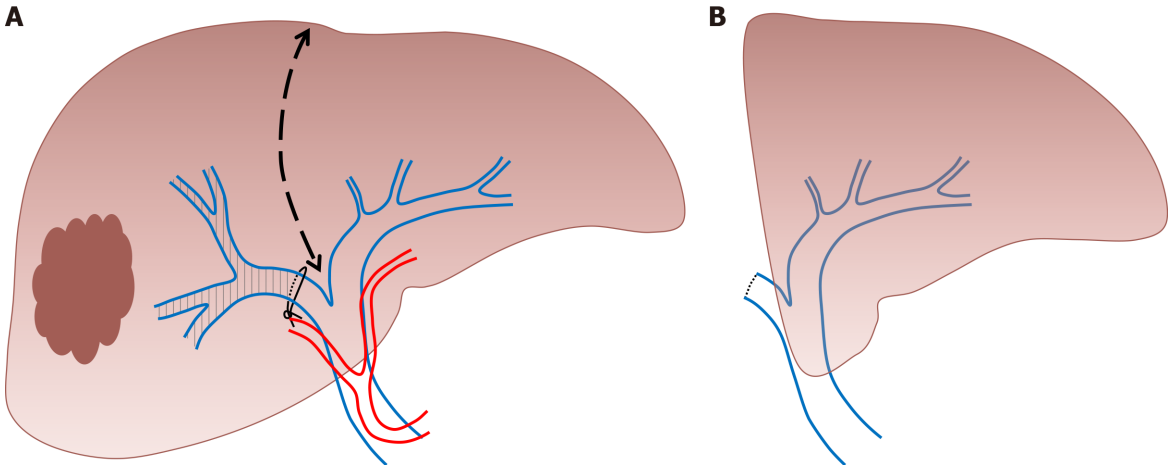

Figure 3 Associating liver partition and portal vein ligation for staged hepatectomy procedure. A: Starts with *in situ* splitting of the liver parenchyma with concomitant right portal vein ligation; B: Ends with right hepatectom.

between the two groups[60]. The time needed to reach final liver volume was shorter in ALPPS than in the TSH approach[60]. In this meta-analysis, ALPPS was associated with a higher incidence of major and overall morbidity and mortality compared to TSH[60]. However, in a recent randomized controlled trial, Hasselgren *et al*[61] observed similar morbidity between ALPPS and classical TSH and an improved survival in the ALPPS group.

To decrease complication rate, a variety of technical modifications have been proposed such as partial-ALPPS, mini-ALPPS, tourniquet-ALPPS, hybrid-ALPPS, microwave ablation-assisted ALPPS and radiofrequency ablation-assisted ALPPS. Huang *et al*[62] suggested in a systematic review that a partial ALPPS technique in which only partial parenchymal sparing is performed during the first surgical step could achieve lower morbidity and mortality rates, reaching the same FLR hypertrophy rate as ALPPS in non-cirrhotic patients.

SEQUENTIAL TRANS-ARTERIAL EMBOLIZATION (TAE) AND PORTAL VEIN EMBOLIZATION

Although PVE remains the gold standard for FLR hypertrophy, two concerns persist with this approach: An insufficient contralateral hypertrophy, particularly in patients with underlying liver disease (steatosis, fibrosis or cirrhosis), and the eventuality of tumor progression while waiting for the non-embolized liver to hypertrophy. In particular, portal flow interruption may induce a compensatory increase in arterial blood flow of embolized segments and result in a paradoxical growth of tumors vascularized by arterial blood flow. In this context, it has been postulated that the addition of trans-arterial embolization (TAE) or trans-arterial chemoembolization (TACE) would produce more rapid and extensive FLR growth (by obtaining

obliteration of intrahepatic arterioportal shunts) and may help to counteract the stimulating effect on tumor growth[63]. Therefore, hepatocellular carcinomas, which are tumors particularly vascularized by arterial blood flow and develop generally in underlying pathological liver parenchyma, are the main target of this combined strategy[64].

During TAE, a catheter is directly inserted *via* either the common femoral or left radial artery and an intra-arterial injection of a combination of microspheres and PVA particles is performed in the arterial branches of the segments to be resected. During TACE, an intra-arterial injection of a cytotoxic drug is performed such as doxorubicin, epirubicin, idarubicin, mitomycin C, or cisplatin, that is emulsified in ethiodized oil (Lipiodol® Ultra-Fluid, Guerbet). This is followed by intra-arterial injection of an embolic agent, such as gelatin sponge, PVA particles, or microspheres[65] (Figure 4). TACE can also be performed using recently developed drug-eluting beads (DEB) that allow the slow release of chemotherapeutic agents, and increase ischemia intensity and duration[65].

A sequential approach, with a time interval of a few days, is recommended to limit the risk of nontumoral liver ischemic necrosis[66] and TAE is mostly performed before PVE[66,67]. Although the number of patients reported in studies that evaluated this approach is limited, observed FLR hypertrophy is generally superior to that observed after isolated PVE. For example, Yoo *et al*[68] reported a statistically significant increase of 7.3% and 5.8% in FLR (over the total liver volume) for sequential TACE/PVE and isolated PVE, respectively.

An important elevation of transaminases is generally observed after this sequential approach without important clinical consequences. In the largest series reporting this approach, Peng *et al*[64] reported 29 procedures without deaths and only one complication and 27 patients (93%) underwent subsequent hepatectomy. Post-hepatectomy morbidity and mortality among these patients was 27.5% and 6.9%, respectively.

Theoretical contraindications of this method include extensive portal thrombus, important portal hypertension or previous biliary surgery (biliodigestive anastomosis) which exposes the patient to hepatic abscess formation after arterial embolization.

LIVER VENOUS DEPRIVATION

This technique consists of performing conventional PVE and ipsilateral hepatic vein obstruction (Figure 5). By associating hepatic vein embolization, the aim is to eliminate any residual portal vein flow and reduce hepatic artery inflow which can further encourage liver regeneration. Initially described as a sequential approach in which hepatic vein embolization is secondarily performed in case of insufficient FLR growth after PVE, it was demonstrated that both procedures (portal and hepatic vein embolization) can be performed simultaneously[69,70]. This novel approach is particularly interesting as it allows important liver regeneration with good tolerance. Although no study comparing ALPPS to LVD is available, it has been suggested that LVD could overcome the limits of ALPPS, abolishing the necessity of two major surgical interventions in close sequence.

Firstly, PVE is performed as previously described. For hepatic vein embolization, a vascular plug is placed in the proximal part of the hepatic vein to avoid migration of embolization agent. The vein is then embolized with a mixture of ethiodized oil and N- butyl cyanoacrylate[71]. The term "extended LVD" is used for concomitant embolization of the right and middle hepatic vein with the right portal branch[57].

The results of this approach on FLR increase are superior to those observed after isolated PVE. In a recent large comparative study, Laurent *et al*[71] observed a FLR volume increase of 28.9% after PVE compared to 61.2% after LVD ($P < 0.0001$). In this study, LVD allowed surgery in 86.4% of patients and no PHLF was reported. Kobayashi *et al*[72] observed similar results with a superior FLR hypertrophy after LVD compared to PVE (35% *vs* 24%, $P = 0.034$). In addition, the tolerance of LVD seems to be similar to the tolerance of isolated PVE[71,72].

RADIATION LOBECTOMY

This recent approach is derived from trans-arterial radioembolization with yttrium-90 [73]. In radiation lobectomy (RL), radioembolization of both the tumor and the non-tumoral liver parenchyma that will be secondarily resected is performed, which

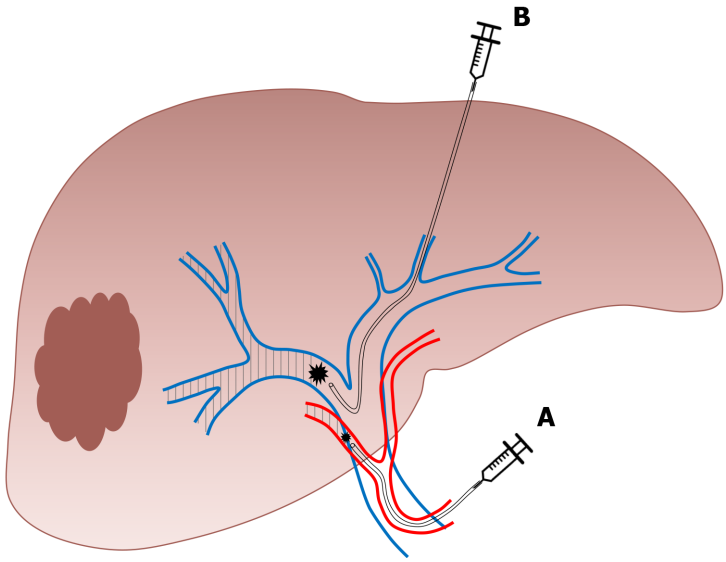

Figure 4 Sequential embolization. A: Trans-arterial embolization; B: Portal vein embolization of the right liver.

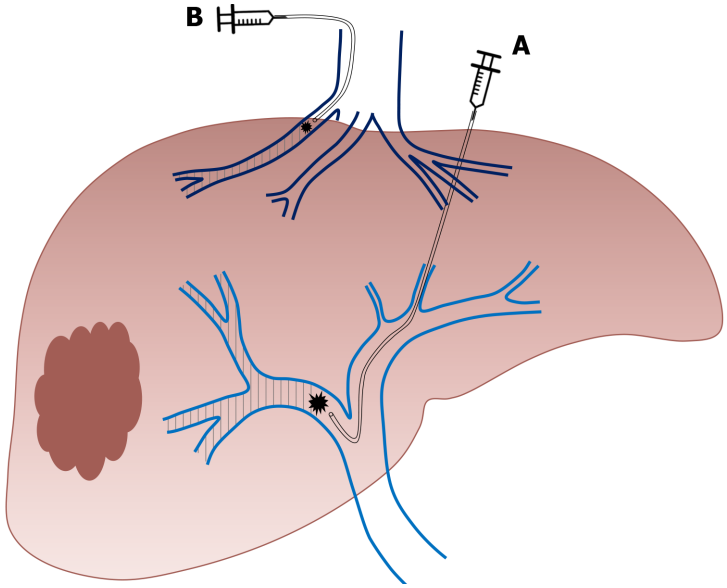

Figure 5 Right liver venous derivation associates in a sequential or concomitant approach. A: Right portal vein embolization; B: Ipsilateral hepatic vein embolization.

requires higher radiation doses[74,75]. This technique allows concomitant tumoral control and FLR increase. One major advantage of this approach is that it could be carried out in patients with portal vein thrombosis[75].

The procedure is well-tolerated[74] with transient moderate adverse events. Results in terms of FLR volume growth are very similar to those observed after PVE. Vouche *et al*[74] reported 45% of FLR hypertrophy and observed a correlation between the presence of a portal vein thrombosis and FLR growth. However, series reporting major liver resection after RL are scarce[76,77]. Andel *et al*[77] recently reported 10 major hepatectomies in patients that were initially treated with RL for insufficient functional FLR. The RL allowed a 41% increase in FLR volume with 84% of FLR function increase (evaluated on scintigraphy). All resections were performed without major intraoperative problems. Only one patient developed a serious complication not directly related to the liver surgery and other complications were mild.

Table 1 Indication, advantages, and disadvantages of existing approaches to induce liver remnant hypertrophy before major liver resection

Approach	Indication	Advantage	Disadvantage
PVE	Insufficient FLR volume	Percutaneous approach	Contraindicated in patients with extensive portal thrombus and important portal hypertension; Could promote tumoral growth within the embolized liver
PVL and two-stage hepatectomy	Insufficient FLR volume and treatment of bilobar liver disease	PVL is performed during the first surgical step (tumoral clearance of the FLR)	Surgical procedure; Morbidity
Associating liver partition and PVL for staged hepatectomy	Insufficient FLR volume +/- treatment of bilobar liver disease	Liver surgery is performed in a short period of time (15 d); First surgical step (PVL and *in situ* splitting of the liver parenchyma) can be associated with tumoral clearance of the FLR	Surgical procedure; Morbidity
Sequential trans arterial embolization and PVE	Insufficient FLR volume in patients with hepatocellular carcinoma	Percutaneous approachMay help to counteract the stimulating effect of PVE on tumor growth	Sequential approach (two procedures) is recommended to limit the risk of nontumoral liver ischemic necrosis; Contraindicated in patients with extensive portal thrombus, important portal hypertension or previous biliary surgery (biliodigestive anastomosis)
Liver venous deprivation	Insufficient FLR volume	Percutaneous approach	Contraindicated in patients with extensive portal thrombus and important portal hypertension; Could promote tumoral growth within the embolized liver
RL	Insufficient FLR volume	Percutaneous approachConcomitant tumoral control and FLR increaseCan be carried out in patients with portal vein thrombosis	Data reporting liver resection after RL is scarce

PVE: Portal vein embolization; FLR: Future liver remnant; PVL: Portal vein ligation; RL: Radiation lobectomy.

CONCLUSION

Careful initial evaluation of FLR volume and function is crucial before planning major liver resection. When required, several approaches are now available to decrease the risk of PHLF (Table 1) and thus postoperative mortality. Although PVE remains the gold standard, recent techniques that are derived from PVE might play an increasingly important role in future years.

REFERENCES

1 **Schreckenbach T**, Liese J, Bechstein WO, Moench C. Posthepatectomy liver failure. *Dig Surg* 2012; **29**: 79-85 [PMID: 22441624 DOI: 10.1159/000335741]

2 **Black DM**, Behrns KE. A scientist revisits the atrophy-hypertrophy complex: hepatic apoptosis and regeneration. *Surg Oncol Clin N Am* 2002; **11**: 849-864 [PMID: 12607575 DOI: 10.1016/s1055-3207(02)00031-5]

3 **Kim RD**, Kim JS, Watanabe G, Mohuczy D, Behrns KE. Liver regeneration and the atrophy-hypertrophy complex. *Semin Intervent Radiol* 2008; **25**: 92-103 [PMID: 21326550 DOI: 10.1055/s-2008-1076679]

4 **Fajardo-Puerta AB**, Mato Prado M, Frampton AE, Jiao LR. Gene of the month: HGF. *J Clin Pathol* 2016; **69**: 575-579 [PMID: 27072686 DOI: 10.1136/jclinpath-2015-203575]

5 **Pack GT**, Islami AH, Hubbard JC, Brasfield RD. Regeneration of human liver after major hepatectomy. *Surgery* 1962; **52**: 617-623 [PMID: 14483060]

6 **Honjo I**, Suzuki T, Ozawa K, Takasan H, Kitamura O. Ligation of a branch of the portal vein for carcinoma of the liver. *Am J Surg* 1975; **130**: 296-302 [PMID: 170837 DOI: 10.1016/0002-9610(75)90389-x]

7 **Kinoshita H**, Sakai K, Hirohashi K, Igawa S, Yamasaki O, Kubo S. Preoperative portal vein embolization for hepatocellular carcinoma. *World J Surg* 1986; **10**: 803-808 [PMID: 3022488 DOI: 10.1007/BF01655244]

8 **Makuuchi M**, Thai BL, Takayasu K, Takayama T, Kosuge T, Gunvén P, Yamazaki S, Hasegawa H, Ozaki H. Preoperative portal embolization to increase safety of major hepatectomy for hilar bile duct carcinoma: a preliminary report. *Surgery* 1990; **107**: 521-527 [PMID: 2333592]

9 **Palmes D**, Spiegel HU. Animal models of liver regeneration. *Biomaterials* 2004; **25**: 1601-1611 [PMID: 14697862 DOI: 10.1016/s0142-9612(03)00508-8]

10 **Taub R**. Liver regeneration: from myth to mechanism. *Nat Rev Mol Cell Biol* 2004; **5**: 836-847 [PMID: 15459664 DOI: 10.1038/nrm1489]

11 **Markiewski MM**, DeAngelis RA, Lambris JD. Liver inflammation and regeneration: two distinct

biological phenomena or parallel pathophysiologic processes? *Mol Immunol* 2006; **43**: 45-56 [PMID: 16002143 DOI: 10.1016/j.molimm.2005.06.019]

12 **Rabes HM**. Kinetics of hepatocellular proliferation as a function of the microvascular structure and functional state of the liver. *Ciba Found Symp* 1977; 31-53 [PMID: 248005 DOI: 10.1002/9780470720363.ch3]

13 **Michalopoulos GK**. Liver regeneration. *J Cell Physiol* 2007; **213**: 286-300 [PMID: 17559071 DOI: 10.1002/jcp.21172]

14 **Rahbari NN**, Garden OJ, Padbury R, Brooke-Smith M, Crawford M, Adam R, Koch M, Makuuchi M, Dematteo RP, Christophi C, Banting S, Usatoff V, Nagino M, Maddern G, Hugh TJ, Vauthey JN, Greig P, Rees M, Yokoyama Y, Fan ST, Nimura Y, Figueras J, Capussotti L, Büchler MW, Weitz J. Posthepatectomy liver failure: a definition and grading by the International Study Group of Liver Surgery (ISGLS). *Surgery* 2011; **149**: 713-724 [PMID: 21236455 DOI: 10.1016/j.surg.2010.10.001]

15 **Dahm F**, Georgiev P, Clavien PA. Small-for-size syndrome after partial liver transplantation: definition, mechanisms of disease and clinical implications. *Am J Transplant* 2005; **5**: 2605-2610 [PMID: 16212618 DOI: 10.1111/j.1600-6143.2005.01081.x]

16 **Tucker ON**, Heaton N. The 'small for size' liver syndrome. *Curr Opin Crit Care* 2005; **11**: 150-155 [PMID: 15758596 DOI: 10.1097/01.ccx.0000157080.11117.45]

17 **Riddiough GE**, Christophi C, Jones RM, Muralidharan V, Perini MV. A systematic review of small for size syndrome after major hepatectomy and liver transplantation. *HPB (Oxford)* 2020; **22**: 487-496 [PMID: 31786053 DOI: 10.1016/j.hpb.2019.10.2445]

18 **Rajakannu M**, Cherqui D, Ciacio O, Golse N, Pittau G, Allard MA, Antonini TM, Coilly A, Sa Cunha A, Castaing D, Samuel D, Guettier C, Adam R, Vibert E. Liver stiffness measurement by transient elastography predicts late posthepatectomy outcomes in patients undergoing resection for hepatocellular carcinoma. *Surgery* 2017; **162**: 766-774 [PMID: 28711320 DOI: 10.1016/j.surg.2017.06.006]

19 **Adams RB**, Aloia TA, Loyer E, Pawlik TM, Taouli B, Vauthey JN; Americas Hepato-Pancreato-Biliary Association; Society of Surgical Oncology; Society for Surgery of the Alimentary Tract. Selection for hepatic resection of colorectal liver metastases: expert consensus statement. *HPB (Oxford)* 2013; **15**: 91-103 [PMID: 23297719 DOI: 10.1111/j.1477-2574.2012.00557.x]

20 **Shindoh J**, Truty MJ, Aloia TA, Curley SA, Zimmitti G, Huang SY, Mahvash A, Gupta S, Wallace MJ, Vauthey JN. Kinetic growth rate after portal vein embolization predicts posthepatectomy outcomes: toward zero liver-related mortality in patients with colorectal liver metastases and small future liver remnant. *J Am Coll Surg* 2013; **216**: 201-209 [PMID: 23219349 DOI: 10.1016/j.jamcollsurg.2012.10.018]

21 **Vauthey JN**, Abdalla EK, Doherty DA, Gertsch P, Fenstermacher MJ, Loyer EM, Lerut J, Materne R, Wang X, Encarnacion A, Herron D, Mathey C, Ferrari G, Charnsangavej C, Do KA, Denys A. Body surface area and body weight predict total liver volume in Western adults. *Liver Transpl* 2002; **8**: 233-240 [PMID: 11910568 DOI: 10.1053/jlts.2002.31654]

22 **Child CG**, Turcotte JG. Surgery and portal hypertension. *Major Probl Clin Surg* 1964; **1**: 1-85 [PMID: 4950264]

23 **Kong FH**, Miao XY, Zou H, Xiong L, Wen Y, Chen B, Liu X, Zhou JJ. End-stage liver disease score and future liver remnant volume predict post-hepatectomy liver failure in hepatocellular carcinoma. *World J Clin Cases* 2019; **7**: 3734-3741 [PMID: 31799298 DOI: 10.12998/wjcc.v7.i22.3734]

24 **Gudowska M**, Gruszewska E, Panasiuk A, Cylwik B, Flisiak R, Świderska M, Szmitkowski M, Chrostek L. Hyaluronic acid concentration in liver diseases. *Clin Exp Med* 2016; **16**: 523-528 [PMID: 26354758 DOI: 10.1007/s10238-015-0388-8]

25 **Imamura H**, Sano K, Sugawara Y, Kokudo N, Makuuchi M. Assessment of hepatic reserve for indication of hepatic resection: decision tree incorporating indocyanine green test. *J Hepatobiliary Pancreat Surg* 2005; **12**: 16-22 [PMID: 15754094 DOI: 10.1007/s00534-004-0965-9]

26 **Weinmann HJ**, Schuhmann-Giampieri G, Schmitt-Willich H, Vogler H, Frenzel T, Gries H. A new lipophilic gadolinium chelate as a tissue-specific contrast medium for MRI. *Magn Reson Med* 1991; **22**: 233-7; discussion 242 [PMID: 1812351 DOI: 10.1002/mrm.1910220214]

27 **de Graaf W**, van Lienden KP, Dinant S, Roelofs JJ, Busch OR, Gouma DJ, Bennink RJ, van Gulik TM. Assessment of future remnant liver function using hepatobiliary scintigraphy in patients undergoing major liver resection. *J Gastrointest Surg* 2010; **14**: 369-378 [PMID: 19937195 DOI: 10.1007/s11605-009-1085-2]

28 **Erdogan D**, Heijnen BH, Bennink RJ, Kok M, Dinant S, Straatsburg IH, Gouma DJ, van Gulik TM. Preoperative assessment of liver function: a comparison of 99mTc-Mebrofenin scintigraphy with indocyanine green clearance test. *Liver Int* 2004; **24**: 117-123 [PMID: 15078475 DOI: 10.1111/j.1478-3231.2004.00901.x]

29 **Cieslak KP**, Runge JH, Heger M, Stoker J, Bennink RJ, van Gulik TM. New perspectives in the assessment of future remnant liver. *Dig Surg* 2014; **31**: 255-268 [PMID: 25322678 DOI: 10.1159/000364836]

30 **Stockmann M**, Lock JF, Riecke B, Heyne K, Martus P, Fricke M, Lehmann S, Niehues SM, Schwabe M, Lemke AJ, Neuhaus P. Prediction of postoperative outcome after hepatectomy with a new bedside test for maximal liver function capacity. *Ann Surg* 2009; **250**: 119-125 [PMID: 19561474 DOI: 10.1097/SLA.0b013e3181ad85b5]

31 **Shinkawa H**, Takemura S, Tanaka S, Kubo S. Portal Vein Embolization: History and Current Indications. *Visc Med* 2017; **33**: 414-417 [PMID: 29344514 DOI: 10.1159/000479474]

32 **Li D**, Madoff DC. Portal vein embolization for induction of selective hepatic hypertrophy prior to major hepatectomy: rationale, techniques, outcomes and future directions. *Cancer Biol Med* 2016; **13**: 426-442 [PMID: 28154774 DOI: 10.20892/j.issn.2095-3941.2016.0083]

33 **Madoff DC**, Abdalla EK, Vauthey JN. Portal vein embolization in preparation for major hepatic resection: evolution of a new standard of care. *J Vasc Interv Radiol* 2005; **16**: 779-790 [PMID: 15947041 DOI: 10.1097/01.RVI.0000159543.28222.73]

34 **Lainas P**, Boudechiche L, Osorio A, Coulomb A, Weber A, Pariente D, Franco D, Dagher I. Liver regeneration and recanalization time course following reversible portal vein embolization. *J Hepatol* 2008; **49**: 354-362 [PMID: 18387688 DOI: 10.1016/j.jhep.2008.01.034]

35 **Tranchart H**, Catherine L, Maitre S, Lainas P, De Laveaucoupet J, Dagher I. Efficient liver regeneration following temporary portal vein embolization with absorbable gelatin sponge powder in humans. *J Vasc Interv Radiol* 2015; **26**: 507-515 [PMID: 25640643 DOI: 10.1016/j.jvir.2014.11.033]

36 **Tranchart H**, Koffi GM, Gaillard M, Lainas P, Poüs C, Gonin P, Nguyen TH, Dubart-Kupperschmitt A, Dagher I. Liver regeneration following repeated reversible portal vein embolization in an experimental model. *Br J Surg* 2016; **103**: 1209-1219 [PMID: 27256140 DOI: 10.1002/bjs.10153]

37 **van Lienden KP**, van den Esschert JW, de Graaf W, Bipat S, Lameris JS, van Gulik TM, van Delden OM. Portal vein embolization before liver resection: a systematic review. *Cardiovasc Intervent Radiol* 2013; **36**: 25-34 [PMID: 22806245 DOI: 10.1007/s00270-012-0440-y]

38 **Abulkhir A**, Limongelli P, Healey AJ, Damrah O, Tait P, Jackson J, Habib N, Jiao LR. Preoperative portal vein embolization for major liver resection: a meta-analysis. *Ann Surg* 2008; **247**: 49-57 [PMID: 18156923 DOI: 10.1097/SLA.0b013e31815f6e5b]

39 **Isfordink CJ**, Samim M, Braat MNGJA, Almalki AM, Hagendoorn J, Borel Rinkes IHM, Molenaar IQ. Portal vein ligation *vs* portal vein embolization for induction of hypertrophy of the future liver remnant: A systematic review and meta-analysis. *Surg Oncol* 2017; **26**: 257-267 [PMID: 28807245 DOI: 10.1016/j.suronc.2017.05.001]

40 **Wajswol E**, Jazmati T, Contractor S, Kumar A. Portal Vein Embolization Utilizing N-Butyl Cyanoacrylate for Contralateral Lobe Hypertrophy Prior to Liver Resection: A Systematic Review and Meta-Analysis. *Cardiovasc Intervent Radiol* 2018; **41**: 1302-1312 [PMID: 29687262 DOI: 10.1007/s00270-018-1964-6]

41 **May BJ**, Madoff DC. Portal vein embolization: rationale, technique, and current application. *Semin Intervent Radiol* 2012; **29**: 81-89 [PMID: 23729977 DOI: 10.1055/s-0032-1312568]

42 **Hoekstra LT**, van Lienden KP, Doets A, Busch OR, Gouma DJ, van Gulik TM. Tumor progression after preoperative portal vein embolization. *Ann Surg* 2012; **256**: 812-7; discussion 817 [PMID: 23095626 DOI: 10.1097/SLA.0b013e3182733f09]

43 **Hoekstra LT**, van Lienden KP, Verheij J, van der Loos CM, Heger M, van Gulik TM. Enhanced tumor growth after portal vein embolization in a rabbit tumor model. *J Surg Res* 2013; **180**: 89-96 [PMID: 23149224 DOI: 10.1016/j.jss.2012.10.032]

44 **Maggiori L**, Bretagnol F, Sibert A, Paradis V, Vilgrain V, Panis Y. Selective portal vein ligation and embolization induce different tumoral responses in the rat liver. *Surgery* 2011; **149**: 496-503 [PMID: 21167542 DOI: 10.1016/j.surg.2010.10.012]

45 **Pamecha V**, Levene A, Grillo F, Woodward N, Dhillon A, Davidson BR. Effect of portal vein embolisation on the growth rate of colorectal liver metastases. *Br J Cancer* 2009; **100**: 617-622 [PMID: 19209170 DOI: 10.1038/sj.bjc.6604872]

46 **Adam R**, Laurent A, Azoulay D, Castaing D, Bismuth H. Two-stage hepatectomy: A planned strategy to treat irresectable liver tumors. *Ann Surg* 2000; **232**: 777-785 [PMID: 11088072 DOI: 10.1097/00000658-200012000-00006]

47 **Kianmanesh R**, Farges O, Abdalla EK, Sauvanet A, Ruszniewski P, Belghiti J. Right portal vein ligation: a new planned two-step all-surgical approach for complete resection of primary gastrointestinal tumors with multiple bilateral liver metastases. *J Am Coll Surg* 2003; **197**: 164-170 [PMID: 12831938 DOI: 10.1016/S1072-7515(03)00334-X]

48 **Jaeck D**, Oussoultzoglou E, Rosso E, Greget M, Weber JC, Bachellier P. A two-stage hepatectomy procedure combined with portal vein embolization to achieve curative resection for initially unresectable multiple and bilobar colorectal liver metastases. *Ann Surg* 2004; **240**: 1037-49; discussion 1049 [PMID: 15570209 DOI: 10.1097/01.sla.0000145965.86383.89]

49 **Broering DC**, Hillert C, Krupski G, Fischer L, Mueller L, Achilles EG, Schulte am Esch J, Rogiers X. Portal vein embolization *vs* portal vein ligation for induction of hypertrophy of the future liver remnant. *J Gastrointest Surg* 2002; **6**: 905-13; discussion 913 [PMID: 12504230 DOI: 10.1016/s1091-255x(02)00122-1]

50 **Aussilhou B**, Lesurtel M, Sauvanet A, Farges O, Dokmak S, Goasguen N, Sibert A, Vilgrain V, Belghiti J. Right portal vein ligation is as efficient as portal vein embolization to induce hypertrophy of the left liver remnant. *J Gastrointest Surg* 2008; **12**: 297-303 [PMID: 18060468 DOI: 10.1007/s11605-007-0410-x]

51 **Capussotti L**, Muratore A, Baracchi F, Lelong B, Ferrero A, Regge D, Delpero JR. Portal vein ligation as an efficient method of increasing the future liver remnant volume in the surgical treatment of colorectal metastases. *Arch Surg* 2008; **143**: 978-82; discussion 982 [PMID: 18936377 DOI: 10.1001/archsurg.143.10.978]

52 **Schnitzbauer AA**, Lang SA, Goessmann H, Nadalin S, Baumgart J, Farkas SA, Fichtner-Feigl S, Lorf T, Goralcyk A, Hörbelt R, Kroemer A, Loss M, Rümmele P, Scherer MN, Padberg W, Königsrainer A, Lang H, Obed A, Schlitt HJ. Right portal vein ligation combined with in situ splitting

induces rapid left lateral liver lobe hypertrophy enabling 2-staged extended right hepatic resection in small-for-size settings. *Ann Surg* 2012; **255**: 405-414 [PMID: 22330038 DOI: 10.1097/SLA.0b013e31824856f5]

53 **Sandström P**, Røsok BI, Sparrelid E, Larsen PN, Larsson AL, Lindell G, Schultz NA, Bjørnbeth BA, Isaksson B, Rizell M, Björnsson B. ALPPS Improves Resectability Compared With Conventional Two-stage Hepatectomy in Patients With Advanced Colorectal Liver Metastasis: Results From a Scandinavian Multicenter Randomized Controlled Trial (LIGRO Trial). *Ann Surg* 2018; **267**: 833-840 [PMID: 28902669 DOI: 10.1097/SLA.0000000000002511]

54 **Schadde E**, Ardiles V, Robles-Campos R, Malago M, Machado M, Hernandez-Alejandro R, Soubrane O, Schnitzbauer AA, Raptis D, Tschuor C, Petrowsky H, De Santibanes E, Clavien PA; ALPPS Registry Group. Early survival and safety of ALPPS: first report of the International ALPPS Registry. *Ann Surg* 2014; **260**: 829-36; discussion 836 [PMID: 25379854 DOI: 10.1097/SLA.0000000000000947]

55 **Knoefel WT**, Gabor I, Rehders A, Alexander A, Krausch M, Schulte am Esch J, Fürst G, Topp SA. In situ liver transection with portal vein ligation for rapid growth of the future liver remnant in two-stage liver resection. *Br J Surg* 2013; **100**: 388-394 [PMID: 23124776 DOI: 10.1002/bjs.8955]

56 **Schadde E**, Schnitzbauer AA, Tschuor C, Raptis DA, Bechstein WO, Clavien PA. Systematic review and meta-analysis of feasibility, safety, and efficacy of a novel procedure: associating liver partition and portal vein ligation for staged hepatectomy. *Ann Surg Oncol* 2015; **22**: 3109-3120 [PMID: 25448799 DOI: 10.1245/s10434-014-4213-5]

57 **Deshayes E**, Schadde E, Piron L, Quenet F, Guiu B. Extended Liver Venous Deprivation Leads to a Higher Increase in Liver Function that ALPPS in Early Assessment: A comment to "Sparrelid E *et al* ". Dynamic Evaluation of Liver Volume and Function in Associating Liver Partition and Portal Vein Ligation for Staged Hepatectomy. *J Gastrointest Surg* 2017; **21**: 1754-1755 [PMID: 28785933 DOI: 10.1007/s11605-017-3508-9]

58 **Sparrelid E**, Jonas E, Tzortzakakis A, Dahlén U, Murquist G, Brismar T, Axelsson R, Isaksson B. Dynamic Evaluation of Liver Volume and Function in Associating Liver Partition and Portal Vein Ligation for Staged Hepatectomy. *J Gastrointest Surg* 2017; **21**: 967-974 [PMID: 28283924 DOI: 10.1007/s11605-017-3389-y]

59 **Adam R**, Imai K, Castro Benitez C, Allard MA, Vibert E, Sa Cunha A, Cherqui D, Baba H, Castaing D. Outcome after associating liver partition and portal vein ligation for staged hepatectomy and conventional two-stage hepatectomy for colorectal liver metastases. *Br J Surg* 2016; **103**: 1521-1529 [PMID: 27517369 DOI: 10.1002/bjs.10256]

60 **Moris D**, Ronnekleiv-Kelly S, Kostakis ID, Tsilimigras DI, Beal EW, Papalampros A, Dimitroulis D, Felekouras E, Pawlik TM. Operative Results and Oncologic Outcomes of Associating Liver Partition and Portal Vein Ligation for Staged Hepatectomy (ALPPS) Versus Two-Stage Hepatectomy (TSH) in Patients with Unresectable Colorectal Liver Metastases: A Systematic Review and Meta-Analysis. *World J Surg* 2018; **42**: 806-815 [PMID: 28798996 DOI: 10.1007/s00268-017-4181-6]

61 **Hasselgren K**, Røsok BI, Larsen PN, Sparrelid E, Lindell G, Schultz NA, Bjørnbeth BA, Isaksson B, Larsson AL, Rizell M, Björnsson B, Sandström P. ALPPS Improves Survival Compared With TSH in Patients Affected of CRLM: Survival Analysis From the Randomized Controlled Trial LIGRO. *Ann Surg* 2021; **273**: 442-448 [PMID: 32049675 DOI: 10.1097/SLA.0000000000003701]

62 **Huang HC**, Bian J, Bai Y, Lu X, Xu YY, Sang XT, Zhao HT. Complete or partial split in associating liver partition and portal vein ligation for staged hepatectomy: A systematic review and meta-analysis. *World J Gastroenterol* 2019; **25**: 6016-6024 [PMID: 31660037 DOI: 10.3748/wjg.v25.i39.6016]

63 **Kim D**, Cornman-Homonoff J, Madoff DC. Preparing for liver surgery with "Alphabet Soup": PVE, ALPPS, TAE-PVE, LVD and RL. *Hepatobiliary Surg Nutr* 2020; **9**: 136-151 [PMID: 32355673 DOI: 10.21037/hbsn.2019.09.10]

64 **Peng PD**, Hyder O, Bloomston M, Marques H, Corona-Villalobos C, Dixon E, Pulitano C, Hirose K, Schulick RD, Barroso E, Aldrighetti L, Choti M, Shen F, Kamel I, Geschwind JF, Pawlik TM. Sequential intra-arterial therapy and portal vein embolization is feasible and safe in patients with advanced hepatic malignancies. *HPB (Oxford)* 2012; **14**: 523-531 [PMID: 22762400 DOI: 10.1111/j.1477-2574.2012.00492.x]

65 **Raoul JL**, Forner A, Bolondi L, Cheung TT, Kloeckner R, de Baere T. Updated use of TACE for hepatocellular carcinoma treatment: How and when to use it based on clinical evidence. *Cancer Treat Rev* 2019; **72**: 28-36 [PMID: 30447470 DOI: 10.1016/j.ctrv.2018.11.002]

66 **Ogata S**, Belghiti J, Farges O, Varma D, Sibert A, Vilgrain V. Sequential arterial and portal vein embolizations before right hepatectomy in patients with cirrhosis and hepatocellular carcinoma. *Br J Surg* 2006; **93**: 1091-1098 [PMID: 16779884 DOI: 10.1002/bjs.5341]

67 **Aoki T**, Imamura H, Hasegawa K, Matsukura A, Sano K, Sugawara Y, Kokudo N, Makuuchi M. Sequential preoperative arterial and portal venous embolizations in patients with hepatocellular carcinoma. *Arch Surg* 2004; **139**: 766-774 [PMID: 15249411 DOI: 10.1001/archsurg.139.7.766]

68 **Yoo H**, Kim JH, Ko GY, Kim KW, Gwon DI, Lee SG, Hwang S. Sequential transcatheter arterial chemoembolization and portal vein embolization *vs* portal vein embolization only before major hepatectomy for patients with hepatocellular carcinoma. *Ann Surg Oncol* 2011; **18**: 1251-1257 [PMID: 21069467 DOI: 10.1245/s10434-010-1423-3]

69 **Hwang S**, Lee SG, Ko GY, Kim BS, Sung KB, Kim MH, Lee SK, Hong HN. Sequential preoperative ipsilateral hepatic vein embolization after portal vein embolization to induce further liver regeneration in patients with hepatobiliary malignancy. *Ann Surg* 2009; **249**: 608-616 [PMID: 19300228 DOI:

10.1097/SLA.0b013e31819ecc5c]

70 **Le Roy B**, Perrey A, Fontarensky M, Gagnière J, Abergel A, Pereira B, Lambert C, Boyer L, Pezet D, Chabrot P, Buc E. Combined Preoperative Portal and Hepatic Vein Embolization (Biembolization) to Improve Liver Regeneration Before Major Liver Resection: A Preliminary Report. *World J Surg* 2017; **41**: 1848-1856 [PMID: 28417185 DOI: 10.1007/s00268-017-4016-5]

71 **Laurent C**, Fernandez B, Marichez A, Adam JP, Papadopoulos P, Lapuyade B, Chiche L. Radiological Simultaneous Portohepatic Vein Embolization (RASPE) Before Major Hepatectomy: A Better Way to Optimize Liver Hypertrophy Compared to Portal Vein Embolization. *Ann Surg* 2020; **272**: 199-205 [PMID: 32675481 DOI: 10.1097/SLA.0000000000003905]

72 **Kobayashi K**, Yamaguchi T, Denys A, Perron L, Halkic N, Demartines N, Melloul E. Liver venous deprivation compared to portal vein embolization to induce hypertrophy of the future liver remnant before major hepatectomy: A single center experience. *Surgery* 2020; **167**: 917-923 [PMID: 32014304 DOI: 10.1016/j.surg.2019.12.006]

73 **Vilgrain V**, Pereira H, Assenat E, Guiu B, Ilonca AD, Pageaux GP, Sibert A, Bouattour M, Lebtahi R, Allaham W, Barraud H, Laurent V, Mathias E, Bronowicki JP, Tasu JP, Perdrisot R, Silvain C, Gerolami R, Mundler O, Seitz JF, Vidal V, Aubé C, Oberti F, Couturier O, Brenot-Rossi I, Raoul JL, Sarran A, Costentin C, Itti E, Luciani A, Adam R, Lewin M, Samuel D, Ronot M, Dinut A, Castera L, Chatellier G; SARAH Trial Group. Efficacy and safety of selective internal radiotherapy with yttrium-90 resin microspheres compared with sorafenib in locally advanced and inoperable hepatocellular carcinoma (SARAH): an open-label randomised controlled phase 3 trial. *Lancet Oncol* 2017; **18**: 1624-1636 [PMID: 29107679 DOI: 10.1016/S1470-2045(17)30683-6]

74 **Vouche M**, Lewandowski RJ, Atassi R, Memon K, Gates VL, Ryu RK, Gaba RC, Mulcahy MF, Baker T, Sato K, Hickey R, Ganger D, Riaz A, Fryer J, Caicedo JC, Abecassis M, Kulik L, Salem R. Radiation lobectomy: time-dependent analysis of future liver remnant volume in unresectable liver cancer as a bridge to resection. *J Hepatol* 2013; **59**: 1029-1036 [PMID: 23811303 DOI: 10.1016/j.jhep.2013.06.015]

75 **Malhotra A**, Liu DM, Talenfeld AD. Radiation Segmentectomy and Radiation Lobectomy: A Practical Review of Techniques. *Tech Vasc Interv Radiol* 2019; **22**: 49-57 [PMID: 31079710 DOI: 10.1053/j.tvir.2019.02.003]

76 **Lewandowski RJ**, Donahue L, Chokechanachaisakul A, Kulik L, Mouli S, Caicedo J, Abecassis M, Fryer J, Salem R, Baker T. (90) Y radiation lobectomy: Outcomes following surgical resection in patients with hepatic tumors and small future liver remnant volumes. *J Surg Oncol* 2016; **114**: 99-105 [PMID: 27103352 DOI: 10.1002/jso.24269]

77 **Andel D**, Dassen MG, Reinders-Hut MTM, Peters NA, Kranenburg OW, Lam MGEH, Hagendoorn J, Rinkes IHMB. Surgical outcomes of major hepatectomy following "radiation lobectomy" for hepatic malignancies and insufficiently functional future liver remnant: initial experience. *Br J Surg* 2020; **107**: e609-e610 [PMID: 32936446 DOI: 10.1002/bjs.11828]

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MINIREVIEWS

Health-related quality of life in autoimmune hepatitis

Romée JALM Snijders, Piotr Milkiewicz, Christoph Schramm, Tom JG Gevers

ORCID number: Romée JALM Snijders 0000-0003-3957-6261; Piotr Milkiewicz 0000-0002-1817-0724; Christoph Schramm 0000-0002-4264-1928; Tom JG Gevers 0000-0002-3070-8443.

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Romée JALM Snijders, Tom JG Gevers, Department of Gastroenterology and Hepatology, Radboudumc, Nijmegen 6525GA, The Netherlands

Romée JALM Snijders, Piotr Milkiewicz, Christoph Schramm, Tom JG Gevers, European Reference Network RARE-LIVER, Hamburg, Germany

Piotr Milkiewicz, Liver and Internal Medicine Unit, Medical University of Warsaw, Warsaw 02-091, Poland

Piotr Milkiewicz, Translational Medicine Group, Pomeranian Medical University, Szczecin 70-204, Poland

Christoph Schramm, First Department of Medicine, University Medical Center Hamburg Eppendorf, Hamburg 20246, Germany

Christoph Schramm, Martin Zeitz Center for Rare Diseases and Hamburg Center for Translational Immunology (HCTI), University Medical Center Hamburg Eppendorf, Hamburg 20246, Germany

Tom JG Gevers, Division of Gastroenterology and Hepatology, Maastricht University Medical Center, Maastricht 6229HX, The Netherlands

Corresponding author: Tom JG Gevers, MD, PhD, Academic Fellow, Doctor, Department of Gastroenterology and Hepatology, Radboudumc, Geert Grooteplein Zuid 10, Nijmegen 6525GA, The Netherlands. tom.gevers@mumc.nl

Abstract

Autoimmune hepatitis (AIH) is a severe chronic autoimmune disease and has a significant impact on the patient's quality of life, in particular regarding psychological problems such as anxiety and depression. Consistent evidence on which patient-related, disease-related or physician-related factors cause health-related quality of life (HRQoL) impairment in patients with AIH is lacking. Current studies on HRQoL in AIH are mainly single-centered, comprising small numbers of patients, and difficult to compare because of the use of different questionnaires, patient populations, and cutoff values. Literature in the pediatric field is sparse, but suggests that children/adolescents with AIH have a lower HRQoL. Knowledge of HRQoL and cohesive factors in AIH are important to improve healthcare for AIH patients, for example by developing an AIH-specific chronic healthcare model. By recognizing the importance of quality of life beyond the concept of biochemical and histological remission, clinicians allow us to seek enhancements and possible interventions in the management of AIH, aiming at

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improved health.

Key Words: Autoimmune hepatitis; Quality of life; Depression; Anxiety; Corticosteroids

Core Tip: Autoimmune hepatitis (AIH) is a severe chronic autoimmune disease and has a significant impact on the patient's quality of life, in particular regarding psychological problems such as anxiety and depression. The health-related quality of life (HRQoL) of patients with AIH can be affected by various patient-related, disease-related, and physician-related factors. In this review we summarized several specific factors that are liable to influence HRQoL in AIH. By recognizing the importance of quality of life beyond the concept of biochemical and histological remission, clinicians allow us to seek enhancements and possible interventions in the management of AIH.

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INTRODUCTION

Autoimmune hepatitis (AIH) is a severe chronic autoimmune disease that occurs mainly in women and affects health-related quality of life (HRQoL) worldwide. The diagnosis of AIH is based on the presence of autoantibodies, typical features on liver histology, and increased immunoglobulin G (IgG) levels[1]. The presentation of AIH is variable, ranging from mild and asymptomatic disease to fulminant hepatic failure. Nonspecific symptoms at presentation are fatigue, anorexia, jaundice, and abdominal pain, whereas others are asymptomatic at disease onset[1]. The majority of patients need lifelong treatment to prevent disease progression to cirrhosis and/or decompensation[2]. Current treatment strategies in AIH include administering corticosteroids (mainly prednisolone) and a long-term corticosteroid-saving regime, including azathioprine (AZA) as first-line treatment[3,4]. Second-line immunosuppressants include mycophenolate mofetil (MMF), calcineurin inhibitors (CNIs), and mercaptopurine and have proven to be effective in mainly uncontrolled studies[5].

The main goal of AIH treatment is to achieve complete biochemical and histological remission without the occurrence of side effects. Alanine aminotransferase, aspartate aminotransferase and IgG serum levels are used as parameters to monitor biochemical response, and current guidelines advocate pursuit of normalization of those parameters as the aim of treatment. As a result, treatment failure, defined as absence of normalization of transaminases, triggers clinical actions such as increase of drug dose or change in drug class. A sole focus on biochemical response is insufficient when managing AIH. From a patient perspective, other aspects that affect HRQoL, including but not limited to side effects, psychological health, and implications of the disease, are just as important.

One of the main objectives relating to AIH according to the International Autoimmune Hepatitis Group (IAIHG), is better assessment of HRQoL in patients. However, literature or guidelines on that topic in AIH are scarce and inconsistent. An update on current literature on HRQoL in AIH, is warranted to reveal the most important research gaps[6]. Understanding which potentially treatable factors are associated with reduced quality of life in patients with AIH is essential for development of interventions targeting well-being. The focus of this paper is to review the current knowledge of HRQoL and associated factors in AIH, to comment on the current status, and to identify future perspectives that may influence and benefit disease management of adult patients with AIH.

METHODOLOGY

We searched the titles, abstracts, and MeSH terms of articles indexed in PubMed using the keywords "autoimmune hepatitis," "AIH," "health-related quality of life," and "quality of life." The search was limited to articles published before January 27, 2021. We included articles based on the following criteria: (1) Full-text articles published in peer-reviewed journals; (2) English or Dutch articles; (3) Publication dates within the last 20 years at the time of the search; and (4) Either adult or pediatric AIH. The search retrieved 116 publications; 39 were evaluated in full-text after screening the titles and abstracts (Figure 1). We also checked the reference lists of the included articles to identify other articles. For the purpose of this review, we primarily focused on articles addressing the role of HRQoL in AIH.

HRQOL IN ADULT PATIENTS WITH AIH

Several studies have reported reduced general or liver-specific HRQoL in AIH patients (Tables 1 and 2)[7-15]. The first study published was conducted in the Netherlands and showed a reduced quality of life in 141 patients with AIH compared with healthy controls, using three instruments, the SF-36 for generic HRQoL, the Multidimensional Fatigue Index-20, and the Liver Disease Symptom Index 2.0, which is a liver-specific questionnaire addressing nine topics. In particular, patients had lower scores in subscales measuring physical problems or general health. Patients with AIH mentioned fatigue more often than healthy controls did[13]. A landmark study performed in Germany compared 102 AIH patients to the German general population and to published data of patients with arthritis using the SF-12[12]. They reported lower mental well-being in patients with AIH compared with both groups, but the physical component score (PCS) was unaffected[12]. A Polish single-center study showed that patients with AIH (n = 140) scored significantly worse in all subscales of the SF-36, except for one measuring the impact of emotional problems on work and daily activities[15]. The majority of the AIH patients in that cohort had cirrhosis (55%), and as in the previously mentioned study, that did not have a significant effect on well-being. A recent Italian multicenter study of chronic liver disease reported that of a total of seven different chronic liver diseases without cirrhosis, patients with AIH had a lower quality of life measured with the EQ-5D VAS score, and experienced difficulties in the self-care domain, even after adjusting for multiple possible confounders, including age, sex, education, and professional status[10]. That was confirmed in a Cuban study in which AIH patients had lower quality of life scores than hepatitis B patients using the disease-specific Chronic Liver Disease Questionnaire (CLDQ)[7]. Only one meta-analysis was performed, including three studies that evaluated HRQoL measured with the SF-36. The analysis confirmed reduction of the PCS and mild reduction of the mental component score in patients with AIH. However, they included only older studies and compared all AIH patients (including Dutch and German patients) to the United States general population norm [16]. Finally, the largest study conducted so far involved multiple health centers in the United Kingdom and confirmed previous results by finding that the HRQoL of patients with AIH (n = 990) was worse than it was in the general population, adjusted for age and gender and using the EQ-5D-5L[14]. Although these studies consistently report a lower HRQoL in AIH, albeit in varying domains, it remains difficult to compare the studies because of the use of different questionnaires (EQ-5D-5L *vs* SF-12 or SF-36 *vs* CLDQ), cutoff values, methodology, and patient populations. Moreover, most studies were conducted at single centers and included small numbers of participants, thereby introducing bias based on the heterogenicity in study populations (*e.g.*, remission status and demographic differences).

HRQOL IN PEDIATRIC PATIENTS WITH AIH

A lower HRQoL was also found in children and adolescents with AIH, although literature in the pediatric field is sparse[17-19]. A study performed in Portugal compared 43 children with AIH to 62 healthy children using the Pediatric Quality of Life Inventory 4.0 (PedsQL 4.0)[17]. They found that especially children with associated comorbidities (*e.g.*, inflammatory bowel disease, hemolytic anemia, and hypothyroidism) had a lower quality of life. That was confirmed in a Brazilian cohort using the same questionnaire[18]. Interestingly, the evaluation of HRQoL in the

Table 1 Overview of the studies assessing aspects of health-related quality of life in autoimmune hepatitis

Ref.	Country	Population (*n*)	Biochemical remission (%)	Cirrhosis (%)	Questionnaire	Factors/results
van der Plas *et al* [13], 2007	The Netherlands	AIH (142), other liver diseases (776)	-	-	SF-36, MFI-20, LDSI	HRQoL impairment; Association with: Fatigue
Afendy *et al*[8], 2009	United States, Italy	AIH (13), other chronic liver diseases (1090)	-	84.6[1]	SF-36	HRQoL impairment; Negative correlation: Age (every scale), female gender (primary predictor of mental health), cirrhosis (every scale, primary predictor of physical health)
Schramm *et al* [12], 2014	Germany	AIH (103)	77	27	SF-12, PHQ-9, GAD-7	HRQoL impairment (total mental score/mental well-being); Association with: depression and anxiety (positive correlation with female gender, corticosteroid use, and concerns about progression of the liver disease)
Takahashi *et al* [11], 2018	Japan	AIH (265), chronic hepatitis C (88)	-	10.6	CLDQ, SF-36	HRQoL impairment; Negative correlation: Age, cirrhosis, comorbid diseases, corticosteroid use (worry domain), disease duration, AST; Positive correlation: platelet count
Wong *et al*[14], 2018	United Kingdom	AIH (990)	56	33	EQ-5D-5L, FIS, CFQ, HADS	HRQoL impairment; Positive correlation: Biochemical remission; Negative correlation: overlap syndromes, corticosteroid use, and calcineurin inhibitor use
Janik *et al*[15], 2019	Poland	AIH (140)	-	55	SF-36, MFIS, PHQ-9, STAI	HRQoL impairment (every scale, except role emotional[2]); Negative correlation: Female gender, depression, trend toward better HRQoL (physical health) with budesonide *vs* prednisone; Association with: Anxiety, depression, and fatigue
Dirks *et al*[9], 2019	Germany	AIH (27), AIH/PBC (8), other liver diseases (97)	-	0	SF-36, FIS, HADS	HRQoL impairment; Association with: Anxiety, depression, and fatigue
Castellanos-Fernández *et al*[7], 2021	Cuba	AIH (22), overlap syndrome of AIH and PBC (7), PBC (14), other liver diseases (500)	-	43.9[3]	FACIT-F, WPAI:SHP, CLDQ	HRQoL impairment; Positive correlation: Male gender, exercising > 90 min/wk; Negative correlation: Fatigue, abdominal pain, anxiety, depression, and extrahepatic comorbidity (diabetes mellitus type 2, sleep apnea)
Cortesi *et al*[10], 2020	Italy	AIH (51), other chronic liver diseases (2911)	-	0	EQ-5D-3L	HRQoL impairment in AIH

[1]Eight patients with Child-Pugh class A and three patients with Child-Pugh class C.
[2]Scale measures the impact of emotional problems on work and daily activities.
[3]Cirrhosis in patients with autoimmune liver diseases (n = 43). AIH: Autoimmune hepatitis; HRQoL: Health-related quality of life; PBC: Primary biliary cholangitis; AST: Aspartate aminotransferase; CFQ: Cognitive failure questionnaire; CLDQ: Chronic liver disease questionnaire; ECR: Experiences in close relationship scale; EQ-5D-5L/3L: European quality of life 5-dimension 5-level/3-level; FACIT-F: Functional assessment of chronic illness therapy-fatigue; FIS: Fatigue impact scale; GAD-7: Generalized anxiety disorder screener; HADS: Hospital anxiety depression scale; LDSI: Liver disease symptom index 2.0; MFI-20: Multidimensional fatigue index-20; PHQ-9: Patient health questionnaire; SF-12: Short-form 12; SF-36: Short-form 36; STAI: State-trait anxiety inventory; WPAI:SHP: Work productivity and activity-specific health problem.

parents differed from the children's self-reports[18]. Only the physical and total scores were significantly lower in patients with AIH based on the parental reports, whereas in the children's reports the emotional, school, physical, and total scores were significantly lower.

Table 2 Overview of the questionnaires assessing aspects of health-related quality of life in autoimmune hepatitis

Questionnaire	Main function	Domains	Items, total score
CFQ[41]	Cognition	Memory, attention, concentration, forgetfulness, word-finding abilities, and confusion	25 items scored 0-4, total score 0-100
CLDQ[42]	Generic HRQoL	Abdominal symptoms, fatigue, systemic symptoms, activity, emotions, and worry	29 items scored 1-7, total score 29-203
ECR[43]	Relationship styles	ECR-anxiety, and ECR-avoidance	12 items scored 1-7, each scale total score 7-42
EQ-5D-5L/EQ-5D-3L/EQ-VAS [44]	Generic HRQoL, EQ-VAS: participants' self-rated health on a visual analog scale	Mobility, self-care, usual activities, pain/discomfort, and anxiety/depression	EQ-5D: 5 items scored 1-5, total score 5-25; EQ-VAS: total score 0-100
FACIT-F[45]	Fatigue	Physical well-being, social well-being, emotional well-being, functional well-being, and a fatigue-specific domain	40 items scored 0-4, total score 0-160
FIS[46]	Fatigue	Cognitive functioning, physical functioning, and psychosocial functioning	40 items scored 0-4, total score 0-160
GAD-7[47]	Anxiety	-	7 items scored 0-3, total score 0-21
HADS[48]	Anxiety, depression	Anxiety, and depression	14 items scored 0-3, total score 0-42
LDSI[49]	Liver disease symptoms	Itch, joint pain, abdominal pain, daytime sleepiness, worry about family situation, decreased appetite, depression, fear of complications, and jaundice (+ symptom hinderance)	18 items scored 1-5, total score 18-90
MFI-20[50]	Fatigue	General fatigue, physical fatigue, reduction in activity, reduction in motivation, and mental fatigue	20 items scored 1-5, each domain total score 4-20
MFIS[46,51]	Fatigue	Physical, cognitive, and psychosocial functioning	21 items scored 0-4, total score 0-84
PHQ-9[52]	Depression	Anhedonia, feeling down, sleep, feeling tired, appetite, feeling bad about self, concentration, activity, and suicidality	9 items scored 0-3, total score 0-27
SF-12[53]	Generic HRQoL	Physical functioning, role limitations due to physical problems, bodily pain, general health, vitality, social functioning, role limitations due to emotional problems, and mental health	12 items scored 1-5, total score 0-100
SF-36[54]	Generic HRQoL	General health, physical and social functioning, bodily pain, role-physical, mental health, role-emotional, and vitality	36 items, total score 0-100
STAI[55]	Anxiety	State anxiety, and trait anxiety	40 items scored 1-4, total score 0-80
WPAI:SHP[56]	Impairment in daily activities and in work	Work productivity impairment, and activity impairment	6 items scored 0-10, total score -

Included in the table are the questionnaires that were employed in the reviewed studies. CFQ: Cognitive Failure Questionnaire; CLDQ: Chronic Liver Disease Questionnaire; ECR: Experiences in Close Relationship Scale; EQ-5D-5L/EQ-5D-3L/EQ-VAS: European Quality of life 5-Dimension 5-Level/3-Level/EQ-visual analog scale; FACIT-F: Functional Assessment of Chronic Illness Therapy-Fatigue; FIS: Fatigue Impact Scale; GAD-7: Generalized Anxiety Disorder Screener; HADS: Hospital Anxiety Depression Scale; LDSI: Liver Disease Symptom Index 2.0); MFI-20: Multidimensional Fatigue Index-20; MFIS: Modified Fatigue Impact Scale); PHQ-9: Patient Health Questionnaire; SF-12: Short-form 12; SF-36: Short-form 36; STAI: State-Trait Anxiety Inventory; WPAI:SHP: Work Productivity and Activity-Specific Health Problem).

DETERMINANTS OF HRQOL IN AIH

The HRQoL of patients with chronic diseases can be affected by various patient-related, disease-related, and physician-related factors. We have summarized the patient-, disease- and physician-related factors that are liable to influence HRQoL in AIH in Figure 2.

Patient-related factors

Patients with AIH are more often diagnosed with symptoms of depression and anxiety compared with the general population or healthy controls[7,9,10,12,15]. Studies by Schramm and Janik *et al*[15] showed a significantly higher percentage of depression

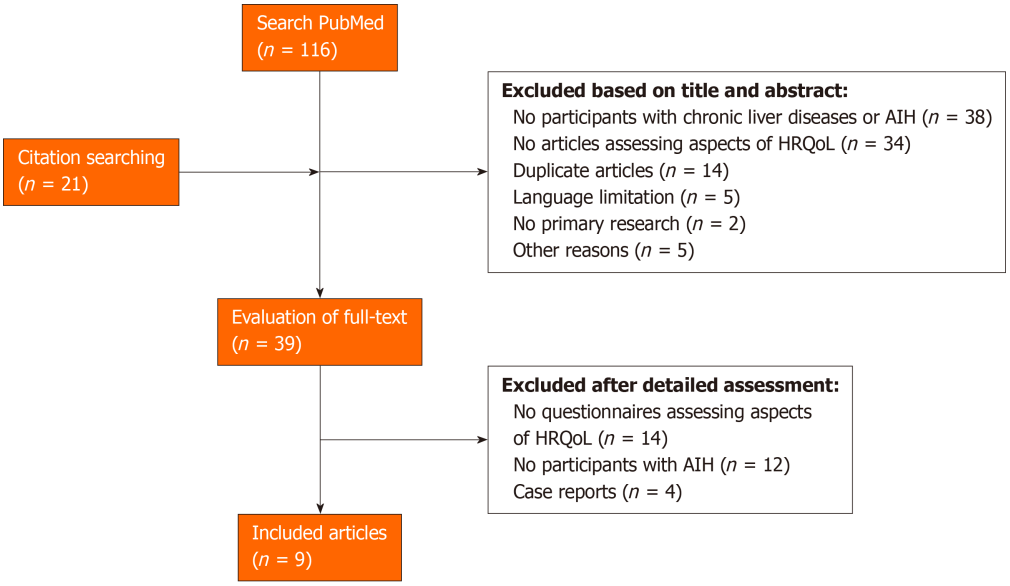

Figure 1 Flowchart of included studies after performing the literature search. AIH: Autoimmune hepatitis; HRQoL: Health-related quality of life.

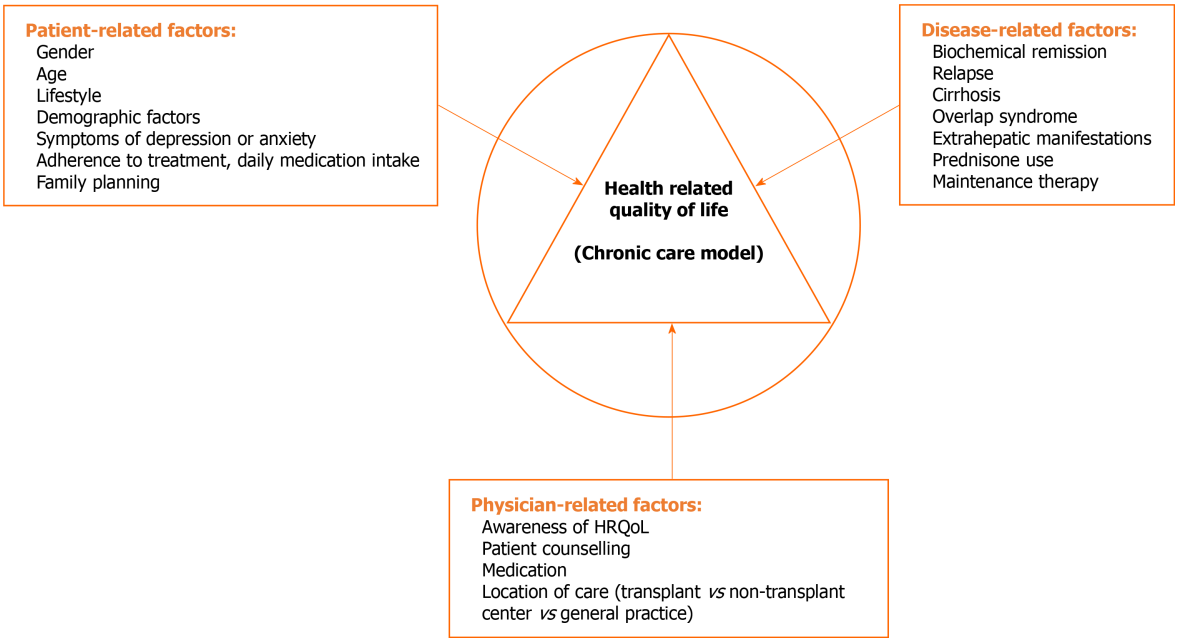

Figure 2 Patient-, disease- and physician-related factors affecting health-related quality of life in patients with autoimmune hepatitis. HRQoL: Health-related quality of life.

and anxiety symptoms, measured with the PHQ-9, GAD-7, or State-Trait Anxiety Inventory[12,15]. Depression was strongly correlated with both physical and mental components of SF-36. Despite biochemical remission in 77% of the patients (n = 103), the occurrence of severe depressive symptoms within the German cohort appeared to be five times as frequent compared with the general population.[12] In addition, even AIH patients without cirrhosis revealed more problems with regard to depression and anxiety compared with the general population[10]. It is interesting to note that psychological stress was also associated with relapses in patients with AIH type 1[20].

Other patient-related factors, particularly age and sex, have been described often in previous studies[7,11,12,14]. Studies in the United Kingdom and Japan reported a negative correlation between age and HRQoL[11,14], but Polish and Cuban studies did not find such a correlation [7,15]. With respect to sex differences, female patients experience more symptoms of depression[12,15] and have a worse quality of life than their male counterparts[7,15]. In our experience, women experience weight increase and other cosmetic changes associated with corticosteroids as a great inconvenience in

particular. In contrast, a study in the United Kingdom study found that the female sex was associated with a higher quality of life, albeit in an unadjusted regression analysis. These inconsistent correlations highlight that we still do not know which patient factors are important when assessing HRQoL in patients with AIH.

For all chronic liver diseases, it holds that lifestyle changes are part of the treatment. While tackling lifestyle is a hot topic in chronic disease, it is infrequently addressed in AIH. However, patients should still be informed about the risk of specific lifestyles, such as overweight, alcohol misuse, and sedentary behavior. Losing weight, more exercise, and a healthier diet contribute to successful management of chronic liver diseases and cirrhosis[21]. Indeed, exercising for more than 90 min/wk is a predictor of a better quality of life in patients with chronic liver diseases (*e.g.*, AIH)[7]. Another study confirmed that an increased body mass index was associated with a lower quality of life in patients with AIH[14]. In addition, alcohol consumption presents a clear risk of the progression of liver fibrosis in chronic liver diseases. Other factors, such as education level, socioeconomic data, smoking, or losing weight, were not frequently mentioned in the described studies. It follows that physicians need to communicate with patients about lifestyle adaptations through motivational interviews.

Coping with chronic conditions and taking medication daily goes hand in hand with discomfort, which potentially results in reduced HRQoL. Patients with more than one chronic disease that take daily medication have a lower quality of life[22]. Adherence to treatment is rarely discussed with patients but has a great impact on well-being and treatment response. A high psychosocial burden has been shown to significantly decrease adherence to treatment and to be associated with poor treatment response[23]. Therefore, prompt recognition of symptoms of depression and anxiety is important to improve patient adherence and lead to better response to treatment. Various factors may influence adherence to drug treatment in adolescents with AIH, particularly depression, anxiety, younger age, sex, prednisone dose, and long-term therapy have been found in previous studies[23-25]. In liver transplant recipients, marital status (if the patient is divorced) and having mental distress are associated with reduced self-reported adherence to immunotherapy[26]. However, information on demographic factors or socioeconomic data, including the status of a relationship and educational level, were not explicitly examined in all previous studies, which would be necessary for more detailed conclusions.

Disease-related factors

As mentioned previously, the main objective in treating AIH is to achieve complete biochemical and histological remission without side effects. While it is plausible that achieving biochemical remission results in better HRQoL, the association has not been studied often. One study found that patients with biochemical remission had a significantly higher quality of life [14]. One could speculate that incomplete biochemical remission causes uncertainty about, and possibly fear of, a relapse, which is understandable given that every relapse increases the risk of decompensated liver failure or the necessity of liver transplantation[27]. Whether this has a role in AIH is unknown at present.

Liver cirrhosis, or an advanced stage of fibrosis in patients with chronic liver disease is a known cause for reduced HRQoL, independent of the underlying liver disease[8, 28,29]. However, studies in patients with AIH demonstrate significant variability regarding the relation between fibrosis and HRQoL. Most studies describe that having liver fibrosis or compensated cirrhosis does not affect patient well-being in general[12, 14,15]. In contrast, another study did find an impaired physical condition in patients with AIH using the same SF-36 questionnaire and an overall lower quality of life using the CLDQ[11]. Plausible explanations for the discrepancy are the use of different general *vs* disease-specific, SF-36 *vs* SF-12 *vs* EQ-5D-5L questionnaires and the inclusion of different AIH populations regarding biochemical remission status and disease duration. Interestingly, none of the cited studies included AIH patients with decompensated cirrhosis in their cohort, which is known to be a major factor for reduced HRQoL in cirrhosis with other etiologies[30,31].

Patients with an overlap syndrome or a variant syndrome of AIH and primary biliary cholangitis (PBC) or primary sclerosing cholangitis (PSC), had a worse quality of life than patients not reporting those comorbidities[7,9,14]. In addition, fatigue is a typical symptom in patients with characteristics of PBC, and is expected to have a negative impact on HRQoL[7,9]. In that context, it is not only essential to treat both AIH and the overlapping syndrome (*i.e.*, PBC or PSC), but also to address associated symptoms (*i.e.*, IBD in PSC, itch in PBC) in the patients[14]. Interestingly, such a correlation was not found in a study in children with autoimmune liver diseases. It

found no differences in HRQoL scores in children with AIH *vs* overlap syndrome or variant syndrome with PSC[19]. Extrahepatic manifestations, for example thyroid disease, insulin-dependent diabetes mellitus, connective tissue disorders, and autoimmune skin disease, are common in AIH and can affect well-being, including fatigue, but the effect on HRQoL is unstudied so far[32].

A large proportion of patients with AIH receive corticosteroid therapy[11,33]. All treatments have specific side effects[34,35], but long-term use of corticosteroids is well-known for its undesirable effects, including osteoporosis, mood swings, depression, obesity, cognitive dysfunction, chronic fatigue, and reduced physical activity[1,5]. The negative impact of the use of corticosteroids on HRQoL was demonstrated in several studies[12,14]. In the United Kingdom cohort, corticosteroids were extensively linked to impaired HRQoL. Even patients who received low-dose of corticosteroids, and independent of their biochemical status, had a lower HRQoL[14]. Schramm *et al*[12] found a significant correlation between corticosteroids and depression. Sockalingam *et al*[23] found that patients with a moderate or high PHQ-9 score of > 10 were administered a significantly higher dose of prednisone compared with patients with a score of < 10. These data give additional support for steroid-free therapy as a treatment goal in every AIH patient to prevent steroid-related complications, and should be attempted within the first year of treatment. Other disease-related factors affecting mental well-being or HRQoL, such as markers of disease activity or disease duration, are so far unknown[12,15].

Currently, AZA is still the primary choice for maintenance therapy, and was not directly associated with a lower quality of life or health utility in a large cross-sectional analysis[14]. It is important to note that the use of AZA is associated with an increased risk of lymphoma and nonmelanoma skin cancer[36,37]. Although lymphoma in the long term is rare, it has to be taken into account that the occurrence of these side effects, or even the patient's concerns, might affect their quality of life. AZA may also cause hair loss that leads to alopecia. The possibility is frequently raised by the female patients and may affect various aspects of quality of life and lead to incompliance. The effect of other prescribed therapies on improving psychosocial outcomes, such as mycophenolate mofetil and mercaptopurine, is unknown. However, calcineurin inhibitors that have undesirable effects may be associated with lower health utility[14].

Physician-related factors

Physician-related factors are usually not addressed in studies and are thus difficult to take into account. Schramm *et al*[12] found that patient concerns about the severity of their disease, and being fearful of cirrhosis (mostly unnecessary) were factors associated with depression and anxiety symptoms. Providing the patient with information on his/her illness or medications and involving the patient in treatment options, can contribute to the patient's well-being. Whether the location of care (*i.e.* transplant *vs* nontransplant center) matters is uncertain. One study showed that there was no difference in health utility between transplant and nontransplant centers[14], and another found that biochemical remission rates were higher in transplant centers compared with nontransplant centers[33]. Both were conducted in the United Kingdom. Extrapolation of the results to other countries is difficult given the differences in health care management among countries.

CONCLUSION

It is clear that patients with AIH experience a lower quality of life and have more psychological problems, such as anxiety and depression, compared with the general population. Consistent evidence on which patient-related, disease-related, or physician-related factors cause HRQoL impairment in patients with AIH is lacking. Most studies did not include information on important socioeconomic, disease behavior, maintenance treatment, or even geographical factors, whereas they are known to affect patient well-being and HRQoL in other chronic liver diseases. In addition, some aspects of AIH are unexplored so far, for example the effect of lifestyle changes, extrahepatic manifestations, and patient counseling on HRQoL. Studies addressing HRQoL in pediatric AIH and their parents/support team are scarce and are desperately needed as a first step to improve their well-being.

Knowledge of HRQoL and associated factors in AIH are important to improve healthcare for AIH patients, for example by incorporating the factors in a chronic healthcare model (CCM). A CCM provides a clear approach for managing chronic diseases, with focus on assessment of the modifiable factors affecting the disease in

order to improve patient well-being. While no studies mentioned a CCM for AIH so far, some studies discussed elements that could be part of a model. For example, Janik *et al*[38] screened AIH patients for moderately severe depression and redirected them to a psychiatrist and psychiatric therapeutic interventions in case of a PHQ ≥ 15 points. Another example are lifestyle interventions for overweight patients[39]. There is also a role for the development of a disease-specific questionnaire for AIH patients, similar to the PBC-40 questionnaire, to measure the patient's perspective of the disease[40]. In what way, a CCM can be developed and implemented that would probably differ from country to country because of differences in health care. However, it is paramount that the AIH-specific CCM incorporate the most important factors of HRQoL in AIH, as discussed in this review.

Finally, HRQoL should not only be targeted in everyday clinical treatment approaches, but also as an important outcome of clinical trials and a research objective per se. Most studies of HRQoL in AIH have been conducted at a single center and comprised small numbers of patients, which underlines the need for collaboration between healthcare centers in different countries. Currently, there is an ongoing multicenter, cross-sectional study of HRQoL in patients with AIH within the European Network for Rare Liver Diseases. Recognizing the importance that quality of life has for the patient beyond the concept of biochemical and histological remission allows us to strive for significant improvements in management of adult and pediatric AIH.

REFERENCES

1 **Manns MP**, Czaja AJ, Gorham JD, Krawitt EL, Mieli-Vergani G, Vergani D, Vierling JM; American Association for the Study of Liver Diseases. Diagnosis and management of autoimmune hepatitis. *Hepatology* 2010; **51**: 2193-2213 [PMID: 20513004 DOI: 10.1002/hep.23584]

2 **Harrison L**, Gleeson D. Stopping immunosuppressive treatment in autoimmune hepatitis (AIH): Is it justified (and in whom and when)? *Liver Int* 2019; **39**: 610-620 [PMID: 30667576 DOI: 10.1111/liv.14051]

3 **Doycheva I**, Watt KD, Gulamhusein AF. Autoimmune hepatitis: Current and future therapeutic options. *Liver Int* 2019; **39**: 1002-1013 [PMID: 30716203 DOI: 10.1111/liv.14062]

4 **Terziroli Beretta-Piccoli B**, Mieli-Vergani G, Vergani D. Autoimmune hepatitis: Standard treatment and systematic review of alternative treatments. *World J Gastroenterol* 2017; **23**: 6030-6048 [PMID: 28970719 DOI: 10.3748/wjg.v23.i33.6030]

5 **European Association for the Study of the Liver**. EASL Clinical Practice Guidelines: Autoimmune hepatitis. *J Hepatol* 2015; **63**: 971-1004 [PMID: 26341719 DOI: 10.1016/j.jhep.2015.06.030]

6 **Dyson JK**, De Martin E, Dalekos GN, Drenth JPH, Herkel J, Hubscher SG, Kelly D, Lenzi M, Milkiewicz P, Oo YH, Heneghan MA, Lohse AW; IAIHG Consortium. Review article: unanswered clinical and research questions in autoimmune hepatitis-conclusions of the International Autoimmune Hepatitis Group Research Workshop. *Aliment Pharmacol Ther* 2019; **49**: 528-536 [PMID: 30671977 DOI: 10.1111/apt.15111]

7 **Castellanos-Fernández MI**, Borges-González SA, Stepanova M, Infante-Velázquez ME, Ruenes-Domech C, González-Suero SM, Dorta-Guridi Z, Arus-Soler ER, Racila A, Younossi ZM. Health-related quality of life in Cuban patients with chronic liver disease: A real-world experience. *Ann Hepatol* 2021; **22**: 100277 [PMID: 33130334 DOI: 10.1016/j.aohep.2020.10.005]

8 **Afendy A**, Kallman JB, Stepanova M, Younoszai Z, Aquino RD, Bianchi G, Marchesini G, Younossi ZM. Predictors of health-related quality of life in patients with chronic liver disease. *Aliment Pharmacol Ther* 2009; **30**: 469-476 [PMID: 19508612 DOI: 10.1111/j.1365-2036.2009.04061.x]

9 **Dirks M**, Haag K, Pflugrad H, Tryc AB, Schuppner R, Wedemeyer H, Potthoff A, Tillmann HL, Sandorski K, Worthmann H, Ding X, Weissenborn K. Neuropsychiatric symptoms in hepatitis C patients resemble those of patients with autoimmune liver disease but are different from those in hepatitis B patients. *J Viral Hepat* 2019; **26**: 422-431 [PMID: 30120896 DOI: 10.1111/jvh.12979]

10 **Cortesi PA**, Conti S, Scalone L, Jaffe A, Ciaccio A, Okolicsanyi S, Rota M, Fabris L, Colledan M, Fagiuoli S, Belli LS, Cesana G, Strazzabosco M, Mantovani LG. Health related quality of life in chronic liver diseases. *Liver Int* 2020; **40**: 2630-2642 [PMID: 32851764 DOI: 10.1111/liv.14647]

11 **Takahashi A**, Moriya K, Ohira H, Arinaga-Hino T, Zeniya M, Torimura T, Abe M, Takaki A, Kang JH, Inui A, Fujisawa T, Yoshizawa K, Suzuki Y, Nakamoto N, Koike K, Yoshiji H, Goto A, Tanaka A, Younossi ZM, Takikawa H; Japan AIH Study Group. Health-related quality of life in patients with autoimmune hepatitis: A questionnaire survey. *PLoS One* 2018; **13**: e0204772 [PMID: 30286131 DOI: 10.1371/journal.pone.0204772]

12 **Schramm C**, Wahl I, Weiler-Normann C, Voigt K, Wiegard C, Glaubke C, Brähler E, Löwe B, Lohse AW, Rose M. Health-related quality of life, depression, and anxiety in patients with autoimmune hepatitis. *J Hepatol* 2014; **60**: 618-624 [PMID: 24240053 DOI: 10.1016/j.jhep.2013.10.035]

13 **van der Plas SM**, Hansen BE, de Boer JB, Stijnen T, Passchier J, de Man RA, Schalm SW. Generic and disease-specific health related quality of life of liver patients with various aetiologies: a survey.

Qual Life Res 2007; **16**: 375-388 [PMID: 17334830 DOI: 10.1007/s11136-006-9131-y]
14 **Wong LL**, Fisher HF, Stocken DD, Rice S, Khanna A, Heneghan MA, Oo YH, Mells G, Kendrick S, Dyson JK, Jones DEJ; UK-AIH Consortium. The Impact of Autoimmune Hepatitis and Its Treatment on Health Utility. *Hepatology* 2018; **68**: 1487-1497 [PMID: 29663477 DOI: 10.1002/hep.30031]
15 **Janik MK**, Wunsch E, Raszeja-Wyszomirska J, Moskwa M, Kruk B, Krawczyk M, Milkiewicz P. Autoimmune hepatitis exerts a profound, negative effect on health-related quality of life: A prospective, single-centre study. *Liver Int* 2019; **39**: 215-221 [PMID: 30204306 DOI: 10.1111/liv.13960]
16 **Honoré LR**, Kjær TW, Kjær MS. Health-related quality of life in patients with autoimmune hepatitis - a systematic review and meta-analysis. *Clin Res Hepatol Gastroenterol* 2018; **42**: e97-e99 [PMID: 30049510 DOI: 10.1016/j.clinre.2018.06.006]
17 **Trevizoli IC**, Pinedo CS, Teles VO, Seixas RBPM, de Carvalho E. Autoimmune Hepatitis in Children and Adolescents: Effect on Quality of Life. *J Pediatr Gastroenterol Nutr* 2018; **66**: 861-865 [PMID: 29470290 DOI: 10.1097/MPG.0000000000001930]
18 **Bozzini AB**, Neder L, Silva CA, Porta G. Decreased health-related quality of life in children and adolescents with autoimmune hepatitis. *J Pediatr (Rio J)* 2019; **95**: 87-93 [PMID: 29331407 DOI: 10.1016/j.jped.2017.10.013]
19 **Gulati R**, Radhakrishnan KR, Hupertz V, Wyllie R, Alkhouri N, Worley S, Feldstein AE. Health-related quality of life in children with autoimmune liver disease. *J Pediatr Gastroenterol Nutr* 2013; **57**: 444-450 [PMID: 23783017 DOI: 10.1097/MPG.0b013e31829ef82c]
20 **Srivastava S**, Boyer JL. Psychological stress is associated with relapse in type 1 autoimmune hepatitis. *Liver Int* 2010; **30**: 1439-1447 [PMID: 20849437 DOI: 10.1111/j.1478-3231.2010.02333.x]
21 **Tandon P**, Berzigotti A. Management of Lifestyle Factors in Individuals with Cirrhosis: A Pragmatic Review. *Semin Liver Dis* 2020; **40**: 20-28 [PMID: 31470455 DOI: 10.1055/s-0039-1696639]
22 **Moussavi S**, Chatterji S, Verdes E, Tandon A, Patel V, Ustun B. Depression, chronic diseases, and decrements in health: results from the World Health Surveys. *Lancet* 2007; **370**: 851-858 [PMID: 17826170 DOI: 10.1016/S0140-6736(07)61415-9]
23 **Sockalingam S**, Blank D, Abdelhamid N, Abbey SE, Hirschfield GM. Identifying opportunities to improve management of autoimmune hepatitis: evaluation of drug adherence and psychosocial factors. *J Hepatol* 2012; **57**: 1299-1304 [PMID: 22871503 DOI: 10.1016/j.jhep.2012.07.032]
24 **Leoni MC**, Amelung L, Lieveld FI, van den Brink J, de Bruijne J, Arends JE, van Erpecum CP, van Erpecum KJ. Adherence to ursodeoxycholic acid therapy in patients with cholestatic and autoimmune liver disease. *Clin Res Hepatol Gastroenterol* 2019; **43**: 37-44 [PMID: 30219692 DOI: 10.1016/j.clinre.2018.08.006]
25 **Gray WN**, Denson LA, Baldassano RN, Hommel KA. Treatment adherence in adolescents with inflammatory bowel disease: the collective impact of barriers to adherence and anxiety/depressive symptoms. *J Pediatr Psychol* 2012; **37**: 282-291 [PMID: 22080456 DOI: 10.1093/jpepsy/jsr092]
26 **Lamba S**, Nagurka R, Desai KK, Chun SJ, Holland B, Koneru B. Self-reported non-adherence to immune-suppressant therapy in liver transplant recipients: demographic, interpersonal, and intrapersonal factors. *Clin Transplant* 2012; **26**: 328-335 [PMID: 21955028 DOI: 10.1111/j.1399-0012.2011.01489.x]
27 **Montano-Loza AJ**, Carpenter HA, Czaja AJ. Consequences of treatment withdrawal in type 1 autoimmune hepatitis. *Liver Int* 2007; **27**: 507-515 [PMID: 17403191 DOI: 10.1111/j.1478-3231.2007.01444.x]
28 **Younossi Z**, Henry L. Overall health-related quality of life in patients with end-stage liver disease. *Clin Liver Dis (Hoboken)* 2015; **6**: 9-14 [PMID: 31040976 DOI: 10.1002/cld.480]
29 **Bonkovsky HL**, Snow KK, Malet PF, Back-Madruga C, Fontana RJ, Sterling RK, Kulig CC, Di Bisceglie AM, Morgan TR, Dienstag JL, Ghany MG, Gretch DR; HALT-C Trial Group. Health-related quality of life in patients with chronic hepatitis C and advanced fibrosis. *J Hepatol* 2007; **46**: 420-431 [PMID: 17196293 DOI: 10.1016/j.jhep.2006.10.009]
30 **van der Plas SM**, Hansen BE, de Boer JB, Stijnen T, Passchier J, de Man RA, Schalm SW. Generic and disease-specific health related quality of life in non-cirrhotic, cirrhotic and transplanted liver patients: a cross-sectional study. *BMC Gastroenterol* 2003; **3**: 33 [PMID: 14617381 DOI: 10.1186/1471-230X-3-33]
31 **Labenz C**, Toenges G, Schattenberg JM, Nagel M, Huber Y, Marquardt JU, Galle PR, Wörns MA. Health-related quality of life in patients with compensated and decompensated liver cirrhosis. *Eur J Intern Med* 2019; **70**: 54-59 [PMID: 31530418 DOI: 10.1016/j.ejim.2019.09.004]
32 **Wong GW**, Yeong T, Lawrence D, Yeoman AD, Verma S, Heneghan MA. Concurrent extrahepatic autoimmunity in autoimmune hepatitis: implications for diagnosis, clinical course and long-term outcomes. *Liver Int* 2017; **37**: 449-457 [PMID: 27541063 DOI: 10.1111/liv.13236]
33 **Dyson JK**, Wong LL, Bigirumurame T, Hirschfield GM, Kendrick S, Oo YH, Lohse AW, Heneghan MA, Jones DEJ; UK-AIH Consortium. Inequity of care provision and outcome disparity in autoimmune hepatitis in the United Kingdom. *Aliment Pharmacol Ther* 2018; **48**: 951-960 [PMID: 30226274 DOI: 10.1111/apt.14968]
34 **Pape S**, Gevers TJG, Vrolijk JM, van Hoek B, Bouma G, van Nieuwkerk CMJ, Taubert R, Jaeckel E, Manns MP, Papp M, Sipeki N, Stickel F, Efe C, Ozaslan E, Purnak T, Nevens F, Kessener DJN, Kahraman A, Wedemeyer H, Hartl J, Schramm C, Lohse AW, Heneghan MA, Drenth JPH. High discontinuation rate of azathioprine in autoimmune hepatitis, independent of time of treatment initiation. *Liver Int* 2020; **40**: 2164-2171 [PMID: 32410363 DOI: 10.1111/liv.14513]

35 **Snijders RJALM**, Pape S, Gevers TJG, Drenth JPH. Discontinuation rate of azathioprine. *Liver Int* 2020; **40**: 2878 [PMID: 32744415 DOI: 10.1111/liv.14615]

36 **Smith MA**, Irving PM, Marinaki AM, Sanderson JD. Review article: malignancy on thiopurine treatment with special reference to inflammatory bowel disease. *Aliment Pharmacol Ther* 2010; **32**: 119-130 [PMID: 20412066 DOI: 10.1111/j.1365-2036.2010.04330.x]

37 **Ngu JH**, Gearry RB, Frampton CM, Stedman CA. Mortality and the risk of malignancy in autoimmune liver diseases: a population-based study in Canterbury, New Zealand. *Hepatology* 2012; **55**: 522-529 [PMID: 21994151 DOI: 10.1002/hep.24743]

38 **Janik MK**, Wunsch E, Moskwa M, Raszeja-Wyszomirska J, Krawczyk M, Milkiewicz P. Depression in patients with autoimmune hepatitis: the need for detailed psychiatric assessment. *Pol Arch Intern Med* 2019; **129**: 645-647 [PMID: 31316046 DOI: 10.20452/pamw.14898]

39 **Rubin RR**, Wadden TA, Bahnson JL, Blackburn GL, Brancati FL, Bray GA, Coday M, Crow SJ, Curtis JM, Dutton G, Egan C, Evans M, Ewing L, Faulconbridge L, Foreyt J, Gaussoin SA, Gregg EW, Hazuda HP, Hill JO, Horton ES, Hubbard VS, Jakicic JM, Jeffery RW, Johnson KC, Kahn SE, Knowler WC, Lang W, Lewis CE, Montez MG, Murillo A, Nathan DM, Patricio J, Peters A, Pi-Sunyer X, Pownall H, Rejeski WJ, Rosenthal RH, Ruelas V, Toledo K, Van Dorsten B, Vitolins M, Williamson D, Wing RR, Yanovski SZ, Zhang P; Look AHEAD Research Group. Impact of intensive lifestyle intervention on depression and health-related quality of life in type 2 diabetes: the Look AHEAD Trial. *Diabetes Care* 2014; **37**: 1544-1553 [PMID: 24855155 DOI: 10.2337/dc13-1928]

40 **Jacoby A**, Rannard A, Buck D, Bhala N, Newton JL, James OF, Jones DE. Development, validation, and evaluation of the PBC-40, a disease specific health related quality of life measure for primary biliary cirrhosis. *Gut* 2005; **54**: 1622-1629 [PMID: 15961522 DOI: 10.1136/gut.2005.065862]

41 **Broadbent DE**, Cooper PF, FitzGerald P, Parkes KR. The Cognitive Failures Questionnaire (CFQ) and its correlates. *Br J Clin Psychol* 1982; **21**: 1-16 [PMID: 7126941 DOI: 10.1111/j.2044-8260.1982.tb01421.x]

42 **Younossi ZM**, Guyatt G, Kiwi M, Boparai N, King D. Development of a disease specific questionnaire to measure health related quality of life in patients with chronic liver disease. *Gut* 1999; **45**: 295-300 [PMID: 10403745 DOI: 10.1136/gut.45.2.295]

43 **Lo C**, Walsh A, Mikulincer M, Gagliese L, Zimmermann C, Rodin G. Measuring attachment security in patients with advanced cancer: psychometric properties of a modified and brief Experiences in Close Relationships scale. *Psychooncology* 2009; **18**: 490-499 [PMID: 18821528 DOI: 10.1002/pon.1417]

44 **Lloyd A**, Pickard AS. The EQ-5D and the EuroQol Group. *Value Health* 2019; **22**: 21-22 [PMID: 30661629 DOI: 10.1016/j.jval.2018.12.002]

45 **Webster K**, Cella D, Yost K. The Functional Assessment of Chronic Illness Therapy (FACIT) Measurement System: properties, applications, and interpretation. *Health Qual Life Outcomes* 2003; **1**: 79 [PMID: 14678568 DOI: 10.1186/1477-7525-1-79]

46 **Fisk JD**, Ritvo PG, Ross L, Haase DA, Marrie TJ, Schlech WF. Measuring the functional impact of fatigue: initial validation of the fatigue impact scale. *Clin Infect Dis* 1994; **18** Suppl 1: S79-S83 [PMID: 8148458 DOI: 10.1093/clinids/18.supplement_1.s79]

47 **Spitzer RL**, Kroenke K, Williams JB, Löwe B. A brief measure for assessing generalized anxiety disorder: the GAD-7. *Arch Intern Med* 2006; **166**: 1092-1097 [PMID: 16717171 DOI: 10.1001/archinte.166.10.1092]

48 **Zigmond AS**, Snaith RP. The hospital anxiety and depression scale. *Acta Psychiatr Scand* 1983; **67**: 361-370 [PMID: 6880820 DOI: 10.1111/j.1600-0447.1983.tb09716.x]

49 **van der Plas SM**, Hansen BE, de Boer JB, Stijnen T, Passchier J, de Man RA, Schalm SW. The Liver Disease Symptom Index 2.0; validation of a disease-specific questionnaire. *Qual Life Res* 2004; **13**: 1469-1481 [PMID: 15503842 DOI: 10.1023/B:QURE.0000040797.17449.c0]

50 **Smets EM**, Garssen B, Bonke B, De Haes JC. The Multidimensional Fatigue Inventory (MFI) psychometric qualities of an instrument to assess fatigue. *J Psychosom Res* 1995; **39**: 315-325 [PMID: 7636775 DOI: 10.1016/0022-3999(94)00125-o]

51 **Kos D**, Kerckhofs E, Nagels G, D'Hooghe BD, Duquet W, Duportail M, Ketelaer P. Assessing fatigue in multiple sclerosis: Dutch modified fatigue impact scale. *Acta Neurol Belg* 2003; **103**: 185-191 [PMID: 15008502]

52 **Kroenke K**, Spitzer RL, Williams JB. The PHQ-9: validity of a brief depression severity measure. *J Gen Intern Med* 2001; **16**: 606-613 [PMID: 11556941 DOI: 10.1046/j.1525-1497.2001.016009606.x]

53 **Ware J Jr**, Kosinski M, Keller SD. A 12-Item Short-Form Health Survey: construction of scales and preliminary tests of reliability and validity. *Med Care* 1996; **34**: 220-233 [PMID: 8628042 DOI: 10.1097/00005650-199603000-00003]

54 **Ware JE Jr**, Sherbourne CD. The MOS 36-item short-form health survey (SF-36). I. Conceptual framework and item selection. *Med Care* 1992; **30**: 473-483 [PMID: 1593914]

55 **Gaudry E**, Vagg P, Spielberger CD. Validation of the State-Trait Distinction in Anxiety Research. *Multivariate Behav Res* 1975; **10**: 331-341 [PMID: 26829634 DOI: 10.1207/s15327906mbr1003_6]

56 **Reilly MC**, Zbrozek AS, Dukes EM. The validity and reproducibility of a work productivity and activity impairment instrument. *Pharmacoeconomics* 1993; **4**: 353-365 [PMID: 10146874 DOI: 10.2165/00019053-199304050-00006]

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MINIREVIEWS

Fungal infections following liver transplantation

Madiha Khalid, Ritesh Neupane, Humayun Anjum, Salim Surani

ORCID number: Madiha Khalid 0000-0002-6338-3729; Ritesh Neupane 0000-0003-3792-5835; Humayun Anjum 0000-0001-7804-4394; Salim Surani 0000-0001-7105-4266.

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Madiha Khalid, Department of Medicine, Orlando Health Medical Center, Orlando, FL 32806, United States

Ritesh Neupane, Department of Medicine, Penn State Health Milton S Hershey Medical Center, Hershey, PA 17033, United States

Humayun Anjum, Department of Medicine, University of North Texas, Denton, TX 76203, United States

Salim Surani, Department of Pulmonary Critical Care and Sleep Medicine, Texas A&M Health Science Center, Corpus Christi, TX 78405, United States

Corresponding author: Salim Surani, FACP, FCCP, MD, MSc, Doctor, Professor, Department of Pulmonary Critical Care and Sleep Medicine, Texas A&M Health Science Center, 701 Ayers Street, Corpus Christi, TX 78405, United States. srsurani@hotmail.com

Abstract

With increasing morbidity and mortality from chronic liver disease and acute liver failure, the need for liver transplantation is on the rise. Most of these patients are extremely vulnerable to infections as they are immune-compromised and have other chronic co-morbid conditions. Despite the recent advances in practice and improvement in diagnostic surveillance and treatment modalities, a major portion of these patients continue to be affected by post-transplant infections. Of these, fungal infections are particularly notorious given their vague and insidious onset and are very challenging to diagnose. This mini-review aims to discuss the incidence of fungal infections following liver transplantation, the different fungi involved, the risk factors, which predispose these patients to such infections, associated diagnostic challenges, and the role of prophylaxis. The population at risk is increasingly old and frail, suffering from various other co-morbid conditions, and needs special attention. To improve care and to decrease the burden of such infections, we need to identify the at-risk population with more robust clinical and diagnostic parameters. A more robust global consensus and stringent guidelines are needed to fight against resistant microbes and maintain the longevity of current antimicrobial therapies.

Key Words: Invasive fungal infections; Liver transplantation; Candidiasis; Antifungal prophylaxis; Aspergillosis; *Cryptococcus*

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Core Tip: Fungal infections post liver transplant remains the predominant source of morbidity and mortality despite the incidence being low. This is because of evasive clinical features coupled with difficulty to isolate and culture these pathogens. Therefore, appropriate patients are selected for prophylactic regimen based on specific risk factors to curb the rise of drug-resistant species. Traditional regimens include fluconazole or liposomal amphotericin with a shift towards echinocandins based on recently published and promising data.

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INTRODUCTION

Liver transplantation is one of the principal treatment modalities for the treatment of many hepatic diseases, mainly but not limited to chronic and end-stage liver disease. Despite advances in the field of transplantation, invasive fungal infections remain a major source of morbidity and mortality. This is attributed to delay in diagnosis, nonspecific clinical features[1], fastidious nature of these organisms, lack of consensus on prophylactic regimens, and rise of antifungal resistant species.

Moreover, with an increase in the number of grafts being offered, there is a trend towards recipients being older, debilitated, and having more non hepatic comorbidities which contributes to the burden and subsequently leads to a higher rate of fungal infections[2].

In this article, we aim to discuss the incidence and trend of invasive fungal infections (IFI) in liver transplant (LT) patients, associated risk factors, diagnostic challenges, and data on prophylaxis.

IFI DEFINITION

IFIs, according to the Invasive Fungal Infections Cooperative Group in Europe and the Mycoses Study Group in the United States, are divided into 3 categories: proven, probable and possible.

Proven IFI is defined as a positive fungal culture or histological proof of fungal or hyphal elements in a sterile site biopsy. This also includes positive cryptococcal antigen in cerebrospinal fluid.

Probable and possible IFIs have a wider definition and inclusion criteria. This is based on several host factors along with various clinical and mycological criteria[3].

Some studies evaluating prophylactic regimens, in this regard have been a focus of criticism as their IFI's were considered colonization rather than infection[4].

INCIDENCE AND RESPONSIBLE FUNGI

The incidence of IFI after LT has decreased in recent years and this is attributable to advancement and improvement in surgical techniques along with more aggressive post-operative care. Previously, in one study by Fung *et al*[5], the incidence of IFI after LT was reported to be 6.6% with a mortality of 54.5%. The ninety-day cumulative mortality after invasive candidiasis has been reported to be 26% and 1-year survival after invasive aspergillosis is about 59% according to TRANSNET in 2010[6].

More recently, according to some cohort studies, the overall incidence of IFI after solid organ transplant is about 1%-4%[7-9]. 1-year cumulative probability of IFI in LT was 1.8%[7]. This shows a promising trend and is related to improvise surgical techniques and timely recognition of risk factors that make certain patients more susceptible to IFIs.

However, in underdeveloped nations, it remains higher at 14.7% with an in-hospital mortality rate of 77%[10]. A future streamlined approach to the problem with specific guidelines might be one of the ways to improve these numbers.

The three major fungi involved are *Candida* spp., *Cryptococcus*, and *Aspergillus* spp. *Candida* predominates with 81% followed by *Aspergillus* (16%) and *Cryptococcus* (3%). Non-*Albicans Candida* accounted for 68% of all *Candida* infections[11]. The rise of resistant non-*Albicans Candida* especially C. parapsilosis was felt to coincide with the increased use of fluconazole[11]. C. parapsilosis is associated with increased mortality in these patients. This increase in resistant fungal species indicates a dire need for a patient-specific prophylactic regimen based on risk factors *vs* a universal approach.

The distribution of the fungal species remains similar in the East with *Candida* representing 64.1% and *Aspergillus* 35.8% of the IFIs in LT patients.

Despite the highly variable clinical presentation, these pathogens most commonly affect the respiratory system followed by renal and gastrointestinal tract[10]. According to a retrospective study in 2015 by Eschenauer and colleagues, intra-abdominal candidiasis (73%) was the most common IFI[12]. The common clinical manifestations of various fungal organisms are shown in Table 1.

TIMING FROM TRANSPLANT TO INFECTION

There has been a shift in the time duration between the developments of IFIs after LT. It was initially thought to occur in the early post-operative phase most commonly within the first couple of months.

Grauhan *et al*[13] in 1994 reported a median time from LT to IFI of 2 mo.

According to Husain *et al*[14] in 2003, the median time to infection for invasive candidiasis was 13.5 d with 72% of the IFIs happening within the first month after LT.

Aspergillus tends to present later as compared to *Candida*. Results from one study by Singh and colleagues in 2003 reported 55% of their *Aspergillus* IFI occurring after 90 d [15] and Gravalda *et al*[16] also described 43% of their IFIs as late onset *Aspergillus*.

In transplant centers with a higher risk of *Aspergillus* based on epidemiology, this delayed time to presentation is important to consider while deciding on the length of prophylactic regimen in high-risk patients. Moreover, clinicians need to be mindful of this time frame while diagnosing an already difficult-to-diagnose disease.

RISK FACTORS

Multiple factors have been observed over time to be associated with the development of fungal infections in LTs. Identifying patients that are at high risk for developing IFI can be of immense help as that can aide in decreasing the diagnostic delay and assure appropriate prophylaxis. By adopting this targeted method of prophylaxis *vs* universal approach, we can also potentially reduce the incidence of drug-resistant fungi, lower the morbidity due to side effects and interactions of these medications particularly with immunosuppressants, and mitigate the overall cost.

Many scientists over the past few decades have worked on identifying these attributes. These can be categorized into pre-operative, operative, and post-operative factors as shown in Table 2. Risk factors for *Aspergillus* specifically seem to depend more on post-operative factors as highlighted in Figure 1.

Collins *et al*[17] in 1994 identified the following as potential risk factors: renal insufficiency, length of transplant operation, rate of re-transplantation, abdominal or intra-thoracic reoperation, and cytomegalovirus infection.

Other studies showed that model for end-stage liver disease (MELD) scores > 25, post-transplant acute kidney injury (Cr > 2 or risk, injury, failure, loss of kidney function, and end-stage n criteria I- or F-) and pre-transplant fungal colonization seem to be the culprits identified with IFIs[11,18].

One of these was an important and common risk factor of daily prophylactic fluconazole dose of < 200 mg, which was thought to cause a rise in drug-resistant non-*Albicans Candida* spp[11].

Although very rare, a French study also identified contamination during organ procurement as a risk factor with a 1.33% prevalence of *Candida* spp. in preservation fluid. This was associated with a higher rate of IFI and impaired survival[19].

Alongside predictable risk factors like diabetes and hemodialysis dependence, Verma *et al*[10] pointed out prior antibiotic use, cerebral and respiratory organ failures, chronic liver failure (CLIF) organ failure/CLIF-consortium acute-on-chronic liver

Table 1 Common clinical manifestations of invasive fungal infection

	Clinical manifestations
Candida	Intra-abdominal abscesses
	Recurrent cholangitis
	Peritonitis
	Fungemia
Aspergillus	Invasive pulmonary *Aspergillosis*
	Brain abscess
	Endophthalmitis
	Osteomyelitis
	Endocarditis
Cryptococcus	CNS infection
	Focal lesions on imaging
	Meningeal enhancement

CNS: Central nervous system.

Table 2 Risk factors for invasive fungal infections

	Risk factors
Pre-operative	SBP prophylaxis with fluoroquinolone
Operative	Retransplantation
	Long transplantation time
	Long transplantation time
	Class 2 partial or complete match
	Donor from male
Post-operative	Post-transplant HD
	High number of RBC units transfused
	Post-transplant bacterial infection
	Cytomegalovirus infection
	Use of muromonab-CD3
	Aspergillus antigenemia

SBP: Spontaneous bacterial prophylaxis; HD: Hemodialysis; RBC: Red blood cells.

failure as predictors of IFIs. Non-survivors in their study also had higher levels of 1.3-beta D glucan (BDG) levels. BDG levels have been studied as a diagnostic marker and look promising.

There has been a general shift in the trend of risk factors over the last 2 decades, which is attributable to better surgical techniques. Singh *et al*[20] studied 190 liver transplants during 1990 and 2000 and demonstrated improvement in length of operation, intraoperative transfusion requirements, use of roux-en-Y biliary anastomosis, re-transplantation, rate of rejection over time, and cold ischemic time. This led to a decrease in the incidence of invasive candidiasis in this study population from 9%-1.7% without any use of antifungal prophylaxis.

In 2015, Eschenauer and colleagues identified bile leaks within the first 30 d post-transplant and living donor liver transplants as new independent risk factors for IFIs. This is because *Candida* has an affinity for growth in the biliary tract. Moreover, living donor liver transplants are highly technical procedures that are not commonly

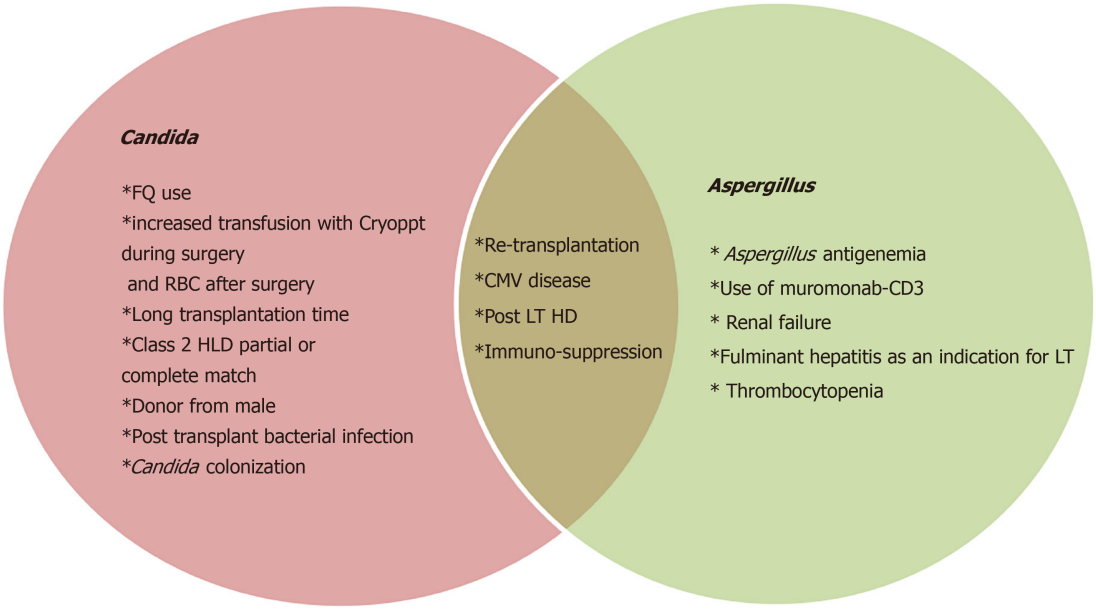

Figure 1 Risk factors for Candida and Aspergillus. FQ: Fluoroquinolone; HLA: Human leukocyte antigen; CMV: Cytomegalovirus; HD: Hemodialysis; LT: Liver transplant.

performed in the United States. The increased length and complexity of these procedures along with higher disruption of the biliary tract is responsible for these findings. The authors recommended instituting antifungal prophylaxis in all living donor liver transplants[12].

A small study recently in 2020 by Jorgenson *et al*[21] studied the effects of pre-transplant roux-en Y gastric bypass on liver transplant outcomes. There were increased rates of fungal infection in patients with bariatric surgery before transplant and might be associated with loss of defense provided by gastric acid. This study is limited by its retrospective nature and its size.

DIAGNOSTIC CHALLENGES

In general, fungal infections do not present themselves vividly and are increasingly difficult to grow in culture media. It makes it even more challenging in patients who have chronic liver disease, are immunosuppressed, or have other underlying comorbidities. They are difficult to detect clinically and also objectively in laboratories. Hence, prevention becomes essential, and it has significantly improved in the last decade with the advancement in surgical techniques, intense pre-operative evaluation, and appropriate use of antifungal prophylactic agents in high-risk patients.

Distinguishing between colonization and true infection can be challenging for the clinician. Apart from 'proven IFI' as discussed above, the other two categories are vague and have plenty of variable factors. In these clinical scenarios, the use of newer diagnostic tools like BDG and galactomannan (GM) can be helpful. Polymerase chain reaction fungal assays are promising but not yet approved by the Food and Drug Administration.

BDG has been studied and looks promising as a diagnostic marker in serum. In a study from 2017, with 271 transplant patients, weekly BDG was tested and monitored for IFIs. 95% of the patients with IFI had positive BDG and a very promising negative predictive value of 96% was seen. The sensitivity of BDG was 75% and specificity was 65%, making it a very good tool to rule out IFIs[22].

The GM test is an enzyme-linked immunosorbent assay that detects the GM antigen released by *Aspergillus* hyphae when they invade host cells.

The patient's epidemiological risk factors should be considered strongly which would help guide better towards increasing clinical suspicion and ordering appropriate tests and guided treatments. Objective risk factors such as the MELD score, the overall duration of need for total parenteral nutrition, length of the operative procedure, and removal of abdominal drains and other catheters or lines should be evaluated[23].

PROPHYLAXIS

Fungal infections in liver transplant recipients are mostly attributed to *Aspergillus* and *Candida*. Three agents are mainly used in prophylaxis–fluconazole, liposomal amphotericin B, and itraconazole. The studies involving these agents have been confounded by the difficulty of differentiating colonization and a true infection, the variability between patient selection, therapeutic agent(s) used in comparison with placebo or each other, and variable duration of treatment.

Data on the effectiveness of antifungal prophylaxis in LT over the past 10 years have been summarized in the Table 3 below.

There have been three meta-analyses as summarized in Table 3. Playford *et al*[24] and Cruciani *et al*[25] published two in 2006, with 10 and 6 studies respectively. These summarized that universal fungal prophylaxis leads to a reduction in proven IFIs without any mortality benefit. This universal approach leads to a significantly higher proportion of episodes of non-*Albicans Candida* infection.

In 2014, Evans *et al*[26] published a meta-analysis of studies on prophylaxis to prevent IFIs after LT and concluded that the odds of proven IFI and IFI related mortality were lower in patients receiving antifungal prophylaxis, even if the overall mortality did not change. It was also demonstrated that the efficacy of fluconazole compared to liposomal amphotericin was similar with the latter having the benefit of not altering the cytochrome P450 system and therefore not affecting the calci-neurin inhibitor levels. However, fluconazole is favored because of its cost-effectiveness and safety profile. This meta-analysis did not reveal any information on echinocandins, however, it was different from their counterparts in that they did a mixed treatment comparison and was more recent of the few meta-analyses already on the subject matter.

Studies since 2014 (after the last meta-analysis) on prophylaxis are summarized in Table 4.

In 2015, Eschenauer and colleagues performed a retrospective study involving liver transplant patients that were divided into three main groups. Group 1 included 145 patients who received targeted prophylaxis with either voriconazole in 54%, fluconazole in 5% or no antifungal which was the case of 38% of these patients. This was compared to a group of 237 patients, who received universal prophylaxis with voriconazole. These regimens were continued for a median time of 11 d in the targeted group and for 6 d in the universal group, with a significant *P* value. There was no statistical difference between incidence of IFI between both groups (6.8% in targeted and 4.2% in universal). Similarly, the *P* value was not statistically significant for the mortality rates over 100 d from IFIs in both groups (10% for targeted and 7% for universal group). They, therefore concluded that targeted approach to antifungal use in liver transplant patients was a safe, cost effective strategy and prevented unnecessary side effects[12].

With regards to echinocandins, Saliba *et al*[27] in 2015 compared micafungin *vs* standard treatment and found them equally effective. Standard therapy was center-specific and included IV fluconazole, liposomal amphotericin, or IV caspofungin.

Similarly, in a study from Spain in 2016, caspofungin was compared to fluconazole in high-risk patients and similar efficacy was reported to prevent global IFIs. In this study caspofungin was related to decrease in breakthrough IFIs and also led to a lower rate of invasive aspergillosis[28].

Echinocandins should be considered as prophylactic agents, where appropriate, especially in areas of increased prevalence of drug-resistant non-*Albicans Candida*. Unfortunately, these too come with a higher price tag compared to fluconazole which can affect their use, especially in non-affluent countries.

According to the Infectious Disease Society of America guidelines, patients who meet 2 or more of the following risk factors to be considered for prophylaxis: creatinine more than 2 mg/dL, need for re-transplantation, choledochojejunostomy, more than 11 h of operative time, need to transfuse with ≥ 40 units of blood products, evidence of fungal colonization in immediate pre and post-operative days. Suggested duration of antifungal use is 14-21 d.

However, since the current data suggest that the incidence and risk of fungal infection overall in the general liver transplantation population is low, these agents should be utilized for higher-risk patients as unguided use is associated with drug-resistant non-*Albicans Candida* infection and higher mortality in these patients[23].

Table 3 Effectiveness of antifungal prophylaxis in liver transplant

Ref.	Trials	Patients	Regimens	Infection reduction (95%CI)	Comments
Cruciani *et al*[25], 2006	6	698	AmB *vs* Pla (1)	Total proven fungal infections RR 0.31 (0.21-0.46), IFI RR 0.33 (0.18-0.59)	Patients receiving prophylaxis had higher number of non-*Albicans* proven fungal infections. Mostly C. glabrata.
			Flu *vs* nonsystemic AF (1)		
			Flu *vs* Pla (2)		
			Itra *vs* Pla(1)		
			Amb-Itra *vs* Flu-itra *vs* Pla (1)		
Playford *et al*[24], 2006	7	793	Flu *vs* Pla (2)	Proven IFI RR 0.39 (0.18-0.85), fungal colonization RR 0.51 (0.41-0.62), fungal colonization with C. glabrata/C. krusei, RR 1.57 (0.76-3.24)	Formulated algorithm in which patients with < 2 RF deemed low risk (4%incidence) for IFI and those with ≥ 2 at high risk (25% incidence) for IFI.
			Flu *vs* nonsystemic AF (2)		
			Itra *vs* Pla (2)		
			AmB *vs* Pla (1)		
Evans *et al* [26], 2014	14	1633	Flu *vs* Pla/nonabs AF (4)	Proven IFI OR 0.37 (0.19-0.72), P = 0.003, Bayesian MTC, AmB *vs* Pla OR 0.21 (0.05-0.71), Flu *vs* Pla OR 0.21 (0.06-0.57)	Benefit of AmB is of similar magnitude to that previously described for fluconazole.
			Itra *vs* Pla (1)		
			AmB *vs* Pla (1)		
			3 arm study with Pla/AmB/Flu (1)		
			Flu *vs* AmB (3)		
			Liposomal + Flu *vs* standard AmB + Flu		
			Itra *vs* Flu (2)		
			Micafungin *vs* standard care (1)		
			Clo *vs* Nys (1)		

AmB: Amphotericin-B; Pla: Placebo; Flu: Fluconazole; AF: Antifungal; Itra: Itraconazole; Nonabs AF: Nonabsorbable antifungal; Nys: Nystatin; Clo: Clotrimazole.

CONCLUSION

Fungal infections following liver transplantation remain an influential cause of morbidity and mortality in these patients, despite the low incidence. Identification of high-risk patients based on risk factors discussed above and starting an appropriate prophylactic antifungal regimen based on epidemiology, calcineurin inhibitor use, and renal function is the first step in avoiding dealing with this evasive disease.

Prophylactic antifungals are generally well tolerated but can lead to drug-resistant *Candida* spp., hence the importance of selecting the appropriate patient and agent. Using BDG as a negative predictive tool and having a high degree of suspicion, even if the time from transplant exceeds 2 mo, can prevent diagnostic delays.

Further randomized controlled trials comparing azoles, amphotericin, and echinocandins are needed to develop an updated standard of care.

Table 4 Studies since 2014 (after the last meta-analysis) on prophylaxis for liver transplant

Ref.	Design	Regimen	Outcomes
Antunes *et al* [29], 2014	Single center. Retrospective (n = 461)	High risk group: AmB *vs* nystatin; Low risk group: nystatin	Higher IFI in high risk patients who did not receive AmB
Winston *et al* [30], 2014	Randomized, double-blind. 2010-2011 (n = 200)	Group 1: Andulafugin; Group 2: Flu	1:1 randomized. Similar cumulative IFI occurrence and equal 3 mo mortality
Saliba *et al*[27], 2015	Randomized, open label. 2009-2012 (n = 347)	Micafungin *vs* center specific standard care (Flu/AmB/Caspo)	Micafungin was non-inferior to standard of care
Giannella *et al* [31], 2015	Prospective, non-randomized. 2009-2013. Safety of high dose AmB (n = 76)	Amb 10 mg/kg Q weekly until hospital discharge for a minimum of 2 wk	10 patients discontinued therapy. (6 for AmB related AEs and 4 for IFI)
Eschenauer *et al*[12], 2015	Single center study. 2008-2012. Effectiveness of targeted prophylaxis (n = 381)	Universal ppx: Vori. Targeted: Group1: Vori, 30 d. Group 2: Flu during icu sta. Group3: No ppx	Cumulative IFI occurrence 5.2% (targeted *vs* universal group). Similar 100 day mortality between targeted and universal ppx gp. 40% breakthrough IFI
Balogh *et al* [32], 2016	Single center study. 2008-2014 (n = 314)	Voriconazole *vs* oral nystatin or Flu	No episodes of IA occurred. No difference in graft and patient survival curves between the two groups
Perrella *et al* [33], 2016	Single center study. 2006-2012. Comparative observational study for targeted prophylaxis (n = 54)	Group 1: AmB 3 mg/kg/day; Group2: Caspofungin 70 mg loading→50 mg/day	No episodes of IFI in both groups
Fortún *et al* [28], 2016	Multicenter. 2005-2012. Comparative observational study for targeted prophylaxis (n = 195)	Group 1: Caspofungin 50 mg/d; Group 2: Flu median 200 mg/day	Similar 6 m IFI occurrence [5.2% b (G1) *vs* 12.2% (G2)]. Reduced risk of IA in LT receiving caspofungin. Similar overall mortality
Chen *et al*[34], 2016	Single center study. 2005-2014. Effectiveness of targeted prophylaxis (n = 402)	Group 1: Anidulafungin 100 mg/day or micafungin 100 mg/day; Group 2: No prophylaxis	High risk patients MELD > 20; Similar IFI occurrence lower cumulative mortality in group 1 (P = 0.001)
Giannella *et al* [35], 2016	Retrospective, single center. 2010-2014. Evaluation of RF for a targeted prophylaxis (n = 303)	Group 1: No RF. No prophylaxis; Group 2: 1RF IC, Flu; Group3: High risk, anti mould agent	Antifungal prophylaxis administered to 45.9% patients. Cumulative IFI prevalence 6.3%. Flu independently associated with IFI development
Lavezzo *et al* [36], 2018	Single center study. 2011-2015. Effectiveness of targeted prophylaxis	Group 1 high risk: AmB; Group 2 low risk: No prophylaxis	Overall IFI prevalence 2.8%. 1 yr mortality higher in prophylaxis group (P = 0.001). 1 yr mortality higher in IFI patients (P < 0.001)
Jorgenson *et al* [37], 2019	Single center study. 2009-2016. Effectiveness of fixed dose prophylaxis (n = 189)	Group 1: Flu 400 mg/day for 14 d for high risk patients; Group 2: unsupervised antifungal protocols	Reduction in 1 yr IFI among high risk group (12.5% *vs* 26.6%). Similar 1 yr patient and graft survival
Kang *et al*[38], 2020	Multicenter, randomized, open label. Living donor LT. 2012-2015 (n = 144)	Group 1: Micafungin 100 mg/d; Group 2: Flu 100-200 mg/day	Group 1 *vs* Group 2: 69 *vs* 75 pts. IFI occurrence in 3 wk: 1/69 *vs* 0/75. Micafungin was noninferior to Flu

AmB: Amphotericin-b; Flu: Fluconazole; Caspo: Caspofungin; AE: Adverse effects; Vori: Voriconazole; ppx: Prophylaxis; gp: Group; IA: Invasive aspergillosis; IC: Invasive candidiasis.

REFERENCES

1 **Liu X**, Ling Z, Li L, Ruan B. Invasive fungal infections in liver transplantation. *Int J Infect Dis* 2011; **15**: e298-e304 [PMID: 21345708 DOI: 10.1016/j.ijid.2011.01.005]

2 **Toniutto P**, Zanetto A, Ferrarese A, Burra P. Current challenges and future directions for liver transplantation. *Liver Int* 2017; **37**: 317-327 [PMID: 27634369 DOI: 10.1111/liv.13255]

3 **De Pauw B**, Walsh TJ, Donnelly JP, Stevens DA, Edwards JE, Calandra T, Pappas PG, Maertens J, Lortholary O, Kauffman CA, Denning DW, Patterson TF, Maschmeyer G, Bille J, Dismukes WE, Herbrecht R, Hope WW, Kibbler CC, Kullberg BJ, Marr KA, Muñoz P, Odds FC, Perfect JR, Restrepo A, Ruhnke M, Segal BH, Sobel JD, Sorrell TC, Viscoli C, Wingard JR, Zaoutis T, Bennett JE; European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group; National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG) Consensus Group. Revised definitions of invasive fungal disease from the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG) Consensus Group. *Clin Infect Dis* 2008; **46**: 1813-1821 [PMID: 18462102 DOI: 10.1086/588660]

4 **Huprikar S**. Reply: Effect of prophylaxis on fungal infection and costs for high-risk liver transplant recipients. *Liver Transpl* 2008; **14**: 709 [PMID: 18433053 DOI: 10.1002/lt.21458]

5 **Fung JJ**. Fungal infection in liver transplantation. *Transpl Infect Dis* 2002; **4** Suppl 3: 18-23 [PMID: 12486788 DOI: 10.1034/j.1399-3062.4.s3.3.x]

6 **Pappas PG**, Alexander BD, Andes DR, Hadley S, Kauffman CA, Freifeld A, Anaissie EJ, Brumble

LM, Herwaldt L, Ito J, Kontoyiannis DP, Lyon GM, Marr KA, Morrison VA, Park BJ, Patterson TF, Perl TM, Oster RA, Schuster MG, Walker R, Walsh TJ, Wannemuehler KA, Chiller TM. Invasive fungal infections among organ transplant recipients: results of the Transplant-Associated Infection Surveillance Network (TRANSNET). *Clin Infect Dis* 2010; **50**: 1101-1111 [PMID: 20218876 DOI: 10.1086/651262]

7 **Hosseini-Moghaddam SM**, Ouédraogo A, Naylor KL, Bota SE, Husain S, Nash DM, Paterson JM. Incidence and outcomes of invasive fungal infection among solid organ transplant recipients: A population-based cohort study. *Transpl Infect Dis* 2020; **22**: e13250 [PMID: 31981389 DOI: 10.1111/tid.13250]

8 **Andes DR**, Safdar N, Baddley JW, Alexander B, Brumble L, Freifeld A, Hadley S, Herwaldt L, Kauffman C, Lyon GM, Morrison V, Patterson T, Perl T, Walker R, Hess T, Chiller T, Pappas PG; TRANSNET Investigators. The epidemiology and outcomes of invasive Candida infections among organ transplant recipients in the United States: results of the Transplant-Associated Infection Surveillance Network (TRANSNET). *Transpl Infect Dis* 2016; **18**: 921-931 [PMID: 27643395 DOI: 10.1111/tid.12613]

9 **van Delden C**, Stampf S, Hirsch HH, Manuel O, Meylan P, Cusini A, Hirzel C, Khanna N, Weisser M, Garzoni C, Boggian K, Berger C, Nadal D, Koller M, Saccilotto R, Mueller NJ; Swiss Transplant Cohort Study. Burden and Timeline of Infectious Diseases in the First Year After Solid Organ Transplantation in the Swiss Transplant Cohort Study. *Clin Infect Dis* 2020; **71**: e159-e169 [PMID: 31915816 DOI: 10.1093/cid/ciz1113]

10 **Verma N**, Singh S, Taneja S, Duseja A, Singh V, Dhiman RK, Chakrabarti A, Chawla YK. Invasive fungal infections amongst patients with acute-on-chronic liver failure at high risk for fungal infections. *Liver Int* 2019; **39**: 503-513 [PMID: 30276951 DOI: 10.1111/liv.13981]

11 **Raghuram A**, Restrepo A, Safadjou S, Cooley J, Orloff M, Hardy D, Butler S, Koval CE. Invasive fungal infections following liver transplantation: incidence, risk factors, survival, and impact of fluconazole-resistant Candida parapsilosis (2003-2007). *Liver Transpl* 2012; **18**: 1100-1109 [PMID: 22577087 DOI: 10.1002/lt.23467]

12 **Eschenauer GA**, Kwak EJ, Humar A, Potoski BA, Clarke LG, Shields RK, Abdel-Massih R, Silveira FP, Vergidis P, Clancy CJ, Nguyen MH. Targeted versus universal antifungal prophylaxis among liver transplant recipients. *Am J Transplant* 2015; **15**: 180-189 [PMID: 25359455 DOI: 10.1111/ajt.12993]

13 **Grauhan O**, Lohmann R, Lemmens P, Schattenfroh N, Keck H, Klein E, Raakow R, Jonas S, Langrehr JM, Bechstein W. Fungal infections in liver transplant recipients. *Langenbecks Arch Chir* 1994; **379**: 372-375 [PMID: 7845165 DOI: 10.1007/BF00191586]

14 **Husain S**, Tollemar J, Dominguez EA, Baumgarten K, Humar A, Paterson DL, Wagener MM, Kusne S, Singh N. Changes in the spectrum and risk factors for invasive candidiasis in liver transplant recipients: prospective, multicenter, case-controlled study. *Transplantation* 2003; **75**: 2023-2029 [PMID: 12829905 DOI: 10.1097/01.TP.0000065178.93741.72]

15 **Singh N**, Avery RK, Munoz P, Pruett TL, Alexander B, Jacobs R, Tollemar JG, Dominguez EA, Yu CM, Paterson DL, Husain S, Kusne S, Linden P. Trends in risk profiles for and mortality associated with invasive aspergillosis among liver transplant recipients. *Clin Infect Dis* 2003; **36**: 46-52 [PMID: 12491201 DOI: 10.1086/345441]

16 **Gavalda J**, Len O, San Juan R, Aguado JM, Fortun J, Lumbreras C, Moreno A, Munoz P, Blanes M, Ramos A, Rufi G, Gurgui M, Torre-Cisneros J, Montejo M, Cuenca-Estrella M, Rodriguez-Tudela JL, Pahissa A; RESITRA (Spanish Network for Research on Infection in Transplantation). Risk factors for invasive aspergillosis in solid-organ transplant recipients: a case-control study. *Clin Infect Dis* 2005; **41**: 52-59 [PMID: 15937763 DOI: 10.1086/430602]

17 **Collins LA**, Samore MH, Roberts MS, Luzzati R, Jenkins RL, Lewis WD, Karchmer AW. Risk factors for invasive fungal infections complicating orthotopic liver transplantation. *J Infect Dis* 1994; **170**: 644-652 [PMID: 8077723]

18 **Utsumi M**, Umeda Y, Yagi T, Nagasaka T, Shinoura S, Yoshida R, Nobuoka D, Kuise T, Fuji T, Takagi K, Takaki A, Fujiwara T. Risk Analysis for Invasive Fungal Infection after Living Donor Liver Transplantation: Which Patient Needs Potent Prophylaxis? *Dig Surg* 2019; **36**: 59-66 [PMID: 29649828 DOI: 10.1159/000486548]

19 **Levesque E**, Paugam-Burtz C, Saliba F, Khoy-Ear L, Merle JC, Jung B, Stecken L, Ferrandiere M, Mihaila L, Botterel F. Fungal complications after Candida preservation fluid contamination in liver transplant recipients. *Transpl Int* 2015; **28**: 1308-1316 [PMID: 26147662 DOI: 10.1111/tri.12633]

20 **Singh N**, Wagener MM, Marino IR, Gayowski T. Trends in invasive fungal infections in liver transplant recipients: correlation with evolution in transplantation practices. *Transplantation* 2002; **73**: 63-67 [PMID: 11792979 DOI: 10.1097/00007890-200201150-00011]

21 **Jorgenson MR**, Gracon AS, Hanlon B, Leverson GE, Parajuli S, Smith JA, Al-Adra DP. Pre-transplant bariatric surgery is associated with increased fungal infection after liver transplant. *Transpl Infect Dis* 2021; **23**: e13484 [PMID: 33012079 DOI: 10.1111/tid.13484]

22 **Levesque E**, Rizk F, Noorah Z, Aït-Ammar N, Cordonnier-Jourdin C, El Anbassi S, Bonnal C, Azoulay D, Merle JC, Botterel F. Detection of (1,3)-β-d-Glucan for the Diagnosis of Invasive Fungal Infection in Liver Transplant Recipients. *Int J Mol Sci* 2017; **18** [PMID: 28422065 DOI: 10.3390/ijms18040862]

23 **Eschenauer GA**, Lam SW, Carver PL. Antifungal prophylaxis in liver transplant recipients. *Liver Transpl* 2009; **15**: 842-858 [PMID: 19642130 DOI: 10.1002/lt.21826]

24 **Playford EG**, Webster AC, Sorrell TC, Craig JC. Systematic review and meta-analysis of antifungal agents for preventing fungal infections in liver transplant recipients. *Eur J Clin Microbiol Infect Dis* 2006; **25**: 549-561 [PMID: 16912905 DOI: 10.1007/s10096-006-0182-3]

25 **Cruciani M**, Mengoli C, Malena M, Bosco O, Serpelloni G, Grossi P. Antifungal prophylaxis in liver transplant patients: a systematic review and meta-analysis. *Liver Transpl* 2006; **12**: 850-858 [PMID: 16628697 DOI: 10.1002/lt.20690]

26 **Evans JD**, Morris PJ, Knight SR. Antifungal prophylaxis in liver transplantation: a systematic review and network meta-analysis. *Am J Transplant* 2014; **14**: 2765-2776 [PMID: 25395336 DOI: 10.1111/ajt.12925]

27 **Saliba F**, Pascher A, Cointault O, Laterre PF, Cervera C, De Waele JJ, Cillo U, Langer RM, Lugano M, Göran-Ericzon B, Phillips S, Tweddle L, Karas A, Brown M, Fischer L; TENPIN (Liver Transplant European Study Into the Prevention of Fungal Infection) Investigators; TENPIN Liver Transplant European Study Into the Prevention of Fungal Infection Investigators. Randomized trial of micafungin for the prevention of invasive fungal infection in high-risk liver transplant recipients. *Clin Infect Dis* 2015; **60**: 997-1006 [PMID: 25520332 DOI: 10.1093/cid/ciu1128]

28 **Fortún J**, Muriel A, Martín-Dávila P, Montejo M, Len O, Torre-Cisneros J, Carratalá J, Muñoz P, Fariñas C, Moreno A, Fresco G, Goikoetxea J, Gavaldá J, Pozo JC, Bodro M, Vena A, Casafont F, Cervera C, Silva JT, Aguado JM; Grupo de Estudio de Infección en Pacientes Trasplantados-Grupo de Estudio de Micología Médica (Sociedad Española de Enfermedades Infecciosas y Microbiología Clínica), and Red Española de Investigación en Patología Infecciosa. Caspofungin versus fluconazole as prophylaxis of invasive fungal infection in high-risk liver transplantation recipients: A propensity score analysis. *Liver Transpl* 2016; **22**: 427-435 [PMID: 26709146 DOI: 10.1002/lt.24391]

29 **Antunes AM**, Teixeira C, Corvo ML, Perdigoto R, Barroso E, Marcelino P. Prophylactic use of liposomal amphotericin B in preventing fungal infections early after liver transplantation: a retrospective, single-center study. *Transplant Proc* 2014; **46**: 3554-3559 [PMID: 25498088 DOI: 10.1016/j.transproceed.2014.06.065]

30 **Winston DJ**, Limaye AP, Pelletier S, Safdar N, Morris MI, Meneses K, Busuttil RW, Singh N. Randomized, double-blind trial of anidulafungin versus fluconazole for prophylaxis of invasive fungal infections in high-risk liver transplant recipients. *Am J Transplant* 2014; **14**: 2758-2764 [PMID: 25376267 DOI: 10.1111/ajt.12963]

31 **Giannella M**, Ercolani G, Cristini F, Morelli M, Bartoletti M, Bertuzzo V, Tedeschi S, Faenza S, Puggioli C, Lewis RE, Pinna AD, Viale P. High-dose weekly liposomal amphotericin b antifungal prophylaxis in patients undergoing liver transplantation: a prospective phase II trial. *Transplantation* 2015; **99**: 848-854 [PMID: 25531982 DOI: 10.1097/TP.0000000000000393]

32 **Balogh J**, Gordon Burroughs S, Boktour M, Patel S, Saharia A, Ochoa RA, McFadden R, Victor DW, Ankoma-Sey V, Galati J, Monsour HP Jr, Fainstein V, Li XC, Grimes KA, Gaber AO, Aloia T, Ghobrial RM. Efficacy and cost-effectiveness of voriconazole prophylaxis for prevention of invasive aspergillosis in high-risk liver transplant recipients. *Liver Transpl* 2016; **22**: 163-170 [PMID: 26515643 DOI: 10.1002/lt.24365]

33 **Perrella A**, Esposito C, Amato G, Perrella O, Migliaccio C, Pisaniello D, Calise F, Cuomo O, Santaniello W. Antifungal prophylaxis with liposomal amphotericin B and caspofungin in high-risk patients after liver transplantation: impact on fungal infections and immune system. *Infect Dis (Lond)* 2016; **48**: 161-166 [PMID: 26513601 DOI: 10.3109/23744235.2015.1100322]

34 **Chen YC**, Huang TS, Wang YC, Cheng CH, Lee CF, Wu TJ, Chou HS, Chan KM, Lee WC, Soong RS. Effect of Prophylactic Antifungal Protocols on the Prognosis of Liver Transplantation: A Propensity Score Matching and Multistate Model Approach. *Biomed Res Int* 2016; **2016**: 6212503 [PMID: 27747235 DOI: 10.1155/2016/6212503]

35 **Giannella M**, Bartoletti M, Morelli M, Cristini F, Tedeschi S, Campoli C, Tumietto F, Bertuzzo V, Ercolani G, Faenza S, Pinna AD, Lewis RE, Viale P. Antifungal prophylaxis in liver transplant recipients: one size does not fit all. *Transpl Infect Dis* 2016; **18**: 538-544 [PMID: 27237076 DOI: 10.1111/tid.12560]

36 **Lavezzo B**, Patrono D, Tandoi F, Martini S, Fop F, Ballerini V, Stratta C, Skurzak S, Lupo F, Strignano P, Donadio PP, Salizzoni M, Romagnoli R, De Rosa FG. A simplified regimen of targeted antifungal prophylaxis in liver transplant recipients: A single-center experience. *Transpl Infect Dis* 2018; **20**: e12859 [PMID: 29427394 DOI: 10.1111/tid.12859]

37 **Jorgenson MR**, Descourouez JL, Marka NA, Leverson GE, Smith JA, Andes DR, Fernandez LA, Foley DP. A targeted fungal prophylaxis protocol with static dosed fluconazole significantly reduces invasive fungal infection after liver transplantation. *Transpl Infect Dis* 2019; **21**: e13156 [PMID: 31390109 DOI: 10.1111/tid.13156]

38 **Kang WH**, Song GW, Lee SG, Suh KS, Lee KW, Yi NJ, Joh JW, Kwon CHD, Kim JM, Choi DL, Kim JD, Kim MS. A Multicenter, Randomized, Open-Label Study to Compare Micafungin with Fluconazole in the Prophylaxis of Invasive Fungal Infections in Living-Donor Liver Transplant Recipients. *J Gastrointest Surg* 2020; **24**: 832-840 [PMID: 31066013 DOI: 10.1007/s11605-019-04241-w]

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MINIREVIEWS

Elastography as a predictor of liver cirrhosis complications after hepatitis C virus eradication in the era of direct-acting antivirals

Lucia Cerrito, Maria Elena Ainora, Alberto Nicoletti, Matteo Garcovich, Laura Riccardi, Maurizio Pompili, Antonio Gasbarrini, Maria Assunta Zocco

ORCID number: Lucia Cerrito 0000-0001-6837-7582; Maria Elena Ainora 0000-0001-5847-1065; Alberto Nicoletti 0000-0003-0658-4310; Matteo Garcovich 0000-0002-5805-7953; Laura Riccardi 0000-0001-6249-0314; Maurizio Pompili 0000-0001-6699-7980; Antonio Gasbarrini 0000-0002-7278-4823; Maria Assunta Zocco 0000-0002-0814-9542.

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Lucia Cerrito, Maria Elena Ainora, Alberto Nicoletti, Matteo Garcovich, Laura Riccardi, Maurizio Pompili, Antonio Gasbarrini, Maria Assunta Zocco, CEMAD Digestive Disease Center, Fondazione Policlinico Universitario "A. Gemelli" IRCCS, Catholic University of Rome (Italy), Rome 00168, Italy

Corresponding author: Maria Assunta Zocco, MD, PhD, Doctor, Senior Researcher, CEMAD Digestive Disease Center, Fondazione Policlinico Universitario "A. Gemelli" IRCCS, Catholic University of Rome (Italy), Largo Agostino Gemelli 8, Rome 00168, Italy.
mariaazocco@hotmail.com

Abstract

Chronic inflammation due to hepatitis C virus (HCV) infection leads to liver fibrosis and rearrangement of liver tissue, which is responsible for the development of portal hypertension (PH) and hepatocellular carcinoma (HCC). The advent of direct-acting antiviral drugs has revolutionized the natural history of HCV infection, providing an overall eradication rate of over 90%. Despite a significant decrease after sustained virological response (SVR), the rate of HCC and liver-related complications is not completely eliminated in patients with advanced liver disease. Although the reasons are still unclear, cirrhosis itself has a residual risk for the development of HCC and other PH-related complications. Ultrasound elastography is a recently developed non-invasive technique for the assessment of liver fibrosis. Following the achievement of SVR, liver stiffness (LS) usually decreases, as a consequence of reduced inflammation and, possibly, fibrosis. Recent studies emphasized the application of LS assessment in the management of patients with SVR in order to define the risk for developing the complications of chronic liver disease (functional decompensation, gastrointestinal bleeding, HCC) and to optimize long-term prognostic outcomes in clinical practice.

Key Words: Direct-acting antiviral agents; Liver stiffness; Portal hypertension; Hepatocellular carcinoma

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Core Tip: Direct-acting antiviral agents lead to hepatitis C virus eradication and to the regression of liver inflammation. However, they do not eliminate the risk of possible portal hypertension-related complications and hepatocellular carcinoma (HCC), increasing the necessity for post-sustained virological response surveillance and the development of non-invasive predictive models to detect the categories of patients requiring more intensive follow-up. Many studies reported a significant reduction in liver fibrosis markers after treatment with direct-acting antiviral drugs. Ultrasound elastography is gaining growing importance as a predictive element in the assessment of the risk of developing esophageal varices or gastrointestinal bleeding, liver functional decompensation and HCC.

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INTRODUCTION

Hepatitis C virus (HCV) infection is one of the major causes of chronic liver disease and a significant cause of morbidity and mortality worldwide[1]. In 2015, it was estimated that over 70 million people were affected, most of whom were unaware of the infection[2]. Chronic inflammation due to HCV infection leads to liver fibrosis and rearrangement of liver tissue, which is responsible for the development of portal hypertension (PH) and other complications. Moreover, inflammation and microenvironmental changes are known risk factors for the occurrence of hepatocellular carcinoma (HCC)[3].

The advent of direct-acting antiviral drugs (DAAs) has revolutionized the natural history of HCV infection, providing an overall eradication rate of over 90% associated with a remarkable safety profile in all stages of chronic liver disease[1].

The achievement of sustained virological response (SVR) prevents the development of cirrhosis in the early stages of the disease and significantly reduces the risk of HCC and PH-related events, such as ascites, hepatic encephalopathy, hepatorenal syndrome, infections and gastrointestinal bleeding, in patients with advanced liver disease[4-6]. However, initial reports have warned of an increased risk of HCC in patients who achieved SVR after treatments with DAAs[7,8]. On the other hand, other studies have shown a protective effect on the development of HCC[9,10]. More recently, a meta-analysis analyzing 41 studies concluded that there is no evidence for increased occurrence or recurrence of HCC in patients treated with DAAs compared with interferon-based therapies[11].

Despite a significant decrease after SVR, the rate of HCC and liver-related complications is not completely eliminated in patients with advanced liver disease. Although the reasons are still unclear, cirrhosis itself has a residual risk for the development of HCC and other PH-related complications[12]. At present, there are no validated predictors to estimate the risk of HCC and PH-related events after HCV eradication.

Ultrasound elastography is a recently developed non-invasive technique for the assessment of liver fibrosis. Vibration controlled transient elastography (VCTE), is the oldest share-wave-based method and the reference standard in this field. The device is equipped with a one-dimensional probe, where a vibrator sends low frequency shear waves through the liver. Wave propagation, evaluated by an ultrasound receiver inside the probe, is directly related to liver tissue elasticity. Since its emergence, this technique has provided a fast point-of-care estimate of liver fibrosis in daily clinical practice, avoiding the complications of liver biopsy[13]. Indeed, several studies using histology as the reference standard defined accurate thresholds that are able to distinguish the different stages of liver fibrosis[14]. In the last few years, new ultrasound based elastographic techniques have been developed. They are embedded into conventional ultrasound devices, allowing visualization of the sampling area. The two main categories are the point shear wave elastography (pSWE) and bidimensional SWE (2D-SWE)[13]. All these devices are able to evaluate the elastic properties of the

liver during real-time B mode imaging. In particular, the ultrasound probe generates short-duration acoustic impulses in a small region of interest that causes soft tissue displacement and shear waves running in the perpendicular plane. Shear wave travelling speed can then be quantified and interpreted as a measurement for liver stiffness (LS)[13].

To date, LS measurement (LSM) is recommended by the European Association for the Study of Liver Disease (EASL) and the American Association for the Study of Liver Disease (AASLD) guidelines for the assessment of liver disease severity in patients with HCV infection eligible for DAAs[1,15]. Following the achievement of SVR, LS usually decreases, as a consequence of reduced inflammation and, possibly, fibrosis [16-19]. Recent studies evaluated the usefulness of LS assessment after HCV eradication and the prediction of HCC and other PH-related complications in patients with advanced liver disease.

In this review, we summarize the current evidence on the role of ultrasound elastography in the prediction of liver-related outcomes of patients with HCV infection treated with DAAs.

DIRECT-ACTING ANTIVIRAL AGENTS AND LIVER FIBROSIS

Despite DAAs being pharmacologically designed only for the eradication of HCV infection and since HCV is directly responsible for liver injury and consequent parenchymal fibrosis, the achievement of both SVR and anti-fibrotic effect results in advantages in terms of prevention of chronic liver disease complications (Table 1).

Different non-invasive methods traditionally used to assess liver fibrosis such as VCTE and the Fibrosis-4 (FIB-4) score (based on patient's age, transaminases levels and platelet count) and aspartate aminotransferase to platelet ratio index (APRI score) have been evaluated for staging chronic liver disease and predicting hepatic fibrosis in patients with HCV infection.

It has been demonstrated that baseline LSM by VCTE together with FIB-4 and APRI score have an important role in the prediction of treatment outcome in the new era of DAAs and could be integrated in pre-treatment assessment as a guide for treatment decisions and optimization of patient management[20,21].

Many authors have documented the improvement of VCTE, FIB-4 and APRI score after DAAs treatment. However, it is not clear if this finding is a true recovery of liver fibrosis or represents only an epiphenomenon of the reduction in liver inflammation resulting in the normalization of blood tests and decrease of LS values[22-25]. The retrospective study by Elsharkawy *et al*[26] analyzed a group of 337 Egyptian patients with chronic genotype 4 HCV infection who underwent sofosbuvir-based treatments. Among the patients evaluated, 29.1% had non-relevant fibrosis (F0-1; VCTE < 7.1 kPa), 17.2% were included in the F2 group (7.1 kPa ≤ VCTE < 9.5 kPa), 8.6% in the F3 group (VCTE ≥ 9.5 kPa) and 45.1% were classified as cirrhotic (F4; ≥ 12.5 kPa). One year after treatment, 77% of responders (with any stage fibrosis) and 81.8% of cirrhotic patients had a valuable recovery in liver fibrosis parameters (measured with FIB-4 and APRI score), due to the increase in platelet count and decrease in transaminase levels together with a reduction in LS values (11.8 ± 8.8 kPa *vs* 14.8 ± 10.7 kPa, $P = 0.000$). A higher number of patients with poor LS improvement after DAAs-therapy was observed in cases with low baseline LS values and infection relapse.

In a group of 42 patients treated with DAAs, Chekuri *et al*[27] demonstrated a significant decrease in LS values at SVR 24 wk after the end of treatment (median values: 10.40 kPa *vs* 7.60 kPa, $P < 0.01$), without significant improvement in the follow-up.

Abdel Alem *et al*[28] used pre-treatment liver fibrosis (measured by VCTE and FIB-4 score) as a predictor of treatment outcome after sofosbuvir-based regimens in 7256 HCV patients (46.6% cirrhotic, 91.4% with SVR12). Both, baseline FIB-4 and VCTE were significantly lower in the group with SVR (2.66 ± 1.98 kPa and 17.8 ± 11.5 kPa, respectively) compared to relapsers (4.02 ± 3.3 kPa and 24.5 ± 13.9 kPa, respectively). Based on these results, the authors concluded that fibrosis stage is a crucial element in the evaluation of treatment outcome and disease prognosis. In particular, a LS value higher than 16.7 kPa resulted as an unfavorable prognostic factor for treatment response (relapse rate 13%), probably related to an impaired immune-mediated HCV clearance that is worsened in advanced liver fibrosis. Similar considerations were drawn by Neukam *et al*[29] in patients treated with pegylated interferon/ribavirin-based therapy associated with NS3/4A protease inhibitor (PR-PI) and patients under DAAs therapy. In the PR-PI group, SVR12 was obtained in 59.6% of patients with LS < 21 kPa and in 46.5% of subjects with LS ≥ 21 kPa ($P = 0.064$); in the DAAs group,

Table 1 Liver stiffness improvement after treatment with direct acting antivirals

Ref.	Study design	Number of Patients	Drugs	Patients with LS improvement (%)	Pre-treatment LS	Post-treatment LS	*P* value	Measurement
Elsharkawy *et al*[26], 2017	Retrospective	337	DAA	81.8% (cirrhotic) 71.7% (non-cirrhotic)	14.8 ± 10.7 kPa	11.8 ± 8.8 kPa	0.000	Fibroscan
Chekuri *et al* [27], 2016	Observational	100	IFN-based and DAA	NA	10.40 kPa	7.60 kPa	< 0.01	Fibroscan
Bachofner *et al* [30], 2017	Multicenter, observational	392	DAA	93%	12.65 kPa	8.55 kPa	< 0.001	Fibroscan
Afdhal *et al* [39], 2017	Prospective	52	DAA	59.6%	15.2 kPa	9.3 kPa (6.7–16.8 kPa)	< 0.0001	Fibroscan
Ravaioli *et al* [68], 2018	Retrospective	139	DAA	44.6% (LS reduction > 30%)	18.6 kPa (15-26.3 kPa)	13.8 kPa (10.4-20.4 kPa)	< 0.001	Fibroscan
Pan *et al*[70], 2018	Retrospective	84	DAA	62%	Fibrosis regression by at least two stages: Cirrhosis group (48%); F3 fibrosis group (39%)		-	Fibroscan

DAA: Direct acting antivirals; IFN: Interferon; LS: Liver stiffness; NA: Not applicable.

SVR12 was reached by 95.3% of patients with LS < 21 kPa and 87.4% of patients with ≥ 21 kPa. Relapse rates after an apparent end-of-treatment response were 4.8% *vs* 17.9% in patients treated with PR-PI and 2.4% *vs* 8.2% in the DAAs group, respectively, for LS < 21 kPa and ≥ 21 kPa. These results suggest that LS evaluation might be useful to avoid HCV-relapse in cirrhotic patients by choosing both the appropriate composition and duration of DAAs-therapy.

Many studies reported a significant reduction in liver fibrosis markers after treatment with DAAs. In particular, Bachofner *et al*[30] highlighted a 32.4% drop in VCTE values from 12.65 kPa to 8.55 kPa ($P < 0.001$), a reduction of FIB-4 from 2.54 to 1.80 ($P < 0.001$) and a decrease of APRI from 1.10 to 0.43 ($P < 0.001$).

DIRECT-ACTING ANTIVIRAL AGENTS AND LIVER CIRRHOSIS RELATED EVENTS

Even though DAA-therapy leads to HCV eradication and to the regression of liver inflammation, it does not eliminate the risk of possible PH-related complications and HCC, increasing the necessity for post-SVR surveillance and the development of non-invasive predictive models to detect the categories of patients requiring more intensive follow-up (Table 2).

To this purpose, Trivedi *et al*[31] suggested a VCTE-based algorithm in order to schedule the controls of patients with SVR after HCV eradication: In the case of mild fibrosis (F1) without liver-related comorbidities, regular monitoring with the primary care physician is indicated; for advanced fibrosis/cirrhosis (F3-4), routine HCC and variceal surveillance is prescribed (six-monthly ultrasound, upper endoscopy every 2-3 years, annual non-invasive fibrosis assessment); for moderate fibrosis (F2) or in the case of concomitant liver-related comorbidities an annual non-invasive fibrosis measurement should be performed.

The importance of liver fibrosis stage in the development of liver-related complications was confirmed by Kozbial *et al*[32], who analyzed 551 patients treated with DAAs for a median period of 65.6 wk: No complications were registered in patients with severe fibrosis, whereas 9.1% of subjects with compensated cirrhosis developed liver-associated complications including HCC (4.1%). Furthermore, the presence of decompensated cirrhosis was markedly associated with the development of complications and mortality.

Even though histology remains the gold standard in evaluating fibrosis, liver biopsy presents some potential obstacles such as patient compliance, severe post-procedural complications, and sampling errors. For this reason, elastography has been proposed as a possible non-invasive alternative to biopsy for patient surveillance after SVR[33-35].

Table 2 Direct-acting antiviral agents and liver cirrhosis related events

Ref.	Study design	Number of patients	Drugs	HCC	Portal hypertension-related complications
Kozbial *et al* [32], 2018	Prospective	551	DAA	16 (4.1%)	Ascites: 3.1%; variceal hemorrhage: 1%; hepatic encephalopathy: 0%
Masuzaki *et al* [36], 2009	Prospective	984	DAA	77 (2.9% *per* 1 person-year); HCC risk: 45.5 times higher in LS > 25 kPa	NA
Afdhal *et al* [39], 2017	Prospective	50	DAA	LS improvement in patients who did not develop HCC during follow-up (42.6% reduction in patients without HCC *vs* 13.6% in HCC group)	24% patients had ≥ 20% decreases in HVPG during treatment (89% subjects with baseline HVPG ≥ 12 mmHg had a ≥ 20% reduction in HVPG after SVR)
Giannini *et al* [51], 2019	Prospective	52	DAA	4 (7.7%)	Clinical decompensation: 0%
Tachi *et al*[58], 2017	Prospective	263	DAA	19 (7.2%)	NA
Foster *et al* [60], 2016	Retrospective, observational	467	DAA	NA	MELD improvement (0.85, SD 2.54); composite adverse outcome in 52.0% (treated) *vs* 61.7% (untreated)
Rinaldi *et al* [63], 2019	Multicenter, prospective	258	DAA	35 (13.6%)	NA
Ravaioli *et al* [68], 2018	Retrospective	139	DAA	20 (14.4%)	NA
Pan *et al*[70], 2018	Retrospective	84	DAA	4 (4.8%)	NA
Toyoda *et al* [75], 2015	Retrospective/prospective	522	IFN-based	18 (1.2% after five yr; 4.3% after ten yr)	NA
D'Ambrosio *et al*[77], 2018	Prospective	38	DAA	5 (13%)	Clinical decompensation: 0%
Lleo *et al*[78], 2019	Prospective	1927	DAA	Previous HCC: 38/161 (recurrence rate: 24.8 *per* 100-yr); No previous HCC: 50/1766 (incidence rate: 2.4 *per* 100-yr)	NA
Hamada *et al* [79], 2018	Retrospective	196	DAA	8 (4.1%)	NA

DAA: Direct acting antivirals; HCC: Hepatocellular carcinoma; HVPG: Hepatic venous pressure gradient; IFN: Interferon; LS: Liver stiffness; MELD: Model for end-stage liver disease; NA: Not applicable; SD: Standard deviation; SVR: Sustained virological response.

VCTE is gaining growing importance as a predictive element in the assessment of the risk of developing esophageal varices or gastrointestinal bleeding, liver functional decompensation and HCC[36]. The retrospective study by Mandorfer *et al*[37] was the first to compare Hepatic Venous Pressure Gradient (HVPG) measurement with VCTE for the assessment of PH and showed a good agreement between the techniques. The authors also observed that a PH decrease after SVR was less likely in subjects with baseline HVPG higher than 16 mmHg and severe liver function impairment.

The review by Garbuzenko *et al*[38] confirmed that staging the severity of PH in cirrhotic subjects and personalized preventive therapy could lead to an increase in both patient survival and treatment effectiveness; particularly, DAAs achieve the amelioration of subclinical PH. In a recent study by Afdhal *et al*[39] of 50 patients with clinically significant PH (presence of esophageal varices, HVPG > 6 mmHg) from different international centers, 89% obtained a HVPG reduction of > 20% and only 3 patients obtained a reduction of portal pressure to less than 12 mmHg.

Paternostro *et al*[40] endorsed spleen stiffness measurement (SSM) through elastography (especially pSWE and 2D-SWE) as an effective tool for high-risk varices assessment in chronic liver disease, especially in distinguishing between small and large varices as confirmed by Sharma *et al*[41]. Previously, both Colecchia *et al*[42] and Fraquelli *et al*[43] had underlined the efficacy of LSM and SSM association in the assessment of HVPG and prediction of gastroesophageal varices in cirrhotic patients, showing a very high sensitivity (98% and 100% in the two studies, respectively), and economic advantages following the implementation of endoscopic screening progr-

ams. However, there are some important limitations related to SSM: It is an operator-dependent measurement and the upper limit of VCTE is fixed to a fibrosis value of 75 kPa that, in the case of severe PH, could be widely exceeded by SSM unlike LSM. Concerning the latter issue, Calvaruso *et al*[44] demonstrated the superior predictive value of SSM for high-risk varices, adopting a modified VCTE unit with a maximum stiffness value of 150 kPa (AUC: 0.80 for SSM *vs* 0.71 for LSM).

It has been demonstrated that the association of LSM with other non-invasive items (*e.g.* platelets, SSM) has a powerful positive predictive value in the detection of esophageal varices: Stefanescu *et al*[45] created a simple diagnostic algorithm with the combination of LSM and SSM (cut-off: 19 kPa and 55 kPa, respectively), thus reaching a 93% sensibility and a 95% positive predictive value.

Wang *et al*[46] observed that the combination of Baveno VI criteria with SSM (with 46 kPa cut-off) might help to avoid 61.6% of esophagogastroduodenoscopies in HBV-related cirrhosis with persistent viral suppression due to antiviral therapy, missing less than 5% high-risk varices.

An interesting analysis by Fofiu *et al*[47] evaluated a score based on the combination of LSM, SSM and spleen size as non-invasive predictors of high-risk varices in compensated cirrhosis, proving a better performance of the association of the three elements compared to each parameter alone. However, a meta-analysis by Ma *et al*[48] found that SSM alone is superior to LSM in predicting any grade esophageal varices, thus turning out to be useful in clinical practice, especially in the case of non-measurable LSM (multifocal HCC, biliary obstruction or liver metastasis).

Semmler *et al*[49] underlined the predictive value of LSM by VCTE included in a non-invasive algorithm together with von Willebrand factor-platelet count ratio as a useful method to define PH, stratify risk categories and predict liver decompensation and HCC development in patients with HCV-related advanced chronic liver disease treated with DAAs. These results could be very interesting in introducing the concept of a tailored follow-up strategy.

It is still not clear if the improvement in non-invasive markers after SVR could be associated to a decline in PH itself. However, in a recent study, Thabut *et al*[50] noted that subjects with previous unfavorable Baveno VI status (LS > 20 kPa, platelets < 150000/mm^3) who experienced platelets increase and/or LS reduction after SVR reached a favorable Baveno VI class, with a subsequent reduction in the probability of PH progression and development of esophageal varices. A decrease of PH has also been demonstrated by Giannini *et al*[51] in a group of 52 patients with advanced fibrosis/cirrhosis at baseline followed for approximately 60 wk after SVR with DAAs. A significant improvement in HVPG was detected, together with a decrease in LS values (from 15.2 kPa at baseline to 9.3 kPa at the end of follow-up), APRI and FIB-4 score, spleen bipolar diameter and an increase in platelet count[37].

As the role of these indices is quite limited, other non-invasive methods have been proposed to detect varices at high risk of bleeding: Considering the worldwide low availability of TE, Jangouk *et al*[52] demonstrated the effectiveness of Baveno VI consensus criteria as a non-invasive method to identify patients with compensated liver cirrhosis and low-risk of varices requiring endoscopic treatment. In particular, the authors highlight the uppermost role of both platelet count (> 150000/mm^3) and MELD score (< 6) in defining a low probability of high-risk varices.

Chen *et al*[53] demonstrated the efficacy and extremely high negative predictive value (97.1% in the study group and 98.1% in the validation cohort) of the association of albumin-bilirubin grade with platelet count (ALBI-PLT score) in the screening of high-risk esophageal varices in subjects with HCC: The 5-year variceal hemorrhage rate was 9.7% in patients with ALBI-PLT score > 2 (decompensated liver disease) as compared to 1.7% in those with a score of 2 (P = 0.007).

Baveno VI guidelines indicate platelet count and VCTE as effective elements in the identification of cirrhotic patients who are at high-risk of developing esophageal varices: Due to the not-always easy access to VCTE (for example, in the case of inmates) or to the unavailability of adequate instrumentation in all hepatological centers, Calvaruso *et al*[54] proposed the "Rete Sicilia Selezione Terapia–HCV" algorithm as an effective and simple tool (based only on blood tests: Platelet count and serum albumin level) that could substitute Baveno VI criteria in the identification of HCV-cirrhotic patients with medium/large varices, thus simplifying the diagnosis of the complications of PH, with a reduction of more than 30% of useless endoscopic exams and diminishing the risk of false-negative results.

The implications of HCV eradication on HCC development are even more complex. Despite the widely demonstrated efficacy of DAAs in both achieving SVR and a reduction in liver fibrosis, there is no corresponding decrease in HCC development risk. These data led to an initial alert claiming the possibility of a DAAs-driven

oncogenic mechanism[7], even if this theory was subsequently proved wrong by other studies[11]. The mechanism of HCC development post SVR is probably sustained by a "point of no-return" in HCV pathogenesis that determines the loss of the potential benefits brought by viral eradication[55]. This evidence highlights the necessity for optimizing regular HCC surveillance with a particular focus on patients with advanced fibrosis or cirrhosis[56]. In fact, even though a decrease in LS values from cirrhosis to advanced fibrosis was observed in some cases after DAAs therapy, patients with SVR maintained an elevated HCC risk[57,58].

Whether the HCC risk of patients with SVR coincides with that of viremic subjects is still a matter of debate. In the case of precariously compensated or decompensated liver function, the achievement of SVR could be useful to reduce the risk of HCC because of the decrease in intrahepatic inflammatory processes, despite the persistence of PH and decompensated liver function (that increase the risk of liver cancer in cirrhotic patients)[59,60].

Both EASL and AASLD guidelines recommend continuing ultrasound surveillance in subjects with advanced fibrosis/cirrhosis despite histological response to treatment and suggest accurate definition of the additional baseline risk-factors profile[61,62].

Rinaldi *et al*[63] assessed the importance of both baseline LS evaluation and ultrasound liver surveillance for the risk of HCC in patients with HCV-related cirrhosis, treated with DAAs: Among 258 subjects enrolled, divided into three groups according to liver fibrosis stage (< 20 kPa, from 20 kPa to 30 kPa, > 30 kPa), 35 developed HCC during follow-up. The group with LS higher than 30 kPa had a statistically significant increase in HCC risk [HR (95%CI): 0.329 (0.131-0.830); $P = 0.019$].

Even though the mechanisms directly involving HCV in both fibrogenesis and oncogenesis have not yet been completely explained, it seems crucial to define the degree of liver fibrosis through VCTE and FIB-4, in order to set appropriate HCC screening and the subsequent therapeutic strategy[64,65].

Many attempts have been made to create prognostic scores to evaluate the risk of HCC development in chronic liver diseases, considering other criteria than PH alone [66]. An interesting example is represented by the King score that includes laboratory parameters (platelet count and bilirubin levels) and gene signature, and classifies cirrhotic patients with HCV infection into three risk categories for functional decompensation, HCC and death. However, it is not clear if this score maintains its predictive efficacy in patients with SVR[67].

Ravaioli *et al*[68] studied 139 cirrhotic patients treated with DAAs, analyzing the difference between LS at baseline and at the end of treatment: They found a lower reduction of LS in patients who developed HCC compared to patients who did not (-18.0% *vs* -28.9%, $P = 0.005$).

Recent studies demonstrated that LS assessment after SVR could be an inaccurate method to define the grade of fibrosis in patients treated with DAAs. In fact, the fast modifications in LS could be determined by both the reduction of liver inflammatory activity and the narrowing of fibrotic septa, without real histological improvement in fibrosis grading as demonstrated by liver biopsy[69-71]. Notwithstanding, LS evaluation by VCTE remains a cornerstone in the assessment of HCC risk after SVR, especially due to its non-invasiveness.

Masuzaki *et al*[36] demonstrated that HCC risk was 45.5 times higher in patients with LS values higher than 25 kPa.

However, it becomes important in the association to other elements in a more complete non-invasive score. Among them, we can include: Age, alcohol abuse, pre-treatment advanced fibrosis/cirrhosis, platelet count, steatosis, diabetes, alfa fetoprotein (AFP), baseline gamma-glutamyltransferase (GGT) levels together with ethnic and environmental factors. All these factors have been studied in patients treated with interferon-based therapies with interesting results[72-76]. During the pre-DAAs era, studies on the complications of liver cirrhosis after HCV-treatment showed that SVR and fibrosis regression did not prevent hepatic carcinogenesis. D'Ambrosio *et al*[77] found that 13% of patients who responded to interferon-based treatments, developed HCC during an 8-year follow-up (17% cumulative probability and 1.2% annual incidence rate) whereas neither variceal-bleeding nor liver-function decompensation occurred. Higher baseline levels of GGT and glycemia were identified as risk factors for HCC development. Similarly, Toyoda *et al*[75] demonstrated that diabetes mellitus and FIB-4 index increase represent risk factors for HCC after SVR with interferon-based regimens, thus suggesting continuing active surveillance in these groups of patients.

In a prospective analysis of 1927 patients with HCV-related cirrhosis, receiving DAAs in ten tertiary Italian liver centers, Lleo *et al*[78] observed a recurrence rate of HCC of 24.8 *per* 100 patients/year and a *de novo* occurrence rate of 2.4 *per* 100

patients/year. They found that treatment failure and high AFP levels represent independent predictors of HCC development, while SVR and absence of PH are associated with a lower HCC incidence, suggesting that HCC risk stratification should rely on the presence of PH and elevated baseline AFP levels.

It has been suggested that PH as a complication of liver fibrosis (more than fibrosis itself) may represent an independent risk factor for HCC[66]. Afdhal *et al*[39] analyzed 50 patients with HCV-related liver cirrhosis treated with DAAs and observed a significant reduction in HVPG values during long-term follow-up after SVR: 24% of all patients and 89% of subjects with baseline HVPG ≥ 12 mmHg who reached SVR had a ≥ 20% reduction in HVPG. With regard to LS, a more evident improvement was observed in patients who did not develop HCC during follow-up (42.6% reduction in patients without HCC *vs* 13.6% in the HCC group), thus proposing a protective role of HVPG and LS against HCC development.

In a recent retrospective study performed in patients with SVR after DAAs, Hamada *et al*[79], identified six variables that could be included in the HCC prediction model: Age, body mass index, platelet count, albumin, AFP, LS and FIB-4 index. Following multivariate analysis they found that age ≥ 75 years, AFP ≥ 6 ng/mL, and LS ≥ 11 kPa were independent risk factors for hepatocarcinogenesis (risk ratio: 35.16, 43.30 and 28.71, respectively; P = 0.001, 0.003 and 0.006, respectively). In particular, patients with LS < 11 kPa had a cumulative HCC incidence of 1.3% at 12 mo, 24 mo, 36 mo and 48 mo, while in the group with LS > 11 kPa the HCC incidence rate was 4.6% at 12 mo and 24 mo, 24.8% at 36 mo and 62.4% at 48 mo.

The role of LSM in the development of a prediction model for HCC has also been emphasized by Feier *et al*[80]. They confirmed that high levels of AFP, transaminases and LS are excellent predictors of HCC but underlined the importance of interquartile range (IQR) in LSMs. This led to the hypothesis of "stiffness shadow" that indicated an inhomogeneous shear stress due to the chaotic tumoral growth in the already hard cirrhotic tissue, with relevant diagnostic repercussions[81,82]. The overall prognostic model combining the four variables demonstrated relevant results both in the training and validation phase with a positive relation with tumor size. The four parameters together showed a 64.5% HCC prediction, with LS alone reaching the highest predictive power. The authors concluded that an elevation in LS values and IQR during follow-up could enhance the diagnostic skill towards early HCC[80].

It is interesting to note that some genetic factors also seem to be involved in hepatocarcinogenesis, despite the lack of clear evidence and the need for further prospective studies.

In their cohort of 200 patients with HCV-related cirrhosis with SVR after DAAs, Simili *et al*[83] noted a strong association of the single-nucleotide polymorphism of interleukin 28 (IL28B–rs12979860) with HCC development (both *de novo* and disease recurrence); furthermore, they observed a relation of HCC with lower levels of serum retinol and the presence of another two polymorphisms: Major histocompatibility complex class I polypeptide-related sequence A gene (*MICA*) and tolloid-like 1. The latter has proven particularly controversial since its oncogenic role was stated by Matsuura *et al*[84] but denied by Degasperi *et al*[85]: The difference between these studies could be ascribed to the different allele frequency or the presence of still unknown cofactors in the two ethnic groups (Japanese and Caucasian) or to discrepancies in the length of the follow-up period.

CONCLUSION

DAAs-therapy has brought about an effective revolution in hepatology resulting in HCV eradication in a wide range of patients and eventually reducing liver fibrosis after SVR. However, these benefits have not erased the risk of developing liver disease-related complications and in particular HCC and PH associated events. For this reason, it is crucial to continue long-term systematic surveillance after HCV eradication focusing on the subjects with a high-risk score.

Due to its accuracy, cost-effectiveness and non-invasiveness, together with specific clinical and laboratory parameters, LSM is gaining a relevant role in the construction of algorithms assessing both liver fibrosis and PH. The potential application of this non-invasive and simple method has been emphasized especially in the management of patients with SVR in order to define the risk to develop the complications of chronic liver disease (functional decompensation, gastrointestinal bleeding, HCC) and optimize long-term prognostic outcomes in clinical practice.

REFERENCES

1 **European Association for the Study of the Liver**; Clinical Practice Guidelines Panel: Chair:; EASL Governing Board representative:; Panel members:. EASL recommendations on treatment of hepatitis C: Final update of the series☆. *J Hepatol* 2020; **73**: 1170-1218 [PMID: 32956768 DOI: 10.1016/j.jhep.2020.08.018]

2 **Polaris Observatory HCV Collaborators**. Global prevalence and genotype distribution of hepatitis C virus infection in 2015: a modelling study. *Lancet Gastroenterol Hepatol* 2017; **2**: 161-176 [PMID: 28404132 DOI: 10.1016/S2468-1253(16)30181-9]

3 **Westbrook RH**, Dusheiko G. Natural history of hepatitis C. *J Hepatol* 2014; **61**: S58-S68 [PMID: 25443346 DOI: 10.1016/j.jhep.2014.07.012]

4 **Bruno S**, Di Marco V, Iavarone M, Roffi L, Crosignani A, Calvaruso V, Aghemo A, Cabibbo G, Viganò M, Boccaccio V, Craxí A, Colombo M, Maisonneuve P. Survival of patients with HCV cirrhosis and sustained virologic response is similar to the general population. *J Hepatol* 2016; **64**: 1217-1223 [PMID: 27059129 DOI: 10.1016/j.jhep.2016.01.034]

5 **van der Meer AJ**, Feld JJ, Hofer H, Almasio PL, Calvaruso V, Fernández-Rodríguez CM, Aleman S, Ganne-Carrié N, D'Ambrosio R, Pol S, Trapero-Marugan M, Maan R, Moreno-Otero R, Mallet V, Hultcrantz R, Weiland O, Rutter K, Di Marco V, Alonso S, Bruno S, Colombo M, de Knegt RJ, Veldt BJ, Hansen BE, Janssen HLA. Risk of cirrhosis-related complications in patients with advanced fibrosis following hepatitis C virus eradication. *J Hepatol* 2017; **66**: 485-493 [PMID: 27780714 DOI: 10.1016/j.jhep.2016.10.017]

6 **Nahon P**, Bourcier V, Layese R, Audureau E, Cagnot C, Marcellin P, Guyader D, Fontaine H, Larrey D, De Lédinghen V, Ouzan D, Zoulim F, Roulot D, Tran A, Bronowicki JP, Zarski JP, Leroy V, Riachi G, Calès P, Péron JM, Alric L, Bourlière M, Mathurin P, Dharancy S, Blanc JF, Abergel A, Serfaty L, Mallat A, Grangé JD, Attali P, Bacq Y, Wartelle C, Dao T, Benhamou Y, Pilette C, Silvain C, Christidis C, Capron D, Bernard-Chabert B, Zucman D, Di Martino V, Thibaut V, Salmon D, Ziol M, Sutton A, Pol S, Roudot-Thoraval F; ANRS CO12 CirVir Group. Eradication of Hepatitis C Virus Infection in Patients With Cirrhosis Reduces Risk of Liver and Non-Liver Complications. *Gastroenterology* 2017; **152**: 142-156.e2 [PMID: 27641509 DOI: 10.1053/j.gastro.2016.09.009]

7 **Reig M**, Mariño Z, Perelló C, Iñarrairaegui M, Ribeiro A, Lens S, Díaz A, Vilana R, Darnell A, Varela M, Sangro B, Calleja JL, Forns X, Bruix J. Unexpected high rate of early tumor recurrence in patients with HCV-related HCC undergoing interferon-free therapy. *J Hepatol* 2016; **65**: 719-726 [PMID: 27084592 DOI: 10.1016/j.jhep.2016.04.008]

8 **Kozbial K**, Moser S, Schwarzer R, Laferl H, Al-Zoairy R, Stauber R, Stättermayer AF, Beinhardt S, Graziadei I, Freissmuth C, Maieron A, Gschwantler M, Strasser M, Peck-Radosalvjevic M, Trauner M, Hofer H, Ferenci P. Unexpected high incidence of hepatocellular carcinoma in cirrhotic patients with sustained virologic response following interferon-free direct-acting antiviral treatment. *J Hepatol* 2016; **65**: 856-858 [PMID: 27318327 DOI: 10.1016/j.jhep.2016.06.009]

9 **ANRS collaborative study group on hepatocellular carcinoma (ANRS CO22 HEPATHER CO12 CirVir and CO23 CUPILT cohorts)**. Lack of evidence of an effect of direct-acting antivirals on the recurrence of hepatocellular carcinoma: Data from three ANRS cohorts. *J Hepatol* 2016; **65**: 734-740 [PMID: 27288051 DOI: 10.1016/j.jhep.2016.05.045]

10 **Romano A**, Angeli P, Piovesan S, Noventa F, Anastassopoulos G, Chemello L, Cavalletto L, Gambato M, Russo FP, Burra P, Vincenzi V, Scotton PG, Panese S, Tempesta D, Bertin T, Carrara M, Carlotto A, Capra F, Carolo G, Scroccaro G, Alberti A. Newly diagnosed hepatocellular carcinoma in patients with advanced hepatitis C treated with DAAs: A prospective population study. *J Hepatol* 2018; **69**: 345-352 [PMID: 29551707 DOI: 10.1016/j.jhep.2018.03.009]

11 **Waziry R**, Hajarizadeh B, Grebely J, Amin J, Law M, Danta M, George J, Dore GJ. Hepatocellular carcinoma risk following direct-acting antiviral HCV therapy: A systematic review, meta-analyses, and meta-regression. *J Hepatol* 2017; **67**: 1204-1212 [PMID: 28802876 DOI: 10.1016/j.jhep.2017.07.025]

12 **Mallet V**, Gilgenkrantz H, Serpaggi J, Verkarre V, Vallet-Pichard A, Fontaine H, Pol S. Brief communication: the relationship of regression of cirrhosis to outcome in chronic hepatitis C. *Ann Intern Med* 2008; **149**: 399-403 [PMID: 18794559 DOI: 10.7326/0003-4819-149-6-200809160-00006]

13 **Dietrich CF**, Bamber J, Berzigotti A, Bota S, Cantisani V, Castera L, Cosgrove D, Ferraioli G, Friedrich-Rust M, Gilja OH, Goertz RS, Karlas T, de Knegt R, de Ledinghen V, Piscaglia F, Procopet B, Saftoiu A, Sidhu PS, Sporea I, Thiele M. EFSUMB Guidelines and Recommendations on the Clinical Use of Liver Ultrasound Elastography, Update 2017 (Long Version). *Ultraschall Med* 2017; **38**: e48 [PMID: 30176678 DOI: 10.1055/a-0641-0076]

14 **Friedrich-Rust M**, Ong MF, Martens S, Sarrazin C, Bojunga J, Zeuzem S, Herrmann E. Performance of transient elastography for the staging of liver fibrosis: a meta-analysis. *Gastroenterology* 2008; **134**: 960-974 [PMID: 18395077 DOI: 10.1053/j.gastro.2008.01.034]

15 **Ghany MG**, Morgan TR; AASLD-IDSA Hepatitis C Guidance Panel. Hepatitis C Guidance 2019 Update: American Association for the Study of Liver Diseases-Infectious Diseases Society of America Recommendations for Testing, Managing, and Treating Hepatitis C Virus Infection. *Hepatology* 2020; **71**: 686-721 [PMID: 31816111 DOI: 10.1002/hep.31060]

16 **Fernandes FF**, Piedade J, Guimaraes L, Nunes EP, Chaves U, Goldenzon RV, Cardoso SW, Duarte J, Grinsztejn B, Veloso VG, Pereira G, Perazzo H. Effectiveness of direct-acting agents for hepatitis C

and liver stiffness changing after sustained virological response. *J Gastroenterol Hepatol* 2019; **34**: 2187-2195 [PMID: 31062880 DOI: 10.1111/jgh.14707]

17 **Pons M**, Rodríguez-Tajes S, Esteban JI, Mariño Z, Vargas V, Lens S, Buti M, Augustin S, Forns X, Mínguez B, Genescà J. Non-invasive prediction of liver-related events in patients with HCV-associated compensated advanced chronic liver disease after oral antivirals. *J Hepatol* 2020; **72**: 472-480 [PMID: 31629779 DOI: 10.1016/j.jhep.2019.10.005]

18 **Alonso López S**, Manzano ML, Gea F, Gutiérrez ML, Ahumada AM, Devesa MJ, Olveira A, Polo BA, Márquez L, Fernández I, Cobo JCR, Rayón L, Riado D, Izquierdo S, Usón C, Real Y, Rincón D, Fernández-Rodríguez CM, Bañares R. A Model Based on Noninvasive Markers Predicts Very Low Hepatocellular Carcinoma Risk After Viral Response in Hepatitis C Virus-Advanced Fibrosis. *Hepatology* 2020; **72**: 1924-1934 [PMID: 33022803 DOI: 10.1002/hep.31588]

19 **Lens S**, Baiges A, Alvarado-Tapias E, LLop E, Martinez J, Fortea JI, Ibáñez-Samaniego L, Mariño Z, Rodríguez-Tajes S, Gallego A, Bañares R, Puente Á, Albillos A, Calleja JL, Torras X, Hernández-Gea V, Bosch J, Villanueva C, García-Pagán JC, Forns X. Clinical outcome and hemodynamic changes following HCV eradication with oral antiviral therapy in patients with clinically significant portal hypertension. *J Hepatol* 2020; **73**: 1415-1424 [PMID: 32535060 DOI: 10.1016/j.jhep.2020.05.050]

20 **Yosry A**, Fouad R, Alem SA, Elsharkawy A, El-Sayed M, Asem N, Hassan E, Ismail A, Esmat G. FibroScan, APRI, FIB4, and GUCI: Role in prediction of fibrosis and response to therapy in Egyptian patients with HCV infection. *Arab J Gastroenterol* 2016; **17**: 78-83 [PMID: 27353055 DOI: 10.1016/j.ajg.2016.05.002]

21 **Bonnard P**, Elsharkawy A, Zalata K, Delarocque-Astagneau E, Biard L, Le Fouler L, Hassan AB, Abdel-Hamid M, El-Daly M, Gamal ME, El Kassas M, Bedossa P, Carrat F, Fontanet A, Esmat G. Comparison of liver biopsy and noninvasive techniques for liver fibrosis assessment in patients infected with HCV-genotype 4 in Egypt. *J Viral Hepat* 2015; **22**: 245-253 [PMID: 25073725 DOI: 10.1111/jvh.12285]

22 **Ogasawara N**, Kobayashi M, Akuta N, Kominami Y, Fujiyama S, Kawamura Y, Sezaki H, Hosaka T, Suzuki F, Saitoh S, Suzuki Y, Arase Y, Ikeda K, Kumada H. Serial changes in liver stiffness and controlled attenuation parameter following direct-acting antiviral therapy against hepatitis C virus genotype 1b. *J Med Virol* 2018; **90**: 313-319 [PMID: 28906010 DOI: 10.1002/jmv.24950]

23 **Tada T**, Kumada T, Toyoda H, Mizuno K, Sone Y, Kataoka S, Hashinokuchi S. Improvement of liver stiffness in patients with hepatitis C virus infection who received direct-acting antiviral therapy and achieved sustained virological response. *J Gastroenterol Hepatol* 2017; **32**: 1982-1988 [PMID: 28299813 DOI: 10.1111/jgh.13788]

24 **Dolmazashvili E**, Abutidze A, Chkhartishvili N, Karchava M, Sharvadze L, Tsertsvadze T. Regression of liver fibrosis over a 24-week period after completing direct-acting antiviral therapy in patients with chronic hepatitis C receiving care within the national hepatitis C elimination program in Georgia: results of hepatology clinic HEPA experience. *Eur J Gastroenterol Hepatol* 2017; **29**: 1223-1230 [PMID: 28857900 DOI: 10.1097/MEG.0000000000000964]

25 **Tachi Y**, Hirai T, Kojima Y, Ishizu Y, Honda T, Kuzuya T, Hayashi K, Ishigami M, Goto H. Liver stiffness reduction correlates with histological characteristics of hepatitis C patients with sustained virological response. *Liver Int* 2018; **38**: 59-67 [PMID: 28557143 DOI: 10.1111/liv.13486]

26 **Elsharkawy A**, Alem SA, Fouad R, El Raziky M, El Akel W, Abdo M, Tantawi O, AbdAllah M, Bourliere M, Esmat G. Changes in liver stiffness measurements and fibrosis scores following sofosbuvir based treatment regimens without interferon. *J Gastroenterol Hepatol* 2017; **32**: 1624-1630 [PMID: 28177543 DOI: 10.1111/jgh.13758]

27 **Chekuri S**, Nickerson J, Bichoupan K, Sefcik R, Doobay K, Chang S, DelBello D, Harty A, Dieterich DT, Perumalswami PV, Branch AD. Liver Stiffness Decreases Rapidly in Response to Successful Hepatitis C Treatment and Then Plateaus. *PLoS One* 2016; **11**: e0159413 [PMID: 27442255 DOI: 10.1371/journal.pone.0159413]

28 **Abdel Alem S**, Elsharkawy A, El Akel W, Abdelaziz AO, Salama RM, El-Sayed MH, El Kassas M, Anees M, Shedeed M, Abdelsalam F, Ziada DH, El Shazly Y, El-Serafy M, Waked I, Esmat G, Doss W. Liver stiffness measurements and FIB-4 are predictors of response to sofosbuvir-based treatment regimens in 7256 chronic HCV patients. *Expert Rev Gastroenterol Hepatol* 2019; **13**: 1009-1016 [PMID: 31418303 DOI: 10.1080/17474124.2019.1653183]

29 **Neukam K**, Morano-Amado LE, Rivero-Juárez A, Macías J, Granados R, Romero-Palacios A, Márquez M, Merino D, Ortega E, Alados-Arboledas JC, Cucurull J, Omar M, Ryan-Murua P, Pineda JA; Grupo de Estudio de Hepatitis Vírica, of the Sociedad Española de Enfermedades Infecciosas y Microbiología Clínica: GEHEP-SEIMC and Grupo de Estudio de Hepatitis Vírica, of the Sociedad Andaluza de Enfermedades Infecciosas y Microbiología Clínica: HEPAVIR/Red de Investigación en SIDA (RIS-HEP07). Liver stiffness predicts the response to direct-acting antiviral-based therapy against chronic hepatitis C in cirrhotic patients. *Eur J Clin Microbiol Infect Dis* 2017; **36**: 853-861 [PMID: 28004322 DOI: 10.1007/s10096-016-2871-x]

30 **Bachofner JA**, Valli PV, Kröger A, Bergamin I, Künzler P, Baserga A, Braun D, Seifert B, Moncsek A, Fehr J, Semela D, Magenta L, Müllhaupt B, Terziroli Beretta-Piccoli B, Mertens JC. Direct antiviral agent treatment of chronic hepatitis C results in rapid regression of transient elastography and fibrosis markers fibrosis-4 score and aspartate aminotransferase-platelet ratio index. *Liver Int* 2017; **37**: 369-376 [PMID: 27678216 DOI: 10.1111/liv.13256]

31 **Trivedi HD**, Lin SC, T Y Lau D. Noninvasive Assessment of Fibrosis Regression in Hepatitis C

Virus Sustained Virologic Responders. *Gastroenterol Hepatol (N Y)* 2017; **13**: 587-595 [PMID: 29391861]

32 **Kozbial K**, Moser S, Al-Zoairy R, Schwarzer R, Datz C, Stauber R, Laferl H, Strasser M, Beinhardt S, Stättermayer AF, Gschwantler M, Zoller H, Maieron A, Graziadei I, Trauner M, Steindl-Munda P, Hofer H, Ferenci P. Follow-up of sustained virological responders with hepatitis C and advanced liver disease after interferon/ribavirin-free treatment. *Liver Int* 2018; **38**: 1028-1035 [PMID: 29136329 DOI: 10.1111/liv.13629]

33 **Kobayashi M**, Suzuki F, Fujiyama S, Kawamura Y, Sezaki H, Hosaka T, Akuta N, Suzuki Y, Saitoh S, Arase Y, Ikeda K, Kumada H. Sustained virologic response by direct antiviral agents reduces the incidence of hepatocellular carcinoma in patients with HCV infection. *J Med Virol* 2017; **89**: 476-483 [PMID: 27531586 DOI: 10.1002/jmv.24663]

34 **Suda T**, Okawa O, Masaoka R, Gyotoku Y, Tokutomi N, Katayama Y, Tamano M. Shear wave elastography in hepatitis C patients before and after antiviral therapy. *World J Hepatol* 2017; **9**: 64-68 [PMID: 28105260 DOI: 10.4254/wjh.v9.i1.64]

35 **Wang JH**, Yen YH, Yao CC, Hung CH, Chen CH, Hu TH, Lee CM, Lu SN. Liver stiffness-based score in hepatoma risk assessment for chronic hepatitis C patients after successful antiviral therapy. *Liver Int* 2016; **36**: 1793-1799 [PMID: 27254286 DOI: 10.1111/liv.13179]

36 **Masuzaki R**, Tateishi R, Yoshida H, Goto E, Sato T, Ohki T, Imamura J, Goto T, Kanai F, Kato N, Ikeda H, Shiina S, Kawabe T, Omata M. Prospective risk assessment for hepatocellular carcinoma development in patients with chronic hepatitis C by transient elastography. *Hepatology* 2009; **49**: 1954-1961 [PMID: 19434742 DOI: 10.1002/hep.22870]

37 **Mandorfer M**, Kozbial K, Schwabl P, Freissmuth C, Schwarzer R, Stern R, Chromy D, Stättermayer AF, Reiberger T, Beinhardt S, Sieghart W, Trauner M, Hofer H, Ferlitsch A, Ferenci P, Peck-Radosavljevic M. Sustained virologic response to interferon-free therapies ameliorates HCV-induced portal hypertension. *J Hepatol* 2016; **65**: 692-699 [PMID: 27242316 DOI: 10.1016/j.jhep.2016.05.027]

38 **Garbuzenko DV**, Arefyev NO. Primary prevention of bleeding from esophageal varices in patients with liver cirrhosis: An update and review of the literature. *J Evid Based Med* 2020; **13**: 313-324 [PMID: 33037792 DOI: 10.1111/jebm.12407]

39 **Afdhal N**, Everson GT, Calleja JL, McCaughan GW, Bosch J, Brainard DM, McHutchison JG, De-Oertel S, An D, Charlton M, Reddy KR, Asselah T, Gane E, Curry MP, Forns X. Effect of viral suppression on hepatic venous pressure gradient in hepatitis C with cirrhosis and portal hypertension. *J Viral Hepat* 2017; **24**: 823-831 [PMID: 28295923 DOI: 10.1111/jvh.12706]

40 **Paternostro R**, Reiberger T, Bucsics T. Elastography-based screening for esophageal varices in patients with advanced chronic liver disease. *World J Gastroenterol* 2019; **25**: 308-329 [PMID: 30686900 DOI: 10.3748/wjg.v25.i3.308]

41 **Sharma P**, Kirnake V, Tyagi P, Bansal N, Singla V, Kumar A, Arora A. Spleen stiffness in patients with cirrhosis in predicting esophageal varices. *Am J Gastroenterol* 2013; **108**: 1101-1107 [PMID: 23629600 DOI: 10.1038/ajg.2013.119]

42 **Colecchia A**, Montrone L, Scaioli E, Bacchi-Reggiani ML, Colli A, Casazza G, Schiumerini R, Turco L, Di Biase AR, Mazzella G, Marzi L, Arena U, Pinzani M, Festi D. Measurement of spleen stiffness to evaluate portal hypertension and the presence of esophageal varices in patients with HCV-related cirrhosis. *Gastroenterology* 2012; **143**: 646-654 [PMID: 22643348 DOI: 10.1053/j.gastro.2012.05.035]

43 **Fraquelli M**, Rigamonti C, Colombo M. Spleen stiffness measured by transient elastography accurately predicts esophageal varices in liver cirrhosis. *Gastroenterology* 2012; **143**: e23; author reply e23-e23; author reply e24 [PMID: 22921672 DOI: 10.1053/j.gastro.2012.07.118]

44 **Calvaruso V**, Bronte F, Conte E, Simone F, Craxì A, Di Marco V. Modified spleen stiffness measurement by transient elastography is associated with presence of large oesophageal varices in patients with compensated hepatitis C virus cirrhosis. *J Viral Hepat* 2013; **20**: 867-874 [PMID: 24304456 DOI: 10.1111/jvh.12114]

45 **Stefanescu H**, Grigorescu M, Lupsor M, Procopet B, Maniu A, Badea R. Spleen stiffness measurement using Fibroscan for the noninvasive assessment of esophageal varices in liver cirrhosis patients. *J Gastroenterol Hepatol* 2011; **26**: 164-170 [PMID: 21175810 DOI: 10.1111/j.1440-1746.2010.06325.x]

46 **Wang H**, Wen B, Chang X, Wu Q, Wen W, Zhou F, Guo Y, Ji Y, Gu Y, Lai Q, He Q, Li J, Chen J, Hou J. Baveno VI criteria and spleen stiffness measurement rule out high-risk varices in virally suppressed HBV-related cirrhosis. *J Hepatol* 2021; **74**: 584-592 [PMID: 33039403 DOI: 10.1016/j.jhep.2020.09.034]

47 **Fofiu R**, Bende F, Popescu A, Şirli R, Lupușoru R, Ghiuchici AM, Sporea I. Spleen and Liver Stiffness for Predicting High-Risk Varices in Patients with Compensated Liver Cirrhosis. *Ultrasound Med Biol* 2021; **47**: 76-83 [PMID: 33067019 DOI: 10.1016/j.ultrasmedbio.2020.09.004]

48 **Ma X**, Wang L, Wu H, Feng Y, Han X, Bu H, Zhu Q. Spleen Stiffness Is Superior to Liver Stiffness for Predicting Esophageal Varices in Chronic Liver Disease: A Meta-Analysis. *PLoS One* 2016; **11**: e0165786 [PMID: 27829057 DOI: 10.1371/journal.pone.0165786]

49 **Semmler G**, Binter T, Kozbial K, Schwabl P, Hametner-Schreil S, Zanetto A, Gavasso S, Chromy D, Bauer DJM, Simbrunner B, Scheiner B, Bucsics T, Stättermayer AF, Pinter M, Steindl-Munda P, Schöfl R, Russo FP, Simioni P, Trauner M, Ferenci P, Reiberger T, Mandorfer M. Noninvasive Risk Stratification After HCV Eradication in Patients With Advanced Chronic Liver Disease. *Hepatology*

2021; **73**: 1275-1289 [PMID: 32659847 DOI: 10.1002/hep.31462]

50 **Thabut D**, Bureau C, Layese R, Bourcier V, Hammouche M, Cagnot C, Marcellin P, Guyader D, Pol S, Larrey D, De Lédinghen V, Ouzan D, Zoulim F, Roulot D, Tran A, Bronowicki JP, Zarski JP, Goria O, Calès P, Péron JM, Alric L, Bourlière M, Mathurin P, Blanc JF, Abergel A, Serfaty L, Mallat A, Grangé JD, Attali P, Bacq Y, Wartelle-Bladou C, Dao T, Pilette C, Silvain C, Christidis C, Capron D, Bernard-Chabert B, Hillaire S, Di Martino V, Sutton A, Audureau E, Roudot-Thoraval F, Nahon P; ANRS CO12 CirVir group. Validation of Baveno VI Criteria for Screening and Surveillance of Esophageal Varices in Patients With Compensated Cirrhosis and a Sustained Response to Antiviral Therapy. *Gastroenterology* 2019; **156**: 997-1009.e5 [PMID: 30768988 DOI: 10.1053/j.gastro.2018.11.053]

51 **Giannini EG**, Crespi M, Demarzo M, Bodini G, Furnari M, Marabotto E, Torre F, Zentilin P, Savarino V. Improvement in hepatitis C virus patients with advanced, compensated liver disease after sustained virological response to direct acting antivirals. *Eur J Clin Invest* 2019; **49**: e13056 [PMID: 30474209 DOI: 10.1111/eci.13056]

52 **Jangouk P**, Turco L, De Oliveira A, Schepis F, Villa E, Garcia-Tsao G. Validating, deconstructing and refining Baveno criteria for ruling out high-risk varices in patients with compensated cirrhosis. *Liver Int* 2017; **37**: 1177-1183 [PMID: 28160373 DOI: 10.1111/liv.13379]

53 **Chen PH**, Hsieh WY, Su CW, Hou MC, Wang YP, Hsin IF, Yang TC, Liao WC, Lin HC, Lee FY, Wu JC. Combination of albumin-bilirubin grade and platelets to predict a compensated patient with hepatocellular carcinoma who does not require endoscopic screening for esophageal varices. *Gastrointest Endosc* 2018; **88**: 230-239.e2 [PMID: 29317268 DOI: 10.1016/j.gie.2017.12.023]

54 **Calvaruso V**, Cacciola I, Licata A, Madonia S, Benigno R, Petta S, Bronte F, Conte E, Malizia G, Bertino G, Distefano M, Montineri A, Digiacomo A, Alaimo G, Cacopardo B, Davì A, Guarneri L, Scalisi I, Colletti P, Cartabellotta F, Portelli V, Prestileo T, Averna A, Iacobello C, Mondello L, Scifo G, Russello M, Squadrito G, Raimondo G, Cammà C, Craxì A, Di Marco V; RESIST-HCV (Rete Sicilia Selezione Terapia–HCV). Is Transient Elastography Needed for Noninvasive Assessment of High-Risk Varices? *Am J Gastroenterol* 2019; **114**: 1275-1282 [PMID: 31135449 DOI: 10.14309/ajg.0000000000000266]

55 **Hamdane N**, Jühling F, Crouchet E, El Saghire H, Thumann C, Oudot MA, Bandiera S, Saviano A, Ponsolles C, Roca Suarez AA, Li S, Fujiwara N, Ono A, Davidson I, Bardeesy N, Schmidl C, Bock C, Schuster C, Lupberger J, Habersetzer F, Doffoël M, Piardi T, Sommacale D, Imamura M, Uchida T, Ohdan H, Aikata H, Chayama K, Boldanova T, Pessaux P, Fuchs BC, Hoshida Y, Zeisel MB, Duong FHT, Baumert TF. HCV-Induced Epigenetic Changes Associated With Liver Cancer Risk Persist After Sustained Virologic Response. *Gastroenterology* 2019; **156**: 2313-2329.e7 [PMID: 30836093 DOI: 10.1053/j.gastro.2019.02.038]

56 **D'Ambrosio R**, Colombo M. Should surveillance for liver cancer be modified in hepatitis C patients after treatment-related cirrhosis regression? *Liver Int* 2016; **36**: 783-790 [PMID: 26936383 DOI: 10.1111/liv.13106]

57 **D'Ambrosio R**, Aghemo A, Fraquelli M, Rumi MG, Donato MF, Paradis V, Bedossa P, Colombo M. The diagnostic accuracy of Fibroscan for cirrhosis is influenced by liver morphometry in HCV patients with a sustained virological response. *J Hepatol* 2013; **59**: 251-256 [PMID: 23528378 DOI: 10.1016/j.jhep.2013.03.013]

58 **Tachi Y**, Hirai T, Kojima Y, Ishizu Y, Honda T, Kuzuya T, Hayashi K, Ishigami M, Goto H. Liver stiffness measurement predicts hepatocellular carcinoma development in patients treated with direct-acting antivirals. *JGH Open* 2017; **1**: 44-49 [PMID: 30483532 DOI: 10.1002/jgh3.12007]

59 **Terrault NA**, Zeuzem S, Di Bisceglie AM, Lim JK, Pockros PJ, Frazier LM, Kuo A, Lok AS, Shiffman ML, Ben Ari Z, Akushevich L, Vainorius M, Sulkowski MS, Fried MW, Nelson DR; HCV-TARGET Study Group. Effectiveness of Ledipasvir-Sofosbuvir Combination in Patients With Hepatitis C Virus Infection and Factors Associated With Sustained Virologic Response. *Gastroenterology* 2016; **151**: 1131-1140.e5 [PMID: 27565882 DOI: 10.1053/j.gastro.2016.08.004]

60 **Foster GR**, Irving WL, Cheung MC, Walker AJ, Hudson BE, Verma S, McLauchlan J, Mutimer DJ, Brown A, Gelson WT, MacDonald DC, Agarwal K; HCV Research, UK. Impact of direct acting antiviral therapy in patients with chronic hepatitis C and decompensated cirrhosis. *J Hepatol* 2016; **64**: 1224-1231 [PMID: 26829205 DOI: 10.1016/j.jhep.2016.01.029]

61 **European Association for the Study of the Liver**. EASL Clinical Practice Guidelines: Management of hepatocellular carcinoma. *J Hepatol* 2018; **69**: 182-236 [PMID: 29628281 DOI: 10.1016/j.jhep.2018.03.019]

62 **Marrero JA**, Kulik LM, Sirlin CB, Zhu AX, Finn RS, Abecassis MM, Roberts LR, Heimbach JK. Diagnosis, Staging, and Management of Hepatocellular Carcinoma: 2018 Practice Guidance by the American Association for the Study of Liver Diseases. *Hepatology* 2018; **68**: 723-750 [PMID: 29624699 DOI: 10.1002/hep.29913]

63 **Rinaldi L**, Guarino M, Perrella A, Pafundi PC, Valente G, Fontanella L, Nevola R, Guerrera B, Iuliano N, Imparato M, Trabucco A, Sasso FC, Morisco F, Ascione A, Piai G, Adinolfi LE. Role of Liver Stiffness Measurement in Predicting HCC Occurrence in Direct-Acting Antivirals Setting: A Real-Life Experience. *Dig Dis Sci* 2019; **64**: 3013-3019 [PMID: 30937719 DOI: 10.1007/s10620-019-05604-8]

64 **Lai MM**. Hepatitis C virus proteins: direct link to hepatic oxidative stress, steatosis, carcinogenesis and more. *Gastroenterology* 2002; **122**: 568-571 [PMID: 11832470 DOI: 10.1053/gast.2002.31474]

65 **Okuda M**, Li K, Beard MR, Showalter LA, Scholle F, Lemon SM, Weinman SA. Mitochondrial

injury, oxidative stress, and antioxidant gene expression are induced by hepatitis C virus core protein. *Gastroenterology* 2002; **122**: 366-375 [PMID: 11832451 DOI: 10.1053/gast.2002.30983]

66 **Ripoll C**, Groszmann RJ, Garcia-Tsao G, Bosch J, Grace N, Burroughs A, Planas R, Escorsell A, Garcia-Pagan JC, Makuch R, Patch D, Matloff DS; Portal Hypertension Collaborative Group. Hepatic venous pressure gradient predicts development of hepatocellular carcinoma independently of severity of cirrhosis. *J Hepatol* 2009; **50**: 923-928 [PMID: 19303163 DOI: 10.1016/j.jhep.2009.01.014]

67 **King LY**, Canasto-Chibuque C, Johnson KB, Yip S, Chen X, Kojima K, Deshmukh M, Venkatesh A, Tan PS, Sun X, Villanueva A, Sangiovanni A, Nair V, Mahajan M, Kobayashi M, Kumada H, Iavarone M, Colombo M, Fiel MI, Friedman SL, Llovet JM, Chung RT, Hoshida Y. A genomic and clinical prognostic index for hepatitis C-related early-stage cirrhosis that predicts clinical deterioration. *Gut* 2015; **64**: 1296-1302 [PMID: 25143343 DOI: 10.1136/gutjnl-2014-307862]

68 **Ravaioli F**, Conti F, Brillanti S, Andreone P, Mazzella G, Buonfiglioli F, Serio I, Verrucchi G, Bacchi Reggiani ML, Colli A, Marasco G, Colecchia A, Festi D. Hepatocellular carcinoma risk assessment by the measurement of liver stiffness variations in HCV cirrhotics treated with direct acting antivirals. *Dig Liver Dis* 2018; **50**: 573-579 [PMID: 29567413 DOI: 10.1016/j.dld.2018.02.010]

69 **Martínez-Campreciós J**, Bonis Puig S, Pons Delgado M, Salcedo Allende MT, Mínguez Rosique B, Genescà Ferrer J. Transient elastography in DAA era. Relation between post-SVR LSM and histology. *J Viral Hepat* 2020; **27**: 453-455 [PMID: 31816146 DOI: 10.1111/jvh.13245]

70 **Pan JJ**, Bao F, Du E, Skillin C, Frenette CT, Waalen J, Alaparthi L, Goodman ZD, Pockros PJ. Morphometry Confirms Fibrosis Regression From Sustained Virologic Response to Direct-Acting Antivirals for Hepatitis C. *Hepatol Commun* 2018; **2**: 1320-1330 [PMID: 30411079 DOI: 10.1002/hep4.1228]

71 **Tachi Y**, Hirai T, Toyoda H, Tada T, Hayashi K, Honda T, Ishigami M, Goto H, Kumada T. Predictive Ability of Laboratory Indices for Liver Fibrosis in Patients with Chronic Hepatitis C after the Eradication of Hepatitis C Virus. *PLoS One* 2015; **10**: e0133515 [PMID: 26214180 DOI: 10.1371/journal.pone.0133515]

72 **Iwasaki Y**, Takaguchi K, Ikeda H, Makino Y, Araki Y, Ando M, Kobashi H, Kobatake T, Tanaka R, Tomita M, Senoh T, Kawaguchi M, Shimoe T, Manabe K, Kita K, Shimamura J, Sakaguchi K, Shiratori Y. Risk factors for hepatocellular carcinoma in Hepatitis C patients with sustained virologic response to interferon therapy. *Liver Int* 2004; **24**: 603-610 [PMID: 15566511 DOI: 10.1111/j.1478-3231.2004.0956.x]

73 **Arase Y**, Kobayashi M, Suzuki F, Suzuki Y, Kawamura Y, Akuta N, Sezaki H, Saito S, Hosaka T, Ikeda K, Kumada H, Kobayashi T. Effect of type 2 diabetes on risk for malignancies includes hepatocellular carcinoma in chronic hepatitis C. *Hepatology* 2013; **57**: 964-973 [PMID: 22991257 DOI: 10.1002/hep.26087]

74 **Nagaoki Y**, Aikata H, Nakano N, Shinohara F, Nakamura Y, Hatooka M, Morio K, Kan H, Fujino H, Kobayashi T, Fukuhara T, Masaki K, Ono A, Nakahara T, Kawaoka T, Miki D, Tsuge M, Hiramatsu A, Imamura M, Takahashi S, Kawakami Y, Ochi H, Chayama K; Hiroshima Liver Study Group. Development of hepatocellular carcinoma in patients with hepatitis C virus infection who achieved sustained virological response following interferon therapy: A large-scale, long-term cohort study. *J Gastroenterol Hepatol* 2016; **31**: 1009-1015 [PMID: 26584407 DOI: 10.1111/jgh.13236]

75 **Toyoda H**, Kumada T, Tada T, Kiriyama S, Tanikawa M, Hisanaga Y, Kanamori A, Kitabatake S, Ito T. Risk factors of hepatocellular carcinoma development in non-cirrhotic patients with sustained virologic response for chronic hepatitis C virus infection. *J Gastroenterol Hepatol* 2015; **30**: 1183-1189 [PMID: 25678094 DOI: 10.1111/jgh.12915]

76 **Fouad R**, Elsharkawy A, Abdel Alem S, El Kassas M, Alboraie M, Sweedy A, Afify S, Abdellatif Z, Khairy M, Esmat G. Clinical impact of serum α-fetoprotein and its relation on changes in liver fibrosis in hepatitis C virus patients receiving direct-acting antivirals. *Eur J Gastroenterol Hepatol* 2019; **31**: 1129-1134 [PMID: 30896550 DOI: 10.1097/MEG.0000000000001400]

77 **D'Ambrosio R**, Aghemo A, Rumi MG, Degasperi E, Sangiovanni A, Maggioni M, Fraquelli M, Perbellini R, Rosenberg W, Bedossa P, Colombo M, Lampertico P. Persistence of hepatocellular carcinoma risk in hepatitis C patients with a response to IFN and cirrhosis regression. *Liver Int* 2018; **38**: 1459-1467 [PMID: 29377616 DOI: 10.1111/liv.13707]

78 **Lleo A**, Aglitti A, Aghemo A, Maisonneuve P, Bruno S, Persico M; collaborators. Predictors of hepatocellular carcinoma in HCV cirrhotic patients treated with direct acting antivirals. *Dig Liver Dis* 2019; **51**: 310-317 [PMID: 30473220 DOI: 10.1016/j.dld.2018.10.014]

79 **Hamada K**, Saitoh S, Nishino N, Fukushima D, Horikawa Y, Nishida S, Honda M. Shear wave elastography predicts hepatocellular carcinoma risk in hepatitis C patients after sustained virological response. *PLoS One* 2018; **13**: e0195173 [PMID: 29672518 DOI: 10.1371/journal.pone.0195173]

80 **Feier D**, Lupsor Platon M, Stefanescu H, Badea R. Transient elastography for the detection of hepatocellular carcinoma in viral C liver cirrhosis. Is there something else than increased liver stiffness? *J Gastrointestin Liver Dis* 2013; **22**: 283-289 [PMID: 24078985]

81 **Kobayashi M**, Suzuki F, Akuta N, Suzuki Y, Sezaki H, Yatsuji H, Kawamura Y, Hosaka T, Kobayashi M, Arase Y, Ikeda K, Mineta R, Iwasaki S, Watahiki S, Miyakawa Y, Kumada H. Development of hepatocellular carcinoma in elderly patients with chronic hepatitis C with or without elevated aspartate and alanine aminotransferase levels. *Scand J Gastroenterol* 2009; **44**: 975-983 [PMID: 19521923 DOI: 10.1080/00365520802588125]

82 **Miyakawa K**, Tarao K, Ohshige K, Morinaga S, Ohkawa S, Okamoto N, Shibuya A, Adachi S,

Miura Y, Fujiyama S, Miyase S, Tomita K. High serum alanine aminotransferase levels for the first three successive years can predict very high incidence of hepatocellular carcinoma in patients with Child Stage A HCV-associated liver cirrhosis. *Scand J Gastroenterol* 2009; **44**: 1340-1348 [PMID: 19891585 DOI: 10.3109/00365520903222681]

83 **Simili A**, Mazzella G, Ravaioli F, Festi D, Bacchi-Reggiani ML, Porro A, Bazzoli F, Azzaroli F. Interleukin 28 Polymorphisms and Hepatocellular Carcinoma Development after Direct Acting Antiviral Therapy for Chronic Hepatitis C. *J Gastrointestin Liver Dis* 2019; **28**: 449-456 [PMID: 31826071 DOI: 10.15403/jgld-309]

84 **Matsuura K**, Sawai H, Ikeo K, Ogawa S, Iio E, Isogawa M, Shimada N, Komori A, Toyoda H, Kumada T, Namisaki T, Yoshiji H, Sakamoto N, Nakagawa M, Asahina Y, Kurosaki M, Izumi N, Enomoto N, Kusakabe A, Kajiwara E, Itoh Y, Ide T, Tamori A, Matsubara M, Kawada N, Shirabe K, Tomita E, Honda M, Kaneko S, Nishina S, Suetsugu A, Hiasa Y, Watanabe H, Genda T, Sakaida I, Nishiguchi S, Takaguchi K, Tanaka E, Sugihara J, Shimada M, Kondo Y, Kawai Y, Kojima K, Nagasaki M, Tokunaga K, Tanaka Y; Japanese Genome-Wide Association Study Group for Viral Hepatitis. Genome-Wide Association Study Identifies TLL1 Variant Associated With Development of Hepatocellular Carcinoma After Eradication of Hepatitis C Virus Infection. *Gastroenterology* 2017; **152**: 1383-1394 [PMID: 28163062 DOI: 10.1053/j.gastro.2017.01.041]

85 **Degasperi E**, Galmozzi E, Facchetti F, Farina E, D'Ambrosio R, Soffredini R, Iavarone M, Lampertico P. TLL1 variants do not predict hepatocellular carcinoma development in HCV cirrhotic patients treated with direct-acting antivirals. *J Viral Hepat* 2019; **26**: 1233-1236 [PMID: 31177595 DOI: 10.1111/jvh.13155]

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MINIREVIEWS

Role of immune dysfunction in drug induced liver injury

Chandrashekaran Girish, Sukumaran Sanjay

ORCID number: Chandrashekaran Girish 0000-0003-1777-5120; Sukumaran Sanjay 0000-0001 8323-8425.

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Chandrashekaran Girish, Sukumaran Sanjay, Department of Pharmacology, Jawaharlal Institute of Postgraduate Medical Education and Research, Puducherry 605006, India

Corresponding author: Chandrashekaran Girish, MSc, PhD, Additional Professor, Department of Pharmacology, Jawaharlal Institute of Postgraduate Medical Education and Research, Puducherry 605006, India. gcnx@rediffmail.com

Abstract

Drug-induced liver injury (DILI) is one of the leading causes of liver failure and withdrawal of drugs from the market. A poor understanding of the precipitating event aetiology and mechanisms of disease progression has rendered the prediction and subsequent treatment intractable. Recent literature suggests that some drugs can alter the liver's repair systems resulting in injury. The pathophysiology of DILI is complex, and immune dysfunction plays an important role in determining the course and severity of the disease. Immune dysfunction is influenced by the host response to drug toxicity. A deeper understanding of these processes may be beneficial in the management of DILI and aid in drug development. This review provides a structured framework presenting DILI in three progressive stages that summarize the interplay between drugs and the host defence networks.

Key Words: Immune dysfunction; Liver damage; Hepatotoxic drugs; Drug-induced liver injury; High mobility group box 1

Core Tip: This review demonstrates the critical role of the immune system in the progression of drug-induced liver injury and also in determining the severity of the damage. Drugs affect the normal functioning of hepatocytes through several direct and indirect mechanisms leading to the dysfunctional immune response. The major effector cells in amplifying liver damage are Kupffer cells, monocytes and neutrophils. Genetic predispositions and environmental factors also make individuals vulnerable to immune dysfunction.

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INTRODUCTION

The liver plays a central role in the complex process of metabolism and elimination of drugs from the body. The liver is equipped with a wide array of detoxification systems that have evolved over time with exposure to xenobiotics. The primary role of this system is to convert a drug to a more hydrophilic form so that it can be eliminated through bile or urine. Despite the liver's detox potential, certain drugs can still cause hepatotoxicity that can range from mild asymptomatic liver damage to liver failure[1,2].

A study showed that, out of the 462 pharmaceuticals withdrawn due to adverse drug reactions between 1953 and 2013, hepatotoxicity ranked first with 81 cases (18%). It is estimated that over 1000 drugs currently available on the market that cause liver damage[3] despite these drugs passing the safety measures of clinical trials before entering the market. Some drugs that are hepatotoxic at doses higher than the therapeutic range can also cause drug-induced liver injury (DILI) at doses within the therapeutic range[2,4-6]. This implies that the dose may not be the only contributing factor.

Despite large number of drugs known to cause liver injury, the incidence of DILI is rare. DILI is reported in 1 in every 10000 to 100000 individuals annually. This suggests that drug-host interactions in these susceptible individuals may play an important role in DILI[7-9]. Recent data shows that this interaction can result in an imbalance between damage and repair mechanisms resulting in DILI with immune dysfunction being cited as an important precipitating event in the pathophysiology of DILI[10-12]. This is supported by evidence from experimental studies. Some drugs that are hepatotoxic in humans do not cause liver damage in animal models, but the administration of these drugs along with low doses of lipopolysaccharide (LPS) result in a similar pattern of liver injury as observed in humans. For example, Trovafloxacin (TVX) is a broad-spectrum fluoroquinolone antibiotic, and a study reported that TVX use caused 140 severe hepatic reactions resulting in 14 cases of liver failure. Examination of the case reports suggest that the duration of TVX therapy in patients does not correlate with the toxic response, so TVX hepatotoxicity is classified as idiosyncratic. In rodent models, TVX did not cause liver damage, even at high doses. However, further studies with a normally nontoxic dose of TVX coupled with LPS induced inflammatory stress caused acute liver injury[13,14].

The upcoming sections provide a structured framework presenting DILI in three progressive stages, summarizing the interplay between drugs and the host defence networks that lead to immune system dysfunction.

STAGES OF DILI

Initiation of DILI

Direct initiation: The metabolism of drugs by phase 1 enzymes results in the production of intermediary metabolites and free radicals, in some instances. These intermediary metabolites may also be unstable and reactive, but they are subsequently neutralized by phase 2 conjugation. DILI is initiated when there is an imbalance between the production of reactive metabolites and their subsequent detoxification[2,5] (Figure 1).

Certain drugs and reactive metabolites can bind to cellular organelles resulting in loss of function and likely cell death. One such case is the damage caused by drugs acting on the endoplasmic reticulum (ER). The ER plays an important role in protein synthesis, folding, assembly, trafficking, and regulation of intracellular calcium homeostasis. Drug related oxidative stress can disturb ER function and lead to the accumulation of unfolded proteins in the ER. This process is termed ER stress. A variety of common drugs cause ER stress, including paracetamol, lopinavir, ritonavir, saquinavir, nelfinavir, atazanavir, and amprenavir[15].

During drug metabolism, free radicals are released that are normally detoxified by cell defence mechanisms. Excessive free radical generation can be caused by enzyme induction or genetic defects in enzyme systems. Free radicals damage the cellular organelles and the lipid bilayer, which results in amplification of damage. Lipid

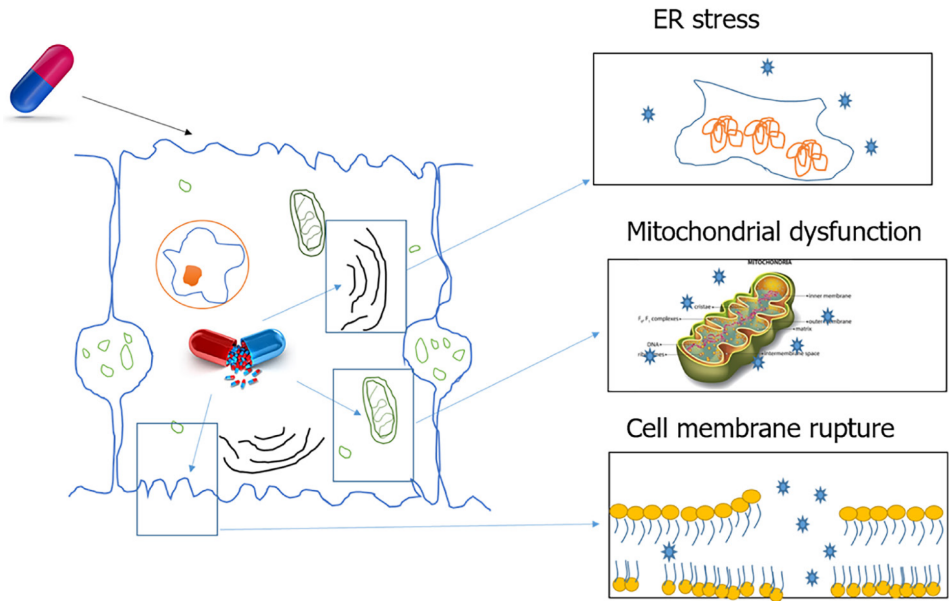

Figure 1 Initiation of drug-induced liver injury - Direct damage by drug and metabolite. Drugs and their metabolites damage organelles and cell membrane of liver cells causing damage. ER: Endoplasmic reticulum.

bilayer damage can lead to the release of cytosolic components and alarmins that attract the liver's resident immune cells. This initial immune response can amplify the sterile damage. Some of the alarmins associated with DILI are high mobility group box 1, S100 proteins, hepatoma-derived growth factor and heat shock proteins[16-20].

Free radicals can also damage the mitochondrial membrane leading to cell dysfunction and death. Mitochondrial dysfunction includes disruption or disturbance to different metabolic pathways and damage to mitochondrial components. In addition, these mitochondrial alterations can have several deleterious consequences, such as oxidative stress, ATP depletion, triglycerides accumulation, and necrotic cell death[21].

Indirect initiation of DILI: There are two main mechanisms of indirect initiation of DILI. Inhibition of efflux transporters. Bile salt export pump (BSEP) is a member of the ABC transporter superfamily located in the canalicular membrane of hepatocytes. BSEP is responsible for the biliary excretion of bile acids. Drug metabolites inhibit BSEP function, resulting in toxicity. One such metabolite, Troglitazone sulphate, a metabolite of troglitazone, inhibits BSEP mediated taurocholate transport which contributes to troglitazone toxicity. Other potent BSEP inhibitors with the potential to cause DILI include cyclosporin A, bosentan, sulindac, rifamycin, and glibenclamide[2, 22].

Enzyme induction: Paracetamol is known to cause liver injury through enzyme induction due to CYP2E1 induction by ethanol. A minor percentage of ethanol is metabolised by CYP2E1. When ethanol and paracetamol are taken simultaneously, ethanol slows the degradation of the CYP enzyme increasing its half-life from 7 h to 37 h. Until ethanol is present in the body more CYP2E1 is induced and a portion is blocked from paracetamol for ethanol metabolism. Once ethanol is completely removed, CYP2E1 enhances paracetamol metabolism resulting in the excess production of toxic intermediary metabolite, NAPQI, causing liver injury[2,23] (Figure 2).

PROGRESSION

The initiation of DILI does not necessarily result in adverse outcomes. In experimental models, the progression of DILI mainly depends on the persistent and recurrent assault by the toxins that deplete the liver's resources leading to irreversible damage. This is unlikely at the therapeutic dose of most drugs, as the liver has highly developed protective and regenerative mechanisms. Experimental and clinical data suggest that a myriad of host and drug-related factors contribute to the progressive dysfunction of survival mechanisms that lead to DILI. This is further complicated by

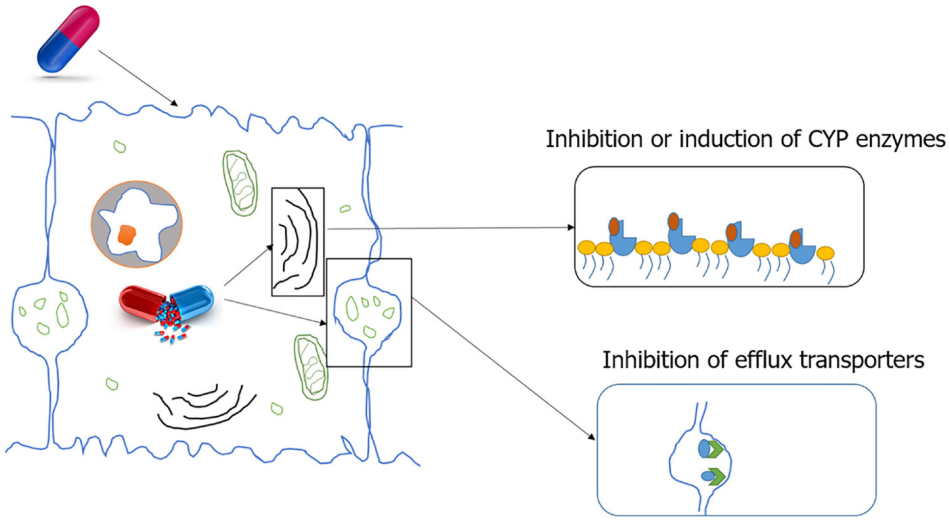

Figure 2 Initiation of drug-induced liver injury - Indirect damage by drugs. Drugs can modulate the functioning of enzymes and transporters involved in drug metabolism and elimination that may lead to toxicity.

the fact that each drug can cause multiple patterns of liver disease, implying an important role for host-drug interactions in the progression of DILI. Immune dysfunction is a major determinant of hepatic cell death and DILI progression[2,4,6,24-26].

This section covers the two main mechanisms of immune reactions induced by drugs and the influence of host factors on them.

Immune allergic DILI

A drug or its metabolites alone cannot activate an immune response due to their small size, but a drug's reactive metabolites or the drug itself can bind to cellular proteins and form protein-drug adducts that elicit an immune response. In normal individuals, this complex is degraded by cellular detoxification but in susceptible individuals, these adducts act as immunogens and are taken up by antigen-presenting cells and presented by major histocompatibility complexes to helper T cells, and further activation by cytokines stimulates an immune response and anti-drug antibodies are also produced, resulting in extensive death of cells where the drug has accumulated[6,27-29] (Figure 3).

It is hypothesized that ER stress is a contributing factor for this type of reaction. Accumulation of drug/metabolite causes ER stress, which results in misfolding of proteins. These misfolded proteins are more susceptible to drug-protein adduct formations that elicit an immune response[15].

An example of this type of reaction is abacavir, a reverse transcriptase inhibitor employed in the treatment of AIDS, which causes a rare, but serious hypersensitivity reaction that resembles an immune allergic drug reaction. Several genetic variants in the HLA regions are identified as risk factors for DILI, the incidence of hypersensitivity reactions to abacavir is markedly elevated in subjects who carry the B*57:01 variant in the human leukocyte antigen B (*HLA-B*) gene. Furthermore, carriers of this genotype are at increased risk of flucloxacillin-induced DILI. Studies have shown an association between HLA-B1*15:01 and amoxicillin/clavulanate DILI. The HLA-B*35:02 allele is reported to have a significant association with minocycline DILI[10,25,30,31]. DILI caused by other drugs such as amoxicillin-clavulanate, lumiracoxib, ticlopidine, lapatinib, and ximelagatran is also associated with HLA genotypes, suggesting an important role of the immune system in DILI[25,31].

Autoimmune DILI

Autoimmune DILI is caused by the release of alarmins from necrotic cells or cells with leaky cell membranes. This results in the activation of innate immune cells. Alarmins are rapidly released following necrotic cell death that are not released by apoptotic cells. The immune system also can be induced to produce and release alarmins to recruit and activate innate immune cells[19,32] (Figure 4).

Mitochondrial dysfunction is reported to play a critical role in the pathogenesis of autoimmune DILI. NSAIDs, such as diclofenac and nimesulide, and other drugs can cause mitochondrial dysfunction that leads to the formation of the mitochondrial

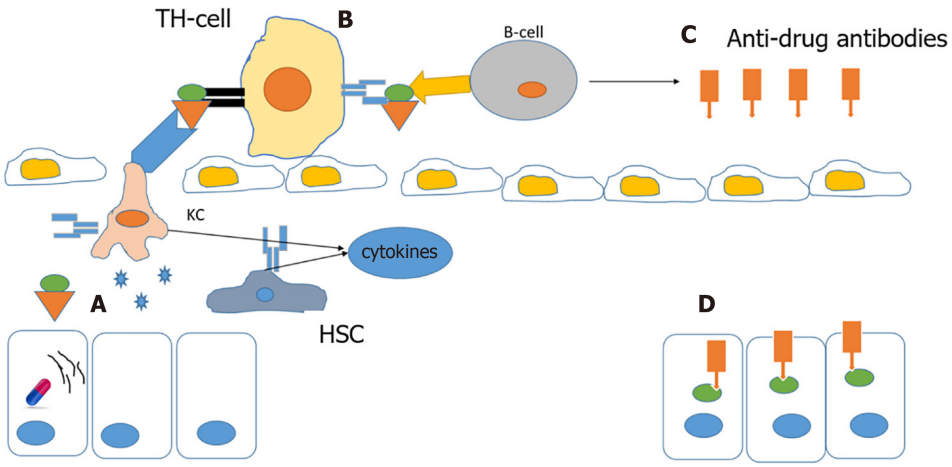

Figure 3 Immune allergic drug-induced liver injury. A: Endoplasmic reticulum stress by drug, causes misfolded protein resulting in cell death and release of stress signals and drug-protein complex. Kupffer cells ingest the drug-protein complex to T-helper cells; B: T-helper cells process it and present it to B-cells; C: B-cells produce anti-drug antibodies; D: These antibodies target the tissues, where drug is accumulated. KC: Kupffer cell; HSC: Hepatic stellate cells.

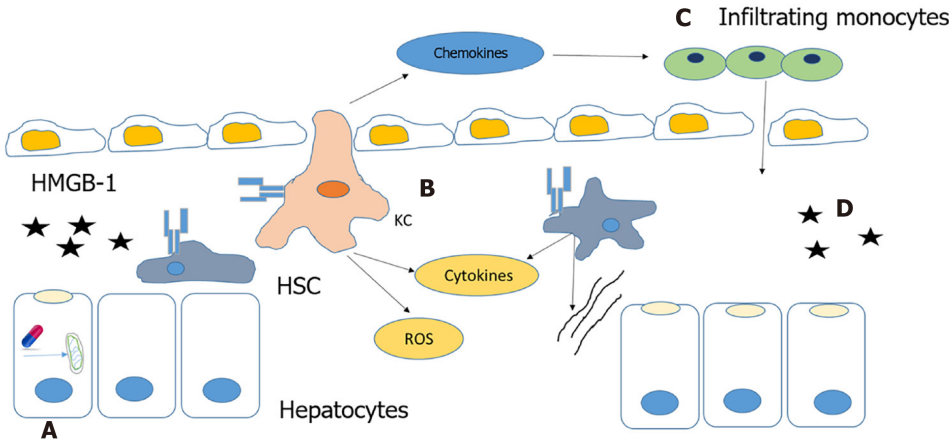

Figure 4 Mechanism of autoimmune drug-induced liver injury. A: Drug causes mitochondrial dysfunction resulting in cell death and release of HMGB-1 and other stress signals; B: Kupffer cells and Stellate cells get activated. Release cytokines, chemokines and toxins; C: Chemokines attract monocytes; D: Amplification of injury and cell death. KC: Kupffer cell; HSC: Hepatic stellate cells; ROS: Reactive oxygen species.

permeability transition pore (MPTP). MPTP formation is induced by increased oxidative stress that results in a dissipation of membrane potential, uncoupling of oxidative phosphorylation leading to necrotic cell death and the release of alarmins[18,21,33].

HMGB-1 is an alarmin released by necrotic cells that binds to TLR4 receptors of kupffer cells (KCs) and hepatic stellate cells (HSC), and activates them. Activated KCs produce mediators that directly induce cell death, such as tumor necrosis factor (TNF)-α, Fas ligand and reactive oxygen species, or indirectly cause death through the recruitment of neutrophils by cytokines and chemokines like IL-1β and CXCL2. Production of chemokine, CCL2 (MCP-1) recruits monocytes from the bone marrow to the liver. These infiltrating monocytes produce inflammatory chemokines resulting in the activation of HSCs and the promotion of fibrosis[18,34].

Host sex and sex hormones influence immune response. Studies have shown that female patients with DILI are at higher risk of developing acute liver failure (ALF) with more severe hepatitis and higher levels of pro-inflammatory cytokines. In a halothane-induced experimental DILI model, oestrogen reduced liver injury while progesterone increased liver damage, both hormones influenced immune response. Another important factor affecting DILI is race. A study reported that African-Americans are at a higher risk of developing chronic DILI, while Asian individuals are at increased risk of ALF, liver-related death, or damage that precipitates a need for liver transplantation[4,7,10,24,35].

ADVERSE OUTCOMES

In normal individuals, DILI resolves completely without any residual liver injury. But there are three major exceptions. They are ALF, cirrhosis and acute-on-chronic liver failure (ACLF). These conditions are relatively rare but severe and may result in death or require a liver transplant.

ALF

Even in the absence of pre-existing liver disease, drugs can cause a rapid loss of liver function either directly, as seen in overdoses, or through inflammatory cell mediated mechanisms such as cytokine overproduction. Drug-induced ALF is defined by the signs or symptoms of hepatic failure and encephalopathy during the course of acute DILI. The time to onset of ALF after the start of a medication can vary from a few days to months, but not exceeding six months[4,24,36-38].

In Western countries, paracetamol overdose is the most common reason behind ALF. In India, anti-TB regimens with isoniazid, rifampicin and pyrazinamide are reported as the leading cause of ALF. Other drugs that are reported to cause ALF include phenytoin, carbamazepine, valproate, nitrofurantoin, propylthiouracil, disulfiram, diclofenac, ketoconazole, flutamide, sulphonamides, terbinafine, fluoroquinolone antibiotics and macrolide antibiotics. Drug-induced ALF is a major cause for withdrawal from the market or restricted use of a medication (troglitazone, bromfenac, nefazodone, halothane, telithromycin). ALF occurs in cases with acute hepatocellular injury with characteristics similar to acute viral hepatitis[10,23,39-41].

Paracetamol is responsible for more than 50% of drug related ALF and about 20% of liver transplant cases in the United States[42]. In case of paracetamol overdose, the drug metabolite NAPQ1 depletes GSH and causes organelle damage, the most significant resulting in mitochondrial stress. Thereby the NAPQ1 accumulation triggers necrosis[43,44]. Hepatocyte necrosis passively releases various DAMPs such as HMGB-1, HSP and DNA fragments. These DAMPs activate the resident immune cells such as Kupffer cells and natural killer (NK) cells. Cytokines and chemokines such as TNF-α, IL-1β and CCL2 produced by the activated immune cells and the DAMPs enter systemic circulation and cause infiltration of neutrophils and monocytes into the liver. In conditions of sterile injury, the immune cells function to clear the dead cells by producing chemokines and free radicals to digest it. Once the cellular debris is cleared the immune cells undergo phenotypic change and support in liver regeneration. However, in case of paracetamol overdose, the overwhelming amount of cellular debris and DAMPs causes excess immune activation, whose products such as superoxide, nitric oxide and peroxynitrite result in further amplification of liver injury leading to massive necrosis and organ failure[45-48].

Cirrhosis

Cirrhosis is characterized by islands or nodules of regenerative parenchymal cells surrounded by excessive deposition of fibrous tissue and portal hypertension. Cirrhosis is rarely the initial manifestation of DILI and is most often a cumulative response to long-term exposure to hepatotoxic drugs. It usually occurs at least six months after starting the drug treatment. The time to onset of cirrhosis due to medications is typically long; at least 6 month after starting the medication but usually several years afterwards. The drugs that are most commonly cause cirrhosis are vitamin A, amiodarone, statins, tamoxifen, valproic acid, fibrates, and methotrexate[4,25,26,49-51]. Drugs such as dantrolene, phenytoin, trazadone and nitrofurantoin are also associated with chronic hepatitis with autoimmune features that may lead to cirrhosis[52-54].

Amiodarone is a benzofuran derivative mainly used in the treatment of arrhythmia. The safety of long-term use of amiodarone is well established however there are several reports of reversible and irreversible liver injury from its long-term use. Even though rare amiodarone can cause asymptomatic continuous liver injury that has histological features similar to alcoholic hepatitis such as nodular formation, fibrosis, steatosis and neutrophil infiltration[55-61]. Due to its lipophilic nature and long half-life, amiodarone accumulates in the hepatocytes affecting cellular organelles such as ER and mitochondria causing misfolding of proteins. Amiodarone affects the cholesterol metabolism by blocking enzymes emopamil binding protein and dehydrocholesterol reductase 24. As cholesterol plays an important role in maintaining membrane fluidity and composition this affects the function of potassium channels and other membrane proteins resulting in "lipid traffic jam"[62-67]. The immune cells in the liver get activated in response to cellular debris, misfolded

proteins and accumulating cholesterol precursors such as desmosterol[63,66,68]. Unless diagnosed in an early stage, this leads to irreversible end stage liver disease[62, 69].

Acute-on-chronic liver failure

Acute-on-chronic liver failure (ACLF) as the name suggests is characterized by ALF due to a different cause in patients with chronic liver disease (compensated) resulting in short term mortality. It consists of two components: a chronic underlying liver disease and an acute trigger[70,71]. Devarbhavi *et al*[72] reported that drugs contributed to 10.5% cases in the Asia-Pacific region. Among these drugs, the most common culprits were complementary and alternative medications (71.7%), followed by anti-TB drug combination therapies (27.3%). Anti-TB drug isoniazid is also observed to cause severe hepatitis that leads to liver failure[72-74].

Studies suggest that excessive focal liver and systemic inflammatory response play a significant role in the development of ACLF. Reports have shown high levels of cytokines in patients with ACLF. This may be due to the activation of monocytes and macrophages in response to DAMPs, microbial toxins or drug adducts[19,75,76].

Paracetamol induced liver failure in patients with alcoholic hepatitis is a typical example of drug induced ACLF. Alcoholic hepatitis is reported in approximately 25% of the cases of ACLF. The trigger due to paracetamol toxicity can occur in two ways- the first is due to direct toxicity by paracetamol and the second due to immune response that is secondary to the hepatocellular damage due to the direct toxicity. The activation of innate immune response due to the paracetamol acute toxicity results in upregulation of cytokine and chemokine production that initiates severe systemic inflammation, liver damage and mortality[70,75,77,78].

The dysregulation in innate immune response plays important roles in disease progression as well as disease severity. In the liver, systemic inflammation plays a significant role in the development and course of chronic alcoholic hepatitis. Similar to the acute toxicity, immune activation in alcoholic liver disease results in activation of resident Kupffer cells and dendritic cells as well as the infiltrating immune cells- monocytes and neutrophils lead to progression towards fibrosis and cirrhosis. This disrupts the liver architecture and function setting stage for liver failure, that can be actuated by an acute trigger[75,78,79].

CONCLUSION

Drugs and their metabolic products can cause liver damage through multiple mechanisms. Under normal conditions, the liver is well equipped to neutralize potential drug-related damage, but in susceptible individuals, this same drug use can result in severe liver injury. This is further amplified by a dysfunctional immune responses that is influenced by host factors like genetics, age and sex. The severe adverse outcomes of DILI are ALF, cirrhosis and acute-on-chronic liver injury. All these injuries are associated with concurrent immune dysfunction. A better understanding of immune mediators may offer new targets for the management of DILI. Individualized therapy that focuses on early detection of risk factors, triggers and stage of the liver injury may play a significant role in effectively attenuating this disorder.

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REFERENCES

1 **Kirchain WR**, Allen RE. Drug-Induced Liver Disease. In: DiPiro JT, Talbert RL, Yee GC, Matzke GR, Wells BG, Posey L. eds. Pharmacotherapy: A pathophysiologic approach, 10e. McGraw-Hill, 2016

2 **Lee WM**. Drug-induced hepatotoxicity. *N Engl J Med* 2003; **349**: 474-485 [PMID: 12890847 DOI: 10.1056/NEJMra021844]

3 **Onakpoya IJ**, Heneghan CJ, Aronson JK. Post-marketing withdrawal of 462 medicinal products because of adverse drug reactions: a systematic review of the world literature. *BMC Med* 2016; **14**: 10 [PMID: 26843061 DOI: 10.1186/s12916-016-0553-2]
4 **Kaplowitz N**. Avoiding idiosyncratic DILI: two is better than one. *Hepatology* 2013; **58**: 15-17 [PMID: 23390057 DOI: 10.1002/hep.26295]
5 **Kaplowitz N**. Drug-induced liver injury. *Clin Infect Dis* 2004; **38** Suppl 2: S44-S48 [PMID: 14986274 DOI: 10.1086/381446]
6 **Waddington JC**, Meng X, Naisbitt DJ, Park BK. Immune drug-induced liver disease and drugs. *Curr Opin Toxicol* 2018; **10**: 46-53 [DOI: 10.1016/j.cotox.2017.12.006]
7 **Bell LN**, Chalasani N. Epidemiology of idiosyncratic drug-induced liver injury. *Semin Liver Dis* 2009; **29**: 337-347 [PMID: 19826967 DOI: 10.1055/s-0029-1240002]
8 **Björnsson ES**. Incidence and outcomes of DILI in Western patients. *Clin Liver Dis (Hoboken)* 2014; **4**: 9-11 [PMID: 30992911 DOI: 10.1002/cld.368]
9 **Devarbhavi H**, Dierkhising R, Kremers WK, Sandeep MS, Karanth D, Adarsh CK. Single-center experience with drug-induced liver injury from India: causes, outcome, prognosis, and predictors of mortality. *Am J Gastroenterol* 2010; **105**: 2396-2404 [PMID: 20648003 DOI: 10.1038/ajg.2010.287]
10 **Chen M**, Suzuki A, Borlak J, Andrade RJ, Lucena MI. Drug-induced liver injury: Interactions between drug properties and host factors. *J Hepatol* 2015; **63**: 503-514 [PMID: 25912521 DOI: 10.1016/j.jhep.2015.04.016]
11 **Williams CD**, Jaeschke H. Role of innate and adaptive immunity during drug-induced liver injury. *Toxicol Res* 2012; **1**: 161 [DOI: 10.1039/C2TX20032E]
12 **Liu ZX**, Kaplowitz N. Role of innate immunity in acetaminophen-induced hepatotoxicity. *Expert Opin Drug Metab Toxicol* 2006; **2**: 493-503 [PMID: 16859400 DOI: 10.1517/17425255.2.4.493]
13 **Shaw PJ**, Ganey PE, Roth RA. Idiosyncratic drug-induced liver injury and the role of inflammatory stress with an emphasis on an animal model of trovafloxacin hepatotoxicity. *Toxicol Sci* 2010; **118**: 7-18 [PMID: 20538741 DOI: 10.1093/toxsci/kfq168]
14 **Oda S**, Yokoi T. [Establishment of animal models of drug-induced liver injury and analysis of possible mechanisms]. *Yakugaku Zasshi* 2015; **135**: 579-588 [PMID: 25832838 DOI: 10.1248/yakushi.14-00249-2]
15 **Kraskiewicz H**, FitzGerald U. InterfERing with endoplasmic reticulum stress. *Trends Pharmacol Sci* 2012; **33**: 53-63 [PMID: 22112465 DOI: 10.1016/j.tips.2011.10.002]
16 **Zhang L**, Wang X, Cueto R, Effi C, Zhang Y, Tan H, Qin X, Ji Y, Yang X, Wang H. Biochemical basis and metabolic interplay of redox regulation. *Redox Biol* 2019; **26**: 101284 [PMID: 31400697 DOI: 10.1016/j.redox.2019.101284]
17 **Stanger BZ**. Cellular homeostasis and repair in the mammalian liver. *Annu Rev Physiol* 2015; **77**: 179-200 [PMID: 25668020 DOI: 10.1146/annurev-physiol-021113-170255]
18 **Yang R**, Tonnesseen TI. DAMPs and sterile inflammation in drug hepatotoxicity. *Hepatol Int* 2019; **13**: 42-50 [PMID: 30474802 DOI: 10.1007/s12072-018-9911-9]
19 **Bianchi ME**. DAMPs, PAMPs and alarmins: all we need to know about danger. *J Leukoc Biol* 2007; **81**: 1-5 [PMID: 17032697 DOI: 10.1189/jlb.0306164]
20 **Zanger UM**, Schwab M. Cytochrome P450 enzymes in drug metabolism: regulation of gene expression, enzyme activities, and impact of genetic variation. *Pharmacol Ther* 2013; **138**: 103-141 [PMID: 23333322 DOI: 10.1016/j.pharmthera.2012.12.007]
21 **Smith RA**, Hartley RC, Cochemé HM, Murphy MP. Mitochondrial pharmacology. *Trends Pharmacol Sci* 2012; **33**: 341-352 [PMID: 22521106 DOI: 10.1016/j.tips.2012.03.010]
22 **Schuetz JD**, Swaan PW, Tweedie DJ. The role of transporters in toxicity and disease. *Drug Metab Dispos* 2014; **42**: 541-545 [PMID: 24598705 DOI: 10.1124/dmd.114.057539]
23 **Larson AM**, Polson J, Fontana RJ, Davern TJ, Lalani E, Hynan LS, Reisch JS, Schiødt FV, Ostapowicz G, Shakil AO, Lee WM; Acute Liver Failure Study Group. Acetaminophen-induced acute liver failure: results of a United States multicenter, prospective study. *Hepatology* 2005; **42**: 1364-1372 [PMID: 16317692 DOI: 10.1002/hep.20948]
24 **Tujios SR**, Lee WM. Acute liver failure induced by idiosyncratic reaction to drugs: Challenges in diagnosis and therapy. *Liver Int* 2018; **38**: 6-14 [PMID: 28771932 DOI: 10.1111/liv.13535]
25 **Russmann S**, Kullak-Ublick GA, Grattagliano I. Current concepts of mechanisms in drug-induced hepatotoxicity. *Curr Med Chem* 2009; **16**: 3041-3053 [PMID: 19689281 DOI: 10.2174/092986709788803097]
26 **Kuzu UB**, Öztaş E, Turhan N, Saygili F, Suna N, Yildiz H, Kaplan M, Akpinar MY, Akdoğan M, Kaçar S, Kiliç ZM, Köksal AŞ, Ödemiş B, Kayaçetin E. Clinical and histological features of idiosyncratic liver injury: Dilemma in diagnosis of autoimmune hepatitis. *Hepatol Res* 2016; **46**: 277-291 [PMID: 25926402 DOI: 10.1111/hepr.12530]
27 **Liu ZX**, Kaplowitz N. Immune Mechanisms in Drug-Induced Hepatotoxicity. In: Gershwin ME, Vierling JM, Manns MP, eds. Liver Immunology. Totowa, NJ: Humana Press; 2007: 363–374 [DOI: 10.1007/978-1-59745-518-3_29]
28 **Uetrecht J**. Mechanistic Studies of Idiosyncratic DILI: Clinical Implications. *Front Pharmacol* 2019; **10**: 837 [PMID: 31402866 DOI: 10.3389/fphar.2019.00837]
29 **Fontana RJ**. Pathogenesis of idiosyncratic drug-induced liver injury and clinical perspectives. *Gastroenterology* 2014; **146**: 914-928 [PMID: 24389305 DOI: 10.1053/j.gastro.2013.12.032]
30 **Mallal S**, Phillips E, Carosi G, Molina JM, Workman C, Tomazic J, Jägel-Guedes E, Rugina S,

Kozyrev O, Cid JF, Hay P, Nolan D, Hughes S, Hughes A, Ryan S, Fitch N, Thorborn D, Benbow A; PREDICT-1 Study Team. HLA-B*5701 screening for hypersensitivity to abacavir. *N Engl J Med* 2008; **358**: 568-579 [PMID: 18256392 DOI: 10.1056/NEJMoa0706135]

31 **Russmann S**, Jetter A, Kullak-Ublick GA. Pharmacogenetics of drug-induced liver injury. *Hepatology* 2010; **52**: 748-761 [PMID: 20607838 DOI: 10.1002/hep.23720]

32 **Sebode M**, Schulz L, Lohse AW. "Autoimmune(-Like)" Drug and Herb Induced Liver Injury: New Insights into Molecular Pathogenesis. *Int J Mol Sci* 2017; **18** [PMID: 28895915 DOI: 10.3390/ijms18091954]

33 **Han D**, Dara L, Win S, Than TA, Yuan L, Abbasi SQ, Liu ZX, Kaplowitz N. Regulation of drug-induced liver injury by signal transduction pathways: critical role of mitochondria. *Trends Pharmacol Sci* 2013; **34**: 243-253 [PMID: 23453390 DOI: 10.1016/j.tips.2013.01.009]

34 **Lotze MT**, Tracey KJ. High-mobility group box 1 protein (HMGB1): nuclear weapon in the immune arsenal. *Nat Rev Immunol* 2005; **5**: 331-342 [PMID: 15803152 DOI: 10.1038/nri1594]

35 **Kullak-Ublick GA**, Andrade RJ, Merz M, End P, Benesic A, Gerbes AL, Aithal GP. Drug-induced liver injury: recent advances in diagnosis and risk assessment. *Gut* 2017; **66**: 1154-1164 [PMID: 28341748 DOI: 10.1136/gutjnl-2016-313369]

36 **Larrey D**, Pageaux GP. Drug-induced acute liver failure. *Eur J Gastroenterol Hepatol* 2005; **17**: 141-143 [PMID: 15674089 DOI: 10.1097/00042737-200502000-00002]

37 **Lee WM**. Drug-induced acute liver failure. *Clin Liver Dis* 2013; **17**: 575-586, viii [PMID: 24099019 DOI: 10.1016/j.cld.2013.07.001]

38 **Argo CK**, Caldwell SH. Editorial: Severe Acute Liver Injury: Cause Connects to Outcome. *Am J Gastroenterol* 2017; **112**: 1397-1399 [PMID: 28874859 DOI: 10.1038/ajg.2017.221]

39 **Koch DG**, Speiser JL, Durkalski V, Fontana RJ, Davern T, McGuire B, Stravitz RT, Larson AM, Liou I, Fix O, Schilsky ML, McCashland T, Hay JE, Murray N, Shaikh OS, Ganger D, Zaman A, Han SB, Chung RT, Brown RS, Munoz S, Reddy KR, Rossaro L, Satyanarayana R, Hanje AJ, Olson J, Subramanian RM, Karvellas C, Hameed B, Sherker AH, Lee WM, Reuben A. The Natural History of Severe Acute Liver Injury. *Am J Gastroenterol* 2017; **112**: 1389-1396 [PMID: 28440304 DOI: 10.1038/ajg.2017.98]

40 **Antoniades CG**, Berry PA, Wendon JA, Vergani D. The importance of immune dysfunction in determining outcome in acute liver failure. *J Hepatol* 2008; **49**: 845-861 [PMID: 18801592 DOI: 10.1016/j.jhep.2008.08.009]

41 **Yang Q**, Shi Y, He J, Chen Z. The evolving story of macrophages in acute liver failure. *Immunol Lett* 2012; **147**: 1-9 [PMID: 22820147 DOI: 10.1016/j.imlet.2012.07.002]

42 **Yoon E**, Babar A, Choudhary M, Kutner M, Pyrsopoulos N. Acetaminophen-Induced Hepatotoxicity: a Comprehensive Update. *J Clin Transl Hepatol* 2016; **4**: 131-142 [PMID: 27350943 DOI: 10.14218/JCTH.2015.00052]

43 **Tittarelli R**, Pellegrini M, Scarpellini MG, Marinelli E, Bruti V, di Luca NM, Busardò FP, Zaami S. Hepatotoxicity of paracetamol and related fatalities. *Eur Rev Med Pharmacol Sci* 2017; **21**: 95-101 [PMID: 28379590]

44 **Rotundo L**, Pyrsopoulos N. Liver injury induced by paracetamol and challenges associated with intentional and unintentional use. *World J Hepatol* 2020; **12**: 125-136 [PMID: 32685105 DOI: 10.4254/wjh.v12.i4.125]

45 **Krenkel O**, Mossanen JC, Tacke F. Immune mechanisms in acetaminophen-induced acute liver failure. *Hepatobiliary Surg Nutr* 2014; **3**: 331-343 [PMID: 25568858 DOI: 10.3978/j.issn.2304-3881.2014.11.01]

46 **Mossanen JC**, Krenkel O, Ergen C, Govaere O, Liepelt A, Puengel T, Heymann F, Kalthoff S, Lefebvre E, Eulberg D, Luedde T, Marx G, Strassburg CP, Roskams T, Trautwein C, Tacke F. Chemokine (C-C motif) receptor 2-positive monocytes aggravate the early phase of acetaminophen-induced acute liver injury. *Hepatology* 2016; **64**: 1667-1682 [PMID: 27302828 DOI: 10.1002/hep.28682]

47 **Yan M**, Huo Y, Yin S, Hu H. Mechanisms of acetaminophen-induced liver injury and its implications for therapeutic interventions. *Redox Biol* 2018; **17**: 274-283 [PMID: 29753208 DOI: 10.1016/j.redox.2018.04.019]

48 **Guo H**, Chen S, Xie M, Zhou C, Zheng M. The complex roles of neutrophils in APAP-induced liver injury. *Cell Prolif* 2021; **54**: e13040 [PMID: 33942422 DOI: 10.1111/cpr.13040]

49 **Dakhoul L**, Ghabril M, Chalasani N. Drug-induced chronic liver injury. *J Hepatol* 2018; **69**: 248-250 [PMID: 29598958 DOI: 10.1016/j.jhep.2018.01.001]

50 **Ortega-Alonso A**, Stephens C, Lucena MI, Andrade RJ. Case Characterization, Clinical Features and Risk Factors in Drug-Induced Liver Injury. *Int J Mol Sci* 2016; **17** [PMID: 27187363 DOI: 10.3390/ijms17050714]

51 **Vaja R**, Ghuman N. Drugs and the liver. *Anaesth Intensive Care Med* 2018; **19**: 30-34 [DOI: 10.1016/j.mpaic.2020.07.001]

52 **Chang CC**, Petrelli M, Tomashefski JF Jr, McCullough AJ. Severe intrahepatic cholestasis caused by amiodarone toxicity after withdrawal of the drug: a case report and review of the literature. *Arch Pathol Lab Med* 1999; **123**: 251-256 [PMID: 10086516 DOI: 10.5858/1999-123-0251-SICCBA]

53 **Fisher K**, Vuppalanchi R, Saxena R. Drug-Induced Liver Injury. *Arch Pathol Lab Med* 2015; **139**: 876-887 [PMID: 26125428 DOI: 10.5858/arpa.2014-0214-RA]

54 **Fernandes NF**, Martin RR, Schenker S. Trazodone-induced hepatotoxicity: a case report with comments on drug-induced hepatotoxicity. *Am J Gastroenterol* 2000; **95**: 532-535 [PMID: 10685763

DOI: 10.1111/j.1572-0241.2000.t01-1-01780.x]
55 **Oikawa H**, Maesawa C, Sato R, Oikawa K, Yamada H, Oriso S, Ono S, Yashima-Abo A, Kotani K, Suzuki K, Masuda T. Liver cirrhosis induced by long-term administration of a daily low dose of amiodarone: a case report. *World J Gastroenterol* 2005; **11**: 5394-5397 [PMID: 16149155 DOI: 10.3748/wjg.v11.i34.5394]
56 **Raja K**, Thung SN, Fiel MI, Chang C. Drug-induced steatohepatitis leading to cirrhosis: long-term toxicity of amiodarone use. *Semin Liver Dis* 2009; **29**: 423-428 [PMID: 19826976 DOI: 10.1055/s-0029-1240011]
57 **Buggey J**, Kappus M, Lagoo AS, Brady CW. Amiodarone-Induced Liver Injury and Cirrhosis. *ACG Case Rep J* 2015; **2**: 116-118 [PMID: 26157932 DOI: 10.14309/crj.2015.23]
58 **Dees A**. A Late Presentation of Amiodarone -Induced Hepatotoxicity. *MOJ Clin Med Case Rep* 2016; **4**
59 **Tsuda T**, Tada H, Tanaka Y, Nishida N, Yoshida T, Sawada T, Sakata K, Hayashi K, Kawashiri MA, Oyama T, Sasaki M, Kurose N, Yamagishi M. Amiodarone-induced reversible and irreversible hepatotoxicity: two case reports. *J Med Case Rep* 2018; **12**: 95 [PMID: 29653592 DOI: 10.1186/s13256-018-1629-8]
60 **Kocak MZ**. Oral Amiodarone-induced liver Injury, especially Gamma Glutamyl Transferase Elevation: A Case Report. *EJMO* 2018; **2**: 117-119 [DOI: 10.14744/ejmo.2017.82474]
61 **Bratton H**, Alomari M, Al Momani LA, Aasen T, Young M. Prolonged Jaundice Secondary to Amiodarone Use: A Case Report and Literature Review. *Cureus* 2019; **11**: e3850 [PMID: 30891390 DOI: 10.7759/cureus.3850]
62 **Erez N**, Hubel E, Avraham R, Cohen R, Fishman S, Bantel H, Manns M, Tirosh B, Zvibel I, Shibolet O. Hepatic Amiodarone Lipotoxicity Is Ameliorated by Genetic and Pharmacological Inhibition of Endoplasmatic Reticulum Stress. *Toxicol Sci* 2017; **159**: 402-412 [PMID: 28962527 DOI: 10.1093/toxsci/kfx143]
63 **Rutkowski DT**. Liver function and dysfunction - a unique window into the physiological reach of ER stress and the unfolded protein response. *FEBS J* 2019; **286**: 356-378 [PMID: 29360258 DOI: 10.1111/febs.14389]
64 **Mansouri A**, Gattolliat CH, Asselah T. Mitochondrial Dysfunction and Signaling in Chronic Liver Diseases. *Gastroenterology* 2018; **155**: 629-647 [PMID: 30012333 DOI: 10.1053/j.gastro.2018.06.083]
65 **Maiers JL**, Malhi H. Endoplasmic Reticulum Stress in Metabolic Liver Diseases and Hepatic Fibrosis. *Semin Liver Dis* 2019; **39**: 235-248 [PMID: 30912096 DOI: 10.1055/s-0039-1681032]
66 **Simonen P**, Li S, Chua NK, Lampi AM, Piironen V, Lommi J, Sinisalo J, Brown AJ, Ikonen E, Gylling H. Amiodarone disrupts cholesterol biosynthesis pathway and causes accumulation of circulating desmosterol by inhibiting 24-dehydrocholesterol reductase. *J Intern Med* 2020; **288**: 560-569 [PMID: 32415867 DOI: 10.1111/joim.13095]
67 **Allen LB**, Genaro-Mattos TC, Anderson A, Porter NA, Mirnics K, Korade Z. Amiodarone Alters Cholesterol Biosynthesis through Tissue-Dependent Inhibition of Emopamil Binding Protein and Dehydrocholesterol Reductase 24. *ACS Chem Neurosci* 2020; **11**: 1413-1423 [PMID: 32286791 DOI: 10.1021/acschemneuro.0c00042]
68 **Njoku DB**. Drug-induced hepatotoxicity: metabolic, genetic and immunological basis. *Int J Mol Sci* 2014; **15**: 6990-7003 [PMID: 24758937 DOI: 10.3390/ijms15046990]
69 **European Association for the Study of the Liver**. Clinical Practice Guideline Panel: Chair:; Panel members; EASL Governing Board representative:. EASL Clinical Practice Guidelines: Drug-induced liver injury. *J Hepatol* 2019; **70**: 1222-1261 [PMID: 30926241 DOI: 10.1016/j.jhep.2019.02.014]
70 **Hernaez R**, Solà E, Moreau R, Ginès P. Acute-on-chronic liver failure: an update. *Gut* 2017; **66**: 541-553 [PMID: 28053053 DOI: 10.1136/gutjnl-2016-312670]
71 **Blasco-Algora S**, Masegosa-Ataz J, Gutiérrez-García ML, Alonso-López S, Fernández-Rodríguez CM. Acute-on-chronic liver failure: Pathogenesis, prognostic factors and management. *World J Gastroenterol* 2015; **21**: 12125-12140 [PMID: 26576097 DOI: 10.3748/wjg.v21.i42.12125]
72 **Devarbhavi H**, Choudhury AK, Sharma MK, Maiwall R, Al Mahtab M, Rahman S, Chawla YK, Dhiman RK, Duseja A, Taneja S, Ning Q, Jia JD, Duan Z, Yu C, Eapen CE, Goel A, Tan SS, Hamid SS, Butt AS, Jafri W, Kim DJ, Hu J, Sood A, Midha V, Shukla A, Ghazinian H, Sahu MK, Treeprasertsuk S, Lee GH, Lim SG, Lesmana LA, Lesmana CR, Shah S, Kalal C, Abbas Z, Sollano JD, Prasad VGM, Payawal DA, Dokmeci AK, Rao PN, Shrestha A, Lau GK, Yuen MF, Saraswat VA, Shiha G, Yokosuka O, Kedarisetty CK, Jain P, Bhatia P, Sarin SK; APASL ACLF working party. Drug-Induced Acute-on-Chronic Liver Failure in Asian Patients. *Am J Gastroenterol* 2019; **114**: 929-937 [PMID: 31021832 DOI: 10.14309/ajg.0000000000000201]
73 **Wang P**, Pradhan K, Zhong XB, Ma X. Isoniazid metabolism and hepatotoxicity. *Acta Pharm Sin B* 2016; **6**: 384-392 [PMID: 27709007 DOI: 10.1016/j.apsb.2016.07.014]
74 **Metushi I**, Uetrecht J, Phillips E. Mechanism of isoniazid-induced hepatotoxicity: then and now. *Br J Clin Pharmacol* 2016; **81**: 1030-1036 [PMID: 26773235 DOI: 10.1111/bcp.12885]
75 **Khanam A**, Kottilil S. Abnormal Innate Immunity in Acute-on-Chronic Liver Failure: Immunotargets for Therapeutics. *Front Immunol* 2020; **11**: 2013 [PMID: 33117329 DOI: 10.3389/fimmu.2020.02013]
76 **Chen P**, Wang YY, Chen C, Guan J, Zhu HH, Chen Z. The immunological roles in acute-on-chronic liver failure: An update. *Hepatobiliary Pancreat Dis Int* 2019; **18**: 403-411 [PMID: 31303562 DOI: 10.1016/j.hbpd.2019.07.003]

77 **Kamath PS**. Acute on chronic liver failure. *Clin Liver Dis (Hoboken)* 2017; **9**: 86-88 [PMID: 30992966 DOI: 10.1002/cld.625]

78 **Gustot T**, Jalan R. Acute-on-chronic liver failure in patients with alcohol-related liver disease. *J Hepatol* 2019; **70**: 319-327 [PMID: 30658733 DOI: 10.1016/j.jhep.2018.12.008]

79 **Arroyo V**, Moreau R, Kamath PS, Jalan R, Ginès P, Nevens F, Fernández J, To U, García-Tsao G, Schnabl B. Acute-on-chronic liver failure in cirrhosis. *Nat Rev Dis Primers* 2016; **2**: 16041 [PMID: 27277335 DOI: 10.1038/nrdp.2016.41]

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MINIREVIEWS

Abnormal liver enzymes: A review for clinicians

M Ammar Kalas, Luis Chavez, Monica Leon, Pahnwat Tonya Taweesedt, Salim Surani

ORCID number: M Ammar Kalas 0000-0002-6230-9172; Luis Chavez 0000-0002-1920-6354; Monica Leon 0000-0001-8652-0725; Pahnwat Tonya Taweesedt 0000-0002-5791-6920; Salim Surani 0000-0001-7105-4266.

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M Ammar Kalas, Luis Chavez, Department of Internal Medicine, Texas Tech University Health Science Center, El Paso, TX 79905, United States

Monica Leon, Department of General Surgery, University of Mexico, Ciudad de Mexico 01120, Mexico

Pahnwat Tonya Taweesedt, Department of Medicine, Corpus Christi Medical Center, Corpus Christi, TX 78412, United States

Salim Surani, Department of Medicine, Texas A&M University, College Station, TX 77843, United States

Salim Surani, Department of Anesthesiology, Mayo Clinic, Rochester, MN 55905, United States

Corresponding author: Salim Surani, FACP, FCCP, MD, MSc, Doctor, Professor, Medicine, Texas A&M University, 400 Bizzell St, College Station, TX 77843, United States. srsurani@hotmail.com

Abstract

Liver biochemical tests are some of the most commonly ordered routine tests in the inpatient and outpatient setting, especially with the automatization of testing in this technological era. These tests include aminotransferases, alkaline phosphatase, gamma-glutamyl transferase, bilirubin, albumin, prothrombin time and international normalized ratio (INR). Abnormal liver biochemical tests can be categorized based on the pattern and the magnitude of aminotransferases elevation. Generally, abnormalities in aminotransferases can be classified into a hepatocellular pattern or cholestatic pattern and can be further sub-classified based on the magnitude of aminotransferase elevation to mild [< 5 × upper limit of normal (ULN)], moderate (> 5-< 15 × ULN) and severe (> 15 × ULN). Hepatocellular pattern causes include but are not limited to; non-alcoholic fatty liver disease/non-alcoholic steatohepatitis, alcohol use, chronic viral hepatitis, liver cirrhosis (variable), autoimmune hepatitis, hemochromatosis, Wilson's disease, alpha-1 antitrypsin deficiency, celiac disease, medication-induced and ischemic hepatitis. Cholestatic pattern causes include but is not limited to; biliary pathology (obstruction, autoimmune), other conditions with hyperbilirubinemia (conjugated and unconjugated). It is crucial to interpret these commonly ordered tests accurately as appropriate further workup, treatment and referral can greatly benefit the patient due to prompt treatment which can improve the natural history of several of the diseases mentioned and possibly reduce the risk of progression to the liver cirrhosis.

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Key Words: Liver function test; Liver enzymes; Hepatitis; Liver biochemical studies; Non-alcoholic steatohepatitis; Hyperbilirubenemia

Core Tip: Liver function test are one of the most commonly ordered tests. With the automation of test and its inclusion in the complete metabolic profile, the knowledge as it pertains to its interpretation is of paramount importance. It is also important for the clinician to understand the difference between cholestatic and hepatocellular abnormalities. This can be of help for the clinician to formulate appropriate further diagnostic workup and plan the treatment.

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INTRODUCTION

Liver biochemical tests are some of the most commonly ordered tests in the United States due to the automation of routine laboratory tests. A United States population-based study of 6823 subjects from 1999 to 2002 showed elevated alanine aminotransferase (ALT) in 8.9% of subjects and aspartate aminotransferase (AST) in 4.9% of subjects.

Another population-based study consisting of 15676 subjects was done from 1988 to 1994 which showed elevation in aminotransferases (either ALT or AST) in 7.9%. In that study, 69% of the elevated aminotransferases results were unexplained[1].

Laboratory tests normal ranges are calculated based on the mean value found amongst a group of healthy individuals +/- 2 standard deviations. Hence 5% of healthy individuals' results lie outside the reference range[2].

As a result of the prevalence of liver biochemical tests ordered and abnormal results, we will be writing this review to increase the knowledge about liver tests to clinicians and improve the interpretation of these tests.

Liver function tests (LFTs) are a term commonly used for aminotransferases, alkaline phosphatase (ALP), bilirubin, and albumin which is somewhat of a misnomer as only bilirubin and albumin represent a synthetic function by the liver[3]. Besides, the liver is crucial in clotting factors production and decreased synthetic function of the liver can result in prothrombin time (PT) prolongation and an increase in the international normalized ratio (INR). Consequently, some of the most widely used scores for predicting mortality in cirrhotic patients such as the Child-Pugh score and model for end stage liver disease-Na (MELD-Na) score do not include AST, ALT, or ALP but rather use INR, bilirubin, and albumin in Child-Pugh score and INR and bilirubin in MELD-Na score.

LIVER BIOCHEMICAL STUDIES

Liver biochemical studies include; ALT, AST, ALP, gamma-glutamyl transferase (GGT), 5'nucleotidase, lactate dehydrogenase (LDH), bilirubin, albumin, PT/INR (Table 1).

Enzymes

ALT is an enzyme that is found primarily in hepatocytes (lower concentrations in cardiac, renal, and muscle tissue) and thus is specific to the hepatocellular injury. ALT levels often fluctuate throughout the d. ALT facilitates the formation of glutamate and pyruvate in the hepatocyte which is important for energy production[4]. The normal range for ALT in males is between 29-33 IU/L and 19-25 IU/L for females.

Table 1 Liver biochemical tests and their respective sites and functions

Interpretation	Test	Site (s)	Function
Hepatocellular integrity	ALT	Hepatocyte (main), cardiac, renal and muscle tissue to smaller extent	Amino acid catabolism. Glutamate and pyruvate production for ATP production
	AST	Hepatocyte, cardiac, muscle and brain tissue	
	LDH	Nonspecific, present widely in the body	Anaerobic glycolysis major enzyme in addition to NADH production. Significant in ischemic hepatitis
Cholestatic pattern	ALP	Hepatobiliary tract, bone, placenta and intestines	Dephosphorylation reactions. Role in bile production
	GGT	Mainly in hepatobiliary tract, present in multiple other organs (nonspecific as an isolate test)	Aids in identification of elevated ALP of biliary origin
	5'nucleotidase	Nonspecific, present widely in the body	Clinical value in hepatobiliary and cholestatic disease specifically when paired with ALP and GGT
	Bilirubin	Serum and liver	End product of heme breakdown. Exists in conjugated and unconjugated form. Elevation in conjugated suggestive of possible cholestasis
Synthetic function	Albumin	Serum	Main protein in the serum, maintains oncotic pressure. Produced by the liver
	PT/INR	Test to measure extrinsic coagulation pathway	Clotting factors primarily produced in the liver. Helpful however does not reflect true coagulation status

ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; LDH: Lactate dehydrogenase; ALP: Alkaline phosphatase; GGT: Gamma-glutamyl transferase; ATP: Adenosine-triphosphate; PT: Prothrombin time; INR: International normalized ratio.

ALT levels have been a point of debate recently as newer studies are suggesting the need for a lower ALT cutoff to increase the sensitivity of the test. It's believed that the current ALT cutoffs were defined by using patients with possible underlying subclinical liver disease and hence decrease the sensitivity of the test. A retrospective study in 2002 evaluated 6835 patients and hypothesized that undiagnosed hepatitis C and non-alcoholic fatty liver disease (NAFLD) are likely to have skewed the studies previously used to determine normal ALT levels based on the 95th or the 97.5th percentile.

Suggested new cut-offs from this study are ALT < 30 in men and < 19 in women. It was found that the sensitivity in detecting hepatitis C virus viremia with the lower cut offs was higher than that of the traditional cut-offs. Nonetheless these values should be cautiously interpreted as body mass index, cholesterol levels and age can affect ALT levels[5].

It is important to note that the reference ranges for labs differs across countries and sometimes even between different centers in the same country.

AST is an enzyme which like ALT is also found in the liver however has also other sites where its presence is not as minimal as ALT. These sites are primarily skeletal muscle, cardiac muscle, renal tissue, and brain. It occurs as 2 isoenzymes that are not differentiated on standard testing and hold little clinical value. AST facilitates amino acid metabolism[6]. When it comes to AST, caution must be practiced when evaluating abnormal levels due to its presence in other tissues. The normal range for AST is < 35 IU/L[7].

ALP is an enzyme that is primarily found in the hepatobiliary tract, bone, placenta, and to a smaller extent in intestinal tissue. ALP is involved in multiple dephosphorylating reactions. The normal range for ALP is between 30-120 IU/L. ALP is generally higher in children and adolescents due to the increased osteoblastic activity associated with the bone growth[8].

GGT is an enzyme that is found in multiple organs in the body including the pancreas, seminal vesicles, kidneys, biliary tract, and liver. Its elevation is usually considered significant for a hepatobiliary disease when accompanied by an elevation in other liver biochemical tests. It is generally elevated in biliary disease, cytochrome-inducing medications, and alcohol abuse. GGT is involved in the glutathione metabolism and production in multiple tissues in the body. Normal GGT levels range between 0-30 IU/L. GGT levels are generally 6-8 times higher in infants[9].

5'nucleotidase is an enzyme that is present in many organs however its clinical value holds significance primarily in hepatobiliary or cholestatic disease. It is generally used as a test to help in evaluating whether an isolated elevated ALP is from a hepatobiliary source *vs* an osseous source. Its primary function is in nucleotide hydrolysis reactions. The normal range for 5'nucleotidase 0.3-3.2 Bodansky units (levels need to be corrected with elevated serum ALP)[10].

LDH is an enzyme that is widely present in the body, it has multiple isoenzymes of which one is primarily excreted/taken up by Kupffer cells in the liver[11]. Hence liver disease/injury can result in elevated LDH. This is non-specific and is rarely used as means of evaluating liver disease. Normal LDH ranges between 140-280 U/L (ranges vary slightly between different labs).

Markers of liver synthetic function

Albumin is one of the major protein constituents in the blood and comprises 50%-60% of total protein in the serum. Albumin synthesis occurs in the liver hence it is considered a marker of the liver's synthetic function. Albumin levels can be influenced by other causes such as systemic inflammation as albumin is a negative inflammatory marker, protein malnutrition, nephrotic syndrome, fluid overload, or protein-losing enteropathy. Albumin has multiple functions such as maintaining serum oncotic pressure and endogenous (*i.e.*, bilirubin) and exogenous (*i.e.*, drugs) substances transport in the blood[12]. Normal albumin levels range between 3.5-5 g/dL.

PT and INR reflect the coagulation cascade and in specific, the extrinsic pathway of the coagulation cascade. The liver is involved in the synthesis of multiple clotting factors including, factors I, II, V, VII, IX, X, XI, and XIII, in addition to protein C, protein S, and anti-thrombin. The reason why PT and INR are primarily elevated rather than activated partial thromboplastin time (aPTT) is due to factor VIII and von Willebrand factor being produced in multiple organs around the body and conceals the aPTT prolongation *in vitro*. Due to deficiency of both pro-coagulant and anticoagulant factors, PT/INR and aPTT are not reliable measures of bleeding risk in cirrhotic patients. Moreover, PT/INR and aPTT are measures of pro-coagulant activity and do not take into consideration defects in anticoagulant pathways. Besides, patients with chronic liver diseases or cirrhosis are likely to have thrombocytopenia due to splenic sequestration and decreased thrombopoietin levels which further increases the risk of bleeding[13].

Bilirubin itself is not a marker of liver synthetic function per se however its excretion and conjugation are closely linked to the liver's conjugating and excreting function. Bilirubin is the end product of heme breakdown and is initially bound to albumin in the serum. In the liver, it is conjugated and excreted in the bile. Elevations in bilirubin levels are further classified as direct hyperbilirubinemia and indirect hyperbilirubinemia. Direct hyperbilirubinemia is generally due to an excretion defect in the liver such as cholestasis or Dubin-Johnson and Rotor syndrome. Indirect hyperbilirubinemia can be due to intrinsic liver injury or hemolysis[14].

PATTERN RECOGNITION AND INTERPRETATION

Pattern recognition and interpretation are crucial in the evaluation of abnormal liver biochemical tests. Patterns can be primarily divided into hepatocellular and cholestatic. These can be subdivided further into; acute (< 6 wk), subacute (6 wk-6 mo), or chronic (> 6 mo).

In hepatocellular pattern, there is a disproportionate rise in ALT and AST in contrast to ALP and GGT. In hepatocellular injury, there is release of aminotransferases from the hepatocytes resulting in elevated serum levels. *R* value is a proposed score aimed to aid physicians in determining the pattern of liver injury based on the upper limit of normal (ULN) of certain enzymes. *R* value = (ALT ÷ ULN ALT)/(ALP ÷ ULN ALP). *R* value > 5 is suggestive of hepatocellular pattern, > 2 to < 5 is suggestive of a mixed pattern, and < 2 suggestive of cholestatic pattern (Table 2)[15].

Hepatocellular pattern

Aminotransferase elevations can be divided into mild, moderate, and severe even though the values for this classification are variable, in this review we will be taking mild as > 2 × - < 5 × ULN lab value, moderate > 5 × - < 15 ×, severe as > 15 × ULN and massive > 10000 IU/L[16]. These values are not accurate measures of the extent of liver injury however can aid in initial workup.

Table 2 R-value calculation and interpretation

***R* value = (ALT ULN ALT)/(ALP ÷ ULN ALP)**	
R value	Interpretation
> 5	Hepatocellular pattern
> 2 but < 5	Mixed pattern
< 2	Cholestatic pattern

ALT: Alanine aminotransferase; ULN: Upper limit of normal; ALP: Alkaline phosphatase.

One of the most commonly known and used ratios is AST:ALT and is generally helpful only for an alcoholic liver disease where AST:ALT > 2. A study done in 1979 among patients with histologic evidence of liver disease demonstrated that 90% of patients with AST:ALT > 2 had alcoholic liver disease and > 96% of patients with AST:ALT > 3 had alcoholic liver disease[17]. This ratio can be explained due to alcohol being a mitochondrial toxin and low pyridoxal phosphate absorption as a result of heavy alcohol use. AST is found in mitochondria and cytoplasm, while ALT is found in cytoplasm but not mitochondria. ALT synthesis is more dependent on pyridoxal phosphate when compared to AST. In alcoholic liver disease, ALT is generally < 300 IU/L and is rarely > 500 IU/L. In situations where ALT > 500 IU/L, even if AST: ALT > 2, other etiologies should be explored. AST:ALT > 1 can be seen in cases of liver cirrhosis. GGT > 2 × the ULN is suggestive of alcohol abuse specifically when paired with AST:ALT > 2, GGT on its own is not a specific indicator of alcohol abuse[1].

Mild elevations in aminotransferases are common to be seen in clinical practice and are generally caused by medications (nontoxic ingestions), alcohol use, and chronic liver diseases such as liver cirrhosis, NAFLD, chronic hepatitis infections (B and C), hemochromatosis, Wilson's disease, autoimmune hepatitis, alpha-1 antitrypsin deficiency (AATD) and celiac disease (CD)[16]. It is advisable in patients with a mild increase in AST and ALT to undergo repeat testing in addition to the investigation of the aforementioned causes.

Moderate and severe elevations of aminotransferases are generally attributed to acute exacerbations of chronic liver diseases (such as exacerbations of hepatitis B virus, Wilson's disease, acute viral hepatitis, autoimmune hepatitis), drug-induced liver injury (DILI), and ischemic liver injury[16]. Also, they can occur in cases of acute biliary obstruction and tend to resolve soon after the obstruction is relieved.

Cholestatic pattern

Elevation of ALP and bilirubin levels often indicate a cholestatic pattern[18]. ALP can be elevated in the presence of liver or bone disease, additionally, it can be elevated due to pregnancy (placenta production). GGT is often used to clarify the origin of ALP elevation. Since ALP is produced in the bile duct epithelia, cholestasis or biliary pathology elevates the enzyme. Both anatomic and autoimmune conditions that affect the biliary system cause a cholestatic pattern. When obstruction of the common bile duct (CBD) is the cause of ALP elevation, the aminotransferases can also be elevated [18].

GGT elevation is also caused by biliary or hepatocyte disease but not bone disease. However, other causes may elevate this enzyme such as drugs (anticonvulsants and oral contraceptives), pulmonary and renal disease. As a marker, it has a high sensitivity for liver disease but low specificity[19,20].

Elevations in bilirubin levels are further classified as direct (conjugated) hyperbilirubinemia and indirect (unconjugated) hyperbilirubinemia. Hemolysis is the most common cause of indirect hyperbilirubinemia followed by Gilbert's syndrome. On the other hand, direct hyperbilirubinemia indicates liver pathology including cholestatic drug reactions, autoimmune cholestatic disease, and biliary obstruction[21].

Further laboratory and imaging studies are essential to work up the causes of a cholestatic pattern[18]. When autoimmune cholestatic liver disease is suspected the presence of anti-neutrophil cytoplasmic antibodies (for primary sclerosing cholangitis) or anti-mitochondrial antibodies (for primary biliary cirrhosis) among other studies help aid in the diagnosis.

COMMON CONDITIONS ASSOCIATED WITH ABNORMAL LIVER ENZYMES

NAFLD is one of the most common liver diseases, a meta-analysis was done in 2016 demonstrated the global prevalence of NAFLD to be approximately 25.24%[22]. Common condition associated with abnormal liver enzyme is shown in Table 3.

Nonalcoholic fatty liver (NAFL) and non-alcoholic steatohepatitis (NASH) are diseases in the same spectrum where NAFL can progress to NASH and subsequently liver cirrhosis if no intervention or modification of risk factors was done[23]. These terms are often used interchangeably however it is important to note that the management is different and accurate assessment should be made. The difference between the two is primarily seen on histology as NAFL has only fatty infiltration without inflammation whereas NASH has marked inflammation. AST and ALT levels can be normal in NAFL and are generally mildly elevated in NASH (ALT > AST). NAFL and NASH are diseases of exclusion and general risk factors are metabolic, such as obesity, dyslipidemia, and diabetes mellitus[23]. It is important to note that NAFL is generally reversible with lifestyle modifications in contrast to NASH (Table 4).

Viral hepatitis can result in a mild increase in aminotransferases, specifically chronic viral hepatitis. Hepatitis B and Hepatitis C infections can generally cause chronic infections and also have a risk for developing liver cirrhosis. In a study done in 1988, patients with chronic viral hepatitis without liver cirrhosis had an AST:ALT < 1 (0.59 average), however those with chronic viral hepatitis and liver cirrhosis had an AST: ALT > 1. This was found to be significant and is important to identify in cases of chronic viral hepatitis to aid in recognizing possible concomitant liver cirrhosis[24]. Nonetheless, caution must be practiced when looking at AST:ALT specifically when alcohol use cannot be excluded. Acute viral hepatitis on the other hand can result in moderate to severe elevation in aminotransferases, often with ALT elevations higher than that of AST. Acute hepatitis C virus can result in marked elevations in aminotransferases however generally the elevation is modest compared to hepatitis A and B. Acutely, elevation in aminotransferases levels peak before bilirubin levels, however, begins declining gradually after in contrast to bilirubin[25]. Acute hepatitis A and B in adults are associated with elevations in bilirubin resulting in jaundice (more common with hepatitis A infection) and ALP. The risk of progression to chronic hepatitis is approximately 10% in hepatitis B patients above the age of 6, hepatitis A is not associated with chronic infection[26].

Hereditary hemochromatosis is an autosomal recessive disease caused by over absorption of iron secondary to abnormal iron sensing in the gastrointestinal tract resulting in iron overload[27]. The 2 most common mutations identified are C282Y and H63D on the hemochromatosis (*HFE*) gene. Non-*HFE* hemochromatosis exists, however in this review we will talk only about *HFE* hemochromatosis.

Hemochromatosis causes mild elevations in aminotransferases (ALT > AST), elevations in ALP and bilirubin can also be seen however liver biochemical tests are non-specific in cases of hemochromatosis[27]. Bilirubin elevation is thought to be a protective mechanism to help mitigate oxidative damage caused by excess iron in the liver. Moreover, a study done in 2004 demonstrated that bilirubin level elevation was found to have a positive correlation with serum iron level[28]. In cases of elevated aminotransferases without a clear cause, it would be wise to check iron studies including iron level, ferritin level, total iron-binding capacity, and transferrin saturation. If results suggestive of iron overload, genetic testing and liver biopsy should be considered.

Wilson's disease is an autosomal recessive disease due to mutations in the ATP7B gene with a prevalence of approximately 1:30000 worldwide, studies have suggested higher prevalence based on gene mutation frequency. The difference between the 2 reported prevalence could be related to the disease's possible low penetrance[29]. Wilson's disease liver presentation is variable and can be from asymptomatic elevation in aminotransferases to acute liver failure (ALF). Aminotransferase elevation is mild in the majority of cases however can be moderate to severe in patients with Wilson's presenting with ALF. 6%-12% of emergent liver transplant referrals are due to Wilson's disease ALF[30]. Markers that aid in the diagnosis of ALF secondary to Wilson's disease are non-immune hemolytic anemia, acute renal failure, AST:ALT > 2.2, and ALP: Bilirubin < 4. Almost all patients presenting with ALF secondary to Wilson's have underlying liver fibrosis or cirrhosis[31,32].

AATD is an autosomal co-dominant disease with an expected prevalence of 3.4 million globally with combinations for severe AATD[33]. However, this number is thought to be under-representative of the actual prevalence[33]. A study done in 1989

Table 3 Common condition with abnormal liver biochemical tests

Condition	AST/ALT	ALP	GGT	Bilirubin	Other
Alcoholic hepatitis	↑↑ AST:ALT > 2	↑	↑	↑	AST/ALT < 500
NAFLD	-/↑ ALT > AST	-/Mild ↑	-/Mild ↑	↑ If progress to cirrhosis	-
Viral hepatitis	↑↑ In acute/↑ in chronic	↑	↑	↑ In chronic	AST:ALT > 1 suggestive of cirrhosis
Hemochromatosis	↑ ALT > AST	↑	↑	↑ Higher levels = higher iron load	↑ Ferritin and transferrin saturation
Wilson's disease	↑/↑↑↑ AST:ALT > 2.2 in ALF	↑	↑	↑	ALP:Bilirubin < 4
AATD	↑ AST > ALT	-	-	-	-
Celiac disease	↑ ALT > AST	-	-	-	-
Autoimmune hepatitis	↑↑	↑	↑	↑	ALP:AST/ALT < 3
DILI	↑↑/↑↑	↑	↑	↑	↑ PT/INR
Cholestasis	↑	↑↑	↑↑	↑	AST:ALT < 1.5 – ExtrahepaticAST:ALT > 1.5 - Intrahepatic

ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; ALP: Alkaline phosphatase; GGT: Gamma-glutamyl transferase; NAFLD: Non-alcohol fatty liver disease; AATD: Alpha-1 antitrypsin deficiency; DILI: Drug induced liver injury; PT: Prothrombin time; INR: International normalized ratio.

Table 4 Non-alcoholic fatty liver disease spectrum

Non-alcoholic fatty liver disease spectrum		
NAFL	Steatosis changes. No cellular ballooning, hepatocyte inflammation or fibrosis	Prevalence of 25% approximately. Reversible
NASH	Steatosis changes. Cellular ballooning and hepatocyte inflammation. No fibrosis	Prevalence of 1.5%-6.45% approximately. Generally irreversible (has been found to be reversible in some patients)
NASH related liver cirrhosis	Hepatocyte destruction and fibrosis	Prevalence of 1%-2% approximately. Irreversible
Healthy liver ←→ NAFL → NASH → NASH related cirrhosis		

NAFL: Non-alcoholic fatty liver; NASH: Non-alcoholic steatohepatitis.

in St. Louis examined 20000 blood bank samples, 700 blood samples came back positive for homozygous PI*Z mutation, however, only 28 of those individuals have been diagnosed with AATD[34]. AATD involves multiple alleles however the alleles thought to be contributing to liver disease are M (maltron) and Z allele. In adults with homozygous PI*Z mutation, 40% were found to have evidence of injury and cirrhosis histologically. Aminotransferases are generally mildly elevated with ALT predominance. Bilirubin levels are elevated in later stages (cirrhosis) along with a decrease in albumin[35].

CD is an autoimmune disease characterized by gluten intolerance which often leads to malabsorption. A study was done where 158 adults recently diagnosed with CD were followed, 42% of patients were found to have mild elevations in aminotransferases. Patients were started on a gluten-free diet and in 95% of cases, the aminotransferases levels normalized at 1 year[36]. Another study was done evaluating patients with chronically elevated aminotransferases, workup on those patients revealed that 9.3% of patients had serological evidence of CD and all but one of the 9.3% had duodenal biopsy findings of CD[37]. Aminotransferases elevation is mild with an AST:ALT < 1, bilirubin levels are generally normal. ALP can be slightly elevated in a subset of patients but is generally normal. Albumin and PT/INR values are not very reliable indicators of hepatic synthetic function in cases of CD as CD is an autoimmune disease, and a state of inflammation could cause a decrease in albumin levels. Moreover, PT/INR values can be elevated due to concomitant vitamin K deficiency secondary to malabsorption[38].

Autoimmune hepatitis is an inflammatory disorder with a female predilection and a prevalence of approximately 1:5000-1:10000 in Europe. At the time of diagnosis, almost 50% of patients have jaundice and approximately 30% have cirrhosis[39,40]. Autoimmune hepatitis affects aminotransferases variably depending on acute *vs* chronic presentations. Acutely, elevations in aminotransferases can be moderate to severe and tend to gradually decline as the disease becomes chronic and/or liver cirrhosis ensues. Bilirubin, ALP, and gamma globulins elevations are also seen in autoimmune hepatitis. ALP:AST or ALT ratio < 3 which is calculated by using the following equation (ALP/ALP ULN)/(AST/AST ULN) (ALT can be used in place of AST for this calculation) and this ratio is thought to be helpful as disproportionate elevation of ALP should prompt exploration of other differentials such as primary biliary cholangitis[41]. Furthermore, it was found that patients with higher elevations in aminotransferases had a better prognosis when compared to those with milder aminotransferase elevations[42].

DILI can cause a multitude of effects on aminotransferases and elevations of aminotransferases can be mild, moderate, or severe. A wide range of medications can cause mild elevations of aminotransferases and those include antibiotics (such as amoxicillin-clavulanic acid, macrolides (cholestatic pattern), ceftriaxone), anticonvulsants (such as Carbamazepine, Phenytoin, Valproic acid, Gabapentin), statins, antituberculosis medications, and herbal supplements. Hence, a thorough history of medication history is crucial in patients with elevated aminotransferases. More commonly, DILI is ALT predominant.

Drugs can also be a cause of moderate to severe aminotransferase elevation with the most commonly implicated drug being acetaminophen. Acetaminophen is advertised as safe with a daily dose < 4000 mg/d[43]. Acetaminophen-induced hepatotoxicity has a prevalence of approximately 30000 cases a year in the United States[44]. Up to 50% of overdoses were found to be unintentional[44]. Studies have been done which showed 6% of acetaminophen prescriptions to be > 4000 mg/d. A study evaluating AST:ALT ratio found that in cases of severe toxicity, an AST:ALT < 0.4 is suggestive of resolving hepatitis and is a positive prognostic marker[45]. Bilirubin, ALP, and PT/INR can all rise in cases of acetaminophen overdose. It is important to note that aminotransferases generally rise 2-3 d after an initial overdose and that an initial normal liver biochemical test does not exclude acetaminophen toxicity[45].

Acute cholecystitis (AC) usually presents as a cholestatic pattern or mixed. The biochemical test abnormalities are associated with obstruction from CBD, reactive hepatitis, fatty liver, direct gallbladder pressure on the biliary tract, or portal tract inflammation[19-21]. Patients with calculous AC may have CBD stones in up to 15% [17]. Gallbladder ultrasound and computed tomography (CT) is not entirely reliable for the diagnosis of CBD stones. Therefore, LFTs may be used for the identification of patients with suspected CBD stones who would benefit from endoscopic retrograde cholangiopancreatography (ERCP) or magnetic resonance cholangiopancreatography (MRCP) which are more sensitive and specific for this condition[18]. Multiple studies have shown mean values of LFTs higher in patients with AC plus CBD stones[18,22]. Bilirubin, AST, ALP, and GGT are the variables mostly studied to predict CBD stones. Ahn *et al*[18] found GGT to be the most reliable variable for CBD stones prediction with a sensitivity of 80.6% and specificity of 75%. Another study found an elevation in ALP to be the most important predictor for CBD stones[21]. Elevated LFTs in patients with AC without CBD stones are more likely to be transient and resolve within 2-7 d after surgery[18].

Ischemic hepatitis (often also referred to as hypoxic liver injury, shock liver, and hypoxic hepatitis) is a clinical condition characterized by acute liver injury causing severe elevation of aminotransferases secondary to hypoperfusion with a prevalence of approximately 2:1000 admissions and 2.5:100 in intensive care unit admissions. Moreover, it was found that approximately 4 out of 10 admissions with severe elevations in aminotransferases had ischemic hepatitis diagnosis. After further analysis, 78.2% of patients with ischemic hepatitis had a preceding acute cardiac event, 23.4% of patients with ischemic hepatitis had a diagnosis of sepsis and 52.9% of patients had a documented episode of hypotension (unspecified duration)[46].

The aminotransferase elevation is generally severe with level > 75 × ULN being suggestive of ischemic hepatitis, AST:ALT > 1 usually due to the location of AST (zone 3) in the liver and ischemic effect on zone 3. Bilirubin rise is not uncommon yet it can bemild and typically < 3 mg/dL. ALP is usually normal and PT/INR can be mildly elevated[47]. Another ratio that was found to be useful is AST:LDH < 1.5 which helps in differentiating ischemic hepatitis from viral hepatitis[48]. The AST:LDH ratio is thought to be due to the rapid and severe rise of LDH in cases of ischemic hepatitis due to hypoperfusion.

ALF is another potential cause of severe elevation in aminotransferases and cautious identification of this condition is crucial as mortality risk is approximately 40%-80% [49]. ALF is defined as the presence of severe liver injury in addition to clinical and laboratory features of liver failure such as hepatic encephalopathy and elevation in INR specifically in an individual with no prior history of liver cirrhosis or liver disease. Etiologies of ALF include but are not limited to Ischemic hepatitis, Budd Chiari syndrome, Wilson's disease, autoimmune hepatitis, acute viral hepatitis, and drug-induced liver disease. Biochemical test evaluation in ALF can be hepatocellular initially and progress to cholestatic in later stages. Labs are typically significant for severe elevation in aminotransferases, mild to moderate elevation in bilirubin and ALP in addition to INR ≥ 1.5, and in some cases LDH elevation[49]. While declining aminotransferases can be suggestive of recovery, this is not an accurate measure of recovery as it could be indicative of worsening liver failure and severe loss of liver mass. It is more appropriate to follow bilirubin, INR, and clinical features (hepatic encephalopathy) in patients with ALF for possible recovery[49].

DIAGNOSTIC TESTS

The initial evaluation of abnormal biochemical tests will be guided by the pattern (hepatocellular, cholestatic, or mixed). As a first step, the clinician should inquire about the use of medication, herbal therapies, drugs, or alcohol consumption. If a hepatocellular pattern is identified, initial serology should be obtained to rule out infectious and autoimmune etiologies. A right upper quadrant ultrasound (RUQ US) is also justified to evaluate for fatty liver. If the previous workup is unrevealing uncommon causes should be worked up (such as Wilson disease, AATD, *etc*.). If the serologic studies and imaging are unremarkable and ALT/AST is persistently elevated, consider a liver biopsy. When ALP is elevated, GGT and 5′ nucleotidase tests are important to identify the source of ALP elevation. If the latter is elevated ALP likely is elevated from hepatobiliary origin. The RUQ US will help to identify ductal dilation or the absence of it. Further workup includes either an MRCP or an ERCP (when ductal dilation is present) or serological studies including AMA if no dilation is identified. Cholestasis can be further divided into intrahepatic or extrahepatic both usually seen with marked elevation of ALP. The workup for extrahepatic cholestasis should aim to rule out choledocholithiasis, malignant obstruction, and biliary strictures. For intrahepatic cholestasis, laboratory works up should aim to rule out primary biliary cholangitis, primary sclerosing cholangitis, sickle cell disease among other causes. In intrahepatic cholestasis imaging or laboratory, workup may not yield a definitive diagnosis and other causes should be considered (*i.e.*, total parenteral nutrition, drugs associated with cholestasis, ischemic, cholestasis of pregnancy, *etc*.)

CONCLUSION

The elevation of liver biochemical studies is a common encounter of all clinicians. The multiple markers used to identify liver injury may be also elevated due to other sources (bone, placenta, kidney, muscle, *etc*.). The biochemical knowledge helps to better understand the behavior of these markers in specific conditions. The proper recognition of hepatocellular or cholestatic pattern prompts further investigations that include imaging and laboratory studies. Other factors highly important to consider when evaluating abnormal liver biochemical patterns are signs and symptoms, medications, degree of liver tests elevation, and other laboratory abnormalities present. Unfortunately, despite the use of additional tests (imaging and laboratory) in some causes the diagnostic is unclear and liver biopsy is recommended.

REFERENCES

1 **Ioannou GN**, Boyko EJ, Lee SP. The prevalence and predictors of elevated serum aminotransferase activity in the United States in 1999-2002. *Am J Gastroenterol* 2006; **101**: 76-82 [PMID: 16405537 DOI: 10.1111/j.1572-0241.2005.00341.x]

2 **Pratt DS**, Kaplan MM. Evaluation of abnormal liver-enzyme results in asymptomatic patients. *N Engl J Med* 2000; **342**: 1266-1271 [PMID: 10781624 DOI: 10.1056/NEJM200004273421707]

3 **Hall P**, Cash J. What is the real function of the liver 'function' tests? *Ulster Med J* 2012; **81**: 30-36

[PMID: 23536736]
4 **Aulbach AD**, Amuzie CJ. Chapter 17 - Biomarkers in Nonclinical Drug Development. In: Faqi AS. A Comprehensive Guide to Toxicology in Nonclinical Drug Development (Second Edition). Second Edition. Boston: Academic Press, 2017: 447-471
5 **Prati D**, Taioli E, Zanella A, Della Torre E, Butelli S, Del Vecchio E, Vianello L, Zanuso F, Mozzi F, Milani S, Conte D, Colombo M, Sirchia G. Updated definitions of healthy ranges for serum alanine aminotransferase levels. *Ann Intern Med* 2002; **137**: 1-10 [PMID: 12093239 DOI: 10.7326/0003-4819-137-1-200207020-00006]
6 **Sparling DW**. Chapter 3 - Bioindicators of Contaminant Exposure. In: Sparling DW. Ecotoxicology Essentials. San Diego: Academic Press, 2016: 45-66
7 **Pagana K**, Pagana T. Mosby's Diagnostic & Laboratory Test Reference. 14th ed. St. Louis, MO: Elsevier
8 **Lowe D**, Sanvictores T, John S. Alkaline Phosphatase. In: StatPearls. Treasure Island. StatPearls Publishing, 2021
9 **Cabrera-Abreu JC**, Green A. Gamma-glutamyltransferase: value of its measurement in paediatrics. *Ann Clin Biochem* 2002; **39**: 22-25 [PMID: 11853185 DOI: 10.1258/0004563021901685]
10 **YOUNG II**. Serum 5-nucleotidase; characterization and evaluation in disease states. *Ann N Y Acad Sci* 1958; **75**: 357-362 [PMID: 13627835 DOI: 10.1111/j.1749-6632.1958.tb36883.x]
11 **Smit MJ**, Duursma AM, Bouma JM, Gruber M. Receptor-mediated endocytosis of lactate dehydrogenase M4 by liver macrophages: a mechanism for elimination of enzymes from plasma. Evidence for competition by creatine kinase MM, adenylate kinase, malate, and alcohol dehydrogenase. *J Biol Chem* 1987; **262**: 13020-13026 [PMID: 2820961]
12 **Moman RN**, Gupta N, Varacallo M. Physiology, Albumin. In: StatPearls. Treasure Island. StatPearls Publishing, 2021
13 **Thachil J**. Relevance of clotting tests in liver disease. *Postgrad Med J* 2008; **84**: 177-181 [PMID: 18424573 DOI: 10.1136/pgmj.2007.066415]
14 **Gopal DV**, Rosen HR. Abnormal findings on liver function tests. Interpreting results to narrow the diagnosis and establish a prognosis. *Postgrad Med* 2000; **107**: 100-102, 105 [PMID: 10689411 DOI: 10.3810/pgm.2000.02.869]
15 **Chalasani NP**, Hayashi PH, Bonkovsky HL, Navarro VJ, Lee WM, Fontana RJ; Practice Parameters Committee of the American College of Gastroenterology. ACG Clinical Guideline: the diagnosis and management of idiosyncratic drug-induced liver injury. *Am J Gastroenterol* 2014; **109**: 950-66; quiz 967 [PMID: 24935270 DOI: 10.1038/ajg.2014.131]
16 **Green RM**, Flamm S. AGA technical review on the evaluation of liver chemistry tests. *Gastroenterology* 2002; **123**: 1367-1384 [PMID: 12360498 DOI: 10.1053/gast.2002.36061]
17 **Cohen JA**, Kaplan MM. The SGOT/SGPT ratio--an indicator of alcoholic liver disease. *Dig Dis Sci* 1979; **24**: 835-838 [PMID: 520102 DOI: 10.1007/BF01324898]
18 **Ahn KS**, Yoon YS, Han HS, Cho JY. Use of Liver Function Tests as First-line Diagnostic Tools for Predicting Common Bile Duct Stones in Acute Cholecystitis Patients. *World J Surg* 2016; **40**: 1925-1931 [PMID: 27094560 DOI: 10.1007/s00268-016-3517-y]
19 **Chang CW**, Chang WH, Lin CC, Chu CH, Wang TE, Shih SC. Acute transient hepatocellular injury in cholelithiasis and cholecystitis without evidence of choledocholithiasis. *World J Gastroenterol* 2009; **15**: 3788-3792 [PMID: 19673021 DOI: 10.3748/wjg.15.3788]
20 **Thapa PB**, Maharjan DK, Suwal B, Byanjankar B, Singh DR. Serum gamma glutamyl transferase and alkaline phosphatase in acute cholecystitis. *J Nepal Health Res Counc* 2010; **8**: 78-81 [PMID: 21876567]
21 **Zgheib H**, Wakil C, Shayya S, Mailhac A, Al-Taki M, El Sayed M, Tamim H. Utility of liver function tests in acute cholecystitis. *Ann Hepatobiliary Pancreat Surg* 2019; **23**: 219-227 [PMID: 31501809 DOI: 10.14701/ahbps.2019.23.3.219]
22 **Younossi ZM**, Koenig AB, Abdelatif D, Fazel Y, Henry L, Wymer M. Global epidemiology of nonalcoholic fatty liver disease-Meta-analytic assessment of prevalence, incidence, and outcomes. *Hepatology* 2016; **64**: 73-84 [PMID: 26707365 DOI: 10.1002/hep.28431]
23 **Chalasani N**, Younossi Z, Lavine JE, Charlton M, Cusi K, Rinella M, Harrison SA, Brunt EM, Sanyal AJ. The diagnosis and management of nonalcoholic fatty liver disease: Practice guidance from the American Association for the Study of Liver Diseases. *Hepatology* 2018; **67**: 328-357 [PMID: 28714183 DOI: 10.1002/hep.29367]
24 **Williams AL**, Hoofnagle JH. Ratio of serum aspartate to alanine aminotransferase in chronic hepatitis. Relationship to cirrhosis. *Gastroenterology* 1988; **95**: 734-739 [PMID: 3135226 DOI: 10.1016/s0016-5085(88)80022-2]
25 **Giannini EG**, Testa R, Savarino V. Liver enzyme alteration: a guide for clinicians. *CMAJ* 2005; **172**: 367-379 [PMID: 15684121 DOI: 10.1503/cmaj.1040752]
26 **Thuener J**. Hepatitis A and B Infections. *Prim Care* 2017; **44**: 621-629 [PMID: 29132524 DOI: 10.1016/j.pop.2017.07.005]
27 **Lin E**, Adams PC. Biochemical liver profile in hemochromatosis. A survey of 100 patients. *J Clin Gastroenterol* 1991; **13**: 316-320 [PMID: 2066547 DOI: 10.1097/00004836-199106000-00013]
28 **Alizadeh BZ**, Njajou OT, Houwing-Duistermaat JJ, de Jong G, Vergeer JM, Hofman A, Pols HA, van Duijn CM. Does bilirubin protect against hemochromatosis gene (HFE) related mortality? *Am J Med Genet A* 2004; **129A**: 39-43 [PMID: 15266614 DOI: 10.1002/ajmg.a.30163]
29 **Coffey AJ**, Durkie M, Hague S, McLay K, Emmerson J, Lo C, Klaffke S, Joyce CJ, Dhawan A,

Hadzic N, Mieli-Vergani G, Kirk R, Elizabeth Allen K, Nicholl D, Wong S, Griffiths W, Smithson S, Giffin N, Taha A, Connolly S, Gillett GT, Tanner S, Bonham J, Sharrack B, Palotie A, Rattray M, Dalton A, Bandmann O. A genetic study of Wilson's disease in the United Kingdom. *Brain* 2013; **136**: 1476-1487 [PMID: 23518715 DOI: 10.1093/brain/awt035]

30 **European Association for Study of Liver**. EASL Clinical Practice Guidelines: Wilson's disease. *J Hepatol* 2012; **56**: 671-685 [PMID: 22340672 DOI: 10.1016/j.jhep.2011.11.007]

31 **Korman JD**, Volenberg I, Balko J, Webster J, Schiodt FV, Squires RH Jr, Fontana RJ, Lee WM, Schilsky ML; Pediatric and Adult Acute Liver Failure Study Groups. Screening for Wilson disease in acute liver failure: a comparison of currently available diagnostic tests. *Hepatology* 2008; **48**: 1167-1174 [PMID: 18798336 DOI: 10.1002/hep.22446]

32 **Schilsky ML**. Wilson Disease: Diagnosis, Treatment, and Follow-up. *Clin Liver Dis* 2017; **21**: 755-767 [PMID: 28987261 DOI: 10.1016/j.cld.2017.06.011]

33 **de Serres FJ**. Worldwide racial and ethnic distribution of alpha1-antitrypsin deficiency: summary of an analysis of published genetic epidemiologic surveys. *Chest* 2002; **122**: 1818-1829 [PMID: 12426287 DOI: 10.1378/chest.122.5.1818]

34 **Silverman EK**, Miletich JP, Pierce JA, Sherman LA, Endicott SK, Broze GJ Jr, Campbell EJ. Alpha-1-antitrypsin deficiency. High prevalence in the St. Louis area determined by direct population screening. *Am Rev Respir Dis* 1989; **140**: 961-966 [PMID: 2679271 DOI: 10.1164/ajrccm/140.4.961]

35 **Bals R**. Alpha-1-antitrypsin deficiency. *Best Pract Res Clin Gastroenterol* 2010; **24**: 629-633 [PMID: 20955965 DOI: 10.1016/j.bpg.2010.08.006]

36 **Bardella MT**, Fraquelli M, Quatrini M, Molteni N, Bianchi P, Conte D. Prevalence of hypertransaminasemia in adult celiac patients and effect of gluten-free diet. *Hepatology* 1995; **22**: 833-836 [PMID: 7657290]

37 **Bardella MT**, Vecchi M, Conte D, Del Ninno E, Fraquelli M, Pacchetti S, Minola E, Landoni M, Cesana BM, De Franchis R. Chronic unexplained hypertransaminasemia may be caused by occult celiac disease. *Hepatology* 1999; **29**: 654-657 [PMID: 10051464 DOI: 10.1002/hep.510290318]

38 **Rubio-Tapia A**, Murray JA. Liver involvement in celiac disease. *Minerva Med* 2008; **99**: 595-604 [PMID: 19034257]

39 **Ngu JH**, Bechly K, Chapman BA, Burt MJ, Barclay ML, Gearry RB, Stedman CA. Population-based epidemiology study of autoimmune hepatitis: a disease of older women? *J Gastroenterol Hepatol* 2010; **25**: 1681-1686 [PMID: 20880179 DOI: 10.1111/j.1440-1746.2010.06384.x]

40 **Werner M**, Prytz H, Ohlsson B, Almer S, Björnsson E, Bergquist A, Wallerstedt S, Sandberg-Gertzén H, Hultcrantz R, Sangfelt P, Weiland O, Danielsson A. Epidemiology and the initial presentation of autoimmune hepatitis in Sweden: a nationwide study. *Scand J Gastroenterol* 2008; **43**: 1232-1240 [PMID: 18609163 DOI: 10.1080/00365520802130183]

41 **Alvarez F**, Berg PA, Bianchi FB, Bianchi L, Burroughs AK, Cancado EL, Chapman RW, Cooksley WG, Czaja AJ, Desmet VJ, Donaldson PT, Eddleston AL, Fainboim L, Heathcote J, Homberg JC, Hoofnagle JH, Kakumu S, Krawitt EL, Mackay IR, MacSween RN, Maddrey WC, Manns MP, McFarlane IG, Meyer zum Büschenfelde KH, Zeniya M. International Autoimmune Hepatitis Group Report: review of criteria for diagnosis of autoimmune hepatitis. *J Hepatol* 1999; **31**: 929-938 [PMID: 10580593 DOI: 10.1016/s0168-8278(99)80297-9]

42 **Al-Chalabi T**, Underhill JA, Portmann BC, McFarlane IG, Heneghan MA. Effects of serum aspartate aminotransferase levels in patients with autoimmune hepatitis influence disease course and outcome. *Clin Gastroenterol Hepatol* 2008; **6**: 1389-95; quiz 1287 [PMID: 18840547 DOI: 10.1016/j.cgh.2008.08.018]

43 **Yoon E**, Babar A, Choudhary M, Kutner M, Pyrsopoulos N. Acetaminophen-Induced Hepatotoxicity: a Comprehensive Update. *J Clin Transl Hepatol* 2016; **4**: 131-142 [PMID: 27350943 DOI: 10.14218/JCTH.2015.00052]

44 **Blieden M**, Paramore LC, Shah D, Ben-Joseph R. A perspective on the epidemiology of acetaminophen exposure and toxicity in the United States. *Expert Rev Clin Pharmacol* 2014; **7**: 341-348 [PMID: 24678654 DOI: 10.1586/17512433.2014.904744]

45 **McGovern AJ**, Vitkovitsky IV, Jones DL, Mullins ME. Can AST/ALT ratio indicate recovery after acute paracetamol poisoning? *Clin Toxicol (Phila)* 2015; **53**: 164-167 [PMID: 25652258 DOI: 10.3109/15563650.2015.1006399]

46 **Tapper EB**, Sengupta N, Bonder A. The Incidence and Outcomes of Ischemic Hepatitis: A Systematic Review with Meta-analysis. *Am J Med* 2015; **128**: 1314-1321 [PMID: 26299319 DOI: 10.1016/j.amjmed.2015.07.033]

47 **Lightsey JM**, Rockey DC. Current concepts in ischemic hepatitis. *Curr Opin Gastroenterol* 2017; **33**: 158-163 [PMID: 28346236 DOI: 10.1097/MOG.0000000000000355]

48 **Cassidy WM**, Reynolds TB. Serum lactic dehydrogenase in the differential diagnosis of acute hepatocellular injury. *J Clin Gastroenterol* 1994; **19**: 118-121 [PMID: 7963356 DOI: 10.1097/00004836-199409000-00008]

49 **Gill RQ**, Sterling RK. Acute liver failure. *J Clin Gastroenterol* 2001; **33**: 191-198 [PMID: 11500606 DOI: 10.1097/00004836-200109000-00005]

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MINIREVIEWS

Hepatopulmonary syndrome: An update

Kejal D Gandhi, Pahnwat Tonya Taweesedt, Munish Sharma, Salim Surani

ORCID number: Kejal D Gandhi 0000-0003-3863-8977; Pahnwat Tonya Taweesedt 0000-0002-5791-6920; Munish Sharma 0000-0002-5881-5742; Salim Surani 0000-0001-7105-4266.

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Kejal D Gandhi, Department of Internal Medicine, Medstar Washington Hospital Center/Georgetown University, Washigton, DC 20010, United States

Pahnwat Tonya Taweesedt, Munish Sharma, Department of Medicine, Corpus Christi Medical Center, Corpus Christi, TX 78412, United States

Salim Surani, Department of Medicine, Texas A&M University, Bryan, TX 78413, United States

Salim Surani, Department of Anesthesiology, Mayo Clinic, Rochester, MN 55905, United States

Corresponding author: Salim Surani, FACP, FCCP, MD, MSc, Doctor, Professor, Department of Medicine, Texas A&M University, 8447 Riverside Pkwy, Bryan, TX 78413, United States. srsurani@hotmail.com

Abstract

Hepatopulmonary syndrome (HPS) is characterized by defects in oxygenation caused by intra-pulmonary vasodilation occurring because of chronic liver disease, portal hypertension, or congenital portosystemic shunts. Clinical implications of portal hypertension are very well-known, however, awareness of its effect on multiple organs such as the lungs are less known. The presence of HPS in chronic liver disease is associated with increased mortality. Medical therapies available for HPS have not been proven effective and definitive treatment for HPS is mainly liver transplantation (LT). LT improves mortality for patients with HPS drastically. This article provides a review on the definition, clinical presentation, diagnosis, and management of HPS.

Key Words: Hepatopulmonary syndrome; Chronic liver disease; Hypoxemia; Intrapulmonary vasodilatation; Liver failure

Core Tip: Hepatopulmonary syndrome (HPS) is a progressive disease, the presence of which in cirrhotic patients worsens their prognosis. Patients with HPS have an increase rate of mortality compared to those without HPS when matched for severity of liver disease, age, sex, and liver transplantation (LT). HPS should be identified in all patients with chronic liver disease and supportive management should be provided until definitive treatment, *e.g.*, LT could be done.

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INTRODUCTION

HPS is a progressive disease associated with worsen prognosis in patients with chronic liver disease. Patients with HPS have an increase rate of mortality compared to those without HPS when matched for severity of liver disease, age, sex, and liver transplantation (LT)[1]. Hepatopulmonary syndrome (HPS) was first described in 1884 by Fluckiger based on observation in a woman with cyanosis, clubbing, and cirrhosis. Later, HPS was coined in 1977 after multiple post-mortem studies showing pulmonary vascular dilation in cirrhotic patients. These studies showed marked peripheral dilation of pulmonary arteries at precapillary and capillary levels, without any obvious lung parenchymal disease. These studies were also remarkable for multiple pleural spider naevi[2].

DEFINITION

HPS is defined as hypoxemia due to pulmonary vascular dilation in the setting of liver disease with or without portal hypertension. Definition and staging of HPS are shown in Table 1 and Table 2.

INCIDENCE/PREVALENCE

HPS has been reported in 5%-35% of patients with end-stage liver disease[3,4]. Studies have shown the presence of HPS in various liver etiologies including cirrhosis, non-cirrhotic portal fibrosis, and extra-hepatic portal vein obstruction[5,6]. Studies showed an increasing prevalence of intrapulmonary shunt in patients with increased severity of cirrhotic disease such as pretransplant patients with Child-Pugh Class C when compared with class A or B[7]. It has also been found to be associated with liver disease severity assessed by MELD score[3].

PATHOPHYSIOLOGY

Chronic liver disease can lead to hypoxemia due to a variety of underlying pathologies. Thus, it is imperative to differentiate between them. For example, HPS is caused by pulmonary vasodilation in the setting of liver disease whereas Porto-pulmonary hypertension, which is very similar in clinical presentation, is defined by pulmonary vasoconstriction causing hypoxemia due to resultant pulmonary hypertension.

The hypoxemia associated with HPS is secondary ventilation-perfusion mismatch caused mainly by diffusion defect in the dilated pulmonary bed: (1) Increased blood flow through the intra-pulmonary vasodilatation (IPVD) through the well-ventilated alveoli results in the passage of mixed venous blood in the pulmonary veins; and (2) Diffusion of oxygen is limited through the dilated pulmonary vessels due to their increased diameters resulting in disequilibrium. Supplemental oxygen increases the partial pressure of oxygen by providing the driving pressure for the oxygen to diffuse across the dilated vessels. Thus, IPVDs act as physiologic shunts more than anatomic shunts as oxygenation improves with external supplementation[8].

The unique pathological feature of HPS is dilatation of pulmonary precapillary and capillary vessels (15-100 μm diameter) along with an absolute increase in the number of dilated vessels. Paraumbilical vein and hepatic artery diameters are significant larger in cirrhotic patients with HPS compared to non-HPS[9]. Lungs and pleural spider nevi are the terms used when these vessels are noted in the lungs and along the pleural surface. Intrahepatic vasculature changes which were reported in HPS include thrombosis in intrahepatic portal venules, fibrous septa with vessels proliferation, and

Table 1 Hepatopulmonary syndrome definition

Index	
Oxygenation	PaO2 < 80 mmHg or A-a gradient (corrected for age) > 15 mmHg or 20 mmHg if age > 64 years while breathing room air
Intrapulmonary vasodilation	Confirmed by contrast-enhance echocardiography or lung perfusion scanning showing brain shunt fraction > 6%
Liver disease	Cirrhosis and/or portal hypertension

Table 2 Staging based on severity of hepatopulmonary syndrome

Stage	Partial pressure of oxygen (mmHg) on room air
Mild	≥ 80
Moderate	≥ 60 to < 80
Severe	≥ 50 to < 60
Very severe	< 50 on room air or < 300 while breathing 100% oxygen

centrilobular venous thickening[9]. Doppler ultrasonography in HPS reveals hepatojugular flow and portal blood flow of less than 10 cm/s[9].

The underlying pathophysiology is not fully proven, however, is thought to be caused by loss of pulmonary capillary vessel tone and inhibition of pulmonary vasoconstrictors. Enhanced production of nitric oxide (NO) is the major factor for pulmonary vasodilatation. NO is produced by the action of NO synthase on l-arginine. NO synthase had three isoforms of which endothelial NO synthase (eNOS) produced by pulmonary endothelial cells is the major source of NO production[10].

In experimental rat models of HPS with common bile duct ligation, proliferating cholangiocytes produces endothelin-1 (ET-1) which activates pulmonary vascular endothelin-B (ETB) receptor which in turn mediates eNOS activation and pulmonary macrophages accumulation. These animal models also showed overall increased expression of ETB receptors and increased circulation of ET-1[11,12].

In humans with HPS, exhaled NO is elevated which is a result of pulmonary vascular production and it normalizes after LT[13,14]. Acute administration of methylene blue, an inhibitor of NOS, transiently improves oxygenation[15].

Bacterial translocation from the gut in the setting of portal hypertension results in pulmonary vascular macrophages has been proposed as a mechanism causing pulmonary vasodilatation[16,17]. A study shows the decrease in this bacterial translocation by norfloxacin and thus, decreasing the severity of HPS[18]. Heme-oxygenase-derived carbon monoxide and tumor necrosis factor-alpha are also observed to contribute to pulmonary vasodilatation and angiogenesis[19,20].

CLINICAL PRESENTATION

Dyspnea on exertion or rest is the most common presenting symptom of HPS. However, dyspnea is very non-specific given it can be present in chronic liver disease due to ascites, volume overload, anemia, or muscle weakness. The presence of platypnea and orthodeoxia are specific for HPS, but not pathognomonic. Platypnea means dyspnea in an upright position which is relieved in the supine position. Orthodeoxia refers to a decrease in partial pressure of oxygen by greater than 4 mmHg or a decrease in oxygen saturation by more than 5% from a supine to upright position [21]. Both platypnea and orthodeoxia are attributed to the ventilation-perfusion mismatch.

Physical signs such as the presence of spider nevi, clubbing, cyanosis along hypoxia are strongly suggestive of HPS. Of these signs, patients with the chronic liver disease having spider nevi have a higher prevalence of HPS compared to those without spider nevi[22].

DIAGNOSIS

Patients with chronic liver disease who has dyspnea, or signs of clubbing, cyanosis, spider nevi should undergo screening and evaluation for HPS. All patients who are candidates for LT are also screened for HPS. Evaluation of HPS includes assessment of hypoxemia and intrapulmonary vasodilation. Exhaled NO is found to be higher in HPS than non-HPS patients which may help with the diagnosis.

ASSESSMENT FOR HYPOXEMIA

Pulse oximetry is used for screening purposes in chronic liver diseases to assess for HPS. All the patients with oxygen saturation < 96% should further undergo arterial blood gas analysis (ABG) to evaluate for underlying hypoxemia[23]. ABG should be drawn in the upright position to evaluate for orthodeoxia. A-a gradient > 15 mmHg or PaO_2 < 80 mmHg is used for evaluation of hypoxemia. A-a gradient is more reliable than the partial pressure of oxygen as it accounts for hyperventilation, which is common in chronic liver disease[24].

The establishment of hypoxemia alone is not enough for the diagnosis of HPS, as it can be seen in other diseases such as Porto-pulmonary hypertension. Diagnosis requires confirmation of intrapulmonary vasodilation.

ASSESSMENT FOR INTRAPULMONARY VASCULAR DILATATIONS

Transthoracic contrast echocardiography (TTCE) is first-line diagnostic tool for IPVDs. IPVDs create a shut wherein 5%-6% of the cardiac output gets shunted. TTCE is performed by injecting the agitated saline into the venous system during the echocardiogram. Agitated saline leads to the formation of bubbles in the right atrium which is then filtered by the pulmonary capillary bed. Pulmonary capillary diameter varies from 8 to 15 μm which does not allow the passage of the microbubbles. The presence of intra-cardiac or intra-pulmonary shunt leads to visualization of microbubbles/contrast in the left heart chambers. The timing of the appearance of these bubbles in the left atrium varies with heart rate, cardiac output, and shunt size. With the intra-pulmonary shunt, the microbubbles or opacification of the left atrium occurs in three to six cardiac cycles after their first appearance in the right atrium. Whereas with the intra-cardiac shunt, this opacification of the left atrium is visualized within the first three cardiac cycles after its first appearance in the right atrium. Thus, TTCE is a sensitive tool for the diagnosis of pulmonary shunt[25].

Transesophageal echocardiography is a more specific alternative to TTCE, however, is generally avoided due to the high risk associated with bleeding from esophageal varices in this patient population[26].

Technetium-99m-labeled macro aggregated albumin is also filtered by the pulmonary capillary bed and can be used to measure shunt fraction by identifying its uptake in the brain and/or kidneys. Under normal circumstances, macro aggregated albumin should not pass the pulmonary capillary bed. However, in presence of right-to-left shunt, the radionuclide is taken up by the brain and kidneys and the percentage uptake can be used to quantify the shunt. In contrast to TTCE, this method does not distinguish between intra-pulmonary and intra-cardiac shunts[27].

Contrast pulmonary angiography is rarely used to visualize the IPVD due to the invasive nature of this procedure. It is generally indicated in patients with suspicion for pulmonary arteriovenous malformations, which rarely occurs in HPS[28]. Contrast-enhanced triple phase multi-detector computed tomography abdominal portosystemic shunts of more than 10 mm in diameter[9].

MANAGEMENT

LT

The only definitive management for HPS is LT. All the patients with the partial pressure of oxygen less than 60 mmHg should be evaluated for LT. Mortality is significantly higher in patients with HPS who do not undergo LT compared to those who undergo LT. A study showed 78% mortality in HPS patients who did not undergo LT compared to 21% mortality in patients who underwent LT[29]. Thus,

patients with HPS are given higher priority for liver transplants compared to other factors. LT has been shown to improve oxygenation and shunt within the first year of transplant[30,31]. A retrospective study with 74 patients showed improvement in PaO_2 from 89% to 94% and a decrease in A-a gradient from 16 to 8 mmHg after transplantation, without significant change in DLCO[32]. A study showed a 76% 5-year survival rate in HPS who underwent LT, which is similar to liver transplant patients without HPS[33].

Oxygen supplementation

All the patients with mild to moderate HPS should be evaluated every 3 to 6 mo with ABG. All patients with oxygen saturation less than 89% or partial pressure of oxygen less than 55 mmHg at rest, exercise and while sleep should be provided supplemental oxygen.

Investigational therapies

Pentoxifylline, a tumor necrosis factor-alpha inhibitor, vasodilator with anti-angiogenesis, showed variable results in oxygenation improvement in HPS[34-36]. Early-stage HPS patients seem to have a favorable outcome, while patients with advanced-stage HPS had unimproved oxygenation and difficulty tolerating pentoxifylline due to gastrointestinal adverse effects. Randomized placebo-controlled trial is needed to prove its result.

Garlic, has allicin which is a potent vasodilator and anti-angiogenesis. It shows significant improvement in gas exchange in small studies, which include one randomized controlled trial[37,38]. Large trials are still required to prove its benefit. Inhaled NO, a vasodilator, showed an improvement of PaO_2 in a recent physiologic study even though prior findings were contradicting[39,40]. Vascular dilatations, pulmonary capillary arteriovenous communication, and blood flow shunting in HPS are thought to be more prominent in lower lung zones due to gravitation and the vasodilators use in HPS are believed to be more potent in upper and mid lung zones. Therefore, ventilation-perfusion mismatch decreased.

Methylene blue causes vasoconstriction by inhibiting NO and may also decrease angiogenesis. It has shown some benefits in improving oxygenation; however, no randomized clinical trial is available to support its use[15]. Another agent that has been shown to reduce pulmonary NO is N(G)-nitro-L-arginine methyl ester. However, it didn't improve arterial oxygenation or ventilation-perfusion mismatch[41].

Sorafenib is a tyrosine kinase inhibitor that can reduce angiogenesis. It significantly decreased alveolar-arterial oxygen gradient in rat model but failed to show benefit in patients with HPS in a randomized-controlled trial[42]. Octreotide, a somatostatin analogue that can inhibit angiogenesis, also showed no benefit in HPS patients in few studies[43].

Mycophenolate mofetil only showed benefit in one case report[44]. Norfloxacin decreases bacterial translocation and reveals benefit in an animal study and a human case report but not in a randomized controlled trial[45]. Other medications including iloprost (vasodilator), paroxetine (NO synthase inhibitor), almitrine bismesylate (pulmonary vasoconstrictor) have been tried without any clear benefit. Letrozole is undergoing an ongoing phase two trial.

The transjugular intrahepatic portosystemic shunt has been proposed to decrease portal hypertension in HPS. A small prospective study showed improvement in gas exchanged, but limited data are available[46,47]. Few case reports regarding embolization of pulmonary vasodilatation have shown improvement in oxygen[28]. All these studies do not have clear establish benefits.

CONCLUSION

All the patients with chronic liver disease with dyspnea should be screened for HPS using ABG. There is no definitive proven treatment plan for HPS except LT. Thus, all patients with HPS should undergo expedited evaluation of LT.

REFERENCES

1 **Yi HM**, Wang GS, Yi SH, Yang Y, Cai CJ, Chen GH. Prospective evaluation of postoperative outcome after liver transplantation in hepatopulmonary syndrome patients. *Chin Med J (Engl)* 2009;

122: 2598-2602 [PMID: 19951576]
2 **Rodríguez-Roisin R**, Agustí AG, Roca J. The hepatopulmonary syndrome: new name, old complexities. *Thorax* 1992; **47**: 897-902 [PMID: 1465744 DOI: 10.1136/thx.47.11.897]
3 **Ferreira PP**, Camara EJ, Paula RL, Zollinger CC, Cavalcanti AR, Bittencourt PL. Prevalence of hepatopulmonary syndrome in patients with decompensated chronic liver disease and its impact on short-term survival. *Arq Gastroenterol* 2008; **45**: 34-37 [PMID: 18425226 DOI: 10.1590/s0004-28032008000100007]
4 **Lv Y**, Fan D. Hepatopulmonary Syndrome. *Dig Dis Sci* 2015; **60**: 1914-1923 [PMID: 25732713 DOI: 10.1007/s10620-015-3593-0]
5 **Gupta D**, Vijaya DR, Gupta R, Dhiman RK, Bhargava M, Verma J, Chawla YK. Prevalence of hepatopulmonary syndrome in cirrhosis and extrahepatic portal venous obstruction. *Am J Gastroenterol* 2001; **96**: 3395-3399 [PMID: 11774955 DOI: 10.1111/j.1572-0241.2001.05274.x]
6 **Anand AC**, Mukherjee D, Rao KS, Seth AK. Hepatopulmonary syndrome: prevalence and clinical profile. *Indian J Gastroenterol* 2001; **20**: 24-27 [PMID: 11206870]
7 **Lee JM**, Choi MS, Lee SC, Park SW, Bae MH, Lee JH, Koh KC, Paik SW, Rhee PL, Kim JJ, Rhee JC. [Prevalence and risk factors of significant intrapulmonary shunt in cirrhotic patients awaiting liver transplantation]. *Taehan Kan Hakhoe Chi* 2002; **8**: 271-276 [PMID: 12499784]
8 **Davis HH 2nd**, Schwartz DJ, Lefrak SS, Susman N, Schainker BA. Alveolar-capillary oxygen disequilibrium in hepatic cirrhosis. *Chest* 1978; **73**: 507-511 [PMID: 630968 DOI: 10.1378/chest.73.4.507]
9 **Lejealle C**, Paradis V, Bruno O, de Raucourt E, Francoz C, Soubrane O, Lebrec D, Bedossa P, Valla D, Mal H, Vilgrain V, Durand F, Rautou PE. Evidence for an Association Between Intrahepatic Vascular Changes and the Development of Hepatopulmonary Syndrome. *Chest* 2019; **155**: 123-136 [PMID: 30292761 DOI: 10.1016/j.chest.2018.09.017]
10 **Rolla G**. Hepatopulmonary syndrome: role of nitric oxide and clinical aspects. *Dig Liver Dis* 2004; **36**: 303-308 [PMID: 15191196 DOI: 10.1016/j.dld.2003.12.016]
11 **Fallon MB**, Abrams GA, McGrath JW, Hou Z, Luo B. Common bile duct ligation in the rat: a model of intrapulmonary vasodilatation and hepatopulmonary syndrome. *Am J Physiol* 1997; **272**: G779-G784 [PMID: 9142908 DOI: 10.1152/ajpgi.1997.272.4.G779]
12 **Zhang J**, Ling Y, Tang L, Luo B, Pollock DM, Fallon MB. Attenuation of experimental hepatopulmonary syndrome in endothelin B receptor-deficient rats. *Am J Physiol Gastrointest Liver Physiol* 2009; **296**: G704-G708 [PMID: 19196949 DOI: 10.1152/ajpgi.90627.2008]
13 **Rolla G**, Brussino L, Colagrande P, Scappaticci E, Morello M, Bergerone S, Ottobrelli A, Cerutti E, Polizzi S, Bucca C. Exhaled nitric oxide and impaired oxygenation in cirrhotic patients before and after liver transplantation. *Ann Intern Med* 1998; **129**: 375-378 [PMID: 9735065 DOI: 10.7326/0003-4819-129-5-199809010-00005]
14 **Rolla G**, Brussino L, Colagrande P, Dutto L, Polizzi S, Scappaticci E, Bergerone S, Morello M, Marzano A, Martinasso G, Salizzoni M, Bucca C. Exhaled nitric oxide and oxygenation abnormalities in hepatic cirrhosis. *Hepatology* 1997; **26**: 842-847 [PMID: 9328302 DOI: 10.1053/jhep.1997.v26.pm0009328302]
15 **Schenk P**, Madl C, Rezaie-Majd S, Lehr S, Müller C. Methylene blue improves the hepatopulmonary syndrome. *Ann Intern Med* 2000; **133**: 701-706 [PMID: 11074903 DOI: 10.7326/0003-4819-133-9-200011070-00012]
16 **Zhang HY**, Han DW, Su AR, Zhang LT, Zhao ZF, Ji JQ, Li BH, Ji C. Intestinal endotoxemia plays a central role in development of hepatopulmonary syndrome in a cirrhotic rat model induced by multiple pathogenic factors. *World J Gastroenterol* 2007; **13**: 6385-6395 [PMID: 18081228 DOI: 10.3748/wjg.v13.i47.6385]
17 **Sztrymf B**, Libert JM, Mougeot C, Lebrec D, Mazmanian M, Humbert M, Herve P. Cirrhotic rats with bacterial translocation have higher incidence and severity of hepatopulmonary syndrome. *J Gastroenterol Hepatol* 2005; **20**: 1538-1544 [PMID: 16174071 DOI: 10.1111/j.1440-1746.2005.03914.x]
18 **Rabiller A**, Nunes H, Lebrec D, Tazi KA, Wartski M, Dulmet E, Libert JM, Mougeot C, Moreau R, Mazmanian M, Humbert M, Hervé P. Prevention of gram-negative translocation reduces the severity of hepatopulmonary syndrome. *Am J Respir Crit Care Med* 2002; **166**: 514-517 [PMID: 12186830 DOI: 10.1164/rccm.200201-027OC]
19 **Arguedas MR**, Drake BB, Kapoor A, Fallon MB. Carboxyhemoglobin levels in cirrhotic patients with and without hepatopulmonary syndrome. *Gastroenterology* 2005; **128**: 328-333 [PMID: 15685544 DOI: 10.1053/j.gastro.2004.11.061]
20 **Luo B**, Liu L, Tang L, Zhang J, Ling Y, Fallon MB. ET-1 and TNF-alpha in HPS: analysis in prehepatic portal hypertension and biliary and nonbiliary cirrhosis in rats. *Am J Physiol Gastrointest Liver Physiol* 2004; **286**: G294-G303 [PMID: 14715521 DOI: 10.1152/ajpgi.00298.2003]
21 **Gómez FP**, Martínez-Pallí G, Barberà JA, Roca J, Navasa M, Rodríguez-Roisin R. Gas exchange mechanism of orthodeoxia in hepatopulmonary syndrome. *Hepatology* 2004; **40**: 660-666 [PMID: 15349905 DOI: 10.1002/hep.20358]
22 **Younis I**, Sarwar S, Butt Z, Tanveer S, Qaadir A, Jadoon NA. Clinical characteristics, predictors, and survival among patients with hepatopulmonary syndrome. *Ann Hepatol* 2015; **14**: 354-360 [PMID: 25864216]
23 **Deibert P**, Allgaier HP, Loesch S, Müller C, Olschewski M, Hamm H, Maier KP, Blum HE. Hepatopulmonary syndrome in patients with chronic liver disease: role of pulse oximetry. *BMC*

Gastroenterol 2006; **6**: 15 [PMID: 16638132 DOI: 10.1186/1471-230X-6-15]
24 **Voiosu A**, Voiosu T, Stănescu CM, Chirilă L, Băicuş C, Voiosu R. Novel predictors of intrapulmonary vascular dilatations in cirrhosis: extending the role of pulse oximetry and echocardiography. *Acta Gastroenterol Belg* 2013; **76**: 241-245 [PMID: 23898563]
25 **Abrams GA**, Jaffe CC, Hoffer PB, Binder HJ, Fallon MB. Diagnostic utility of contrast echocardiography and lung perfusion scan in patients with hepatopulmonary syndrome. *Gastroenterology* 1995; **109**: 1283-1288 [PMID: 7557096 DOI: 10.1016/0016-5085(95)90589-8]
26 **Aller R**, Moya JL, Moreira V, Boixeda D, Cano A, Picher J, García-Rull S, de Luis DA. Diagnosis of hepatopulmonary syndrome with contrast transesophageal echocardiography: advantages over contrast transthoracic echocardiography. *Dig Dis Sci* 1999; **44**: 1243-1248 [PMID: 10389704 DOI: 10.1023/a:1026657114256]
27 **Mimidis KP**, Vassilakos PI, Mastorakou AN, Spiropoulos KV, Lambropoulou-Karatza CA, Thomopoulos KC, Tepetes KN, Nikolopoulou VN. Evaluation of contrast echocardiography and lung perfusion scan in detecting intrapulmonary vascular dilatation in normoxemic patients with early liver cirrhosis. *Hepatogastroenterology* 1998; **45**: 2303-2307 [PMID: 9951913]
28 **Ryu JK**, Oh JH. Hepatopulmonary syndrome: angiography and therapeutic embolization. *Clin Imaging* 2003; **27**: 97-100 [PMID: 12639774 DOI: 10.1016/s0899-7071(02)00511-9]
29 **Swanson KL**, Wiesner RH, Krowka MJ. Natural history of hepatopulmonary syndrome: Impact of liver transplantation. *Hepatology* 2005; **41**: 1122-1129 [PMID: 15828054 DOI: 10.1002/hep.20658]
30 **Eriksson LS**, Söderman C, Ericzon BG, Eleborg L, Wahren J, Hedenstierna G. Normalization of ventilation/perfusion relationships after liver transplantation in patients with decompensated cirrhosis: evidence for a hepatopulmonary syndrome. *Hepatology* 1990; **12**: 1350-1357 [PMID: 2258151 DOI: 10.1002/hep.1840120616]
31 **Gupta S**, Castel H, Rao RV, Picard M, Lilly L, Faughnan ME, Pomier-Layrargues G. Improved survival after liver transplantation in patients with hepatopulmonary syndrome. *Am J Transplant* 2010; **10**: 354-363 [PMID: 19775311 DOI: 10.1111/j.1600-6143.2009.02822.x]
32 **Battaglia SE**, Pretto JJ, Irving LB, Jones RM, Angus PW. Resolution of gas exchange abnormalities and intrapulmonary shunting following liver transplantation. *Hepatology* 1997; **25**: 1228-1232 [PMID: 9141442 DOI: 10.1002/hep.510250527]
33 **Deberaldini M**, Arcanjo AB, Melo E, da Silva RF, Felício HC, Arroyo PC Jr, Duca WJ, Cordeiro JA, da Silva RC. Hepatopulmonary syndrome: morbidity and survival after liver transplantation. *Transplant Proc* 2008; **40**: 3512-3516 [PMID: 19100426 DOI: 10.1016/j.transproceed.2008.08.134]
34 **Zhang J**, Ling Y, Tang L, Luo B, Chacko BK, Patel RP, Fallon MB. Pentoxifylline attenuation of experimental hepatopulmonary syndrome. *J Appl Physiol (1985)* 2007; **102**: 949-955 [PMID: 17110505 DOI: 10.1152/japplphysiol.01048.2006]
35 **Gupta LB**, Kumar A, Jaiswal AK, Yusuf J, Mehta V, Tyagi S, Tempe DK, Sharma BC, Sarin SK. Pentoxifylline therapy for hepatopulmonary syndrome: a pilot study. *Arch Intern Med* 2008; **168**: 1820-1823 [PMID: 18779471 DOI: 10.1001/archinte.168.16.1820]
36 **Tanikella R**, Philips GM, Faulk DK, Kawut SM, Fallon MB. Pilot study of pentoxifylline in hepatopulmonary syndrome. *Liver Transpl* 2008; **14**: 1199-1203 [PMID: 18668653 DOI: 10.1002/lt.21482]
37 **Abrams GA**, Fallon MB. Treatment of hepatopulmonary syndrome with Allium sativum L. (garlic): a pilot trial. *J Clin Gastroenterol* 1998; **27**: 232-235 [PMID: 9802451 DOI: 10.1097/00004836-199810000-00010]
38 **De BK**, Dutta D, Pal SK, Gangopadhyay S, Das Baksi S, Pani A. The role of garlic in hepatopulmonary syndrome: a randomized controlled trial. *Can J Gastroenterol* 2010; **24**: 183-188 [PMID: 20352147 DOI: 10.1155/2010/349076]
39 **Jounieaux V**, Leleu O, Mayeux I. Cardiopulmonary effects of nitric oxide inhalation and methylene blue injection in hepatopulmonary syndrome. *Intensive Care Med* 2001; **27**: 1103-1104 [PMID: 11497151 DOI: 10.1007/s001340100967]
40 **Gupta S**, Tang R, Al-Hesayen A. Inhaled nitric oxide improves the hepatopulmonary syndrome: a physiologic analysis. *Thorax* 2021; **76**: 1142-1145 [PMID: 33859047 DOI: 10.1136/thoraxjnl-2020-216128]
41 **Gómez FP**, Barberà JA, Roca J, Burgos F, Gistau C, Rodríguez-Roisin R. Effects of nebulized N(G)-nitro-L-arginine methyl ester in patients with hepatopulmonary syndrome. *Hepatology* 2006; **43**: 1084-1091 [PMID: 16628648 DOI: 10.1002/hep.21141]
42 **Kawut SM**, Ellenberg SS, Krowka MJ, Goldberg D, Vargas H, Koch D, Sharkoski T, Al-Naamani N, Fox A, Brown R, Levitsky J, Oh JK, Lin G, Song N, Mottram C, Doyle MF, Kaplan DE, Gupta S, Fallon MB. Sorafenib in Hepatopulmonary Syndrome: A Randomized, Double-Blind, Placebo-Controlled Trial. *Liver Transpl* 2019; **25**: 1155-1164 [PMID: 30816637 DOI: 10.1002/lt.25438]
43 **Söderman C**, Juhlin-Dannfelt A, Lagerstrand L, Eriksson LS. Ventilation-perfusion relationships and central haemodynamics in patients with cirrhosis. Effects of a somatostatin analogue. *J Hepatol* 1994; **21**: 52-57 [PMID: 7963422 DOI: 10.1016/s0168-8278(94)80136-3]
44 **Moreira Silva H**, Reis G, Guedes M, Cleto E, Vizcaíno JR, Kelly D, Gennery AR, Santos Silva E. A case of hepatopulmonary syndrome solved by mycophenolate mofetil (an inhibitor of angiogenesis and nitric oxide production). *J Hepatol* 2013; **58**: 630-633 [PMID: 23104163 DOI: 10.1016/j.jhep.2012.10.021]
45 **Gupta S**, Faughnan ME, Lilly L, Hutchison S, Fowler R, Bayoumi AM. Norfloxacin therapy for hepatopulmonary syndrome: a pilot randomized controlled trial. *Clin Gastroenterol Hepatol* 2010; **8**:

1095-1098 [PMID: 20816858 DOI: 10.1016/j.cgh.2010.08.011]

46 **Tsauo J**, Zhao H, Zhang X, Ma H, Jiang M, Weng N, Li X. Effect of Transjugular Intrahepatic Portosystemic Shunt Creation on Pulmonary Gas Exchange in Patients with Hepatopulmonary Syndrome: A Prospective Study. *J Vasc Interv Radiol* 2019; **30**: 170-177 [PMID: 30717947 DOI: 10.1016/j.jvir.2018.09.017]

47 **Lasch HM**, Fried MW, Zacks SL, Odell P, Johnson MW, Gerber DA, Sandhu FS, Fair JH, Shrestha R. Use of transjugular intrahepatic portosystemic shunt as a bridge to liver transplantation in a patient with severe hepatopulmonary syndrome. *Liver Transpl* 2001; **7**: 147-149 [PMID: 11172400 DOI: 10.1053/jlts.2001.21287]

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MINIREVIEWS

Mitochondrial hepatopathy: Respiratory chain disorders- 'breathing in and out of the liver'

Amrit Gopan, Moinak Sen Sarma

ORCID number: Amrit Gopan 0000-0001-8344-1298; Moinak S Sarma 0000-0003-2015-4069.

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Amrit Gopan, Department of Gastroenterology, Seth G.S Medical College and K.E.M Hospital, Mumbai 400012, India

Moinak Sen Sarma, Department of Pediatric Gastroenterology, Sanjay Gandhi Postgraduate Institute of Medical Sciences, Lucknow 226014, India

Corresponding author: Moinak Sen Sarma, DM, Associate Professor, Department of Pediatric Gastroenterology, Sanjay Gandhi Postgraduate Institute of Medical Sciences, Rae Bareilly Road, Lucknow 226014, India. moinaksen@gmail.com

Abstract

Mitochondria, the powerhouse of a cell, are closely linked to the pathophysiology of various common as well as not so uncommon disorders of the liver and beyond. Evolution supports a prokaryotic descent, and, unsurprisingly, the organelle is worthy of being labeled an organism in itself. Since highly metabolically active organs require a continuous feed of energy, any dysfunction in the structure and function of mitochondria can have variable impact, with the worse end of the spectrum producing catastrophic consequences with a multisystem predisposition. Though categorized a hepatopathy, mitochondrial respiratory chain defects are not limited to the liver in time and space. The liver involvement is also variable in clinical presentation as well as in age of onset, from acute liver failure, cholestasis, or chronic liver disease. Other organs like eye, muscle, central and peripheral nervous system, gastrointestinal tract, hematological, endocrine, and renal systems are also variably involved. Diagnosis hinges on recognition of subtle clinical clues, screening metabolic investigations, evaluation of the extrahepatic involvement, and role of genetics and tissue diagnosis. Treatment is aimed at both circumventing the acute metabolic crisis and long-term management including nutritional rehabilitation. This review lists and discusses the burden of mitochondrial respiratory chain defects, including various settings when to suspect, their evolution with time, including certain specific disorders, their tiered evaluation with diagnostic algorithms, management dilemmas, role of liver transplantation, and the future research tools.

Key Words: Mitochondrial hepatopathy; Respiratory chain defects; Maternal inheritance; Neonatal liver failure; DNA depletion syndrome; Pearson syndrome

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Core Tip: Liver disease with multi-system involvement should arouse the suspicion for mitochondrial respiratory chain hepatopathies. These disorders are predominantly autosomal recessive with some having a maternal inheritance. Presence of lactic acidosis without hypoglycemia is an important clue. A tiered evaluation yields the most data, with the final step being a genetic and enzyme analysis from tissue of interest. Treatment is largely supportive with blood transfusions, correction of acidosis and shock, providing cofactors and salvage therapies, with liver transplantation in a select group. A periodic follow-up is mandatory for monitoring evolution of disease including "migration" to other systems.

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INTRODUCTION

Mitochondria are intracellular organelles, with a double lamellar covering outer membrane serving as a corset that holds the highly convoluted inner membrane in place (Figure 1). The inter-membrane space is the first of two liquid components within the mitochondria mainly participating in the exchange of lipids, proteins, and metal ions and also signaling cascades[1]. The second space is the soluble matrix, lying within the inner membrane and the hub of various metabolically active processes, most notably the tricarboxylic acid cycle, fatty acid oxidation, and urea synthesis. The inner membrane is folded into multiple cristae, which are shelf like projections into the matrix. The number of cristae are reflective of a metabolically active state, with a more active tissue having numerous mitochondria with many cristae, an ideal comparison being striated muscle tissue against adipocytes. This inner membrane of the mitochondria houses the respiratory chain comprised of electron carriers (complexes I, II, III, and IV, cytochrome c, coenzyme Q) and complex V, which is the hydrogen adenosine triphosphatases complex (Figure 2). All metabolic processes within the matrix generate reducing equivalents in the form of electrons (carried as NADPH2), which pass through these complexes, entering it at various points. While doing so, from one complex to another, it also results in proton (H^+) flow from matrix to intermembrane space leading to its pooling up and a chemical gradient that then flows down the potential *via* the complex V, which utilizes the energy to generate adenosine triphosphate from adenosine diphosphate, the ultimate objective of this intricately woven complex process called oxidative phosphorylation[2].

MITOCHONDRIAL GENOME AND ITS IMPLICATIONS

From the perspective of evolution, the classical endosymbiont theory proposes that mitochondria are actually prokaryotes within eukaryotic cells and hence have a genome of their own[3]. Mitochondrial genome consists of a circular double stranded DNA made of 16569 base pairs organized to make up 37 genes. Of these, 13 genes are exclusively for synthesis of proteins that are part of the respiratory chain. The other 24 genes are required for mitochondrial DNA (mtDNA) translation process (22 genes for an equal number of transfer RNA and two for ribosomal RNA synthesis). There are three major differences between mitochondrial and Mendelian inheritance: Maternal inheritance, heteroplasmy and threshold effect, and mitotic segregation. Maternal inheritance in simple terms means that the mtDNA and its aberrations are transferred from mother (ovum) to its offspring (zygote), as there is hardly any mitochondria left in the sperm, which concentrates itself to fill its entire cytoplasm with the energy dense nucleus. However, there are a few exceptions, as reported in skeletal muscle defects linked to mitochondrial inheritance that are transmitted by father to offspring [4]. It is essential to understand that all characteristics encoded by mtDNA are maternally inherited but all mitochondrial diseases are not maternally inherited.

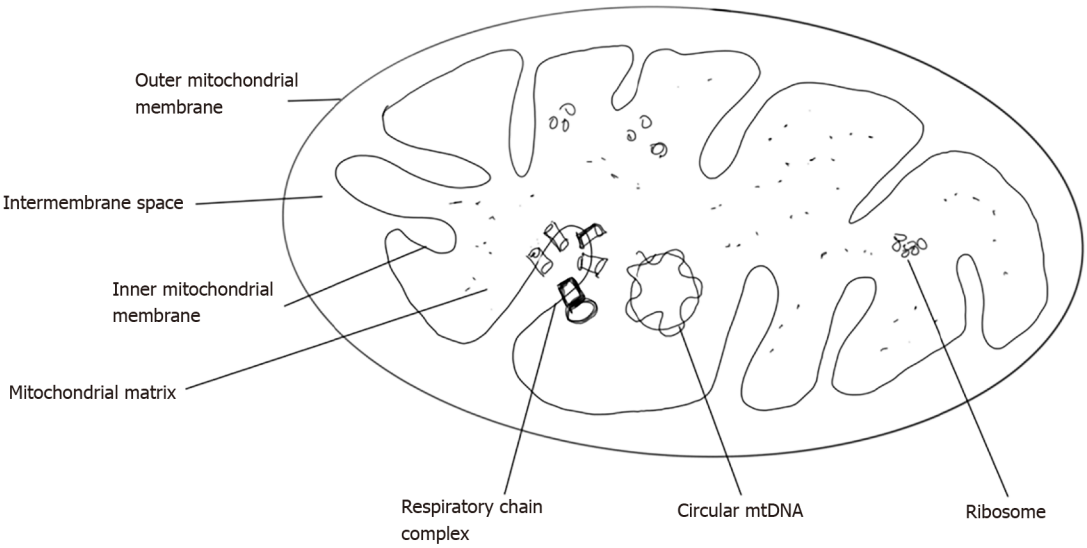

Figure 1 Diagrammatic representation of structure of mitochondria. mtDNA: Mitochondrial DNA.

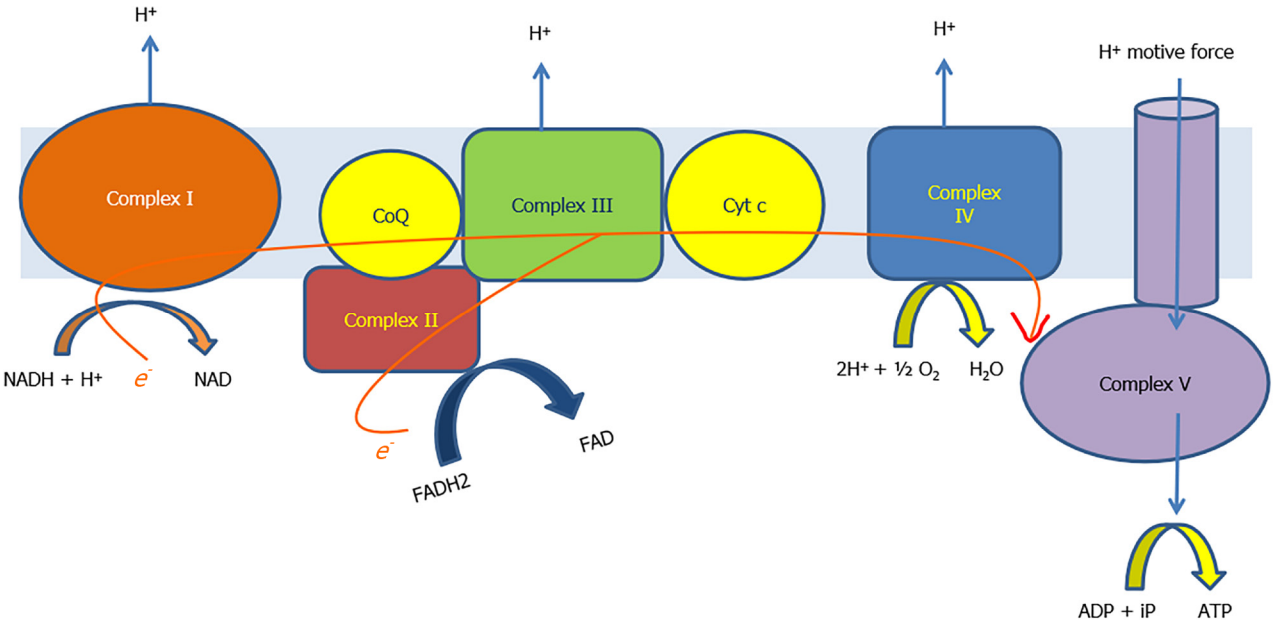

Figure 2 The electron transport chain formed by the respiratory chain complexes and process of oxidative phosphorylation. ADP: Adenosine diphosphate; ATP: Adenosine triphosphate; CoQ: Coenzyme Q; Cyt c: Cytochrome c; FAD: Flavin adenine dinucleotide; FADH2: Reduced form of FAD; NAD: Nicotinamide adenine dinucleotide; NADH: Reduced form of NAD.

Nuclear DNA (nuDNA) encodes most of the metabolic processes occurring in the mitochondria. NuDNA also encodes many enzymes and cofactors required for maintenance of mtDNA as well as approximately 70 respiratory chain subunits[5]. Figures 3 and 4 describe the way of inheritance and the mathematics of genetics in mitochondrial diseases. In normal persons, all mtDNA are identical, a state known as homoplasmy. Presence of both mutated and non-mutated wild type mtDNA containing mitochondria together in a cell is cellular heteroplasmy, while having 2 types of mtDNA within a single mitochondrion is organellar heteroplasmy. A particular number of abnormal mtDNA burden should exist for disease phenotype to manifest, a phenomenon known as threshold effect. This effect is seen at different levels of mutated mtDNA in various organs, the lowest threshold (and hence maximum susceptibility) being in organs dependent highly on oxidative metabolism like brain, heart, skeletal muscle, retina, and endocrine organs. Another interesting phenomenon is "skewed heteroplasmy" where some organs selectively have a higher burden of abnormal mitochondria, exemplified by mitochondrial diabetes, cardiomy-

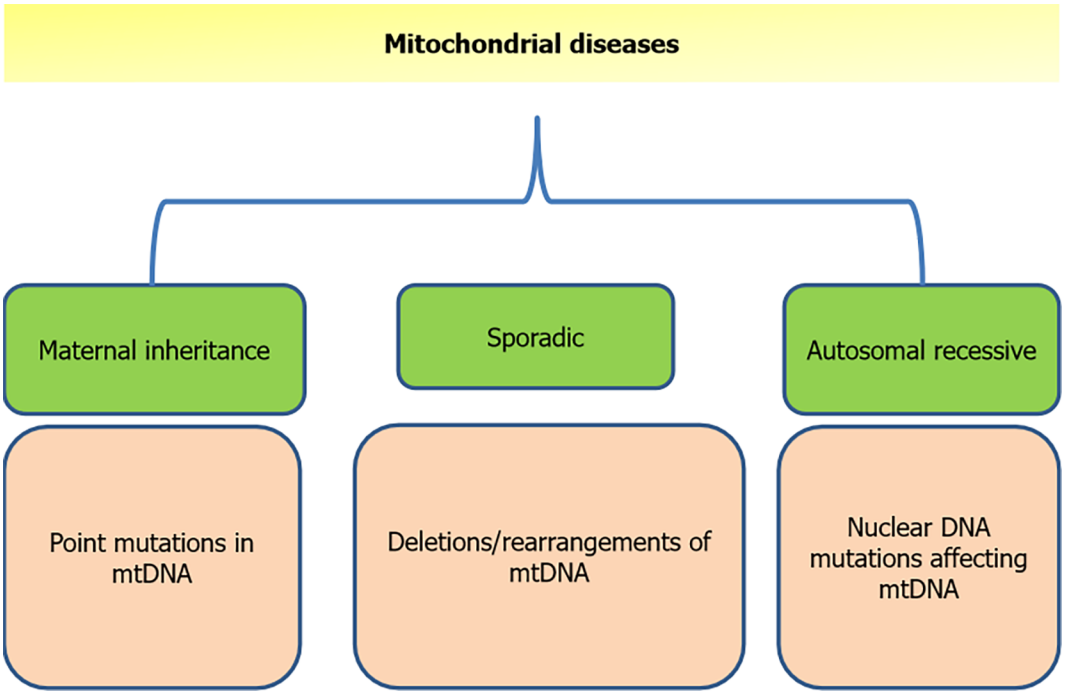

Figure 3 Various modes of inheritance of mitochondrial disease. mtDNA: Mitochondrial DNA.

The numbers game

No. of mitochondrial genes: 37

No. of genes encoding proteins required for translation of mtDNA : 24

tRNA – 22

rRNA – 2

No. of genes encoding for final structuro-functional proteins in mitochondria: 13

Complex 1 subunits – 7

Complex 3 subunit –1

Cyt c oxidase subunits – 3

ATP synthase subunits - 2

No. of gene products in mitochondria: 900

Gene products encoded by nuDNA: 863

Figure 4 Comparison of mitochondrial and nuclear DNA influence in genetics of mitochondria. mtDNA: Mitochondrial DNA; rRNA: Ribosomal RNA; tRNA: Transfer RNA.

opathies, and deafness[6-8]. Mitotic segregation effect refers to the random distribution of mitochondria at end of cell division, which can segregate mutated and non-mutated mtDNA in a variable manner into the two daughter cells. This may result in a daughter cell phenotype that is diseased (due to presence of more abnormal mitochondria), *i.e.* more abnormal than the originator cell in the subsequent divisions. With age, abnormal cells may predominate, explaining age related unmasking of diseases.

THE PROBLEM STATEMENT: EPIDEMIOLOGY

Prevalence of respiratory chain defects is variable across geographical lines as well as across eras. A large study examining birth prevalence of mitochondrial respiratory

chain disorders (RCDs) up to 16 years of age puts the figure at 5/100000 births[9]. This would mean that for every 20000 births in a particular time period, 1 child has the probability of getting affected by a respiratory chain defect of any type till he or she reaches the age of 16. The same study extrapolated the prevalence as 13.1/100000 births with onset at any age when seen together in the light of another study by Chinnery *et al*[10]. According to the Swedish registry, in a population study identifying mitochondrial encephalomyopathies, 20% had liver involvement[11]. In a 5 year French study of 1041 children, 22 (10%) of the 234 patients with respiratory chain defects had hepatopathy[12]. We would, however, add a word of caution that these figures can be an underrepresentation of true values in view of the heterogeneity of presentation and difficulty in diagnosis of mitochondrial respiratory chain defects.

CLASSIFICATION OF MITOCHONDRIAL HEPATOPATHIES AND STATUS OF RESPIRATORY CHAIN DISORDERS

Mitochondrial disorders are characterized by their variability in presentation and predilection for more than one organ system simultaneously or separated in time.

Sokol and Treem proposed classifying these disorders as primary and secondary depending on whether defect is inherently present in the mitochondria and leads to liver dysfunction or there is secondary involvement of mitochondria in the form of injury or alteration in non-mitochondrial genetics. There are two broad types of mitochondrial hepatopathies, one which affects the respiratory chain present on the inner mitochondrial membrane and the other includes fatty acid oxidation defects, which are related to the process within the mitochondrial matrix. The RCDs can also be divided into those arising due to defective mtDNA and those due to defect/mutation in nuDNA. Among the diseases affecting mtDNA, the affliction can be in the form of either mutations or an overall depletion of quantity of mtDNA compared to nuDNA in a cell/tissue. Figure 5 shows a simplified way of classification of mitochondrial hepatopathies (MH), and a tabular representation of primary MH individual disorders is shown in Table 1. It is worthwhile to note that usually mitochondrial disorders with primary myopathic involvement have mutations in mtDNA, while those with primary hepatic involvement have mutations in nuDNA affecting mitochondrial processes, with some exceptions[13]. Since we are discussing respiratory chain disorders not confined to liver but to include the gastrointestinal tract, we will include one prototype non-hepatic RCD affecting the gastrointestinal (GI) tract, mitochondrial neurogastrointestinal encephalomyopathy (MNGIE), in our review. This review does not cover non-RCD mitochondrial hepatopathies (fatty acid oxidation disorders and others) and GI manifestations of non-RCD, non-hepatic mitochondrial disorders.

CLINICAL PRESENTATION

Mitochondrial disorders are often called mitochondrial multiorgan disorder syndrome (MIMODS) in view of their heterogenous presentation affecting the nervous system (central and peripheral), eyes, ears, endocrine system, kidneys, heart and blood vessels, bone marrow, lungs, and also the intestinal tract, apart from affecting the liver (hepatopathy). The liver involvement is also variable in clinical presentation as well as in age of onset, from acute liver failure, cholestasis, or as chronic liver disease. A graphical summary of all mitochondrial RCDs affecting the liver is represented in Figure 6. Each of the individual disorders is briefly discussed.

Neonatal liver failure

Neonatal liver failure is a catastrophic event, and there are few disorders that present in the first few months as liver failure. Neonatal acute liver failure (ALF) is distinct from pediatric and adult liver failures in that it can include causes that have underlying cirrhosis. Also, the cut-off for coagulopathy is proposed as an international normalized ratio (INR) of ≥ 3 for newborns, as normal INR can be up to 2 in this age [14]. The four main causes of neonatal liver failure are: (1) Gestational alloimmune liver disease (neonatal hemochromatosis); (2) Viral infections (herpes simplex); (3) Hemophagocytic lymphohistiocytosis (primary-familial/secondary to infections); and (4) Mitochondrial hepatopathies (respiratory chain defects).

Table 1 Various mitochondrial primary respiratory chain disorders

Disorder	Mutation/defective gene	Location of defect	Affected proteins/consequence
Neonatal liver failure: (1) Complex I deficiency; (2) Complex III deficiency; (3) Complex IV deficiency; and (4) Multiple complex deficiencies	ACAD9; BCS1L; SCO1	nuDNA	Respective complexes deficiency as per name
Delayed onset liver failure: Alper's Huttenlocher syndrome	POLG mutation	nuDNA	Defective mtDNA polymerase; mtDNA depletion
MtDNA depletion syndrome	DGUOK; TK-2; MPV 17; POLG	All nuDNA	Decreased deoxyribonucleotide concentrations within mitochondria
Mitochondrial neuro-gastrointestinal encephalomyelopathy	TYMP	nuDNA	Markedly low levels of thymidine phosphorylase activity
Pearson marrow pancreas syndrome	4000-5000 bp deletions in mtDNA; tRNA gene of mtDNA	Both mtDNA	Complex I, IV, V
Navajo neurohepatopathy	MPV 17 mutations	nuDNA	mtDNA depletion
Villous atrophy with hepatic involvement	Rearrangement defect/deletion-duplications in mtDNA	mtDNA	Complex III deficiency

nuDNA: Nuclear DNA; mtDNA: Mitochondrial DNA.

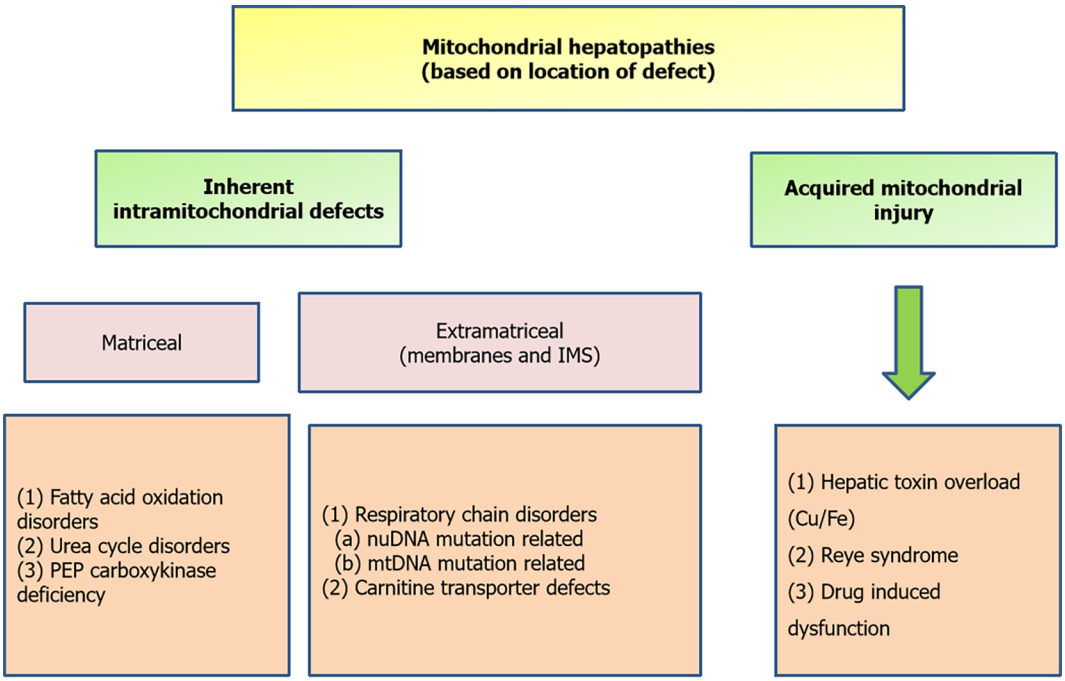

Figure 5 Simplified way of classification of mitochondrial hepatopathies based on location of defect. IMS: Intermembrane space; mtDNA: Mitochondrial DNA; PEP: Phosphoenolpyruvate.

Apart from these, galactosemia, tyrosinemia, and hereditary fructose intolerance can present as ALF in early infantile period and rarely in neonatal age[15]. The key here is to keep mitochondrial hepatopathy (RCD and non-RCD) as one of the differentials of acute liver failure in a newborn/early infantile period, though it accounts for < 5% in neonatal ALF series[14]. Multi-system involvement, especially with neurological symptoms in form of lethargy, floppy tone, vomiting, poor suck, and seizures, are diagnostic clues. Some patients are apparently normal until a viral illness or an unknown inciting event seems to trigger a downhill course either hepatic or neurological or both. Infants with mitochondrial hepatopathies are seen to have a low birth weight in up to 23%, and associated intrauterine growth retardation is seen in 16%, likely due to insult beginning from intrauterine period[16]. Laboratory findings of metabolic acidosis, elevated lactate levels, high lactate to pyruvate ratio often more than 30 mol/mol, elevated ketone bodies betahydroxybutyrate, and betahydroxybu-

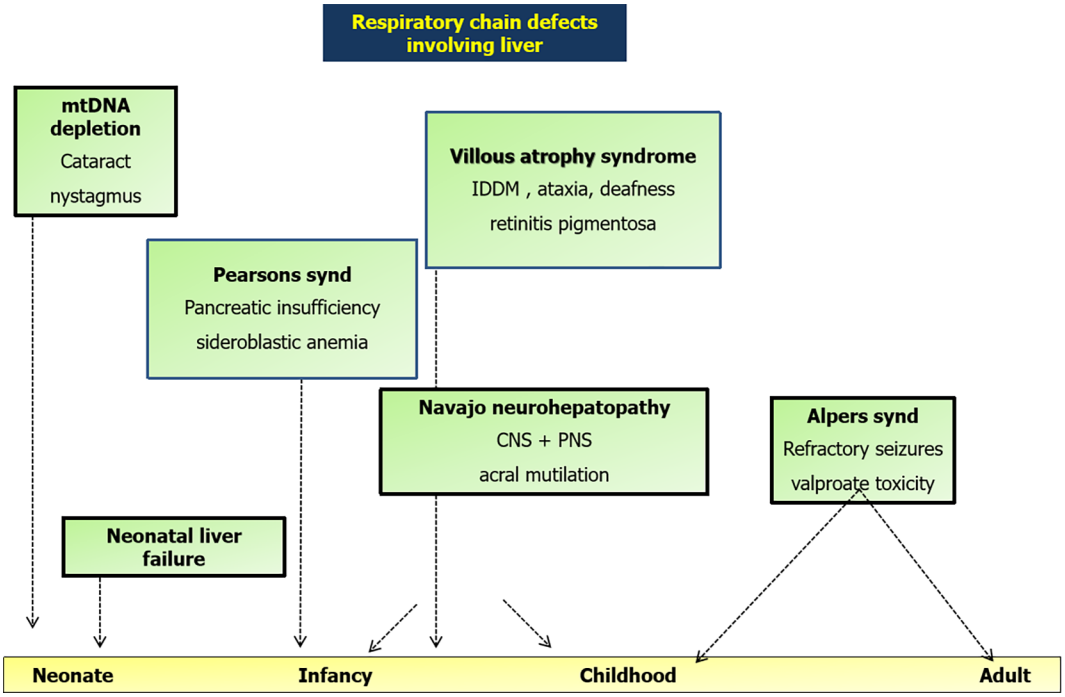

Figure 6 Graphical summary of various respiratory chain disorders involving liver on a timeline with key features. CNS: Central nervous system; IDDM: Insulin dependent diabetes mellitus; mtDNA: Mitochondrial DNA; PNS: Peripheral nervous system.

tyrate to acetoacetate ratio > 2 mol/mol are corroborative, but absence does not rule out the diagnosis. Liver biopsy findings may yield micro or macrovesicular steatosis, which reflects impaired energy metabolism. Liver or muscle tissue respiratory chain analysis shows decreased levels of complexes I, III, or IV. Liver biopsy is often done post-mortem due to the inability to do so percutaneously in view of coagulopathy. Treatment including liver transplant is discussed subsequently.

Delayed onset liver disease: Alpers Huttenlocher syndrome

This syndrome presents anywhere from 2 mo to 8 years of age, predominantly in late infancy to childhood (Figure 7 graphical summary). The diagnostic criteria include[17]: (1) Presence of refractory seizures including focal seizures; (2) Infection triggered psychomotor regression that is episodic in nature; and (3) Liver dysfunction with or without liver failure. Liver involvement is in the form of hepatomegaly, jaundice, coagulopathy, and episodes of hypoglycemia. Gastrointestinal involvement mainly due to the muscle impairment results in progressive feeding difficulty and gastroesophageal reflux, progressing to intractable vomiting. One series of 5 patients with Alpers Huttenlocher syndrome (AHS) showed mean age of liver disease presentation of 35 mo, and all died over a mean 4.6 wk period, due to progressive liver failure[18]. Autopsy findings across series show macrovesicular steatosis, massive hepatocyte dropout, proliferating bile ductular elements replacing hepatocytes, and often cirrhosis [17,18]. Valproate is known to precipitate liver failure in these patients when given for the frequently associated seizure disorder, which often demands use of more than one anticonvulsant. This is possibly because of depletion of respiratory chain enzyme activity by the drug and inability to increase metabolic rate by the DNA polymerase subunit gamma (POLG) deficient cells[19]. Valproate increases glycolysis, likely an indirect clue of impaired mitochondrial function as shown in yeast and mouse liver models[20]. POLG mutation subtype and zygosity influence outcome, with worst outcomes shown in compound heterozygous mutations for A467T and W748S[21].

Liver failure management, addressing feeding issues often mandating percutaneous endoscopic gastrostomy tube insertion, seizure control, and use of respiratory aids like continuous positive airway pressure in view of progressive motor impairment are cornerstones of management. Liver transplantation is often contraindicated in view of the multisystem involvement.

MtDNA depletion syndrome

DNA depletion is distinct from DNA deletion. MtDNA depletion refers to a state when a cell contains less than normal mtDNA per unit nuDNA. Depletion diseases are

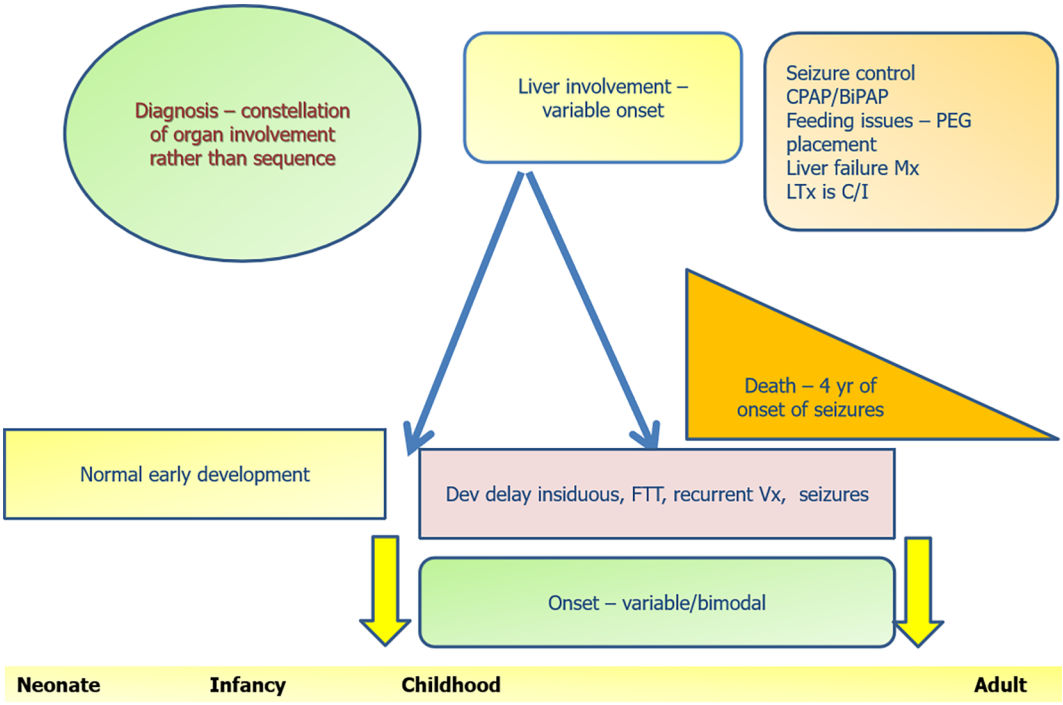

Figure 7 Graphical summary of Alpers Huttenlocher syndrome and its natural history. BiPAP: Bilevel positive airway pressure; C/I: Contraindicated; CPAP: Continuous positive airway pressure; FTT: Failure to thrive; LTx: Liver transplantation; Mx: Management; PEG: Percutaneous endoscopic gastrostomy; Vx: Vomiting.

much more severe and earlier in onset compared to deletion diseases[22]. NuDNA encodes for processes within the mitochondria including production and stability of mtDNA. Mutations in nuDNA may result in low levels of or increased destruction of DNA pool essential for mtDNA synthesis[23], thereby reducing the concentration of mtDNA in the cell or tissue as a whole. The end result is suboptimal mitochondrial function. DGUOK mutations lead to predominant neuro-hepatopathy, while TK2 mutations lead to predominant myopathy[23]. As an illustration to above statements, TK2 induced depletions present early in the first few years with myopathy, feeding difficulty, hypotonia, and respiratory failure as a terminal event. However, multiple TK2 deletions present as proximal myopathy and chronic progressive external ophthalmoplegia later in life[24]. Two additional genes, POLG coding for mtDNA polymerase and MPV17, have been described in hepatocerebral form of MDS. POLG mutations in older children have been associated with AHS as already described. It should be understood that MDS and AHS both have mtDNA depletion. AHS got its name earlier and was later found to have its molecular basis as mtDNA depletion, and it characteristically refers to a comparatively delayed onset (> 2 mo age), compared to MDS, which has its onset in the first few weeks of life. The other mutation in nuclear gene MPV17 leads to decreased synthesis of an unknown inner mitochondrial membrane protein that possibly has a role in oxidative phosphorylation, and knockout mice (-/-) have shown impaired oxidative phosphorylation and also mtDNA depletion [25].

Liver failure in infancy is the common presentation of the hepatopathic form. There is notably an overlap between the hepatopathic form of MDS and neonatal liver failure presentation of RCD. The difference exists in the fact that the former has mtDNA quantity that is < 10% of nuDNA, and there is no sequence alteration in mtDNA.

Pearson syndrome

Pearson syndrome is one among three mitochondrial diseases (Kearne Sayre syndrome and chronic progressive external ophthalmoplegia being the other two) associated with a single large deletion in mtDNA[26]. This is a multi-systemic fatal disorder with involvement of exocrine pancreas, eyes, skin, hematological system, liver, and kidneys[22]. MtDNA rearrangements form the etiological basis, and it is associated with large 4-5 kbp deletions in a large proportion of cases. All respiratory chain complexes can suffer a decreased synthesis, with complex I most severely affected. Refractory anemia with ring sideroblasts occurs in infancy with vacuolization in bone marrow of myeloid and erythroid precursors[27]. Elevated plasma alanine and

fumaric acid levels are discriminating from other non-mitochondrial bone marrow failure syndromes[28], though neither specific for Pearson syndrome nor distinguishing it from other mitochondrial disorders. Hematological manifestations may occur alone or in combination with renal tubular dysfunction (Fanconi syndrome) and hepatic failure. If the patient survives this phase of hematological symptoms, its intensity begins to decrease[29], and symptoms change from hematological to a phenotype of severe pancreatic insufficiency in late infancy to early childhood, during the same time which villous atrophy is found to appear. Eye involvement is in form of pigmentary retinopathy and external ophthalmoplegia and appears in early to late childhood. Liver involvement is in form of hepatomegaly with cirrhosis, cholestatic jaundice, elevated liver enzymes, and progressive liver failure leading to death in early childhood similar to what is seen in MDS[30]. Recent series have shown age of death ranging from 5 to 11 years, and mortality is worse in Pearson syndrome compared to other single large mitochondrial deletions[28,31]. A graphical summary outlining the natural history is shown in Figure 8. Supportive therapy with packed red cell transfusions for anemia, granulocyte colony stimulated factor for neutropenia, and bicarbonate for metabolic acidosis forms the basis of care.

Navajo neurohepatopathy

This is an autosomal recessive disease prevalent in southwestern United States. The genetic defect is a nuclear gene MPV17 (chromosome 2p24)[32], whose product is located on the inner mitochondrial membrane and is responsible for mtDNA maintenance and regulation of oxidative phosphorylation. Hence, there is impaired pool of mtDNA and disrupted oxidative phosphorylation. While earlier only neurological manifestations were known and this entity was called Navajo neuropathy, liver manifestations in form of jaundice, failure to thrive, and liver failure were recognized to be part of the same disease spectrum prompting a change in name to Navajo neurohepatopathy[33]. Clinical features are outlined in Figure 9 graphical summary. All the three subtypes have occurred in same kindred, underscoring the pattern of mitochondrial inheritance.

Villous atrophy syndrome

This disorder was described in 1994 by Cormier-Daire *et al*[34] in 2 unrelated children presenting as chronic diarrhea in infancy with villous atrophy. The defect was identified as mtDNA rearrangements in the form of deletion-duplications. Hepatomegaly and steatosis on biopsy with mildly deranged transaminases was the liver manifestation. Both children survived the diarrheal phase, which subsided by early childhood, including a reversal in histology (Figure 10: Graphical summary). However, the phenotype then changed to neuromuscular and ophthalmic involvement and death by the end of first decade. Complex III defect was detected on muscle biopsy after the advent of neuromuscular symptoms and was normal in lymphocytes. Intravenous dextrose for resuscitation should not be used in high rates as it may lead to worsening of metabolic acidosis.

Mitochondrial neurogastrointestinal encephalomyopathy

This entity is discussed purely as a prototype for GI (non-hepatic) manifestations of mitochondrial disorders, and also since it is a respiratory chain disorder, though not classically a "hepatopathy", and additionally as it is rewarding to diagnose in view of available therapy[35]. It is to be understood that RCD and non RCD mitochondrial diseases can have some or the other GI manifestation (Table 2). MNGIE was earlier known as polyneuropathy, ophthalmoplegia, leukoencephalopathy and intestinal pseudo-obstruction, oculogastrointestinal encephalopathy syndrome, or oculogastrointestinal muscular distrophy[36]. The current nomenclature was given by Hirano *et al*[37].

MNGIE occurs due to mutation in a nuclear gene encoding TYMP, encoding thymidine phosphorylase, deficiency of which leads to toxic accumulation of pyrimidine nucleosides thymidine and deoxyuridine. This impairs mtDNA synthesis thereby leading to a mtDNA depletion state. Clinical symptoms of MNGIE usually begin between the first and fifth decades of life and before 20 years of age in approximately 60%. GI dysmotility is one of the most important features in form of dysphagia, gastroparesis, and pseudo-obstruction leading to consequences like small bowel bacterial overgrowth, nutritional deficiencies, and severe weight loss[38]. Hepatic steatosis, hepatomegaly, elevated transaminases, and cirrhosis have also been described[38,39].

Table 2 Gastrointestinal manifestations of mitochondrial respiratory chain defects

Site	Manifestation
Oral cavity and esophagus	Sicca syndrome; Dry mouth; Dysphagia
Stomach	Vomiting; Reflux; Pseudo-obstruction
Small bowel and large bowel	Pseudo-obstruction; Diarrhea; Megacolon; Constipation
Extra-luminal/miscellaneous	Poor appetite; Pancreatitis; Pancreatic cysts

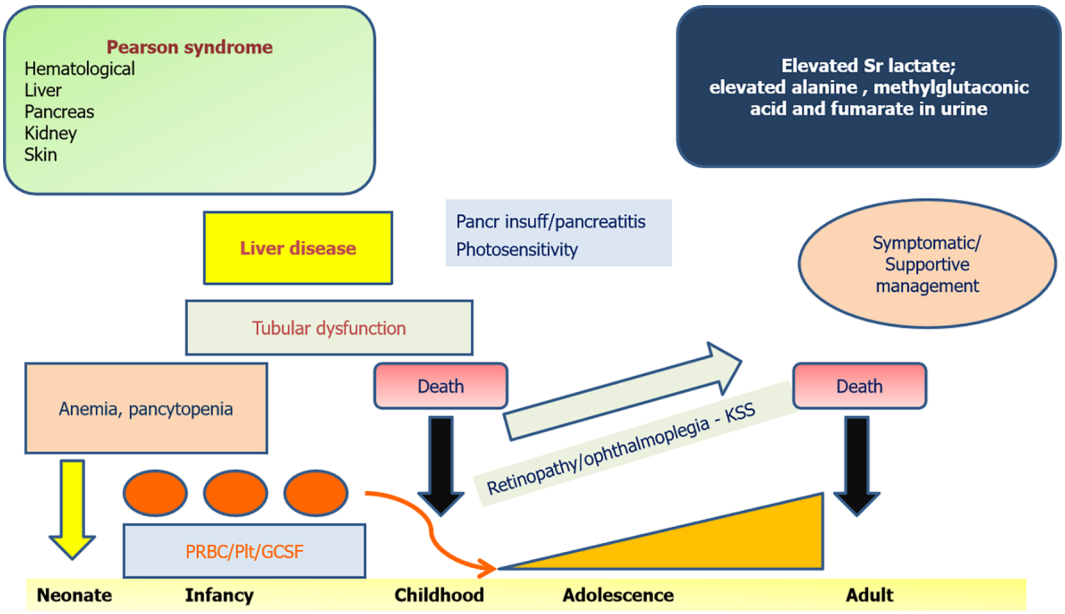

Figure 8 Graphical summary of Pearson marrow pancreas syndrome and its natural history. GCSF: Granulocyte colony stimulation factor; insuff: Insufficiency; KSS: Kearns Sayre syndrome; Pancr: Pancreatic; Plt: Platelets; PRBC: Packed red blood cells.

A diagnostic delay of about 5 to 10 years can occur in view of multisystem and complex clinical presentation[40,41]. Often there are unnecessary exploratory surgeries for the GI symptoms before being diagnosed as pseudo-obstruction[36]. Neurological involvement is mainly in form of peripheral neuropathy (demyelination with or without axonal neuropathy)[38], oculoparesis, with subtle central nervous system manifestations due to subcortical white matter involvement, and magnetic resonance imaging changes showing leukoencephalopathy. Muscle biopsies may show ragged red fibers due to proliferation of abnormal mitochondria. Current diagnostic methods employ testing for plasma thymidine and deoxyuridine levels (> 3 μmol/L and > 5 μmol/L, respectively)[42] or elevated urinary concentrations[43] and thymidine phosphorylase activity in leucocytes (< 10% of healthy controls)[43]. TYMP gene (nuDNA) mutations and also consequent mtDNA abnormalities can be identified on Sanger sequencing and Southern blot assays[44]. A graphical summary is as shown in Figure 11.

Symptomatic management remains the cornerstone. Experimental therapies include hemodialysis and peritoneal dialysis[43], platelet transfusions, hematopoietic stem cell transplant, enzyme replacement, and liver transplant[45,46]. All above therapies concentrate on 2 aspects: To reduce the toxic load of nucleosides and to replace the enzyme thymidine phosphorylase.

SETTINGS TO SUSPECT RCD AND DIAGNOSTIC EVALUATION

The settings of when to suspect a mitochondrial hepatopathy are shown in Figure 12.

Individual disorders discussed above and their graphical summaries outlined give specific information. The diagnostic evaluation of mitochondrial disorders follows once a clinical suspicion is raised, and in this section we highlight general steps towards approaching to diagnose a mitochondrial RCD[30]. Parallel evaluation of

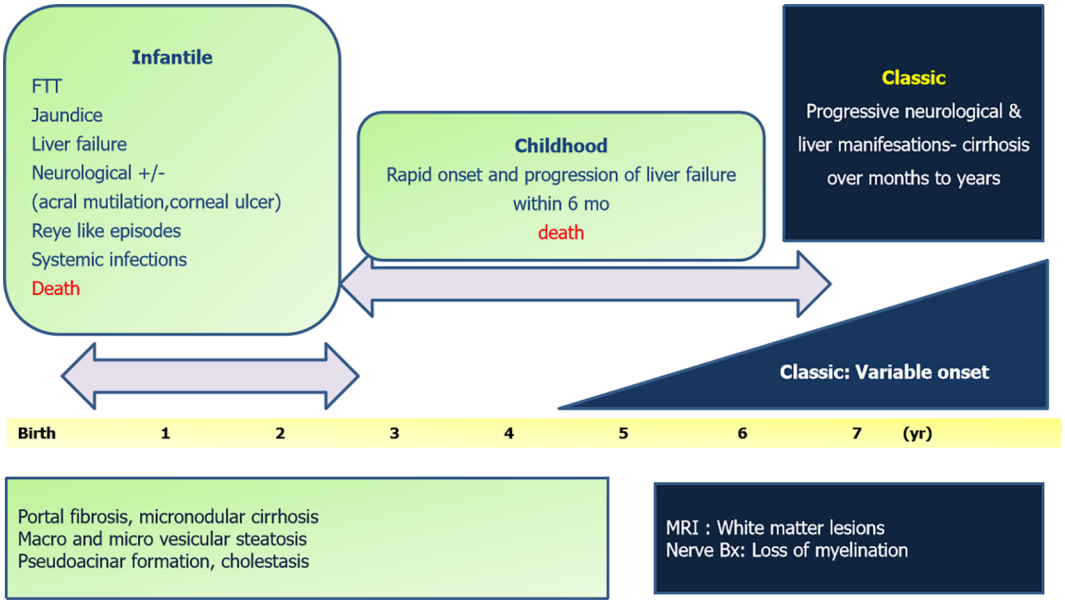

Figure 9 Graphical summary of Navajo neurohepatopathy. Bx: Biopsy; FTT: Failure to thrive.

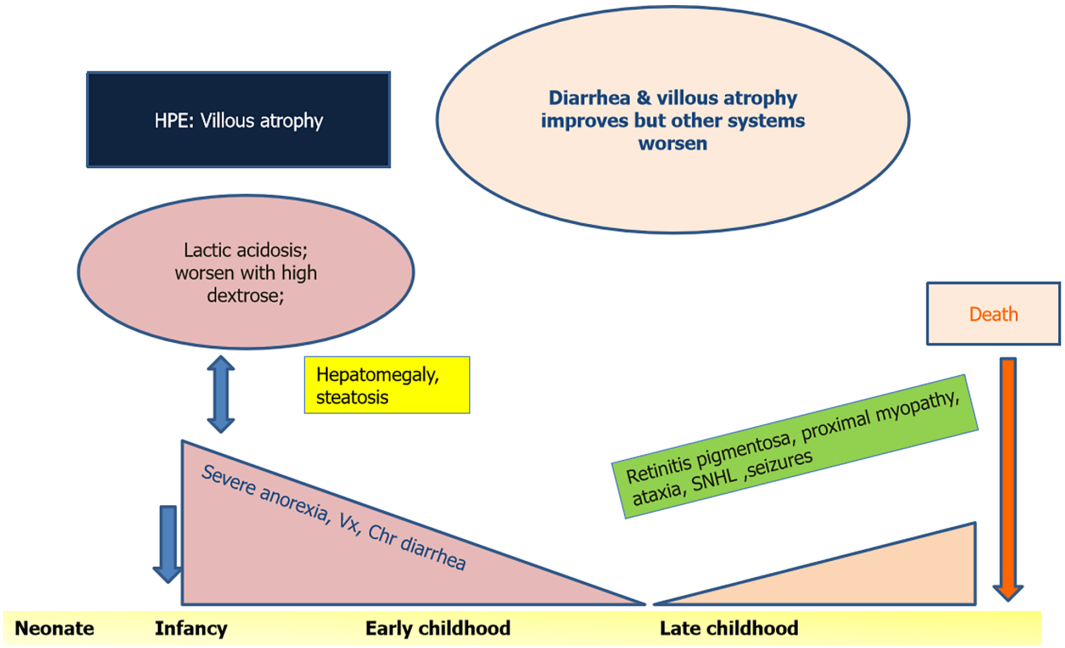

Figure 10 Graphical summary of villous atrophy syndrome and its natural history. HPE: Histopathological examination; SNHL: Sensorineural hearing loss; Vx: Vomiting.

extra-hepatic and extra-GI symptoms, if present, need to be carried out for "mapping" the disease and for aid in management and improving quality of life. Table 3 elucidates a stepwise evaluation algorithm[30]. Diagnostic steps proceed from non-invasive, easily available, and less expensive investigations to more complex elaborate tests, some of which are available in the research setting only. Level-1 entails workup for basic metabolic causes including checking for hypoglycemia and, if present, whether it is ketotic or non-ketotic. Fatty acid oxidation defects (but not RCDs) are known to have non-ketotic hypoglycemic episodes. Lactate levels more than 2.1 mmol/L (mmol) are significant, and this may often not be observed when not in a metabolic crisis. Notably, lactate may not be elevated much in POLG1 mutations[47]. Normal lactate to pyruvate ratio is less than 20 mol/mol. However, the value often rises above 30 and is typical though not exclusive to RCDs and is discriminatory from pyruvate metabolism defects[48]. Similarly, 3-hydroxy butyrate to acetoacetate ratio is normally less than 4, and values above this should arouse a suspicion of mitochondrial

Table 3 Stepwise evaluation of mitochondrial hepatopathies (respiratory chain disorder/non- respiratory chain disorders)

Steps	Description	Additional action
Level-1 (body fluids)	**Basic:** CBC, INR, AFP, CPK, NH3, sugars, phosphorous, urine ketones. **Advanced:** Lactate: Pyruvate (1 h post feeds); Ketone Body ratio, 3OH-butyrate: Acetoacetate; Serum acylcarnitine profile; Urine organic acidogram; Serum aminoacidogram; 3 Methyl Glutaconic acid in serum/urine; CSF lactate: Pyruvate, CSF alanine, protein; Plasma thymidine (MNGIE); Leucocyte CoQ levels	**Parallel level-1:** Evaluate other involved systems: CNS: MRI/MR-Spectroscopy, EEG; Eye: Fundus evaluation, clinical evaluation for ophthalmoplegias; Hearing screen; Heart: 2D-Echo, ECG; Renal: urine electrolytes, proteins, amino acids; Muscle: Muscle biopsy (Level-1 in case of primary muscle involvement, level-3 otherwise); Endocrine: HbA1c, 8 AM cortisol; Pancreas: Fecal elastase
Level-2 (genetics)	**Common genes genotyping:** POLG-1; DGUOK; MPV-17; SUCLG-1; TRMU; C10ORF2/Twinkle; CPT-1; mtDNA point mutations	**Alternative level-2:** Next generation sequencing/clinical exome sequencing for simultaneous evaluation of all mitochondrial DNA and nuclear DNA
Level-3 (invasive)	**Tissue diagnosis:** (1) Liver biopsy: Light microscopy including oil red O stain for steatosis; Electron microscopy for structural mitochondrial alterations; Frozen tissue analysis for respiratory chain enzymes, DNA quantification. (2) Muscle biopsy: Frozen tissue analysis as above; Blue native page analysis. (3) Skin biopsy: Same as muscle biopsy	**Key points to note during level-3 evaluation:** Biopsy specimens for electron microscopy need to be preserved in glutaraldehyde and not formalin; It is possible that one invasive test may not give a clue and one has to proceed for an additional invasive test. This is usually because of heteroplasmy. Often liver biopsy molecular analysis provides a final definitive answer; Combination of level-1, level-2 and level-3 studies are sometimes needed to provide comprehensive management and for prognostication

2D Echo: Two-dimensional echocardiography; AFP: Alpha-fetoprotein; CBC: Complete blood count; CNS: Central nervous system; CoQ: Coenzyme Q; CPK: Creatine phosphokinase; CSF: Cerebrospinal fluid; EEG: Electroencephalogram; HbA1c: Glycosylated hemoglobin; INR: International normalized ratio; MRI: Magnetic resonance imaging; NH3: Serum ammonia levels; POLG: DNA polymerase subunit gamma; RCD: Respiratory chain disorders.

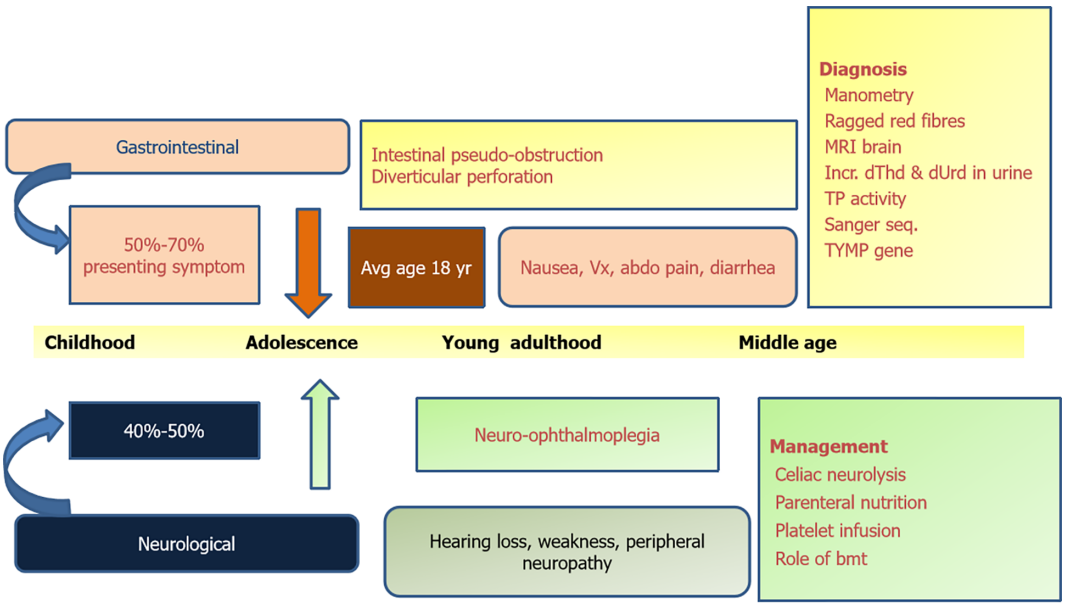

Figure 11 Graphical summary of mitochondrial neurogastrointestinal encephalomyopathy. BMT: Bone marrow transplantation; dThd: Thymidine; dUrd: Deoxy uridine levels; MRI: Magnetic resonance imaging; TP: Thymidine phosphorylase. Vx: Vomiting.

dysfunction. Urine organic acids like lactate, succinate, fumarate, malate, and 3-methyl-glutaconic are seen elevated in Pearson syndrome. Serum alanine elevation is also a clue; however, it is often more elevated in pyruvate dehydrogenase deficiency than in RCDs[48]. Creatine kinase elevation and concomitant low levels of phospho-creatine in brain and muscle tissue are seen in RCDs[49]. Branched chain amino acid to glutamine ratios were highest in RCDs and lowest in pyruvate dehydrogenase deficiency compared to controls, according to one study[48].

Table 4 helps differentiate the common metabolic disorders encountered in the pediatric patient and how to filter out RCD.

Role of genetic testing

Genetic studies are confirmatory but have a high turnaround time of 4-6 wk. They may not also be available freely at all centers or in resource poor settings. Timely referral to tertiary care centers for management is advisable. A major limitation is selection of the gene panel testing for the phenotypic presentation. In products of consanguineous

Table 4 Biochemical differentiation between various metabolic hepatopathies (respiratory chain disorder *vs* non respiratory chain disorder comparison)

	Acidosis	Urine ketones	Blood sugar	Serum lactate	Serum ammonia
RCD	++	++	Normal	++++	±
FAOD	++	Nil (non-ketotic)	Low (hypoglycemia)	+	+
OA	+++ (persistent)	++/+++	Low/normal/high	Normal	++
UCD	Normal	Normal	Normal	Normal	++++

FAOD: Fatty acid oxidation defects; OA: Organic acidemias; RCD: Respiratory chain defects; UCD: Urea cycle defects.

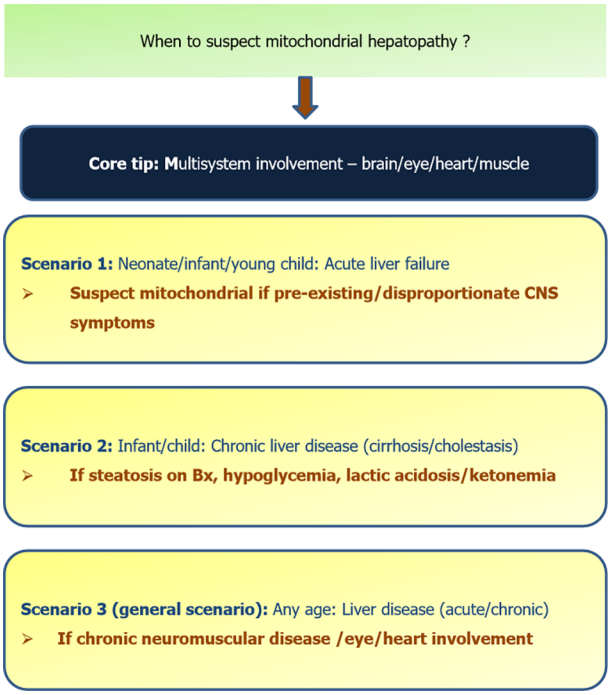

Figure 12 Scenarios when to suspect mitochondrial hepatopathy. Bx: Biopsy; CNS: Central nervous system.

union and multiple affected siblings, genetic evaluation is better guided, and it is possible to identify the index patient's chromosomal region containing the abnormality by linkage analysis. Targeted gene analysis is performed if the phenotype matches the previous cases in a family and there is an already identified mutation responsible for the clinical features in those particular kindred. Whole exome analysis for nuDNA and mtDNA is preferred otherwise as an alternative step in case there are no previously affected siblings or if the phenotype does not classically match previously described entities[50]. Targeted analysis can be performed using Sanger method of few genes, while whole exome sequencing refers to a massive parallel sequencing technique of multiple genes or the entire exome using next generation sequencing[51]. Another method to short-list genes for analysis is to study the expression profiles of RNA or specific proteins or polypeptides levels encoded by the gene(s) of interest, especially after a biochemical diagnosis is made. As an example, complex I deficiency can be caused by any of the multiple mtDNA and nuDNA responsible for each of its subunits. To identify a particular gene (of the multiple encoding ones) responsible for causing overall complex I deficiency in an index patient, analyzing the distribution or expression of proteins or RNA and its deviation from healthy controls or known standards can help pinpoint which gene may be defective. Once a specific change is identified, either in RNA expression, which can be detected by microarray assays, or in enzyme levels or stress protein expression, which can be identified by immunoblotting using an antibody panel, indirectly "reverse identification" of the causative gene(s) is facilitated[52]. This of course is only possible when, with time, all genes encoding each subunit of large proteins are identified so

that such indirect, simpler, and time saving methods may be employed. Tables 1 and 3 list genes implicated in mitochondrial RCDs. Figure 13 suggests a two-step strategy of genetic evaluation in mitochondrial RCDs and also a few phenotypes for which specific genes should be tested.

It is pertinent to note that mitochondrial hepatopathies, unlike other metabolic disorders, require analysis of mtDNA in addition to nuDNA defects. Hence, when screening genetics do not yield the diagnosis and the next step of whole exome sequencing is being undertaken, it is essential to specify to the testing lab that mtDNA analysis be included in addition to the wide gamut of nuDNA being tested. While for nuDNA analysis, the tissue of interest may not be specific and whole blood sample may serve the purpose, for mtDNA molecular analysis, specific tissues (liver, muscle) may be required, mainly because of the phenomenon of heteroplasmy.

MtDNA depletions are diagnosed by first isolating the DNA of the tissue biopsied, which is then subjected to electrophoresis and blotting followed by hybridization with probes specific for mtDNA and nuDNA both. The relative levels of autoradiographic signals emitted post hybridization are detected for mtDNA and nuDNA, and this helps in diagnosing mtDNA depletions. MtDNA deletions and point mutations on the other hand can be detected by single strand conformational polymorphisms[53,54].

Next generation sequencing by parallel exome sequencing undertaken with blood or any tissue is limited by its inability to detect mutations in the non-exonic region, like untranslated regions or intronic splice sites. It is also not adept in diagnosing trinucleotide repeat sequences, complex genetic inheritance like synergistic contribution of nuDNA and mtDNA to cause a particular disease, and epigenetic effects[51].

Role of tissue biopsies

Tissue biopsies are important despite having readily evident biochemical abnormalities; only one-third to one-half of mitochondrial disorders have identifiable mutations despite extensive exome sequencing of known genetic defects[51,55]. That is to say, all genes related to mitochondrial disorders have not yet been identified. Biopsies from the most involved site are more likely to yield the diagnosis[56]. Respiratory chain enzymes can be analyzed and activity quantified on tissue biopsy specimens. Quantitative Southern blot analysis or real-time quantitative polymerase chain reaction to detect mtDNA depletion can be done in liver biopsy specimens. Skin biopsy for cultured skin fibroblasts can be stored indefinitely and retrieved for re-culture once newer diagnostic modalities are available. It is simpler to perform and less invasive compared to muscle biopsy. However, the downside is that not all diseases are detectable on skin fibroblast analysis[47].

How to select which tissue to test is an important question that the clinician must be aware. Most mitochondrial disorders involve the muscle, and hence muscle is one of the most useful sites for analysis of enzymes, metabolites, and even molecular DNA studies. While earlier 1-5 g of muscle tissue was required for respiratory chain enzyme assays, now even 100-200 mg of skeletal muscle tissue (usually quadriceps or soleus) is sufficient especially in young children, which then yields a mitochondrial enriched fraction of 400-500 μg of protein, enough to characterize the respiratory chain enzyme deficiencies[57]. Muscle biopsies may be analyzed either as frozen or fresh samples. Samples once collected should be snap frozen immediately bedside or in the procedure room at -80 °C till analysis of mitochondrial enzymes[53]. Fresh muscle samples should not be frozen and transported in cool buffer solution, which offers the advantage of analysis of the entire mitochondrial energy generation system in addition to mitochondrial enzymes being studied in frozen samples[58].

Those diseases that have primary liver involvement and no apparent muscle involvement, especially the ones with liver failure phenotype, liver tissue of up to 10 mg can be more yielding than muscle. Cardiac tissue requirement, when indicated, is even less, about 1-2 mg, obtained by endomyocardial biopsy[53]. These invasive techniques may be gradually substituted by molecular DNA techniques done on whole blood and cater to only research purpose over time as cost and availability of next generation sequencing is eased.

MANAGEMENT OF MITOCHONDRIAL RESPIRATORY CHAIN DEFECTS

There are three aspects to management: Firstly, acute management of crisis, second is general management of children with metabolic liver disease, followed by specific treatment if available including the role of liver transplant. An additional important component relates to parental counseling and to bust myths and avoid patients to

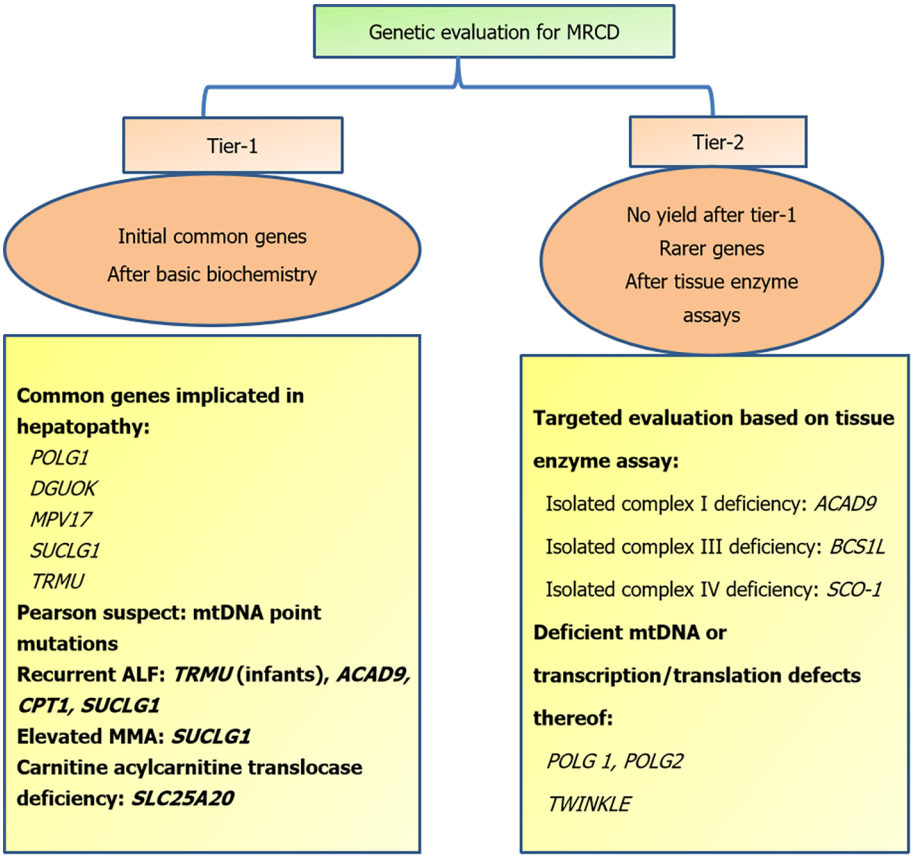

Figure 13 Step-wise strategy of genetic evaluation in mitochondrial respiratory chain defects. MRCD: Mitochondrial respiratory chain disorders; ALF: Acute liver failure; MMA: Methylmalonic acid.

resort to non-scientific therapies and refraining from standard of care.

Acute crisis management

Treatment of acute liver failure and progressive liver disease remains unsatisfactory. The aim of therapy is mainly mitigating, postponing, or circumventing damage to the respiratory chain. The basic steps and precautions are outlined as in Table 5. Bicarbonate infusions when used for a longer time may itself worsen cerebral function. An alternative is dichloroacetate, which inhibits pyruvate dehydrogenase kinase, hence favoring persistent levels of active pyruvate dehydrogenase and hence preventing pyruvate accumulation and pyruvate to lactate conversion.

Other supportive therapy is to give packed red cell and platelet transfusions for anemia and thrombocytopenia and pancreatic enzyme replacement in case of insufficiency.

General considerations for managing a child with mitochondrial hepatopathy

Once the acute crisis is settled, it is important to improve the nutrition of the child as malnutrition itself can lead to secondary mitochondrial dysfunction[59]. These children often have increased caloric needs and an inability to maintain it owing to either repeated sickness bouts or a general anorexia associated with liver disorders. The issues of swallowing difficulties, impaired gut motility, and gastro-esophageal reflux need to be addressed often to the extent of placement of feeding tubes (orogastric or nasogastric), percutaneous endoscopic feeding gastrostomy, or using parenteral nutrition therapy. Nutritional improvement has led to improved quality of life and an increase in developmental quotients in these children[60]. Ketogenic diet may be useful in some mitochondrial disorders but may worsen fatty acid oxidation defects and should be avoided in them. Exercise helps in reducing the burden of abnormal mitochondria[61]. It is useful to do so regularly, under supervision in a graded manner, and to have a meal prior to exercise[62].

Pharmacotherapy

A combination of drugs is often empirically administered to suspected mitochondrial

Table 5 Management during evaluation in acute phase

Following thumb rules while attending to a patient with suspected mitochondrial disorder
Monitor closely for hypoglycemia and acidosis
Avoid lactated ringer's solution for fluid administration: Worsens acidosis
Bicarbonate infusions as 1st line of defense
Avoid propofol for sedation/anesthesia
Avoid fasting > 12 h; avoid high rate glucose only infusions
Avoid drugs that are toxic to mitochondria: Chloramphenicol, valproate, aminoglycosides, phenytoin, carbamazapine, phenobarbital, statins, linezolid
Avoid drugs precipitating hepatopathy/liver dysfunction

disorders and comprises: Coenzyme Q, carnitine, thiamine, riboflavin, vitamins C and E, and creatine. Of all, Coenzyme Q shows promise and along with B vitamins remains the most common combination as part of cocktail therapy[63]. The various drugs and their pediatric dosages are outlined in Table 6[13,63,63].

Role of organ transplant

Multisystem involvement in mitochondrial hepatopathies often precludes performing a liver transplant. However, in hepatocerebral form of DGUOK defects when detected in infancy without neurological involvement, liver transplant has shown to be effective. Those with neurological involvement do not benefit from liver transplant [65]. Overall post-transplant survival is less with RCDs than non-RCDs. Sokal *et al*[66] reported 8 cases with a survival of 50% post transplantation for RCDs. In an elaborate compilation of 40 cases with mitochondrial RCDs across various centers at different time points, it was noted that 22 (55%) patients died within 24 mo post-transplant[67]. Early postoperative multi-organ failure and neuro-degeneration followed by respiratory complications and severe pulmonary hypertension were the cause of death in these patients. The same group recognized that those diagnosed pre-transplant had a higher survival (58%) than those recognized to have RCD after transplant (29%). Thus, the emphasis is on early recognition of the diagnosis and a thorough evaluation for extra-hepatic manifestations, adding investigations like magnetic resonance imaging of the brain and echocardiography.

MNGIE stands out as the single mitochondrial disorder for which replacement of the missing enzyme thymidine phosphorylase by stem cell transplantation can be curative and lead to improvement in long term outcomes. While earlier enzyme levels were artificially increased using repeated platelet transfusions[40], stem cell transplant has come up as a definitive modality[68,69].

Myths in mitochondrial disorders

Immunizations are not contraindicated in children with mitochondrial diseases. This is to be emphasized because of certain misconceptions that immunization may lead to autism in children with mitochondrial diseases for which there is no evidence[64]. The other important aspect that needs to be clarified is that there is no role of hyperbaric therapy in treatment of MH and in fact may lead to oxygen toxicity. Vagus nerve stimulation may not be very helpful in controlling refractory seizures in children with MH[63].

CONCLUSION

In a nutshell: (1) Liver along with other system involvement may not be just sepsis – think of mitochondrial respiratory chain hepatopathy; (2) Lactic acidosis without hypoglycemia is an important clue, avoid ringer lactate and drugs causing hepatopathy; (3) Evaluation should be done in a tiered manner – genetic evaluation and enzyme analysis from tissue of interest; (4) Treatment is largely supportive with transfusions, correction of acidosis, shock, and providing cofactors/salvage therapies; (5) Liver transplantation needs to be considered in only a select group and may worsen disease despite adequate precautions; and (6) Periodic follow-up is mandatory for monitoring evolution of disease including "migration" to other organ systems.

Table 6 Pharmacotherapy used for mitochondrial diseases

Drug	Pediatric dose	Remark
Coenzyme Q: (1) Ubiquinol form; (2) Ubiquinone form	2-8 mg/kg/d in BD dosing; 10-30 mg/kg/d BD dosing	Preferably had after meals; Most effective and most used therapy; Free radical scavenger; Bypasses complex I
Idebenone	5 mg/kg/d	Synthetic form of CoQ; Penetrates blood-brain barrier
L-carnitine	10-100 mg/kg/d IV or oral divided 3 times/d	Avoid in long chain FAO-Ds: May lead to cardiac arrhythmias
Creatine	0.1 g/kg PO, OD	Used for repletion of muscle phosphocreatine levels
L-arginine	500 mg/kg IV per day for 1-3 d followed by 150-300 mg/kg oral daily in BD dosing	Used for acute stroke; Watch for hypotension while infusion; Evidence is anecdotal
Thiamine	100 mg/d	Cofactor of PDH; useful for thiamine responsive PDH deficiency; Helpful in leigh disease
Riboflavin	50-400 mg/d	Give at night time before sleep; Shown to be useful in ACAD9 mutations; Flavin precursor for complex I & II
Vitamin C	5 mg/kg/d OD	Antioxidant; Artificial electron acceptor
Vitamin E	Variable dosing, up to 25 IU/kg/d OD (avoid > 400 IU/d)	Absorption better when taken with meals
Dichloroacetate	25-50 mg/kg/d	Improves lactic acidosis

BD: Twice daily; CoQ: Coenzyme Q; FAO-D: Fatty acid oxidation defects; IV: Intravenous; PDH: Pyruvate dehydrogenase; PO: Per oral; OD: Once daily.

REFERENCES

1 **Herrmann JM**, Riemer J. The intermembrane space of mitochondria. *Antioxid Redox Signal* 2010; **13**: 1341-1358 [PMID: 20367280 DOI: 10.1089/ars.2009.3063]
2 **Lee WS**, Sokol RJ. Mitochondrial hepatopathies: advances in genetics and pathogenesis. *Hepatology* 2007; **45**: 1555-1565 [PMID: 17538929 DOI: 10.1002/hep.21710]
3 **Gray MW**. Mitochondrial evolution. *Cold Spring Harb Perspect Biol* 2012; **4**: a011403 [PMID: 22952398 DOI: 10.1101/cshperspect.a011403]
4 **Schwartz M**, Vissing J. Paternal inheritance of mitochondrial DNA. *N Engl J Med* 2002; **347**: 576-580 [PMID: 12192017 DOI: 10.1056/NEJMoa020350]
5 **Di Mauro S**, Schon EA. Mitochondrial Respiratory-Chain Diseases. *N Engl J Med* 2003; **348**: 2656-2668 [DOI: 10.1056/NEJMra022567]
6 **Kadowaki T**, Kadowaki H, Mori Y, Tobe K, Sakuta R, Suzuki Y, Tanabe Y, Sakura H, Awata T, Goto Y. A subtype of diabetes mellitus associated with a mutation of mitochondrial DNA. *N Engl J Med* 1994; **330**: 962-968 [PMID: 8121460 DOI: 10.1056/NEJM199404073301403]
7 **Meyers DE**, Basha HI, Koenig MK. Mitochondrial cardiomyopathy: pathophysiology, diagnosis, and management. *Tex Heart Inst J* 2013; **40**: 385-394 [PMID: 24082366]
8 **Fischel-Ghodsian N**. Mitochondrial mutations and hearing loss: paradigm for mitochondrial genetics. *Am J Hum Genet* 1998; **62**: 15-19 [PMID: 9443888 DOI: 10.1086/301695]
9 **Skladal D**, Halliday J, Thorburn DR. Minimum birth prevalence of mitochondrial respiratory chain disorders in children. *Brain* 2003; **126**: 1905-1912 [PMID: 12805096 DOI: 10.1093/brain/awg170]
10 **Chinnery PF**, Johnson MA, Wardell TM, Singh-Kler R, Hayes C, Brown DT, Taylor RW, Bindoff LA, Turnbull DM. The epidemiology of pathogenic mitochondrial DNA mutations. *Ann Neurol* 2000; **48**: 188-193 [PMID: 10939569]
11 **Darin N**, Oldfors A, Moslemi AR, Holme E, Tulinius M. The incidence of mitochondrial encephalomyopathies in childhood: clinical features and morphological, biochemical, and DNA abnormalities. *Ann Neurol* 2001; **49**: 377-383 [PMID: 11261513 DOI: 10.1002/ana.75]
12 **Cormier-Daire V**, Chretien D, Rustin P, Rötig A, Dubuisson C, Jacquemin E, Hadchouel M, Bernard O, Munnich A. Neonatal and delayed-onset liver involvement in disorders of oxidative phosphorylation. *J Pediatr* 1997; **130**: 817-822 [PMID: 9152294 DOI: 10.1016/s0022-3476(97)80027-3]
13 **Lee WS**, Sokol RJ. Liver disease in mitochondrial disorders. *Semin Liver Dis* 2007; **27**: 259-273 [PMID: 17682973 DOI: 10.1055/s-2007-985071]
14 **Taylor SA**, Whitington PF. Neonatal acute liver failure. *Liver Transpl* 2016; **22**: 677-685 [PMID: 26946058 DOI: 10.1002/lt.24433]
15 **Durand P**, Debray D, Mandel R, Baujard C, Branchereau S, Gauthier F, Jacquemin E, Devictor D. Acute liver failure in infancy: a 14-year experience of a pediatric liver transplantation center. *J Pediatr* 2001; **139**: 871-876 [PMID: 11743517 DOI: 10.1067/mpd.2001.119989]
16 **von Kleist-Retzow JC**, Cormier-Daire V, Viot G, Goldenberg A, Mardach B, Amiel J, Saada P, Dumez Y, Brunelle F, Saudubray JM, Chrétien D, Rötig A, Rustin P, Munnich A, De Lonlay P. Antenatal manifestations of mitochondrial respiratory chain deficiency. *J Pediatr* 2003; **143**: 208-212

[PMID: 12970634 DOI: 10.1067/S0022-3476(03)00130-6]
17 **Harding BN**. Progressive neuronal degeneration of childhood with liver disease (Alpers-Huttenlocher syndrome): a personal review. *J Child Neurol* 1990; **5**: 273-287 [PMID: 2246481 DOI: 10.1177/088307389000500402]
18 **Narkewicz MR**, Sokol RJ, Beckwith B, Sondheimer J, Silverman A. Liver involvement in Alpers disease. *J Pediatr* 1991; **119**: 260-267 [PMID: 1861211 DOI: 10.1016/s0022-3476(05)80736-x]
19 **Sitarz KS**, Elliott HR, Karaman BS, Relton C, Chinnery PF, Horvath R. Valproic acid triggers increased mitochondrial biogenesis in POLG-deficient fibroblasts. *Mol Genet Metab* 2014; **112**: 57-63 [PMID: 24725338 DOI: 10.1016/j.ymgme.2014.03.006]
20 **Salsaa M**, Pereira B, Liu J, Yu W, Jadhav S, Hüttemann M, Greenberg ML. Valproate inhibits mitochondrial bioenergetics and increases glycolysis in Saccharomyces cerevisiae. *Sci Rep* 2020; **10**: 11785 [PMID: 32678210 DOI: 10.1038/s41598-020-68725-5]
21 **Tzoulis C**, Engelsen BA, Telstad W, Aasly J, Zeviani M, Winterthun S, Ferrari G, Aarseth JH, Bindoff LA. The spectrum of clinical disease caused by the A467T and W748S POLG mutations: a study of 26 cases. *Brain* 2006; **129**: 1685-1692 [PMID: 16638794 DOI: 10.1093/brain/awl097]
22 **Rötig A**, Cormier V, Blanche S, Bonnefont JP, Ledeist F, Romero N, Schmitz J, Rustin P, Fischer A, Saudubray JM. Pearson's marrow-pancreas syndrome. A multisystem mitochondrial disorder in infancy. *J Clin Invest* 1990; **86**: 1601-1608 [PMID: 2243133 DOI: 10.1172/JCI114881]
23 **Mandel H**, Szargel R, Labay V, Elpeleg O, Saada A, Shalata A, Anbinder Y, Berkowitz D, Hartman C, Barak M, Eriksson S, Cohen N. The deoxyguanosine kinase gene is mutated in individuals with depleted hepatocerebral mitochondrial DNA. *Nat Genet* 2001; **29**: 337-341 [PMID: 11687800 DOI: 10.1038/ng746]
24 **Tyynismaa H**, Sun R, Ahola-Erkkilä S, Almusa H, Pöyhönen R, Korpela M, Honkaniemi J, Isohanni P, Paetau A, Wang L, Suomalainen A. Thymidine kinase 2 mutations in autosomal recessive progressive external ophthalmoplegia with multiple mitochondrial DNA deletions. *Hum Mol Genet* 2012; **21**: 66-75 [PMID: 21937588 DOI: 10.1093/hmg/ddr438]
25 **Spinazzola A**, Viscomi C, Fernandez-Vizarra E, Carrara F, D'Adamo P, Calvo S, Marsano RM, Donnini C, Weiher H, Strisciuglio P, Parini R, Sarzi E, Chan A, DiMauro S, Rötig A, Gasparini P, Ferrero I, Mootha VK, Tiranti V, Zeviani M. MPV17 encodes an inner mitochondrial membrane protein and is mutated in infantile hepatic mitochondrial DNA depletion. *Nat Genet* 2006; **38**: 570-575 [PMID: 16582910 DOI: 10.1038/ng1765]
26 **Saneto RP**. Mitochondrial diseases: expanding the diagnosis in the era of genetic testing. *J Transl Genet Genom* 2020; **4**: 384-428 [PMID: 33426505 DOI: 10.20517/jtgg.2020.40]
27 **Dror Y**. Inherited Bone Marrow Failure Syndromes. In: Hoffman R, Benz Jr E, Silberstein L, Weitz J, Heslop H, Anastasi J, Salama M, Abutalib SA, editors. Hematology: Basic Principles and Practice. Elsevier Science, 2017: 350-393 [DOI: 10.1016/B978-0-323-35762-3.00029-9]
28 **Broomfield A**, Sweeney MG, Woodward CE, Fratter C, Morris AM, Leonard JV, Abulhoul L, Grunewald S, Clayton PT, Hanna MG, Poulton J, Rahman S. Paediatric single mitochondrial DNA deletion disorders: an overlapping spectrum of disease. *J Inherit Metab Dis* 2015; **38**: 445-457 [PMID: 25352051 DOI: 10.1007/s10545-014-9778-4]
29 **Muraki K**, Nishimura S, Goto Y, Nonaka I, Sakura N, Ueda K. The association between haematological manifestation and mtDNA deletions in Pearson syndrome. *J Inherit Metab Dis* 1997; **20**: 697-703 [PMID: 9323565 DOI: 10.1023/a:1005378527077]
30 **Molleston JP**, Sokol RJ, Karnsakul W, Miethke A, Horslen S, Magee JC, Romero R, Squires RH, Van Hove JL; Childhood Liver Disease Research Education Network (ChiLDREN). Evaluation of the child with suspected mitochondrial liver disease. *J Pediatr Gastroenterol Nutr* 2013; **57**: 269-276 [PMID: 23783016 DOI: 10.1097/MPG.0b013e31829ef67a]
31 **Farruggia P**, Di Cataldo A, Pinto RM, Palmisani E, Macaluso A, Valvo LL, Cantarini ME, Tornesello A, Corti P, Fioredda F, Varotto S, Martire B, Moroni I, Puccio G, Russo G, Dufour C, Pillon M. Pearson Syndrome: A Retrospective Cohort Study from the Marrow Failure Study Group of A.I.E.O.P. (Associazione Italiana Emato-Oncologia Pediatrica). *JIMD Rep* 2016; **26**: 37-43 [PMID: 26238250 DOI: 10.1007/8904_2015_470]
32 **Karadimas CL**, Vu TH, Holve SA, Chronopoulou P, Quinzii C, Johnsen SD, Kurth J, Eggers E, Palenzuela L, Tanji K, Bonilla E, De Vivo DC, DiMauro S, Hirano M. Navajo neurohepatopathy is caused by a mutation in the MPV17 gene. *Am J Hum Genet* 2006; **79**: 544-548 [PMID: 16909392 DOI: 10.1086/506913]
33 **Holve S**, Hu D, Shub M, Tyson RW, Sokol RJ. Liver disease in Navajo neuropathy. *J Pediatr* 1999; **135**: 482-493 [PMID: 10518083 DOI: 10.1016/s0022-3476(99)70172-1]
34 **Cormier-Daire V**, Bonnefont JP, Rustin P, Maurage C, Ogler H, Schmitz J, Ricour C, Saudubray JM, Munnich A, Rötig A. Mitochondrial DNA rearrangements with onset as chronic diarrhea with villous atrophy. *J Pediatr* 1994; **124**: 63-70 [PMID: 8283377 DOI: 10.1016/s0022-3476(94)70255-1]
35 **Finsterer J**, Frank M. Gastrointestinal manifestations of mitochondrial disorders: a systematic review. *Therap Adv Gastroenterol* 2017; **10**: 142-154 [PMID: 28286566 DOI: 10.1177/1756283X16666806]
36 **Pacitti D**, Levene M, Garone C, Nirmalananthan N, Bax BE. Mitochondrial Neurogastrointestinal Encephalomyopathy: Into the Fourth Decade, What We Have Learned So Far. *Front Genet* 2018; **9**: 669 [PMID: 30627136 DOI: 10.3389/fgene.2018.00669]
37 **Hirano M**, Silvestri G, Blake DM, Lombes A, Minetti C, Bonilla E, Hays AP, Lovelace RE, Butler I, Bertorini TE. Mitochondrial neurogastrointestinal encephalomyopathy (MNGIE): clinical,

biochemical, and genetic features of an autosomal recessive mitochondrial disorder. *Neurology* 1994; **44**: 721-727 [PMID: 8164833 DOI: 10.1212/wnl.44.4.721]

38 **Garone C**, Tadesse S, Hirano M. Clinical and genetic spectrum of mitochondrial neurogastrointestinal encephalomyopathy. *Brain* 2011; **134**: 3326-3332 [PMID: 21933806 DOI: 10.1093/brain/awr245]

39 **Finkenstedt A**, Schranz M, Bösch S, Karall D, Bürgi SS, Ensinger C, Drach M, Mayr JA, Janecke AR, Vogel W, Nachbaur D, Zoller H. MNGIE Syndrome: Liver Cirrhosis Should Be Ruled Out Prior to Bone Marrow Transplantation. *JIMD Rep* 2013; **10**: 41-44 [PMID: 23430799 DOI: 10.1007/8904_2012_199]

40 **Lara MC**, Weiss B, Illa I, Madoz P, Massuet L, Andreu AL, Valentino ML, Anikster Y, Hirano M, Martí R. Infusion of platelets transiently reduces nucleoside overload in MNGIE. *Neurology* 2006; **67**: 1461-1463 [PMID: 16971699 DOI: 10.1212/01.wnl.0000239824.95411.52]

41 **Taanman JW**, Daras M, Albrecht J, Davie CA, Mallam EA, Muddle JR, Weatherall M, Warner TT, Schapira AH, Ginsberg L. Characterization of a novel TYMP splice site mutation associated with mitochondrial neurogastrointestinal encephalomyopathy (MNGIE). *Neuromuscul Disord* 2009; **19**: 151-154 [PMID: 19056268 DOI: 10.1016/j.nmd.2008.11.002]

42 **Martí R**, Nishigaki Y, Hirano M. Elevated plasma deoxyuridine in patients with thymidine phosphorylase deficiency. *Biochem Biophys Res Commun* 2003; **303**: 14-18 [PMID: 12646159 DOI: 10.1016/s0006-291x(03)00294-8]

43 **Spinazzola A**, Marti R, Nishino I, Andreu AL, Naini A, Tadesse S, Pela I, Zammarchi E, Donati MA, Oliver JA, Hirano M. Altered thymidine metabolism due to defects of thymidine phosphorylase. *J Biol Chem* 2002; **277**: 4128-4133 [PMID: 11733540 DOI: 10.1074/jbc.M111028200]

44 **Nishino I**, Spinazzola A, Hirano M. MNGIE: from nuclear DNA to mitochondrial DNA. *Neuromuscul Disord* 2001; **11**: 7-10 [PMID: 11166160 DOI: 10.1016/s0960-8966(00)00159-0]

45 **De Giorgio R**, Pironi L, Rinaldi R, Boschetti E, Caporali L, Capristo M, Casali C, Cenacchi G, Contin M, D'Angelo R, D'Errico A, Gramegna LL, Lodi R, Maresca A, Mohamed S, Morelli MC, Papa V, Tonon C, Tugnoli V, Carelli V, D'Alessandro R, Pinna AD. Liver transplantation for mitochondrial neurogastrointestinal encephalomyopathy. *Ann Neurol* 2016; **80**: 448-455 [PMID: 27421916 DOI: 10.1002/ana.24724]

46 **D'Angelo R**, Rinaldi R, Pironi L, Dotti MT, Pinna AD, Boschetti E, Capristo M, Mohamed S, Contin M, Caporali L, Carelli V, De Giorgio R. Liver transplant reverses biochemical imbalance in mitochondrial neurogastrointestinal encephalomyopathy. *Mitochondrion* 2017; **34**: 101-102 [PMID: 28263873 DOI: 10.1016/j.mito.2017.02.006]

47 **Mitochondrial Medicine Society's Committee on Diagnosis**, Haas RH, Parikh S, Falk MJ, Saneto RP, Wolf NI, Darin N, Wong LJ, Cohen BH, Naviaux RK. The in-depth evaluation of suspected mitochondrial disease. *Mol Genet Metab* 2008; **94**: 16-37 [PMID: 18243024 DOI: 10.1016/j.ymgme.2007.11.018]

48 **Clarke C**, Xiao R, Place E, Zhang Z, Sondheimer N, Bennett M, Yudkoff M, Falk MJ. Mitochondrial respiratory chain disease discrimination by retrospective cohort analysis of blood metabolites. *Mol Genet Metab* 2013; **110**: 145-152 [PMID: 23920046 DOI: 10.1016/j.ymgme.2013.07.011]

49 **Atkuri KR**, Cowan TM, Kwan T, Ng A, Herzenberg LA, Enns GM. Inherited disorders affecting mitochondrial function are associated with glutathione deficiency and hypocitrullinemia. *Proc Natl Acad Sci U S A* 2009; **106**: 3941-3945 [PMID: 19223582 DOI: 10.1073/pnas.0813409106]

50 **ACMG Board of Directors**. Points to consider in the clinical application of genomic sequencing. *Genet Med* 2012; **14**: 759-761 [PMID: 22863877 DOI: 10.1038/gim.2012.74]

51 **McCormick E**, Place E, Falk MJ. Molecular genetic testing for mitochondrial disease: from one generation to the next. *Neurotherapeutics* 2013; **10**: 251-261 [PMID: 23269497 DOI: 10.1007/s13311-012-0174-1]

52 **Thorburn DR**, Sugiana C, Salemi R, Kirby DM, Worgan L, Ohtake A, Ryan MT. Biochemical and molecular diagnosis of mitochondrial respiratory chain disorders. *Biochim Biophys Acta* 2004; **1659**: 121-128 [PMID: 15576043 DOI: 10.1016/j.bbabio.2004.08.006]

53 **Rustin P**, Chretien D, Bourgeron T, Gérard B, Rötig A, Saudubray JM, Munnich A. Biochemical and molecular investigations in respiratory chain deficiencies. *Clin Chim Acta* 1994; **228**: 35-51 [PMID: 7955428 DOI: 10.1016/0009-8981(94)90055-8]

54 **Munnich A**, Rötig A, Chretien D, Saudubray JM, Cormier V, Rustin P. Clinical presentations and laboratory investigations in respiratory chain deficiency. *Eur J Pediatr* 1996; **155**: 262-274 [PMID: 8777918 DOI: 10.1007/BF02002711]

55 **Calvo SE**, Compton AG, Hershman SG, Lim SC, Lieber DS, Tucker EJ, Laskowski A, Garone C, Liu S, Jaffe DB, Christodoulou J, Fletcher JM, Bruno DL, Goldblatt J, Dimauro S, Thorburn DR, Mootha VK. Molecular diagnosis of infantile mitochondrial disease with targeted next-generation sequencing. *Sci Transl Med* 2012; **4**: 118ra10 [PMID: 22277967 DOI: 10.1126/scitranslmed.3003310]

56 **Munnich A**, Rustin P. Clinical spectrum and diagnosis of mitochondrial disorders. *Am J Med Genet* 2001; **106**: 4-17 [PMID: 11579420 DOI: 10.1002/ajmg.1391]

57 **Janssen AJ**, Smeitink JA, van den Heuvel LP. Some practical aspects of providing a diagnostic service for respiratory chain defects. *Ann Clin Biochem* 2003; **40**: 3-8 [PMID: 12542905 DOI: 10.1258/000456303321016114]

58 **Lian C**, Cao S, Zeng W, Li Y, Su J, Li J, Zhao S, Wu L, Tao J, Zhou J, Chen X, Peng C. RJT-101, a novel camptothecin derivative, is highly effective in the treatment of melanoma through DNA damage by targeting topoisomerase 1. *Biochem Pharmacol* 2020; **171**: 113716 [PMID: 31751535 DOI:

10.1016/j.bcp.2019.113716]
59 **Wortmann SB**, Zweers-van Essen H, Rodenburg RJ, van den Heuvel LP, de Vries MC, Rasmussen-Conrad E, Smeitink JA, Morava E. Mitochondrial energy production correlates with the age-related BMI. *Pediatr Res* 2009; **65**: 103-108 [PMID: 19096353 DOI: 10.1203/PDR.0b013e31818d1c8a]
60 **Choi HS**, Lee YM. Enteral Tube Feeding in Paediatric Mitochondrial Diseases. *Sci Rep* 2017; **7**: 16909 [PMID: 29203845 DOI: 10.1038/s41598-017-17256-7]
61 **Tarnopolsky MA**. Mitochondrial DNA shifting in older adults following resistance exercise training. *Appl Physiol Nutr Metab* 2009; **34**: 348-354 [PMID: 19448697 DOI: 10.1139/H09-022]
62 **Jeppesen TD**, Schwartz M, Olsen DB, Wibrand F, Krag T, Dunø M, Hauerslev S, Vissing J. Aerobic training is safe and improves exercise capacity in patients with mitochondrial myopathy. *Brain* 2006; **129**: 3402-3412 [PMID: 16815877 DOI: 10.1093/brain/awl149]
63 **Parikh S**, Saneto R, Falk MJ, Anselm I, Cohen BH, Haas R, Medicine Society TM. A modern approach to the treatment of mitochondrial disease. *Curr Treat Options Neurol* 2009; **11**: 414-430 [PMID: 19891905 DOI: 10.1007/s11940-009-0046-0]
64 **Dimauro S**, Rustin P. A critical approach to the therapy of mitochondrial respiratory chain and oxidative phosphorylation diseases. *Biochim Biophys Acta* 2009; **1792**: 1159-1167 [PMID: 19026744 DOI: 10.1016/j.bbadis.2008.10.015]
65 **Dimmock DP**, Dunn JK, Feigenbaum A, Rupar A, Horvath R, Freisinger P, Mousson de Camaret B, Wong LJ, Scaglia F. Abnormal neurological features predict poor survival and should preclude liver transplantation in patients with deoxyguanosine kinase deficiency. *Liver Transpl* 2008; **14**: 1480-1485 [PMID: 18825706 DOI: 10.1002/lt.21556]
66 **Sokal EM**, Sokol R, Cormier V, Lacaille F, McKiernan P, Van Spronsen FJ, Bernard O, Saudubray JM. Liver transplantation in mitochondrial respiratory chain disorders. *Eur J Pediatr* 1999; **158** Suppl 2: S81-S84 [PMID: 10603105 DOI: 10.1007/pl00014328]
67 **De Greef E**, Christodoulou J, Alexander IE, Shun A, O'Loughlin EV, Thorburn DR, Jermyn V, Stormon MO. Mitochondrial respiratory chain hepatopathies: role of liver transplantation. A case series of five patients. *JIMD Rep* 2012; **4**: 5-11 [PMID: 23430890 DOI: 10.1007/8904_2011_29]
68 **Hirano M**, Martí R, Casali C, Tadesse S, Uldrick T, Fine B, Escolar DM, Valentino ML, Nishino I, Hesdorffer C, Schwartz J, Hawks RG, Martone DL, Cairo MS, DiMauro S, Stanzani M, Garvin JH Jr, Savage DG. Allogeneic stem cell transplantation corrects biochemical derangements in MNGIE. *Neurology* 2006; **67**: 1458-1460 [PMID: 16971696 DOI: 10.1212/01.wnl.0000240853.97716.24]
69 **Rahman S**, Hargreaves IP. Allogeneic stem cell transplantation corrects biochemical derangements in MNGIE. *Neurology* 2007; **68**: 1872; author reply 1872; discussion 1872-1872; author reply 1872; discussion 1873 [PMID: 17515557 DOI: 10.1212/01.wnl.0000265356.98295.be]

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MINIREVIEWS

Cystic fibrosis associated liver disease in children

Joseph J Valamparampil, Girish L Gupte

ORCID number: Joseph J Valamparampil 0000-0002-8114-2523; Girish L Gupte 0000-0002-9026-1583.

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Joseph J Valamparampil, Girish L Gupte, Liver Unit, Birmingham Children's Hospital, Birmingham B4 6NH, United Kingdom

Corresponding author: Girish L Gupte, MD, Consultant Physician-Scientist, Liver Unit, Birmingham Children's Hospital, Steelhouse Lane, Birmingham B4 6NH, United Kingdom. girishgupte@nhs.net

Abstract

Cystic fibrosis (CF) is an autosomal recessive disorder caused by mutations in the CF transmembrane conductance regulator gene. CF liver disease develops in 5%-10% of patients with CF and is the third leading cause of death among patients with CF after pulmonary disease or lung transplant complications. We review the pathogenesis, clinical presentations, complications, diagnostic evaluation, effect of medical therapies especially CF transmembrane conductance regulator modulators and liver transplantation in CF associated liver disease.

Key Words: Cystic fibrosis liver disease; Portal hypertension; Cirrhosis; Liver transplantation; Cystic fibrosis transmembrane conductance regulator modulators; Distal intestinal obstructive syndrome

Core Tip: Cystic fibrosis(CF) liver disease is caused by abnormal cholangiocyte function, altered biliary secretion and abnormal innate immune response with abnormal response to endotoxins. CF liver disease can present with a wide variety of clinical features from a heterogenous liver on ultrasound, to life threatening gastrointestinal bleeds secondary to portal hypertension. Novel treatment strategies directly targeting the ion channel abnormality-cystic fibrosis transmembrane conductance regulator modulators are available and has significantly improved the clinical status and life expectancy of the cystic fibrosis patients.

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INTRODUCTION

Cystic fibrosis (CF) the most frequent fatal autosomal recessive disorder in Caucasians, is caused by autosomal recessive disease caused by mutations in the CF transmembrane conductance regulator (*CFTR*) gene on the long arm of chromosome 7 with more than 2000 variants reported[1]. F508del variant resulting from deletion of three nucleotides that leads to loss of a single phenylalanine residue at codon 508, accounts for approximately 70% mutations[2]. CFTR protein is found in the epithelial cells of lungs, sweat glands, liver pancreas and intestine. Liver disease is one of the classic phenotypes of CF.

CF liver disease (CFLD) usually develops within the first 20 years of life and has a stable non-progressive or mildly progressive course in later life[3,4]. Most children with CF will have some degree of steatosis but clinically significant liver disease develops in < 10% of pediatric CF patients usually by 10 years of age. CF related cirrhosis is a disease of the childhood and adolescence while predominant biliary involvement mimicking sclerosing cholangitis mostly occurs in adulthood[5]. The diagnosis of liver disease has profound implications in short and long term prognosis in CF patients and is the third leading cause of mortality in CF. Analysis of a large cohort of patients from the CF Foundation Patient Registry database showed that in CF patients with liver disease, the estimated 10-year cumulative rate of any adverse liver-related outcomes was approximately 20%[2]. Liver disease with cirrhosis and or portal hypertension has been classified as severe CFLD.

PATHOPHYSIOLOGY

CFLD is a genetic disorder of cholangiocyte transport protein defect, resulting in chronic cholangiopathy caused by reduced ductal bile flow generation and reduction in biliary chloride and bicarbonate secretion caused by the dysfunction of CFTR[6,7]. But this mechanism alone cannot explain CFLD, because CFTR deficiency is present in all patients while CFLD occurs only in a small population of CF patients and has varying clinical manifestations and severity. As described below, a combination of factors including CFTR genotype, non-CFTR genetic variability, abnormal intracellular interactions, abnormal cholangiocyte function, altered biliary secretion, pathologic stimulation of innate immune response with abnormal response to endotoxins lead to CFLD.

Abnormal cholangiocyte function and altered biliary secretion

Abnormal CFTR results in inhibition of cyclic adenosine monophosphate dependent chloride and bicarbonate secretion. This reduces the bile flow and alkalinity resulting in the biliary epithelial damages deriving from the retention of cytotoxic bile acids and xenobiotics and from the reduction in natural defenses against microbiologic pathogens. The response to chronic epithelial damage and the progression in the liver damage depends on the immunogenetic response of the individual and on other modifier genes.

Abnormal protein-protein interactions

CFTR mediated liver injury is also postulated to be caused by ability to regulate the function of other proteins by physically associating in macromolecular complexes at the membrane (protein-protein interaction)[8,9]. CFTR interacting proteins are located not only in the plasma membrane but also in nucleus, endoplasmic reticulum, Golgi apparatus, trafficking vesicles, proteasomes and cytoskeleton[9]. For example, the interaction of CFTR with proteins regulating the function of non-receptor tyrosine kinase Rous sarcoma oncogene cellular homologue can modulate innate immune responses in cholangiocytes[8]. Dysfunction of interactions can have systemic consequences resulting from the perturbation of the interconnected cellular networks accounting for some of the phenotypic variation in CF[8].

Abnormal innate inflammatory response

The conventional theory of CFLD postulates that biliary epithelial CFTR dysfunction causes alterations in the volume and composition of bile, resulting in loss of protective effect of biliary bicarbonate and mucus and an accumulation of toxic bile acids causing damage to the epithelium by initiating an inflammatory response[8]. But it is now postulated that the abnormal inflammatory response is due to lack of tolerance in the innate immune system[7]. CFTR is a now thought as a regulator of cholangiocyte

innate immune responses and defective CFTR results in aberrant activation of Src tyrosine kinase causing upregulation of innate inflammatory responses *via* the Toll-like receptor 4/NF-κB axis[7,10]. This results in lack of tolerance of biliary epithelium to endotoxin (*e.g.* pathogen-associated molecular patterns) from bile and intestine, leading to a para-inflammatory process in the biliary epithelium with the release of cyto/chemokines and the infiltration of the portal spaces with inflammatory cells[7, 10].

Gut dysbiosis and role of gut-liver axis

There is a substantial reduction in the richness and diversity of gut bacteria in patients with CF from early childhood until late adolescence and the changes deviate progressively farther from the path of healthy controls with increasing age[11]. Gut dysbiosis results in reduction in anti-inflammatory short-chain fatty acids, altered ratios of arachidonic acid/Linoleic acid and arachidonic acid/docosahexaenoic acid leading to increased gut inflammation[8,12]. This causes increased permeability of intestinal epithelia, increasing the exposure of biliary epithelial cholangiocytes to endotoxins, perpetuating the inflammatory cascade[8,12]. But it is not certain if intestinal inflammation is caused by the altered microbiota in CF or is the consequence of an altered environment[8,12].

Genetics

There is massive heterogenicity in CFTR phenotype among patients with CFLD and CFTR genotype-phenotype correlations are generally weak. The functional consequences of CF-causing variants have been grouped into six classes[1,13] (Figure 1). Mutations in classes I and II are also known as minimal function mutations since they demonstrate no to very little CFTR function, while those in classes IV, V, and VI are known as residual function mutations since they demonstrate some CFTR function, although it is lower compared to the wild type CFTR[14]. CFLD is mostly occurs in pancreatic insufficient patients with biallelic loss-of-function mutations in CFTR (class I, II, or III mutations on both allele)[1,3]. It has been shown that non-CFTR genetic variability also contributes to risk for severe liver disease[15]. This might be one of the reasons in variability of phenotype even between siblings inheriting the same mutations. Though many candidate genes have been postulated, in a large study *SERPINA1* (coding for alpha1-antitrypsin) Z allele was significantly associated with CFLD and portal hypertension[16].

CLINICAL FEATURES

The prevalence of CFLD varies widely in children and adolescents, based upon the diagnostic criteria used ranging from < 5% to 68%[17,18]. CFLD is more common and the median age of diagnosis is earlier in males[19]. Liver involvement in CF may be subclinical until diffuse liver damage occurs. Liver involvement can vary from mild elevation of aminotransferases to cirrhosis with synthetic failure and portal hypertension. The degree of liver involvement and the rate of progression of liver disease varies significantly among individuals. The awareness of CFLD and its clinical implications has increased as evidenced by an early diagnosis and a drop in the median time at diagnosis from adolescence to < 3 years of age[17,18].

Risk factors for CFLD include male sex, presence of severe mutations, presence of *SERPINA 1Z* allele, history of meconium ileus, exocrine pancreatic insufficiency and CF-related diabetes[20]. The most common clinical feature is asymptomatic hepatomegaly detected by clinical examination or ultrasonography[18]. Pancreatic insufficiency occurs in 99% of patients with CFLD[19]. Liver involvement in CF can be classified into two broad categories based on the presence of cirrhosis/portal hypertension (Table 1).

Liver disease without portal hypertension

Cholestasis: Neonatal/infantile cholestasis is the earliest manifestation of liver involvement in CF, but is very rare (< 2%). It is important to exclude other common causes of neonatal cholestasis like biliary atresia and also to consider the diagnosis of CF in infants who present with cholestasis[21].

Abnormal liver enzymes: The commonly noticed abnormalities include intermittent rise in serum transaminases (aspartate aminotransferase (AST) and alanine aminotransferase (ALT)) and/or increased serum levels of alkaline phosphatase (ALP)

Table 1 Spectrum of cystic fibrosis liver disease in children

Spectrum of cystic fibrosis liver disease in children
Liver
Neonatal cholestasis
Pre-clinical
Elevated aminotransferases
Increased GGT
Steatosis
Portal hypertension including non-cirrhotic portal hypertension
Cirrhosis
Focal biliary
Multi-lobular
Gallbladder and biliary system
Cholelithiasis
Abnormal size/function
Intra and extrahepatic biliary strictures (sclerosing cholangitis)

GGT: Gamma glutamyl transferase.

and gamma glutamyl transferase (GGT). Elevated liver enzymes can precede clinical and radiological abnormalities by several years. Bile duct damages can be demonstrated even in asymptomatic cases[22]. About 53%–93% of patients with CF have at least one abnormal value of AST/ALT, while over one-third have abnormal levels of GGT by 21 years of age[23]. CFLD patients with cirrhosis with portal hypertension can have normal liver biochemistry and synthetic function. Fluctuations in liver biochemistry is common and can be due to medications, infection or malnutrition.

Steatosis: Steatosis is common in CF patients, seen in upto 70% children undergoing liver biopsies[24]. The etiology is uncertain, but postulated to be due to malnutrition, deficiencies of essential fatty acid, carnitine and choline[24,25]. Steatosis in CF patients can also be caused by impaired glucose tolerance, diabetes mellites, hypertriglyceridemia and obesity[23]. Significant steatosis has become uncommon due to earlier diagnosis of CFLD and appropriate nutritional management. Alcohol consumption should be considered in adolescent CF patients with steatosis. Steatosis in CF was previously thought to be a benign condition, but with the emergence of nonalcoholic steatohepatitis as a leading cause of cirrhosis and understanding of the pathology, this might no longer be the case. Other signs of chronic liver disease or portal hypertension are usually not present.

Gallbladder and biliary tract involvement: Abnormalities of gallbladder (GB) can be present in children with CF. Micro-GB has been described in up to 33% of patients and GB might even be absent in CF patients[26]. Abnormal function of gallbladder and gallstones can also present. Black pigmented stones are more commonly found in patients with CF compared to cholesterol gallstones which are common in general population[26]. Symptomatic GB disease (4%) and need for cholecystectomy is common in adults[26].

Intra- or extrahepatic biliary strictures and segmental dilation has been reported in children with CF. Bile duct strictures and associated complications frequently occur even in patients with mild variants of CF. Magnetic resonance (MR) cholangiography data has shown that up to 70% of patients can have abnormalities of biliary tree regardless of biochemical or clinical evidence of liver disease and can mimic primary sclerosing cholangitis[24,26]. There is no correlation between severity of liver disease, abnormal liver tests and the presence of biliary strictures[24,26].

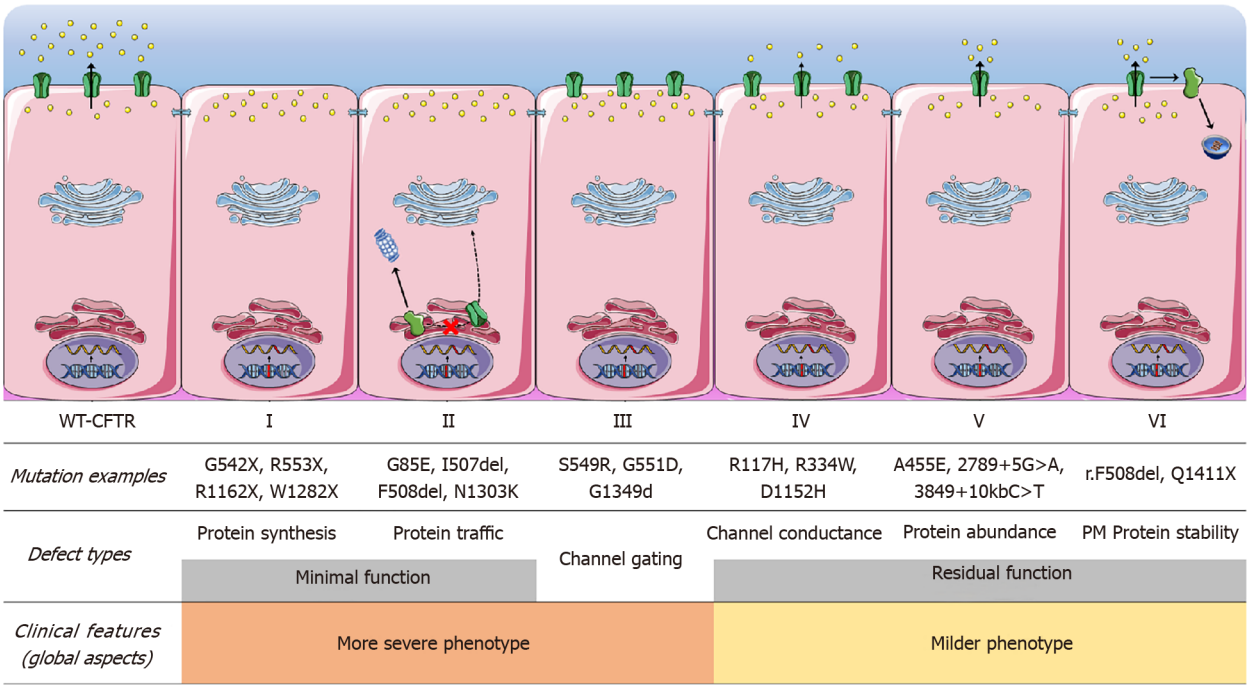

Figure 1 The functional consequences of cystic fibrosis-causing variants have been grouped into six classes. Class I mutations lead to no protein synthesis or translation of shortened, truncated forms. They result from splice site abnormalities, frameshifts due to deletions or insertions, or nonsense mutations, which generate premature termination codons. Class II mutations lead to a misfolding protein that fails to achieve conformational stability in the endoplasmic reticulum and then does not traffic to the plasma membrane (PM), being instead prematurely degraded by proteasomes. Class III mutations lead to a gating channel defect due to impaired response to agonists, although the protein is present at the PM. Class IV mutations lead to a channel conductance defect with a significant reduction in cystic fibrosis transmembrane conductance regulator (CFTR)-dependent chloride transport. Class V mutations lead to a reduction in protein abundance of functional CFTR due to reduced synthesis or inefficient protein maturation. They result from alternative splicing, promoter or missense mutations. Class VI mutations lead to reduced protein stability at the PM, which results in increased endocytosis and degradation by lysosomes, and reduced recycling to the PM. PM: Plasma membrane.

Liver disease with cirrhosis/portal hypertension (severe CFLD)

Portal hypertension: Variceal bleed can occur with or without cirrhosis and frequently occurs in the context of preserved hepatic synthetic function. Varices can be seen in 10-70% with CFLD and may be present at diagnosis of CFLD in 25%[19,27,28]. Isolated gastric varices may be seen in 15%[19]. Variceal bleed can be the sentinel event in CFLD leading to the diagnosis of portal hypertension/cirrhosis in up to 50% and may also be fatal, either from bleed itself or by precipitating liver failure. The age at first bleed can range from 10-30 years and recurrent bleeds can also occur[4]. Variceal bleed is associated with 5 fold risk of liver transplantation (LT)[2]. Thrombocytopenia has been postulated as a marker of severe CFLD with portal hypertension, so decreasing or persistently low platelet counts should prompt evaluation for portal hypertension [19]. Non cirrhotic portal hypertension can also occur in CFLD[28,29]. This has been postulated to be due to perisinusoidal portal venopathy caused by inflammation and fibrosis[24,29].

Focal biliary cirrhosis: Focal biliary cirrhosis is characterized by focal areas of scarring and furrowing in the liver with large areas of normal preserved hepatic architecture in between. Histologically, it is characterized by cholestasis, significant focal fibrosis, plugging of bile ducts with eosinophilic material, bile duct proliferation and expansion of portal tract leading to the postulation that bile duct plugging is the causative factor.

Focal biliary cirrhosis is clinically silent without any abnormalities on physical examination and normal liver biochemistry. Radiological imaging is also frequently noncontributory. Postmortem studies have shown that the incidence of focal biliary cirrhosis increases with advanced age- 11% in infants, 27% at 1 year and 25%–70% of adults[24]. Only a small subset of patients will progress to more severe liver disease and eventually multilobular cirrhosis, but the factors causing this is not known.

Multilobular cirrhosis: Biliary cirrhosis with portal hypertension is the most severe clinical manifestation of CFLD. Clinically, liver is multilobulated and firm- extensive lobulation is characteristic of CF cirrhosis. Signs of chronic liver disease such as clubbing, spider angioma, and palmar erythema may be present but is uncommon and

often occurs late in the disease course. There are no clinical or biochemical abnormalities or radiological features that consistently predict the presence of cirrhosis or risk of development of portal hypertension[28]. Majority of the morbidity due cirrhosis is caused by complications arising from portal hypertension. Hepatic encephalopathy is rare complication of cirrhosis per se in CFLD and mostly has occurred after therapeutic portosystemic shunting for management of portal hypertension[24]. Hepatic decompensation as evidenced by progressive decrease in albumin levels and development of ascites represents poor prognosis and necessitates LT evaluation.

Patients with cirrhosis are at risk of significant malnutrition as compared to CF patients without liver disease. This is due to anorexia, micronutrient deficiency, early satiety due to organomegaly and increased catabolism. In a study comparing CFLD patients with CF patients without liver disease, body fat measurements, including triceps, subscapular, and supra-iliac skinfold measures, were significantly less in the CFLD patients[27]. However, weight, height and mid upper arm circumference were not different between the two groups[27].

EVALUATION

Liver enzymes (AST, ALT, GGT) are poor predictors or indicators of cirrhosis or the risk of development of cirrhosis or CFLD and are neither sensitive or specific. There is poor correlation of liver enzymes with histologic findings, with 25% of CFLD patients with biopsy proven severe liver fibrosis having normal ALT levels[28]. But patients presenting with significant or persistently elevated liver biochemistries warrant further investigation for evidence of CFLD and other etiologies (Table 2). Persistently elevated GGT might be a pointer to biliary disease (*e.g.*, sclerosing cholangitis). Thrombocytopenia with splenomegaly is suggestive of development of portal hypertension. The synthetic function of liver (clotting, albumin) should be checked in all patients with suspected CFLD. If deranged after correcting nutritional (poor diet, vitamin deficiency) defects, should be thoroughly investigated.

Imaging

Ultrasound (US) of the hepatobiliary system with Doppler measurements of hepatic vasculature is non-invasive and may be a valuable marker of early CFLD[30]. Partial or complete hyper echogenicity liver, suggestive of steatosis is the most common US finding in CF[31]. Another fatty infiltration pattern, pseudomasses, seen as lobulated fatty structures of 1–2cm causing heterogeneity in the liver parenchyma is typical of CF[31]. Focal biliary cirrhosis appears sonographically as regions of increased echogenicity in periportal areas[31,32]. Cirrhotic liver has a nodular appearance with a coarsened echotexture[32]. Right hepatic lobe atrophy and hypertrophy of the caudate and lateral segments of the left lobe may be seen[32]. Splenomegaly, portosystemic shunts, hepatofugal flow in portal vein, and ascites can be seen with portal hypertension.

Abnormal echogenicity frequently precedes biochemical/clinical evidence of liver disease, with one study showing that two thirds of the children with abnormal liver echotexture and 50% with portal hypertension had no biochemical/clinical evidence of CFLD at the time when US changes were first noted[30]. Heterogeneous pattern of liver has been shown to be associated with higher risk of development of advanced liver disease in CF patients[30,33]. However, there is significant intra/ interobserver variability in US imaging and children with normal hepatic US can have advanced fibrosis, so a normal US does not exclude significant liver fibrosis or CFLD[3].

Assessment of the intra and extrahepatic biliary tree is better with MR cholangiography. The typical appearances include strictures, beading, narrowing, or dilatation of the intrahepatic ducts; diffuse narrowing or focal stricture of the common bile duct; and calculi[32].

Liver biopsy

Liver biopsy (LB) the gold standard in diagnosing fibrosis and cirrhosis, but is difficult to perform in CF patients because of the invasive nature and presence of associated comorbidities. Also because of the patchy distribution of lesions in CFLD, LB may underestimate the severity of lesions[25]. LB should be reserved for evaluation for other potential causes of fibrosis (autoimmune hepatitis, Wilson's disease, hepatotrophic infections) or drug-induced liver injury.

Table 2 Causes of acute or chronic liver disease in cystic fibrosis patients showing hepatic abnormalities

Condition	Investigation
Acute/chronic viral hepatitis	Serology for HAV, HBV, HCV, EBV, CMV, adenovirus, HHV 6, parvovirus
α1 antitrypsin deficiency	Serum α1 antitrypsin level, including phenotype
Autoimmune hepatitis	Non-organ specific autoantibodies (SMA, anti-LKM1, LC1)
Celiac disease	Total IgA, IgA anti-tissue transglutaminase
Wilson disease	Ceruloplasmin, serum copper, 24 h urinary copper
Drug induced liver injury	Antibiotics (cyclines, macrolides, amoxicillin-based, and cephalosporins) & antifungals (azoles and polyenes)
Genetic hemochromatosis (adults)	Iron, Ferritin, Transferrin binding capacity
Other causes of steatosis	Malnutrition, diabetes, obesity

This table is modified from Debray *et al*[25]. HAV: Hepatitis A virus; HBV: Hepatitis B virus; HCV: Hepatitis C virus; CMV: Cytomegalovirus; EBV: Epstein-Barr virus; HHV6: Herpes hominis virus type 6; SMA: Smooth muscle antibody; LKM1: Liver kidney microsomal type 1; LC: Liver cytosol type 1; IgA: Immunoglobulin A.

Noninvasive tests of fibrosis and liver disease

The early detection and monitoring of fibrosis, assessment of stage of fibrosis and progression to CFLD is challenging because routinely available tests to measure liver damage can often be normal even in advanced cirrhosis and liver biopsy is invasive with potential risk of complications. Non-invasive tests are divided into direct and indirect markers of liver fibrosis and imaging modalities as outlined in Table 3.

Direct markers are components of extracellular matrix degradation or fibrogenesis in serum include Matrix Metalloproteinase-9, tissue inhibitor of metalloproteinase-1 and 2, procollagen III peptide, collagen type-IV, hyaluronic acid, laminin, prolyl hydroxylase and YKL-40. These are not readily available in the routine clinical setting, are costly and are not validated in large scale studies. Indirect markers are serum-based tests and consist of readily available biochemical surrogates and clinical risk factors (AST, ALT, platelet count, age) for liver fibrosis. These include aspartate aminotransferase to platelet ratio index (APRI) and Fibrosis-4 (Fib-4). Stonebraker *et al* [19] demonstrated in a large pediatric cohort (n = 497) with CFLD and portal hypertension that APRI and Fib-4 values could differentiate patients who developed complications of portal hypertension and were significantly different in CFLD patients with and without oesophageal varices.

Advanced imaging modalities which quantify liver stiffness as a marker of fibrosis such as transient elastography (TE, Fibroscan®), acoustic radiation force impulse and MR elastography have been shown to accurately reflect advanced liver disease/end-stage fibrosis in CF. Liver stiffness as measured by TE had high diagnostic accuracy and was increased in CFLD compared to CF patients without liver disease[34]. Serial monitoring using TE is more useful as progressive enhancement of liver stiffness as this might reflect progression of liver disease thereby facilitating early detection[34, 35]. MR elastography is currently the most accurate noninvasive method across the spectrum of liver fibrosis and offers promise in the assessment of response to antifibrotic drugs but is not well studied in the context of CF liver disease[36].

Noninvasive methods are valuable for excluding advanced fibrosis or cirrhosis, but are not sufficiently predictive when used in isolation and have not yet been demonstrated to accurately reflect fibrosis change in response to treatment, limiting their role in disease monitoring[36]. Combination of serum markers with liver stiffness analysis might improve the sensitivity and negative predictive value without altering the specificity[34]. The negative predictive value of noninvasive tests is generally very high, allowing the clinician to be confident that advanced fibrosis or cirrhosis has been excluded.

DIFFERENTIAL DIAGNOSIS

The wide spectrum, variability of presentation at different age groups, presence of confounding factors and the absence of specific markers or tests makes it difficult to diagnose CFLD. The common differential diagnosis to be considered in CFLD are

Table 3 Examples of noninvasive monitoring of liver fibrosis in pediatric cystic fibrosis liver disease

Non-invasive marker	Ref.	Outcome measured	AUC	Sensitivity	Specificity	Comments
Indirect markers of liver fibrosis						
APRI	Leung *et al*[37]	CFLD diagnosis and severe CFLD	0.81	73%	70%	APRI score cut-off > 0.264; Predict CFLD and significant fibrosis in CFLD with a high degree of accuracy
FIB-4	Leung *et al*[37]	Portal hypertension	0.91	78%	93%	FIB-4 cutoff 0.358
Direct markers of liver fibrosis						
TIMP-1	Pereira *et al*[38]	CFLD diagnosis	0.76	64%	83%	Significantly increased in CFLD *vs* no-CFLD
Prolyl hydroxylase	Pereira *et al*[38]	CFLD diagnosis		60%	91%	Negative correlation between serum TIMP-1 levels and the stage of histological fibrosis; Prolyl hydroxylase useful in distinguishing CFLD patients with early fibrogenesis *vs* extensive fibrosis; Not able to differentiate CFLD versus no-CFLD
TIMP-2	Rath *et al* [38]	CFLD diagnosis	0.69	-	-	
m-RNA's	Cook *et al* [39]	CFLD diagnosis	0.78	47%	94%	Able to differentiate between CFLD versus no-CFLD but quantify not fibrosis stage; Pathological significance not yet certain, more studies needed
Imaging methods						
Transient elastography	Witters *et al*[40]	Liver stiffness	0.86	63%	87%	Less inter and intra-observer variability; Easy to learn and perform; Regular measurements for serial follow-up feasible
	Rath *et al* [34]	Liver stiffness	0.68	-	-	Few centres have access to technology
MR elastography	Palermo *et al*[41]	Liver stiffness	-	100%	100%	Small study, paucity of data; Shear stiffness significantly elevated in CF patients with cirrhosis; Costly with limited availability

AUC: Area under the curve; APRI: Aspartate aminotransferase to platelet ratio index; CFLD: Cystic fibrosis associated liver disease; Fib-4: Fibrosis-4; TIMP: Tissue inhibitor of metalloproteinase; m-RNA: Messenger ribonucleic acid; MR: Magnetic resonance.

listed in Table 2.

DIAGNOSTIC CRITERIA OF CFLD

The commonly used diagnostic criteria are described in Table 4.

MANAGEMENT

Management of CFLD should be done by a multi-disciplinary team and is mainly supportive since there is no effective therapy to treat or prevent progression of fibrosis, portal hypertension, or cirrhosis in CFLD. The CF foundation guidelines recommends annual screening for CFLD in children with examination of abdomen (hepatosplenomegaly), biochemical evaluation (bilirubin, AST, ALT, GGT, ALP, albumin, prothrombin time, platelet count), abdominal US and pulse oximetry (screening for hepatopulmonary syndrome)[25]. Salicylic acid and non-steroid anti-inflammatory drugs are contraindicated once CFLD is diagnosed and vaccination against hepatitis A and B should be done.

Ursodeoxycholic acid (UDCA) is recommended for all children diagnosed with CFLD at 20 mg/kg/d divided twice daily initially and increased up to 30 mg/kg/d [25]. A Cochrane review[42] had shown that there were only few trials assessing the effectiveness of UDCA with poor quality of evidence and there was no data on the effect of UDCA on long term outcomes including need for LT or mortality. Hence, the long term continuation of UDCA should be individualized.

Table 4 Diagnostic criteria of cystic fibrosis liver disease

Debray *et al*[25]	CF foundation classification[24]
Hepatomegaly and/or splenomegaly- increased liver span at midclavicular line and spleen size in longitudinal coronal plane for age and sex, confirmed by ultrasonography	CF related liver disease with cirrhosis/portal hypertension (based on clinical exam/imaging, histology, laparoscopy)
Abnormalities of liver function tests-elevated AST and ALT and GGT levels above the upper limit of normal with at least at 3 consecutive determinations over 12 months after excluding other causes of liver diseases	Liver involvement without cirrhosis/portal hypertension consisting of at least one of the following: (1) Persistent AST, ALT, GGT > 2 times upper limit of normal; (2) Intermittent elevations of the above laboratory values; (3) Steatosis (histologic determination); (4) Fibrosis (histologic determination); (5) Cholangiopathy (based on ultrasound, MRI, CT, ERCP); and (6) Ultrasound abnormalities not consistent with cirrhosis
Ultrasonographic evidence of coarseness, nodularity, increased echogenicity, or portal hypertension	Preclinical: No evidence of liver disease on clinical examination, imaging or laboratory values
Liver biopsy showing cirrhosis	

AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; GGT: Gamma glutamyl transpeptidase; CF: Cystic fibrosis; MRI: Magnetic resonance imaging; CT: Computed tomography; ERCP: Endoscopic retrograde cholangiopancreatography.

Nutrition

Optimal nutrition is the cornerstone of CFLD management. Malnutrition in CF is multifactorial including malabsorption due to pancreatic insufficiency, recurrent infections, chronic inflammation, chronic liver disease and anorexia. Nutrition should be managed by experienced CF dietetic team. It is recommended that CFLD patients increase energy intake to 150% of Recommended Daily Allowance preferably by increasing proportion of fat to 40%–50% of the energy content of the feed or diet, with supplementation in medium chain triglycerides and special attention to polyunsaturated fatty acids[25].

About 3 g/kg/d of protein and sufficient pancreatic enzymes to allow optimal absorption of long-chain triglycerides and essential fatty acids is also recommended. High dose oral fat soluble vitamin supplements is recommended- vitamin A (5000–15000 international units daily), vitamin E (alpha tocopherol 100–500 mg daily), vitamin D (alphacalcidiol 50 ng/kg to maximum of 1 μg) and vitamin K (1–10 mg daily)[25]. Plasma levels of vitamins (A, D and E) and prothrombin time needs to be closely monitored to prevent toxicity or deficiencies.

Salt supplementation should be avoided in CF patients with cirrhosis and portal hypertension due to the risk of development of ascites. If adequate caloric intake cannot be achieved orally, nasogastric feeding may be required to ensure adequate caloric intake. CFTR modulator therapy has resulted in less pulmonary exacerbations, decrease in levels of inflammatory makers, better body mass index and pancreatic function resulting in better overall nutritional status[14].

Management of esophageal varices

Management of varices in CFLD is complicated by the fact that non-selective beta-blocker (propranolol or carvedilol) might be contraindicated due to the associated lung disease and repeated general anesthesia required for screening of therapeutic endoscopic procedures may also reduce lung function and predispose to infections. Primary variceal prophylaxis in CFLD most commonly involves endoscopic variceal band ligation, but there is lack of quality evidence in children[24].

Variceal bleeding in the absence of decompensated cirrhosis in CFLD is most commonly managed by therapeutic endoscopy (band ligation +/- sclerotherapy)[4]. Sclerotherapy is useful if variceal band ligation is unsuccessful or gastric varices are present. Patients with refractory life threatening bleeds might require transjugular intrahepatic portosystemic shunt (TIPSS) or in rare circumstances surgical portosystemic shunting as an lifesaving procedure. Careful evaluation of liver disease and lung disease is necessary before proceeding with an elective TIPSS procedure. In a study[4] specifically analyzing outcomes of variceal bleeds in CFLD, out of 35 bleeding episodes, 30 were controlled by endoscopic procedures, while 11% (4 episodes) required either TIPSS, surgical shunts procedures.

Liver transplantation

LT evaluation should be offered for CFLD patients with intractable complications of portal hypertension and/or end stage liver disease since LT confers significant

survival advantage[43]. The main indications of isolated LT in CFLD is listed in Table 5. Poor growth and nutrition as an indication remains controversial because studies have not shown consistent improvement after LT[43]. LT should be considered when nutritional deficiencies are believed to be sequelae of advanced liver disease and portal hypertensive enteropathy impacting clinical outcomes[43]. Lung function may improve, remain stable or deteriorate after LT and any short term advantage with improvement of lung function is lost within 3 years of LT[44,45]. So, rapidly deteriorating lung function alone should not be an indication for isolated LT in stable CFLD [46].

Long term outcomes after LT are lower in children with CFLD as compared to other etiologies[44]. Table 6 illustrates details of few published series on LT in CFLD in children. For those patients with end-stage liver disease and significant pulmonary complications, combined liver-lung or liver-heart-lung transplantation may be considered, but outcomes are worse compared to isolated LT[45,46].

Pre-transplant considerations

Careful assessment of liver disease, pulmonary function, nutritional status and type of transplant to be performed should be done by an experienced multidisciplinary team. Concomitant causes or other etiologies of liver injury as listed in Table 3 should be ruled out before LT is considered. Alpha-1-antitrypsin level and genotype, screening for autoimmune hepatitis and Wilson's disease should be done as a part of the workup especially if the child is seen for the first time in a LT center. CFLD patients being considered for LT should have endoscopic variceal surveillance and possibly coordinated with bronchoscopy and dental procedures as part of the LT evaluation to minimize the number anaesthetic procedures[43]. Careful evaluation of cardiac function should be done since patients with cardiomyopathy or severe pulmonary hypertension may require combined heart, lung, and liver transplantation.

A thorough evaluation by a pediatric pulmonologist with CF and lung transplantation expertise should be a part of the LT assessment, irrespective of the forced expiratory volume in one second (FEV1). Analysis of United Network for Organ Sharing data from 1987 through 2009 suggested that patients with a predicted forced vital capacity (FVC) > 75% and FEV1 > 60% (possibly even ≥ 40%) may be safely offered isolated LT[50]. The possibility of progressive deterioration in lung function after LT should be communicated to the family. The most difficult group to decide is patients who require LT but present with borderline (FEV1 40%-60% predicted) and/or rapidly declining (10% FEV1 predicted/year) pulmonary function[43].

Microbial considerations, such as multidrug resistant bacterial infections and history of recurrent/ invasive fungal infections are critical since post-transplant sepsis is a leading cause of mortality[43,50]. Flexible bronchoscopy with bronchioalveolar lavage with cultures for mycobacteria, fungus, and quantitative bacterial analysis from at least 2 locations within each lung is recommended[43]. The presence of multidrug resistant *Mycobacterium abscessus* in the lungs, even with well-preserved pulmonary function, carries a high risk of mortality in the first year after transplant and needs to be considered carefully before recommendation for LT[43].

Patients should be evaluated for nasal polyps and chronic sinusitis and treated immediately if identified[43]. CF–related diabetes should be evaluated and well controlled prior to LT. Dietetic and nutritional assessment is an integral part of the evaluation.

Post-transplant considerations

Immunosuppression after LT in patients with CF will vary from center to center but typically consists of triple drug therapy with tacrolimus, steroids and mycophenolate mofetil/azathioprine. Close collaboration between the CF, transplant and infectious diseases teams is crucial because of the increased risk of mortality from infections. Early mortality (< 6 mo) post-LT is due to disseminated aspergillosis/candidiasis, and sepsis with gram-negative enteric bacteria and staphylococcus aureus while later deaths are a result of progressive pulmonary disease[43]. Post-transplant antibiotic prophylaxis in our unit consists of fluconazole for candida species, acyclovir for herpes simplex virus, valganciclovir for cytomegalovirus and trimethoprim-sulfamethoxazole for *Pneumocystis jiroveci*. Distal intestinal obstructive syndrome (DIOS) causing acute potentially life-threatening intestinal obstruction can develop post- transplant in >20% of pediatric patients[49]. In the pre-transplant period, DIOS occurs typically in older CF patients in adolescence and adulthood, in those with advanced liver disease, severe CFTR mutations, pancreatic insufficiency and diabetes mellitus. In our unit, patients are categorized into low risk (no episodes of DIOS in previous 5 years) and high risk (episodes of DIOS in previous 5 years and previous abdominal surgery) before LT.

Table 5 Indications and contraindications for liver transplantation in cystic fibrosis liver disease (Modified from Freeman *et al*[43])

Indications and contraindications	
Indications	
Strong	(1) Progressive hepatic dysfunction with hypoalbuminemia and coagulopathy (Coagulopathy not corrected by vitamin K, cholestasis not attributed to other causes); (2) Complications of portal hypertension (Intractable/recurrent variceal bleeding which is not controlled by medical or endoscopic management); (3) Hepatopulmonary and porto-pulmonary syndrome; (4) Overt hepatic encephalopathy; and (5) Hepatorenal syndrome
Controversial	(1) Deteriorating pulmonary function (FEV1/FVC <50%) with increased frequency and severity of pulmonary infective episodes requiring hospitalization; and (2) Severe malnutrition, unresponsive to intensive nutritional support
Contraindications	
Absolute	(1) Extrahepatic malignancies not amenable to curative therapy; (2) Multiorgan disease for which transplant would not be considered life-sustaining; (3) Uncontrolled systemic or pulmonary infection, active exacerbation, or veno-arterial extracorporeal membrane oxygenation; and (4) Severe porto-pulmonary hypertension nonresponsive to medical management
Relative	(1) Hepatocellular carcinoma; (2) Noncompliance or psychosocial concerns unamenable to transplant; (3) Uncontrollable CF-related diabetes; (4) Substance abuse; (5) Severe cardiopulmonary disease; and (6) Infection/colonization with multi-resistant organism (*e.g.*, *Burkholderia cenocepacia* and *Mycobacterium abscessus*)

FEV1: Forced expiratory volume in one second; FVC: Forced vital capacity.

Table 6 Liver transplantation in cystic fibrosis liver disease - data from few published series

Ref.	Type	Number of pediatric recipients	Type of transplants	Males	Mean age at isolated liver transplantation (yr)	Lung function after Liver transplantation	5-year survival
Milkiewicz *et al*[45], 2002	Single center	9	Liver; Liver-lung -heart	Not available	15	Improved	Not available
Fridell *et al* [21], 2003	Single center	12	Liver	83%	10 ± 4.5	Improved or remained unchanged	75%
Molmenti *et al* [47], 2003	Single center	10	Liver	90%	9.7 (1.23–19)	Not available	60%
Mendizabal *et al*[44], 2011	Analysis of United Network for Organ Sharing database	148	Liver; Liver-lung (3.4%)	62%	11 ± 4.7	Not available	86%
Miguel *et al* [48], 2011	Single center	11	Liver	67%	12 (5.4–17)	Worsened or remained unchanged	> 85%
Dowman *et al* [49], 2012	Single center	19	Liver	Not available	11.8 (9.5–16.5)	Stable/improved initially, deteriorated > 5 years after transplant	> 60%

Our pre and post-LT protocol for prevention and treatment of DIOS is given in Table 7. High risk patients should be counselled for loop ileostomy formation at transplant assessment.

CFTR modulators

CFTR modulator drugs enhance or even restore the expression, function, and stability of a defective CFTR by different mechanisms[14,51] (Table 8). These treatments target the underlying cause of CF and is classified into five main groups depending on their effects on CFTR mutations[14,51] (Table 8). Different CFTR genetic variants can benefit from the same type of modulator and this is the base of a new system recently introduced to classify and group common and rare CFTR variants based on their response to modulators called 'theratyping'.

The first United States Food and Drug Administration (FDA) approved drug was ivacaftor (Kalydeco, Vertex Pharmaceuticals)[14,51]. Other FDA approved CF modulators combinations are lumacaftor/ivacaftor (Orkambi®, Vertex Pharmaceuticals), tezacaftor/ivacaftor (Symdeko® or Symkevi®, Vertex Pharmaceuticals) for patients aged ≥ 12 years who are F508del-homozygous or F508del-heterozygous with a residual function mutation[14,20]. Lumacaftor/ivacaftor has been approved for F508del homozygous patients aged ≥ 2 years[14]. The triple combination elexacaftor/ivacaftor/tezacaftor (Trikafta™, Vertex Pharmaceuticals) has been by the FDA for the

Table 7 Pre and post-transplant protocol for prevention and treatment of distal intestinal obstructive syndrome

Pre and post-transplant protocol	
Low risk	(1) 600 mg N-acetyl-cysteine in 120 mL water orally/nasogastric tube twice/day. Senna twice daily; (2) 2 liters of Klean prep per day post-transplant; (3) Consider early nasogastric tube in patients with delayed gastric emptying studies pre-operatively; (4) All patients in intensive care unit should only receive only elemental feed *via* nasogastric tube as this does not require pancreatic enzyme replacement. Once transferred to ward, can be restarted on regular feeding and pancreatic enzyme supplements; (5) Try and reduce opiates early during hospital stay; and (6) Treat all patients with proton pump inhibitors.
High risk	(1) As per low risk management; and (2) High risk of developing DIOS and subsequent surgical gut decompression is associated with a high mortality. So these patients should receive a prophylactic loop ileostomy.
Treatment of DIOS	(1) Stop feeding, nasogastric tube on free drainage and intravenous fluids; (2) 100 mL gastrografin in 400 mL water enterally and repeat after 6 h; (3) Subsequent management is with Klean prep in 1 L water over 1 h *via* oral/nasogastric tube and can be repeated up to 4 times every 24 h until bowel movement is achieved; and (4) If no improvement after 48 h, then it is unlikely to resolve without surgery to decompress the gut and also consider total parenteral nutrition.

DIOS: Distal intestinal obstructive syndrome.

Table 8 Cystic fibrosis transmembrane conductance regulator modulators

Type of modulator	Mechanism of action	Mutation class in which drug is effective	Example	Clinical effects/present status of modulator
Potentiators	Restore or even enhance the channel open probability, thus allowing for CFTR-dependent anion conductance	Classes III and IV	Ivacaftor	Improvement in lung function, pancreatic function and body mass index
Correctors	Rescue folding, processing and trafficking to the plasma membrane of a CFTR mutant. Enhance protein conformational stability during the endoplasmic reticulum folding process	Class II	Lumacaftor; Tezacaftor; Posenacaftor; Elexacaftor	Significant improvement in lung function when used with Ivacaftor
Stabilizers	Anchor CFTR at the plasma membrane, thus preventing its removal and degradation by lysosomes	Class VI	Cavosonstat	First CFTR stabilizer studied in clinical trials- studies terminated because of lack of clinical efficacy
Read-through agents	Induce ribosomal over-reading of premature termination codon, enabling the incorporation of a foreign amino acid in place and continued translation to the normal end of the transcript	Class I	Ataluren (PTC124)	Clinical trials terminated
Amplifiers	Increase expression of CFTR mRNA and thus biosynthesis of the CFTR protein	Class V	Nesolicaftor (PTI-428)	Clinical trial planned

CFTR: Cystic fibrosis transmembrane conductance regulator; mRNA: Messenger RNA.

treatment of CF patients aged ≥ 12 years with F508del mutation in at least one allele, benefiting 90% of CF population[14,51].

CF MODULATORS AND LIVER

Abnormal elevation aminotransaminases (> 8 times upper limit of normal, more commonly in pediatric patients) and bilirubin (> 3 times upper limit of normal) has been reported 3%-15% of patients on CFTR modulators[52-54]. Lumacaftor/ivacaftor was shown to have less hepatic steatosis as assessed by MR imaging proton density fat fraction in a small cohort[55]. In a study[56] of 117 patients with CFTR gating mutations (partially F508 del heterozygous) treatment with Ivacaftor partially restored disrupted FGF19-regulated bile acid homeostasis. Worsening of liver function and liver failure leading to death has been reported in CF patients with pre-existing cirrhosis and portal hypertension receiving lumacaftor/ivacaftor.

Recommendations for dose adjustment are based on Child Pugh classification: no dose adjustment for Child-Pugh Class A but dose reduction is recommended for Child-Pugh Class B and C. This is applicable to adults and no specific recommendations exist in the literature for children with CFLD. Lumacaftor/ivacaftor should be used with caution in patients with advanced liver disease and only if the benefits are expected to outweigh the risks.

Because an association with liver injury cannot be excluded, assessments of liver function tests (ALT, AST and bilirubin) are recommended before initiation, a month after starting the treatment and every 3 mo during the first year of treatment, and annually thereafter. For patients with a history of ALT, AST, or bilirubin elevations, more frequent monitoring should be considered in collaboration with a pediatric hepatology centre. In the event of significant elevation of ALT or AST, with or without elevated bilirubin [either ALT or AST > 5× the upper limit of normal (ULN), or ALT or AST > 3× ULN with bilirubin > 2× ULN and/or clinical jaundice], dosing with CFTR modulators should be discontinued and closely followed up until the abnormalities resolve. A thorough investigation of potential causes should be conducted and patients should be followed closely for clinical progression. Following resolution of transaminase elevations, the benefits and risks of resuming CFTR modulators should be considered.

Metabolism of CFTR inhibitors is by the CYP450 enzyme pathway. Hence concomitant use of lumacaftor/ivacaftor with these immunosuppressants is not recommended at present as they may reduce efficacy of immunosuppressants by induction of the CYP3A pathway. Given the fact that respiratory function may eventually worsen after LT, CFTR modulators might need to be initiated post-transplant due to significant beneficial effects on lung function, nutritional status and decreased pulmonary exacerbations[43].

CONCLUSION

CFLD is the most important non-pulmonary cause of death in CF. CFLD is has a wide spectrum from asymptomatic elevation of liver enzymes to severe disease with portal hypertension and cirrhosis with synthetic failure. The degree of liver involvement and the rate of progression of liver disease varies significantly among individuals. There are no specific clinical features or tests for prediction or early detection of CFLD, so regular screening is essential for CF patients. Currently, there is no medical therapy to prevent or treat or CFLD. With the advent of CFTR modulators, improvement in medical management has resulted in significantly improved life expectancy in patients with CF and this will have implications in the management of CFLD in future. The long term effects of CFTR modulators on CFLD and liver function is not known, but will hopefully have a beneficial effect. LT is indicated in patients with CFLD with severe portal hypertension or impaired synthetic function of liver either alone or in combination with lung transplantation.

REFERENCES

1 **Cutting GR**, Engelhardt J, Zeitlin PL. Genetics and pathophysiology of Cystic Fibrosis. In: Wilmott R, Bush A, Deterding R, Ratjen F, Sly P, Zar H, Li AP, editors. Kendig's Disorders of the Respiratory Tract in Children. Philadelphia: Elsevier, 2019: 757-768

2 **Ye W**, Narkewicz MR, Leung DH, Karnsakul W, Murray KF, Alonso EM, Magee JC, Schwarzenberg SJ, Weymann A, Molleston JP; CFLDnet research group. Variceal Hemorrhage and Adverse Liver Outcomes in Patients With Cystic Fibrosis Cirrhosis. *J Pediatr Gastroenterol Nutr* 2018; **66**: 122-127 [PMID: 28906321 DOI: 10.1097/MPG.0000000000001728]

3 **Lindblad A**, Glaumann H, Strandvik B. Natural history of liver disease in cystic fibrosis. *Hepatology* 1999; **30**: 1151-1158 [PMID: 10534335 DOI: 10.1002/hep.510300527]

4 **Gooding I**, Dondos V, Gyi KM, Hodson M, Westaby D. Variceal hemorrhage and cystic fibrosis: outcomes and implications for liver transplantation. *Liver Transpl* 2005; **11**: 1522-1526 [PMID: 16258952 DOI: 10.1002/Lt.20465]

5 **Leung DH**, Narkewicz MR. Cystic Fibrosis-related cirrhosis. *J Cyst Fibros* 2017; **16** Suppl 2: S50-S61 [PMID: 28986027 DOI: 10.1016/j.jcf.2017.07.002]

6 **Staufer K**, Halilbasic E, Trauner M, Kazemi-Shirazi L. Cystic fibrosis related liver disease--another black box in hepatology. *Int J Mol Sci* 2014; **15**: 13529-13549 [PMID: 25093717 DOI: 10.3390/ijms150813529]

7 **Strazzabosco M**, Fiorotto R, Cadamuro M, Spirli C, Mariotti V, Kaffe E, Scirpo R, Fabris L. Pathophysiologic implications of innate immunity and autoinflammation in the biliary epithelium. *Biochim Biophys Acta Mol Basis Dis* 2018; **1864**: 1374-1379 [PMID: 28754453 DOI: 10.1016/j.bbadis.2017.07.023]

8 **Fiorotto R**, Strazzabosco M. Pathophysiology of Cystic Fibrosis Liver Disease: A Channelopathy Leading to Alterations in Innate Immunity and in Microbiota. *Cell Mol Gastroenterol Hepatol* 2019; **8**: 197-207 [PMID: 31075352 DOI: 10.1016/j.jcmgh.2019.04.013]

9 **Lim SH**, Legere EA, Snider J, Stagljar I. Recent Progress in CFTR Interactome Mapping and Its

Importance for Cystic Fibrosis. *Front Pharmacol* 2017; **8**: 997 [PMID: 29403380 DOI: 10.3389/fphar.2017.00997]

10 **Fiorotto R**, Scirpo R, Trauner M, Fabris L, Hoque R, Spirli C, Strazzabosco M. Loss of CFTR affects biliary epithelium innate immunity and causes TLR4-NF-κB-mediated inflammatory response in mice. *Gastroenterology* 2011; **141**: 1498-1508, 1508.e1 [PMID: 21712022 DOI: 10.1053/j.gastro.2011.06.052]

11 **Nielsen S**, Needham B, Leach ST, Day AS, Jaffe A, Thomas T, Ooi CY. Disrupted progression of the intestinal microbiota with age in children with cystic fibrosis. *Sci Rep* 2016; **6**: 24857 [PMID: 27143104 DOI: 10.1038/srep24857]

12 **Garg M**, Ooi CY. The Enigmatic Gut in Cystic Fibrosis: Linking Inflammation, Dysbiosis, and the Increased Risk of Malignancy. *Curr Gastroenterol Rep* 2017; **19**: 6 [PMID: 28155088 DOI: 10.1007/s11894-017-0546-0]

13 **Ratjen F**, Bell SC, Rowe SM, Goss CH, Quittner AL, Bush A. Cystic fibrosis. *Nat Rev Dis Primers* 2015; **1**: 15010 [PMID: 27189798 DOI: 10.1038/nrdp.2015.10]

14 **Lopes-Pacheco M**. CFTR Modulators: The Changing Face of Cystic Fibrosis in the Era of Precision Medicine. *Front Pharmacol* 2019; **10**: 1662 [PMID: 32153386 DOI: 10.3389/fphar.2019.01662]

15 **Debray D**, Corvol H, Housset C. Modifier genes in cystic fibrosis-related liver disease. *Curr Opin Gastroenterol* 2019; **35**: 88-92 [PMID: 30585791 DOI: 10.1097/MOG.0000000000000508]

16 **Bartlett JR**, Friedman KJ, Ling SC, Pace RG, Bell SC, Bourke B, Castaldo G, Castellani C, Cipolli M, Colombo C, Colombo JL, Debray D, Fernandez A, Lacaille F, Macek M Jr, Rowland M, Salvatore F, Taylor CJ, Wainwright C, Wilschanski M, Zemková D, Hannah WB, Phillips MJ, Corey M, Zielenski J, Dorfman R, Wang Y, Zou F, Silverman LM, Drumm ML, Wright FA, Lange EM, Durie PR, Knowles MR; Gene Modifier Study Group. Genetic modifiers of liver disease in cystic fibrosis. *JAMA* 2009; **302**: 1076-1083 [PMID: 19738092 DOI: 10.1001/jama.2009.1295]

17 **Lamireau T**, Monnereau S, Martin S, Marcotte JE, Winnock M, Alvarez F. Epidemiology of liver disease in cystic fibrosis: a longitudinal study. *J Hepatol* 2004; **41**: 920-925 [PMID: 15582124 DOI: 10.1016/j.jhep.2004.08.006]

18 **Dos Santos ALM**, de Melo Santos H, Nogueira MB, Távora HTO, de Lourdes Jaborandy Paim da Cunha M, de Melo Seixas RBP, de Freitas Velloso Monte L, de Carvalho E. Cystic Fibrosis: Clinical Phenotypes in Children and Adolescents. *Pediatr Gastroenterol Hepatol Nutr* 2018; **21**: 306-314 [PMID: 30345244 DOI: 10.5223/pghn.2018.21.4.306]

19 **Stonebraker JR**, Ooi CY, Pace RG, Corvol H, Knowles MR, Durie PR, Ling SC. Features of Severe Liver Disease With Portal Hypertension in Patients With Cystic Fibrosis. *Clin Gastroenterol Hepatol* 2016; **14**: 1207-1215.e3 [PMID: 27062904 DOI: 10.1016/j.cgh.2016.03.041]

20 **Staufer K**. Current Treatment Options for Cystic Fibrosis-Related Liver Disease. *Int J Mol Sci* 2020; **21** [PMID: 33202578 DOI: 10.3390/ijms21228586]

21 **Fridell JA**, Bond GJ, Mazariegos GV, Orenstein DM, Jain A, Sindhi R, Finder JD, Molmenti E, Reyes J. Liver transplantation in children with cystic fibrosis: a long-term longitudinal review of a single center's experience. *J Pediatr Surg* 2003; **38**: 1152-1156 [PMID: 12891484 DOI: 10.1016/s0022-3468(03)00260-4]

22 **Strazzabosco M**, Fabris L, Spirli C. Pathophysiology of cholangiopathies. *J Clin Gastroenterol* 2005; **39**: S90-S102 [PMID: 15758666 DOI: 10.1097/01.mcg.0000155549.29643.ad]

23 **Sakiani S**, Kleiner DE, Heller T, Koh C. Hepatic Manifestations of Cystic Fibrosis. *Clin Liver Dis* 2019; **23**: 263-277 [PMID: 30947876 DOI: 10.1016/j.cld.2018.12.008]

24 **Flass T**, Narkewicz MR. Cirrhosis and other liver disease in cystic fibrosis. *J Cyst Fibros* 2013; **12**: 116-124 [PMID: 23266093 DOI: 10.1016/j.jcf.2012.11.010]

25 **Debray D**, Kelly D, Houwen R, Strandvik B, Colombo C. Best practice guidance for the diagnosis and management of cystic fibrosis-associated liver disease. *J Cyst Fibros* 2011; **10** Suppl 2: S29-S36 [PMID: 21658639 DOI: 10.1016/S1569-1993(11)60006-4]

26 **Assis DN**, Debray D. Gallbladder and bile duct disease in Cystic Fibrosis. *J Cyst Fibros* 2017; **16** Suppl 2: S62-S69 [PMID: 28986023 DOI: 10.1016/j.jcf.2017.07.006]

27 **Rowland M**, Gallagher CG, O'Laoide R, Canny G, Broderick A, Hayes R, Greally P, Slattery D, Daly L, Durie P, Bourke B. Outcome in cystic fibrosis liver disease. *Am J Gastroenterol* 2011; **106**: 104-109 [PMID: 20736939 DOI: 10.1038/ajg.2010.316]

28 **Lewindon PJ**, Shepherd RW, Walsh MJ, Greer RM, Williamson R, Pereira TN, Frawley K, Bell SC, Smith JL, Ramm GA. Importance of hepatic fibrosis in cystic fibrosis and the predictive value of liver biopsy. *Hepatology* 2011; **53**: 193-201 [PMID: 21254170 DOI: 10.1002/hep.24014]

29 **Witters P**, Libbrecht L, Roskams T, Boeck KD, Dupont L, Proesmans M, Vermeulen F, Strandvik B, Lindblad A, Stéphenne X, Sokal E, Gosseye S, Heye S, Maleux G, Aerts R, Monbaliu D, Pirenne J, Hoffman I, Nevens F, Cassiman D. Noncirrhotic presinusoidal portal hypertension is common in cystic fibrosis-associated liver disease. *Hepatology* 2011; **53**: 1064-1065 [PMID: 21374682 DOI: 10.1002/hep.24183]

30 **Lenaerts C**, Lapierre C, Patriquin H, Bureau N, Lepage G, Harel F, Marcotte J, Roy CC. Surveillance for cystic fibrosis-associated hepatobiliary disease: early ultrasound changes and predisposing factors. *J Pediatr* 2003; **143**: 343-350 [PMID: 14517517 DOI: 10.1067/S0022-3476(03)00329-9]

31 **Akata D**, Akhan O. Liver manifestations of cystic fibrosis. *Eur J Radiol* 2007; **61**: 11-17 [PMID: 17174503 DOI: 10.1016/j.ejrad.2006.11.008]

32 **Fields TM**, Michel SJ, Butler CL, Kriss VM, Albers SL. Abdominal manifestations of cystic fibrosis in older children and adults. *AJR Am J Roentgenol* 2006; **187**: 1199-1203 [PMID: 17056906 DOI:

10.2214/AJR.05.0327]

33 **Siegel MJ**, Freeman AJ, Ye W, Palermo JJ, Molleston JP, Paranjape SM, Stoll J, Leung DH, Masand P, Karmazyn B, Harned R, Ling SC, Navarro OM, Karnsakul W, Alazraki A, Schwarzenberg SJ, Seidel FG, Towbin A, Alonso EM, Nicholas JL, Murray KF, Otto RK, Sherker AH, Magee JC, Narkewicz MR; CFLD Network. Heterogeneous Liver on Research Ultrasound Identifies Children with Cystic Fibrosis at High Risk of Advanced Liver Disease: Interim Results of a Prospective Observational Case-Controlled Study. *J Pediatr* 2020; **219**: 62-69.e4 [PMID: 32061406 DOI: 10.1016/j.jpeds.2019.12.033]

34 **Rath T**, Menendez KM, Kügler M, Hage L, Wenzel C, Schulz R, Graf J, Nährlich L, Roeb E, Roderfeld M. TIMP-1/-2 and transient elastography allow non invasive diagnosis of cystic fibrosis associated liver disease. *Dig Liver Dis* 2012; **44**: 780-787 [PMID: 22652148 DOI: 10.1016/j.dld.2012.04.008]

35 **Gominon AL**, Frison E, Hiriart JB, Vergniol J, Clouzeau H, Enaud R, Bui S, Fayon M, de Ledinghen V, Lamireau T. Assessment of Liver Disease Progression in Cystic Fibrosis Using Transient Elastography. *J Pediatr Gastroenterol Nutr* 2018; **66**: 455-460 [PMID: 29112089 DOI: 10.1097/MPG.0000000000001822]

36 **Loomba R**, Adams LA. Advances in non-invasive assessment of hepatic fibrosis. *Gut* 2020; **69**: 1343-1352 [PMID: 32066623 DOI: 10.1136/gutjnl-2018-317593]

37 **Leung DH**, Khan M, Minard CG, Guffey D, Ramm LE, Clouston AD, Miller G, Lewindon PJ, Shepherd RW, Ramm GA. Aspartate aminotransferase to platelet ratio and fibrosis-4 as biomarkers in biopsy-validated pediatric cystic fibrosis liver disease. *Hepatology* 2015; **62**: 1576-1583 [PMID: 26223427 DOI: 10.1002/hep.28016]

38 **Pereira TN**, Lewindon PJ, Smith JL, Murphy TL, Lincoln DJ, Shepherd RW, Ramm GA. Serum markers of hepatic fibrogenesis in cystic fibrosis liver disease. *J Hepatol* 2004; **41**: 576-583 [PMID: 15464237 DOI: 10.1016/j.jhep.2004.06.032]

39 **Cook NL**, Pereira TN, Lewindon PJ, Shepherd RW, Ramm GA. Circulating microRNAs as noninvasive diagnostic biomarkers of liver disease in children with cystic fibrosis. *J Pediatr Gastroenterol Nutr* 2015; **60**: 247-254 [PMID: 25625579 DOI: 10.1097/MPG.0000000000000600]

40 **Witters P**, De Boeck K, Dupont L, Proesmans M, Vermeulen F, Servaes R, Verslype C, Laleman W, Nevens F, Hoffman I, Cassiman D. Non-invasive liver elastography (Fibroscan) for detection of cystic fibrosis-associated liver disease. *J Cyst Fibros* 2009; **8**: 392-399 [PMID: 19733131 DOI: 10.1016/j.jcf.2009.08.001]

41 **Palermo J**, Kotyk JJ, Siegel MJ. Magnetic resonance elastography for assessment of cystic fibrosis liver disease. *Gastroenterology* 2011; **140**: S-686

42 **Cheng K**, Ashby D, Smyth RL. Ursodeoxycholic acid for cystic fibrosis-related liver disease. *Cochrane Database Syst Rev* 2014; CD000222 [PMID: 25501301 DOI: 10.1002/14651858.CD000222.pub3]

43 **Freeman AJ**, Sellers ZM, Mazariegos G, Kelly A, Saiman L, Mallory G, Ling SC, Narkewicz MR, Leung DH. A Multidisciplinary Approach to Pretransplant and Posttransplant Management of Cystic Fibrosis-Associated Liver Disease. *Liver Transpl* 2019; **25**: 640-657 [PMID: 30697907 DOI: 10.1002/lt.25421]

44 **Mendizabal M**, Reddy KR, Cassuto J, Olthoff KM, Faust TW, Makar GA, Rand EB, Shaked A, Abt PL. Liver transplantation in patients with cystic fibrosis: analysis of United Network for Organ Sharing data. *Liver Transpl* 2011; **17**: 243-250 [PMID: 21384506 DOI: 10.1002/lt.22240]

45 **Milkiewicz P**, Skiba G, Kelly D, Weller P, Bonser R, Gur U, Mirza D, Buckels J, Stableforth D, Elias E. Transplantation for cystic fibrosis: outcome following early liver transplantation. *J Gastroenterol Hepatol* 2002; **17**: 208-213 [PMID: 11966953 DOI: 10.1046/j.1440-1746.2002.02671.x]

46 **Morrell MR**, Kiel SC, Pilewski JM. Organ Transplantation for Cystic Fibrosis. *Semin Respir Crit Care Med* 2019; **40**: 842-856 [PMID: 31887769 DOI: 10.1055/s-0039-3399554]

47 **Molmenti EP**, Squires RH, Nagata D, Roden JS, Molmenti H, Fasola CG, Prestidge C, D'Amico L, Casey D, Sanchez EQ, Goldstein RM, Levy MF, Benser M, McPhail W, Andrews W, Andersen JA, Klintmalm GB. Liver transplantation for cholestasis associated with cystic fibrosis in the pediatric population. *Pediatr Transplant* 2003; **7**: 93-97 [PMID: 12654048 DOI: 10.1034/j.1399-3046.2003.00021.x]

48 **Miguel M**, Andres AM, Lopez-Santamaria M, Barrena S, Hierro L, Hernandez F, Ramírez M, Frauca E, Encinas JL, Lopez-Fernandez S, Jara P, Tovar JA. Liver transplantation in children with cystic fibrosis: experience in our centre and preliminary results with a combined en bloc liver-pancreas graft. *Eur J Pediatr Surg* 2012; **22**: 60-66 [PMID: 22161075 DOI: 10.1055/s-0031-1291288]

49 **Dowman JK**, Watson D, Loganathan S, Gunson BK, Hodson J, Mirza DF, Clarke J, Lloyd C, Honeybourne D, Whitehouse JL, Nash EF, Kelly D, van Mourik I, Newsome PN. Long-term impact of liver transplantation on respiratory function and nutritional status in children and adults with cystic fibrosis. *Am J Transplant* 2012; **12**: 954-964 [PMID: 22225648 DOI: 10.1111/j.1600-6143.2011.03904.x]

50 **Arnon R**, Annunziato RA, Miloh T, Padilla M, Sogawa H, Batemarco L, Willis A, Suchy F, Kerkar N. Liver and combined lung and liver transplantation for cystic fibrosis: analysis of the UNOS database. *Pediatr Transplant* 2011; **15**: 254-264 [PMID: 21219560 DOI: 10.1111/j.1399-3046.2010.01460.x]

51 **Cuevas-Ocaña S**, Laselva O, Avolio J, Nenna R. The era of CFTR modulators: improvements made and remaining challenges. *Breathe (Sheff)* 2020; **16**: 200016 [PMID: 33304402 DOI: 10.1183/20734735.0016-2020]

52 **Davies JC**, Cunningham S, Harris WT, Lapey A, Regelmann WE, Sawicki GS, Southern KW, Robertson S, Green Y, Cooke J, Rosenfeld M; KIWI Study Group. Safety, pharmacokinetics, and pharmacodynamics of ivacaftor in patients aged 2-5 years with cystic fibrosis and a CFTR gating mutation (KIWI): an open-label, single-arm study. *Lancet Respir Med* 2016; **4**: 107-115 [PMID: 26803277 DOI: 10.1016/S2213-2600(15)00545-7]

53 **Keating D**, Marigowda G, Burr L, Daines C, Mall MA, McKone EF, Ramsey BW, Rowe SM, Sass LA, Tullis E, McKee CM, Moskowitz SM, Robertson S, Savage J, Simard C, Van Goor F, Waltz D, Xuan F, Young T, Taylor-Cousar JL; VX16-445-001 Study Group. VX-445-Tezacaftor-Ivacaftor in Patients with Cystic Fibrosis and One or Two Phe508del Alleles. *N Engl J Med* 2018; **379**: 1612-1620 [PMID: 30334692 DOI: 10.1056/NEJMoa1807120]

54 **Ratjen F**, Hug C, Marigowda G, Tian S, Huang X, Stanojevic S, Milla CE, Robinson PD, Waltz D, Davies JC; VX14-809-109 investigator group. Efficacy and safety of lumacaftor and ivacaftor in patients aged 6-11 years with cystic fibrosis homozygous for F508del-CFTR: a randomised, placebo-controlled phase 3 trial. *Lancet Respir Med* 2017; **5**: 557-567 [PMID: 28606620 DOI: 10.1016/S2213-2600(17)30215-1]

55 **Kutney K**, Donnola SB, Flask CA, Gubitosi-Klug R, O'Riordan M, McBennett K, Sferra TJ, Kaminski B. Lumacaftor/ivacaftor therapy is associated with reduced hepatic steatosis in cystic fibrosis patients. *World J Hepatol* 2019; **11**: 761-772 [PMID: 31966908 DOI: 10.4254/wjh.v11.i12.761]

56 **van de Peppel IP**, Doktorova M, Berkers G, de Jonge HR, Houwen RHJ, Verkade HJ, Jonker JW, Bodewes FAJA. IVACAFTOR restores FGF19 regulated bile acid homeostasis in cystic fibrosis patients with an S1251N or a G551D gating mutation. *J Cyst Fibros* 2019; **18**: 286-293 [PMID: 30279125 DOI: 10.1016/j.jcf.2018.09.001]

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ORIGINAL ARTICLE

Case Control Study

Tumor characteristics of hepatocellular carcinoma after direct-acting antiviral treatment for hepatitis C: Comparative analysis with antiviral therapy-naive patients

Magdy Fouad, Mohamed El Kassas, Elham Ahmed, Reem El Sheemy

ORCID number: Magdy Fouad 0000-0001-8065-5581; Mohamed El Kassas 0000-0002-3396-6894; Elham Ahmed 0000-0002-7182-3245; Reem El Sheemy 0000-0001-8158-8878.

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Magdy Fouad, Department of Tropical Medicine and Gastroenterology, Minia University, Minia 61519, Egypt

Mohamed El Kassas, Department of Endemic Medicine, Faculty of Medicine, Helwan University, Cairo 11795, Egypt

Elham Ahmed, Department of Internal Medicine, Minia University, Minia 61519, Egypt

Reem El Sheemy, Department of Tropical Medicine, Minia University, Minia 61111, Egypt

Corresponding author: Mohamed El Kassas, MD, Associate Professor, Department of Endemic Medicine, Faculty of Medicine, Helwan University, Ain Helwan, Cairo 11795, Egypt.
m_elkassas@hq.helwan.edu.eg

Abstract

BACKGROUND

Insufficient and contradictory data are available about the relation between direct-acting antivirals (DAAs) and hepatocellular carcinoma (HCC) development in patients with hepatitis C virus (HCV).

AIM

To analyze differences in basic clinical, radiological, and laboratory characteristics in addition to tumor behavior upon HCC diagnosis between patients with and without a previous history of DAAs exposure.

METHODS

This multicenter case-control study included 497 patients with chronic HCV-related HCC, allocated into one of two groups according to their history of antiviral treatment for their HCV.

RESULTS

Group I included 151 HCC patients with a history of DAAs, while 346 patients who had never been treated with DAAs were assigned to group II. A significant difference was observed between both groups regarding basic assessment scores (Child, MELD, and BCLC), which tended to have more advanced liver disease and HCC stage upon diagnosis in group I. However, serum albumin was

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significantly affected, and serum α-fetoprotein was significantly higher in group II ($P < 0.001$). In addition, group I showed significant HCC multicentricity than group II, while the incidence of portal vein thrombosis was significantly higher in group I ($P < 0.001$).

CONCLUSION
The basic clinical scores and laboratory characteristics of HCC patients are advanced in patients who are naïve to DAAs treatment; however, HCC behavior is more aggressive in DAA-treated patients.

Key Words: Hepatocellular carcinoma; Direct-acting antiviral treatment; Hepatitis C; Tumor behavior; Occurrence

Core Tip: Despite the introduction of newer direct-acting antivirals (DAAs), hepatitis C virus (HCV)-related hepatocellular carcinoma (HCC) will continue to be a significant public health concern in the coming decades. Post-treatment HCV-related HCC has been discovered to be an emerging issue due to unmet needs for early HCC identification and intervention. In addition, we found that aggressive tumors were more common in DAAs exposed patients, which needs to be investigated further in prospective studies with larger cohorts and necessitates proactive screening for HCC in HCV-treated patients *via* public or private pharmacovigilance programs.

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INTRODUCTION

Hepatocellular carcinoma (HCC) is the sixth most common cancer and the third most common cause of cancer-related mortality worldwide[1]. In Egypt, HCC is a significant public health problem responsible for 33.63% and 13.54% of all cancers in males and females, respectively[2]. On the other hand, hepatitis C virus infection (HCV) is considered a leading cause of chronic liver disease in Europe, the United States, and many other countries, including Egypt[3,4]. The risk of HCC development in HCV-related liver cirrhosis is 2% to 8% per year[5]. Multiple studies and meta-analyses demonstrated during the era of interferon (IFN)-based therapy that HCV eradication decreased the risk of hepatocarcinogenesis regardless of fibrosis stage[6,7]. Furthermore, these studies showed that the achievement of sustained virologic response (SVR) after IFN based treatment is directly related to reduced incidence of HCC and increased survival rates[8].

In 2014, the introduction of the more effective direct-acting antivirals (DAAs) for HCV treatment was generally expected to benefit all patients, including those who were not permitted to be treated with IFN-based therapy[7]. However, unexpectedly, the clinical use of DAAs has evoked a significant dilemma about the relationship between DAAs and the development of HCC. Some studies have suggested a direct relation between DAAs and the development of HCC, while others have insisted that DAAs are protective against HCC development[7].

In 2016, the first report in this context showed an unexpectedly high recurrence rate of previously treated HCC after DAAs exposure[6]. This initial report was followed by another retrospective study conducted in Italy which included 344 patients with HCV-related cirrhosis who received different DAA regimens; 91% achieved SVR. The patients were followed for 24 wk. The study revealed a 29% recurrence rate for those with a history of HCC and a 3.16% incidence rate (de novo HCCs) in those without a history of prior HCC irrespective of the used DAA regimen[9].

In addition to HCC recurrence, the different biological behavior of HCC in DAAs exposed patients, and the pattern of recurrence after DAA treatment has also been reported in studies coming from various countries. For example, in 2017, Reig and his colleagues reported more aggressive HCC recurrence after DAA treatment, as defined by an advanced Barcelona Clinic Liver Cancer (BCLC) stage[6]. Moreover, Renzulli *et al*[10] found a more aggressive HCC recurrence pattern with vascular invasion evidence after DAA therapy.

This study aimed to analyze differences in basic clinical, radiological, and laboratory characteristics and tumor behavior upon HCC diagnosis between patients with and without a previous history of DAAs exposure.

MATERIALS AND METHODS

Study design

The current study is a multicenter retrospective case-control study designed to compare the basic demographic, laboratory, and radiological criteria of HCC in patients with a history of DAAs treatment for their chronic HCV infection compared to HCC patients with no previous history of HCV antiviral treatment. Patients were recruited from December 2016 to April 2019 from Minia university hospital and Minia fever hospital, Minia, Egypt. Study patients were assigned to one of 2 groups according to previous DAAs exposure. The first group included 151 HCC patients who were previously treated with DAAs (Group I). According to a standardized treatment protocol, all patients were treated in one of the specialized viral hepatitis treatment centers affiliated to the Egyptian National Committee for Control of Viral Hepatitis. Group II included 346 HCC patients with the first presentation as HCC and no history of antiviral treatment for their HCV infection. Patients with combined HBV or HIV infections and patients with extrahepatic malignancies were excluded from the study.

Methods

All patients were recruited and diagnosed according to EASL guidelines and updated AASLD practice guidelines for managing HCC and BCLC guidelines[11-13]. In addition, baseline demographic, clinical, laboratory, and radiological criteria were studied. The Child-Turcotte Pugh score (CTP), Model for End-stage Liver Disease (MELD) score, BCLC score, and FIB 4 as a non-invasive marker for fibrosis were calculated and presented.

Lines of treatment for HCV have been verified as well as the viral response. In addition, all baseline characteristics, laboratory, radiological and medical scores were compared between the two groups.

The study was performed according to the ethical guidelines of the 1975 Declaration of Helsinki after approval from the Research Ethics Committee for human subject research at the Faculty of Medicine, Minia University (Serial: 165: 2/2019) on Feb 25, 2019. In addition, written informed consent was obtained from all participants before enrolment in the study.

Statistical analysis

Statistical analyses were performed using IBM SPSS advanced statistics, version 26 (SPSS Inc., Chicago, Illinois, USA. Numerical data were presented as mean ± SD and median (range), whereas categorical data were presented as number (percent). The Mann-Whitney U-test and the χ^2-test are used when appropriate. Statistical significance is considered if P value is less than or equal to 0.05.

RESULTS

This study included 497 patients with chronic HCV-related HCC, allocated into one of two groups according to their history of antiviral treatment for their HCV. Group I included 151 patients with chronic HCV and HCC who were previously treated with DAAs. Group II included 346 patients representing all patients recruited in the same period with HCV-related HCC and age and sex-matched with group I. Most of the studied patients in both groups were males: (76.2%) and (72.0%) (P value 0.33), with a mean age of 60.2 years and 59.8 years in groups I and II, respectively (P value 0.70) (Table 1). Regarding the received DAAs regimen in group I patients, 44.4% of patients

Table 1 Basic demographic data and underlying liver status in both groups

	Group I	Group II	*P* value
	HCC with previous DAAs (*n* = 151)	HCC without previous DAAS (*n* = 346)	
Age (mean ± SD)	60.17 ± 7.75	59.84 ± 9.12	0.70
Gender			0.33
Female	36 (23.8)	97 (28.0)	
Male	115 (76.2)	249 (72.0)	
Residence			0.28
Rural	131 (86.8)	287 (82.9)	
Urban	20 (13.2)	59 (17.1)	
BCLC			< 0.001[a]
0	5 (3.3)	15 (4.3)	
A	47 (31.1)	134 (38.7)	
B	17 (11.3)	68 (19.7)	
C	49 (32.5)	50 (14.5)	
D	33 (21.9)	79 (22.8)	
MELD (mean ± SD)	14.35 ± 5.041	36.10 ± 30.22	< 0.001[a]
CTP score			0.04
A	50 (33.1)	88 (25.4)	
B	65(43.0)	138 (39.9)	
C	36 (23.8)	120 (34.7)	
FIB4			< 0.001[c]
mean ± SD	3.25 ± 9.87	7.11 ± 7.68	
Median	0.023	4.49	
IQR	4.51	6.2	
HCC detection time after stop of DAAS	Range: 1-72 moMedian: 8 mo	-	-

[a]*P* < 0.05.
[c]*P* < 0.001.
HCC: Hepatocellular carcinoma; MELD: Model for end stage liver disease; CTP: Child Turcotte-pough; BCLC: Barcelona cancer liver clinic.

received sofosbuvir/daclatasvir (SOF/DAC), 40.1% received SOF/DAC/RBV, 13.2% received SOF/RBV, and only 2% received SOF/RBV/PEG IFN. Figure 1 shows patients' distribution among various treatment regimens and treatment duration, in addition to treatment viral response.

Notably, significant differences were observed between the two groups regarding the case assessment scores that reflect the severity of the underlying liver condition upon HCC discovery. A total of 34.7% of patients in group II were CTP class C, and only 23.8% of group I patients were class C. Mean MELD score in group I was 14, while the mean MELD in group II was 36 (*P* value < 0.001). Moreover, a significant difference was observed in the BCLC score (*P* value < 0.001). A significant difference was encountered in FIB4 as a method for non-invasive fibrosis assessment with a mean FIB4 of 3.25 in group I, compared to 7.11 in group II (*P* value < 0.001). Basic demographic data and underlying liver status in both groups are detailed in Table 1. The time between stopping DAAs and the development of HCC ranged from 1 to 72 mo with a median of 8 mo.

When comparing both groups' clinical data, no significant differences were observed except in the current smoking status, which was significantly increased in group I compared to the other group (*P* value 0.005). On the other hand, a significant history of blood transfusion was observed in patients with no previous history of DAAs (*P* value 0.01); cellular decompensation in the form of hepatic encephalopathy is

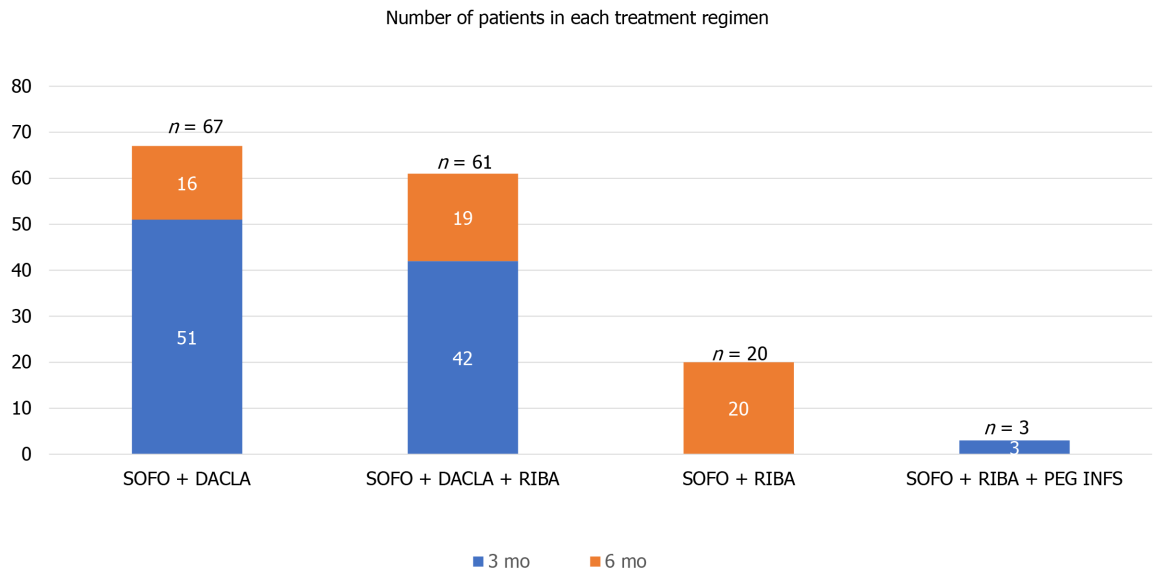

Figure 1 Patients in group I distribution among different treatment regimen. SOFO: Sofosbuvir; DACLA: Daclatasvir; RIBA: Ribavirin; PEG INFS: Pegylated interferon.

significantly observed in patients with no previous history of DAAs (*P* value 0.01). Detailed clinical data of the two studied groups are well presented in Table 2.

Comparing laboratory data in both groups, hemoglobin level and total leukocytic count were significantly different (*P* values are 0.02 and 0.004, respectively). Median ALT in group I was 77 IU in comparison to 54 IU in group II (*P* value 0.001). Mean albumin in groups I and II was (3.3 and 2.9 respectively) (*P* value 0.004), and mean urea in groups I and II was (40 and 54 respectively) (*P* value 0.04). Median AFP in group I was 184 in comparison to 60 in group II (*P* value < 0.001). An illustrated comparison of all laboratory data is presented in Table 3.

Regarding the radiological characters of HCC in both groups, HCC in group I patients was more multifocal (53%) in comparison to (25%) in group II (*P* value < 0.001). Moreover, HCCs in group I patients tended to present with a bigger tumor size at the initial presentation than group II patients. More precisely, less than 1% of group I patients were presented with tumors less than 2 cm, while more than 15% of group II patients presented with tumors less than 2 cm (*P* value < 0.001), indicating more aggressive tumor behavior associated with the previous history of DAAs. The right lobe was the dominant victim in both groups. Early vascular invasion was significantly higher in group I compared to group II as evidenced radiologically by portal vein thrombosis (PVT), which present in 45% of group I patients and only 21% of group II patients (*P* value < 0.001), all radiological data for HCCs in the studied patients are detailed in Table 4.

DISCUSSION

Chronic HCV infection is a significant risk factor for developing liver cirrhosis in approximately 20%-30% of patients with subsequent increased risk for HCC development in those patients with an estimated annual incidence of 3.5%[14]. This risk is shown to be lower in patients with chronic HCV infection without cirrhosis and in patients who succeeded in achieving eradication, as proved by their SVR[15]. Despite the notable decrease in the overall incidence of HCV infection, its prevalence in HCC patients is still high[16]. Surprisingly, HCC development's risk is continuous in HCV-induced liver cirrhosis even after viral eradication and SVR achievement[16]. During the interferon-based treatment era, successful viral eradication decreases the risk for HCC and improvement in the fibrosis stage[9].

The emergence of DAAs with their extended patient spectrum, improved efficacy, and safety profile increased our expectations regarding a decrease in HCC occurrence and recurrence. However, unpleasant data from new studies showed that DAAs might encourage tumor occurrence in patients with cirrhosis or recurrence in patients with previously treated HCC[9,17]. The same was reported in some studies regarding HCC

Table 2 Clinical presentation in both groups

	Group I	Group II	*P* value
	HCC with previous DAAs (*n* = 151)	HCC without previous DAAS (*n* = 346)	
Hypertension	56 (37.1)	135 (39.0)	0.68
DM	56 (37.1)	113 (32.7)	0.33
Smoking	73 (48.3)	121 (35.0)	0.005[a]
Surgical operations	32 (21.2)	101 (29.2)	0.06
Blood transfusion	23 (15.2)	87 (25.1)	0.01[a]
Jaundice	60 (39.7)	154 (44.5)	0.32
Ascites	90 (59.6)	197 (56.9)	0.58
LL edema	48 (31.8)	143 (41.3)	0.07
Hepatic encephalopathy	14 (9.3)	61 (17.6)	0.01[a]

[a]$P < 0.05$.
HCC: Hepatocellular carcinoma; DM: Diabetes mellitus; LL: Lower limb.

Table 3 Comparison of laboratory data in both groups

	Group I	Group II	*P* value
	HCC with previous DAAs (*n* = 151)	HCC without previous DAAS (*n* = 346)	
HB (mean ± SD)	10.41 ± 1.88	10.78 ± 1.99	0.02[a]
TLC (mean ± SD)	6.55 ± 6.20	7.74 ± 8.60	0.004[b]
PLATELETS (mean ± SD)	147.28 ± 79.76	135.95 ± 61.17	0.22
TBIL (median)	3.07	2.5	0.93
DBIL (median)	0.7	0.9	0.84
ALB (mean ± SD)	3.32 ± 1.47	2.98 ± 0.85	0.004[b]
INR	1.31 ± 0.35	1.44 ± 0.47	0.4
ALT (median)	77	54	0.001[c]
AST (median)	76	70	0.62
CREAT (mean ± SD)	1.21 ± 0.45	1.43 ± 3.67	0.15
UREA (mean ± SD)	40.81 ± 16.01	54.93 ± 46.17	0.04[a]
AFP (median)	184.0	60.0	< 0.001[c]

[a]$P < 0.05$.
[b]$P < 0.01$.
[c]$P < 0.001$.
HCC: Hepatocellular carcinoma; CBC: Complete blood picture; TLC: Total leucocytic count; TBIL: Total bilirubin; DBIL: Direct bilirubin; ALB: Albumin, ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; AFP: Alfa feto protein.

recurrence after initial management upon treatment with DAAs[18].

This study stands at the current dilemma between DAAs' benefits and drawbacks; studying the basic characteristics of HCC patients previously treated with DAAs and comparing them with HCC patients never treated with DAAs provides the central part of this controversy.

In our study, significant differences were found in the CTP, MELD, and BCLC scores in HCC patients without DAAs and those who received DAAs; these findings are contrary to what proved by Abdelaziz *et al*[19], who found matching between patients with HCC and previous DAAs and HCC without DAAs regarding CTP score. In accordance with our results, a large study from Pakistan reported a raised neutrophil to lymphocyte ratio and younger patient age with more aggressive tumor

Table 4 Radiological characters of hepatocellular carcinoma in both groups

	Group I	Group II	*P* value[c]
	HCC with previous DAAs (*n* = 151)	HCC without previous DAAS (*n* = 346)	
Number			< 0.001
Single	71 (47.0)	259 (74.9)	
Multiple	80 (53.0)	87 (25.1)	
Size			< 0.001
Less than 2 cm	1 (0.7)	54 (15.6)	
2.5 cm	91(60.3)	177 (51.2)	
Greater than 5 cm	59 (39.1)	115 (33.2)	
Site			0.001
Bilobar	21 (13.9)	29 (8.4)	
Lt lobe	24 (15.9)	23 (6.6)	
RT lobe	106 (70.2)	294 (84.9)	
PVT	68 (45.0)	75 (21.7)	< 0.001
Splenomegaly			< 0.001
Average	38 (25.2)	98 (28.3)	
Mild	113 (74.8)	214 (61.8)	
Moderate	0 (0.0)	34 (9.8)	

[c]*P* < 0.001.

HCC: Hepatocellular carcinoma; DAAs: Directly acting antiviral agents; PVT: Portal vein thrombosis.

behavior in HCV-treated HCC patients[20].

The pattern of HCC invasion either locally inside the liver manifested by multiplicity and larger size or vascularly manifested by PVT is significantly increased with the previous history of DAAs, suggesting a possible DAAs role in such aggressive behavior. In accordance with the current study, Reig *et al*[6] stated the increased aggressiveness of HCC, but in recurrent cases, he omitted de novo HCC in his study. Also, Renzulli *et al*[10] noticed a faster rate of development of HCC after DAA therapy with an aggressive course of microvascular invasion. Similarly, Faillaci *et al*[21] proved that DAAs are associated with increased aggressiveness and tumor recurrence growth. Another study done by Romano *et al*[21] demonstrated an aggressive behavior of tumors after DAA in the form of a higher number of nodules and extrahepatic metastases, suggesting that such patients' tumor growth is faster than usual. Many theories have been proposed to explain this unexpected event; some researchers have related the development of HCC to baseline risk factors such as advanced fibrosis grade, HBV co-infection, or age[7]. Another theory proposes that DAAs cause immune surveillance mechanisms to become dysregulated due to the rapid viral clearance, and this behavior has been confirmed by several investigations [16,19]. With the downregulation of type II and III IFNs, their receptors, and IFN-stimulated genes, this dysregulation may result in the re-establishment of innate immunity. Due to the anti-angiogenic and anti-proliferative capabilities of IFN, which DAAs lack, a reduction in IFN activation may promote the proliferation of malignant cells. Furthermore, after HCV eradication, one of the immune system alterations observed is a decrease in the number of cytotoxic activity of natural killer cells in the liver, favoring a faster progression of HCC foci[7,22].

A significant difference was observed in AFP levels between the two groups, explained mainly by the invasive pattern and prominent vascular invasion in group I, and this is in agreement with Abdelaziz *et al*[20].

The strengths of our study include its design and the large number of included subjects. Limitations include the exclusive existence of genotype four patients because of its prevalence in Egypt and lack of tight evaluation of other risk factors for HCC, like non-alcoholic fatty liver disease and aflatoxin effect, and the lack of further longitudinal follow up of the studied cohort.

CONCLUSION

In conclusion, despite the introduction of newer DAAs, HCV-related HCC will continue to be a significant public health concern in the coming period. Post-treatment HCV-related HCC has been discovered to be an emerging issue due to unmet needs for early HCC identification and intervention. In this study, more aggressive tumor behavior was encountered in DAAs exposed patients. Such finding needs to be investigated further in prospective studies with larger cohorts and more longitudinal follow-up for comparing survival and necessitates proactive screening for HCC in HCV-treated patients *via* public or private pharmacovigilance programs. Furthermore, anti-HCV therapy in HCC patients should be postponed until a consistent risk-benefit ratio is established through further research.

ARTICLE HIGHLIGHTS

Research background

The evidence on the link between direct-acting antivirals (DAAs) and the development of hepatocellular carcinoma (HCC) in hepatitis C virus (HCV) patients is insufficient and conflicting.

Research motivation

Due to unmet needs for early HCC detection and care, post-treatment HCV-related HCC is an increasing concern.

Research objectives

To compare fundamental clinical, radiographic, and laboratory features and tumor behavior in individuals with and without a history of DAAs exposure after HCC diagnosis.

Research methods

A multicenter case-control study including 497 patients with chronic HCV-related HCC, allocated into one of two groups according to their history of antiviral treatment for their HCV.

Research results

Group I consisted of 151 HCC patients who had previously been treated with DAAs, while group II included 346 patients who had never been treated with DAAs. Regarding basic assessment scores (Child, MELD, and BCLC), there was a substantial difference between the two groups, with group I showing a tendency for more advanced liver disease and HCC stage at diagnosis. However, serum albumin levels were considerably lower in group II, and serum-fetoprotein levels were significantly greater (P = 0.001). In addition, HCC multicentricity was substantially higher in group I than in group II, and the rate of portal vein thrombosis was significantly higher in group I (P = 0.001).

Research conclusions

HCC patients who are naïve to DAAs have more advanced clinical scores and laboratory features than those who have never been treated with DAAs; yet, HCC behavior is more aggressive in DAA-treated patients.

Research perspectives

The findings of this study warrant additional investigation in prospective trials with larger cohorts and longer follow-up for comparing survival and proactive screening for HCC in HCV-treated patients through public or private pharmacovigilance programs.

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REFERENCES

1 **Tabrizian P**, Roayaie S, Schwartz ME. Current management of hepatocellular carcinoma. *World J Gastroenterol* 2014; **20**: 10223-10237 [PMID: 25132740 DOI: 10.3748/wjg.v20.i30.10223]

2 **Holah NS**, El-Azab DS, Aiad HAE, Sweed DMM. The Diagnostic Role of SPINK1 in Differentiating Hepatocellular Carcinoma From Nonmalignant Lesions. *Appl Immunohistochem Mol Morphol* 2017; **25**: 703-711 [PMID: 27028242 DOI: 10.1097/PAI.0000000000000363]

3 **Messina JP**, Humphreys I, Flaxman A, Brown A, Cooke GS, Pybus OG, Barnes E. Global distribution and prevalence of hepatitis C virus genotypes. *Hepatology* 2015; **61**: 77-87 [PMID: 25069599 DOI: 10.1002/hep.27259]

4 **Kandeel A**, Genedy M, El-Refai S, Funk AL, Fontanet A, Talaat M. The prevalence of hepatitis C virus infection in Egypt 2015: implications for future policy on prevention and treatment. *Liver Int* 2017; **37**: 45-53 [PMID: 27275625 DOI: 10.1111/liv.13186]

5 **Ioannou GN**, Splan MF, Weiss NS, McDonald GB, Beretta L, Lee SP. Incidence and predictors of hepatocellular carcinoma in patients with cirrhosis. *Clin Gastroenterol Hepatol* 2007; **5**: 938-945, 945.e1 [PMID: 17509946 DOI: 10.1016/j.cgh.2007.02.039]

6 **Reig M**, Mariño Z, Perelló C, Iñarrairaegui M, Ribeiro A, Lens S, Díaz A, Vilana R, Darnell A, Varela M, Sangro B, Calleja JL, Forns X, Bruix J. Unexpected high rate of early tumor recurrence in patients with HCV-related HCC undergoing interferon-free therapy. *J Hepatol* 2016; **65**: 719-726 [PMID: 27084592 DOI: 10.1016/j.jhep.2016.04.008]

7 **El Kassas M**, Elbaz T, Salaheldin M, Abdelsalam L, Kaseb A, Esmat G. Impact of treating chronic hepatitis C infection with direct-acting antivirals on the risk of hepatocellular carcinoma: The debate continues - A mini-review. *J Adv Res* 2019; **17**: 43-48 [PMID: 31193326 DOI: 10.1016/j.jare.2019.03.001]

8 **Tanimoto Y**, Tashiro H, Aikata H, Amano H, Oshita A, Kobayashi T, Kuroda S, Tazawa H, Takahashi S, Itamoto T, Chayama K, Ohdan H. Impact of pegylated interferon therapy on outcomes of patients with hepatitis C virus-related hepatocellular carcinoma after curative hepatic resection. *Ann Surg Oncol* 2012; **19**: 418-425 [PMID: 21710324 DOI: 10.1245/s10434-011-1866-1]

9 **Conti F**, Buonfiglioli F, Scuteri A, Crespi C, Bolondi L, Caraceni P, Foschi FG, Lenzi M, Mazzella G, Verucchi G, Andreone P, Brillanti S. Early occurrence and recurrence of hepatocellular carcinoma in HCV-related cirrhosis treated with direct-acting antivirals. *J Hepatol* 2016; **65**: 727-733 [PMID: 27349488 DOI: 10.1016/j.jhep.2016.06.015]

10 **Renzulli M**, Buonfiglioli F, Conti F, Brocchi S, Serio I, Foschi FG, Caraceni P, Mazzella G, Verucchi G, Golfieri R, Andreone P, Brillanti S. Imaging features of microvascular invasion in hepatocellular carcinoma developed after direct-acting antiviral therapy in HCV-related cirrhosis. *Eur Radiol* 2018; **28**: 506-513 [PMID: 28894901 DOI: 10.1007/s00330-017-5033-3]

11 **Bruix J**, Sherman M, Llovet JM, Beaugrand M, Lencioni R, Burroughs AK, Christensen E, Pagliaro L, Colombo M, Rodés J; EASL Panel of Experts on HCC. Clinical management of hepatocellular carcinoma. Conclusions of the Barcelona-2000 EASL conference. European Association for the Study of the Liver. *J Hepatol* 2001; **35**: 421-430 [PMID: 11592607 DOI: 10.1016/s0168-8278(01)00130-1]

12 **Bruix J**, Sherman M; American Association for the Study of Liver Diseases. Management of hepatocellular carcinoma: an update. *Hepatology* 2011; **53**: 1020-1022 [PMID: 21374666 DOI: 10.1002/hep.24199.]

13 **Llovet JM**, Bustamante J, Castells A, Vilana R, Ayuso Mdel C, Sala M, Brú C, Rodés J, Bruix J. Natural history of untreated nonsurgical hepatocellular carcinoma: rationale for the design and evaluation of therapeutic trials. *Hepatology* 1999; **29**: 62-67 [PMID: 9862851 DOI: 10.1002/hep.510290145]

14 **Lok AS**, Seeff LB, Morgan TR, di Bisceglie AM, Sterling RK, Curto TM, Everson GT, Lindsay KL, Lee WM, Bonkovsky HL, Dienstag JL, Ghany MG, Morishima C, Goodman ZD; HALT-C Trial Group. Incidence of hepatocellular carcinoma and associated risk factors in hepatitis C-related advanced liver disease. *Gastroenterology* 2009; **136**: 138-148 [PMID: 18848939 DOI: 10.1053/j.gastro.2008.09.014.]

15 **Swain MG**, Lai MY, Shiffman ML, Cooksley WG, Zeuzem S, Dieterich DT, Abergel A, Pessôa MG, Lin A, Tietz A, Connell EV, Diago M. A sustained virologic response is durable in patients with chronic hepatitis C treated with peginterferon alfa-2a and ribavirin. *Gastroenterology* 2010; **139**: 1593-1601 [PMID: 20637202 DOI: 10.1053/j.gastro.2010.07.009]

16 **Robert S B Jr**. The Possible Association Between DAA Treatment for HCV Infection and HCC Recurrence. *Gastroenterol Hepatol (N Y)* 2016; **12**: 776-779 [PMID: 28035203]

17 **Tayyab GUN**, Rasool S, Nasir B, Rubi G, Abou-Samra AB, Butt AA. Hepatocellular carcinoma occurs frequently and early after treatment in HCV genotype 3 infected persons treated with DAA regimens. *BMC Gastroenterol* 2020; **20**: 93 [PMID: 32252635 DOI: 10.1186/s12876-020-01249-4]

18 **Sapena V**, Enea M, Torres F, Celsa C, Rios J, Rizzo GEM, Nahon P, Mariño Z, Tateishi R, Minami T, Sangiovanni A, Forns X, Toyoda H, Brillanti S, Conti F, Degasperi E, Yu ML, Tsai PC, Jean K, El Kassas M, Shousha HI, Omar A, Zavaglia C, Nagata H, Nakagawa M, Asahina Y, Singal AG, Murphy C, Kohla M, Masetti C, Dufour JF, Merchante N, Cavalletto L, Chemello LL, Pol S, Crespo J, Calleja JL, Villani R, Serviddio G, Zanetto A, Shalaby S, Russo FP, Bielen R, Trevisani F, Cammà C, Bruix J, Cabibbo G, Reig M. Hepatocellular carcinoma recurrence after direct-acting antiviral therapy: an individual patient data meta-analysis. *Gut* 2021 [DOI: 10.1136/gutjnl-2020-323663]

19 **Abdelaziz AO**, Nabil MM, Abdelmaksoud AH, Shousha HI, Hashem MB, Hassan EM, Salah A,

Omran DA, Elbaz TM. Tumor behavior of hepatocellular carcinoma after hepatitis C treatment by direct-acting antivirals: comparative analysis with non-direct-acting antivirals-treated patients. *Eur J Gastroenterol Hepatol* 2019; **31**: 75-79 [PMID: 30199473 DOI: 10.1097/MEG.0000000000001264]

20 **Khalid J**, Umar M, Ur-Rehman T, Ali M, Khan GM. Tumor aggression among hepatitis-C related hepatocellular carcinoma patients: an observational study regarding the impact of anti-HCV therapy. *Infect Agent Cancer* 2020; **15**: 35 [PMID: 32508980 DOI: 10.1186/s13027-020-00300-z]

21 **Faillaci F**, Marzi L, Critelli R, Milosa F, Schepis F, Turola E, Andreani S, Vandelli G, Bernabucci V, Lei B, D'Ambrosio F, Bristot L, Cavalletto L, Chemello L, Sighinolfi P, Manni P, Maiorana A, Caporali C, Bianchini M, Marsico M, Turco L, de Maria N, Del Buono M, Todesca P, di Lena L, Romagnoli D, Magistri P, di Benedetto F, Bruno S, Taliani G, Giannelli G, Martinez-Chantar ML, Villa E. Liver Angiopoietin-2 Is a Key Predictor of De Novo or Recurrent Hepatocellular Cancer After Hepatitis C Virus Direct-Acting Antivirals. *Hepatology* 2018; **68**: 1010-1024 [PMID: 29604220 DOI: 10.1002/hep.29911]

22 **Innes H**, Barclay ST, Hayes PC, Fraser A, Dillon JF, Stanley A, Bathgate A, McDonald SA, Goldberg D, Valerio H, Fox R, Kennedy N, Bramley P, Hutchinson SJ. The risk of hepatocellular carcinoma in cirrhotic patients with hepatitis C and sustained viral response: Role of the treatment regimen. *J Hepatol* 2018; **68**: 646-654 [PMID: 29155019 DOI: 10.1016/j.jhep.2017.10.033]

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ORIGINAL ARTICLE

Case Control Study

Circulating microRNA 9-3p and serum endocan as potential biomarkers for hepatitis C virus-related hepatocellular carcinoma

Amany Mohamed Salah Eldin Wahb, Mohamed El Kassas, Ahmed Kamal Khamis, Mostafa Elhelbawy, Nesreen Elhelbawy, Mona Salah Eldin Habieb

ORCID number: Amany Mohamed Salah Eldin Wahb 0000-0001-7063-4285; Mohamed El Kassas 0000-0002-3396-6894; Ahmed Kamal Khamis 0000-0002-9326-662X; Mostafa Elhelbawy 0000-0002-6945-6205; Nesreen Elhelbawy 0000-0002-9231-0342; Mona Salah Eldin Habieb 0000-0002-4433-0584.

Author contributions: Wahb A designed the research; Wahb A and Habieb M performed the laboratory work, performed the RT-qPCR work and drafted the manuscript; El Kassas M, Khamis A and Elhelbawy M performed the case selection and the clinical and radiological evaluations; Habieb M supplied reagents; Elhelbawy N performed the data analysis, biological parameter assessment and performed the RT-qPCR work; all authors contributed equally to the manuscript drafting and revision; All authors approved the final version of the manuscript.

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Amany Mohamed Salah Eldin Wahb, Department of Medical Biochemistry and Molecular Biology, Faculty of Medicine, Helwan University, Cairo 11795, Egypt

Mohamed El Kassas, Department of Endemic Medicine, Faculty of Medicine, Helwan University, Cairo 11795, Egypt

Ahmed Kamal Khamis, Mostafa Elhelbawy, Department of Hepatology and Gastroenterology, National Liver Institute, Menoufia University, Shebin Elkom 32512, Menoufia, Egypt

Nesreen Elhelbawy, Mona Salah Eldin Habieb, Department of Medical Biochemistry and Molecular Biology, Faculty of Medicine, Menoufia University, Shebin Elkoum 3511, Egypt

Corresponding author: Mohamed El Kassas, MD, Associate Professor, Department of Endemic Medicine, Faculty of Medicine, Helwan University, Ain Helwan, Cairo 11795, Egypt.
m_elkassas@hq.helwan.edu.eg

Abstract

BACKGROUND

The high mortality rate of hepatocellular carcinoma (HCC) in Egypt is due mainly to the increasing prevalence of hepatitis C virus infection (HCV) and late diagnosis of the carcinoma. MicroRNAs (miRNA), which regulate tumor proliferation and metastasis in HCC, may serve as a useful diagnostic approach for the early detection of HCC, thus decreasing its mortality. Meanwhile, endocan is a protein with angiogenic and inflammatory properties that are associated with tumor progression and poor outcomes.

AIM

To analyze the levels of miRNA 9-3p and endocan in HCV-infected HCC patients and correlate them with clinicopathological parameters.

METHODS

We compared levels of endocan and circulating miRNA 9-3p from 35 HCV-related HCC patients to 33 patients with HCV-induced chronic liver disease and 32 age and gender matched healthy controls recruited from inpatient and outpatient clinics of the National Liver Institute, Menoufia University, Egypt in the period from January to March 2021 in a case-control study. Serum samples from all groups were analyzed for HCV. Endocan was measured by enzyme-

study.

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linked immunosorbent assays, and the expression levels of circulating miRNA 9-3p were measured by real-time quantitative reverse transcriptase PCR.

RESULTS
The levels of circulating miRNA 9-3p were significantly lower in the HCC group compared to the chronic liver disease ($P < 0.001$) and control ($P < 0.001$) groups, while levels in the chronic liver disease were significantly lower than those in the control group ($P < 0.001$). The levels of serum endocan were significantly higher in the HCC group compared to the chronic liver disease ($P < 0.001$) and control ($P < 0.001$) groups. Moreover miRNA 9-3p and endocan performed better than α-fetoprotein in discriminating HCC patients from cirrhosis and healthy patients. The levels of miRNA 9-3p were significantly inversely correlated to vascular invasion ($P = 0.002$), stage of advancement of Barcelona Clinical Liver Cancer ($P < 0.001$) and the metastatic site ($P < 0.001$) of the HCC group.

CONCLUSION
Circulating miRNA 9-3p and endocan can be used as novel biomarkers for the early diagnosis of HCV-related HCC.

Key Words: MicroRNA 9-3p; Hepatocellular carcinoma; Endocan; Diagnostic; Biomarker; Egypt

Core Tip: The level of circulating microRNA 9-3p was significantly decreased in hepatocellular carcinoma (HCC) patients than in chronic liver disease and control groups. The level of serum endocan was significantly increased in HCC patients than in the cirrhotic and control groups, and there was better diagnostic performance of microRNA 9-3p and endocan than α-fetoprotein. The levels of microRNA 9-3p have a significant inverse correlation with endocan and vascular invasion and advanced stage of Barcelona Clinical Liver Cancer in the HCC group. Circulating microRNA 9-3p and endocan could be novel biomarkers for early diagnosis of hepatitis C virus-related HCC patients.

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INTRODUCTION

Worldwide, hepatocellular carcinoma (HCC) is the sixth most prevalent cancer and the third leading cause of cancer deaths[1]. In Egypt, HCC is a significant health problem, as it is the most prevalent and second most prevalent cancer in males and females, respectively. It is the most prevalent malignancy in general, accounting for 32.35% of the total cancer deaths[2,3]. One reason for these high prevalence rates is the high prevalence of hepatitis C in Egypt[4].

Moreover, HCC has been attributed to molecular aberrations, such as errors in regulation of gene expression, which may result in translational repression and/or degradation[5,6]. To improve the overall survival from HCC, extensive research is needed, focusing particularly on more accurate and monitored management of the disease[7].

MicroRNAs (miRNAs) are small (approximately 22 nucleotides long), endogenous, non-protein coding RNAs that are key post-transcriptional regulators of gene expression[8]. miRNAs regulate different cellular pathways, including the cell cycle, cell proliferation and apoptosis. Dysregulation of miRNAs can therefore impact cellular processes involved in tumorigenesis and cancer. Thus, serum miRNAs may serve as non-invasive biomarkers for the diagnosis of cancer[9].

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Three genes encode miRNA 9-3p: *MIR9-1*, *MIR9-2* and *MIR9-3*, located on chromosomes 1 (1q22), 5 (5q14.3) and 15 (15q26.1), respectively[10]. miRNA 9-3p is expressed abnormally in various types of human cancer, suggesting that it is functionally versatile[11]. miRNA 9-3p has been identified to have a tumor suppressive role by targeting an oncogenic tafazzin expression in HCC cells[12].

Endocan is a 50 kDa soluble proteoglycan that circulates freely in the bloodstream of healthy individuals and is expressed by the vascular endothelium. It has angiogenic and inflammatory properties that may affect vascular permeability, thus it plays crucial roles in regulating major physiological and pathophysiological processes, such as cell adhesion, inflammation and tumor progression[13]. Endocan expression is upregulated in cancer cells derived from the lung, kidney, brain, astrocytes and liver [14].

A single study has reported that miRNA 9-3p expression decreases in bladder cancer patients, resulting in reduced inhibition of endocan, thus increasing its expression, which promotes cell proliferation[15]. These results indicate that the gene encoding endocan is a target of miRNA 9-3p. To the best of our knowledge, simultaneous assessment of the roles of both miRNA 9-3p and inflammatory role of endocan that may induce tumorigenesis and tumor progression have not been evaluated in HCV-related HCC, which is triggered by the viral inflammation. Thus, the current work aimed to study the diagnostic value of circulating levels of miRNA 9-3p and endocan in HCV-related HCC patients and to correlate them with clinicopathological parameters.

MATERIALS AND METHODS

Study subjects

This case-control study included a total of 100 subjects recruited from inpatient and outpatient clinics of the National Liver Institute, Menoufia University, Egypt in the period from January to March 2021. Participants were categorized into three groups: Group I: 35 patients with HCV-related HCC; Group II: 33 patients with chronic liver disease due to chronic HCV; and Group III: 32 healthy and free of viral infection volunteers of matched age and gender. Patients were selected based on restrictive inclusion criteria including patients whose age was more than 18 years with confirmed HCV infection by both HCV antibody (anti-HCV) detection and positive HCV RNA. In Group I, HCC was diagnosed (triphasic spiral computed tomography or dynamic magnetic resonance imaging together with elevated α-fetoprotein and/or liver biopsy), and its stage was identified according to the Barcelona Clinical Liver Cancer (BCLC) system[16]. In Group II, chronic liver disease was diagnosed based on history, clinical examination, laboratory results and imaging that included abdominal ultrasonography and computed tomography. Liver disease severity was assessed by the Child-Pugh score. Patients with positive hepatitis B surface antigen and/or hepatitis B c antibody, secondary liver cancer, other malignancies, chronic hepatitis or cirrhosis due to any cause other than HCV infection, significant associated comorbidities (such as renal failure or heart failure) and those receiving chemotherapy, radiotherapy or on immunosuppression medication were excluded. Detailed histories of all participants were taken, and they all underwent physical examination, liver imaging (abdominal ultrasound) and routine laboratory tests that included complete blood counts, kidney and liver function tests [albumin, total bilirubin, direct bilirubin, alanine aminotransferase (ALT) and aspartate aminotransferase (AST)], serum levels of α-fetoprotein, and serological tests for hepatitis B virus and HCV. Serum samples from Groups I and II were analyzed for HCV by reverse transcriptase PCR (RT-PCR).

Laboratory procedures

Seven milliliters of venous blood were withdrawn by venipuncture; of this, 5 mL were transferred into a plain tube, left to clot and centrifuged for 10 min at 4000 rpm. The serum obtained was stored at -80 °C until subsequent analyses for serum α-fetoprotein levels, liver function tests, hepatitis viral markers and endocan levels. The remaining 2 mL of blood were placed into an EDTA containing tube for HCV RT-PCR and miRNA 9-3p expression analysis. Anti-HCV levels were determined by electrochemiluminescence immunoassay using the Cobas immunoassay analyzer.

The hepatitis B surface antigen in serum was determined using (Sorin Biomedica Co. kits, Italy). Serum levels of ALT and AST were determined by the kinetic UV optimized method of the IFCC (ELTEC Kit, England). Serum levels of total bilirubin were measured using the DIAMOND diagnostics Kit, Germany. Serum albumin levels

were quantified using a colorimetric method of enhanced specificity of bromocresol green (DIAMOND diagnostics Kit, Germany). Prothrombin time was determined by the STA-Stago Compact computed tomography autoanalyzer. Serum α-fetoprotein levels were measured by enzyme-linked immunosorbent assays using the IMMULITE 1000 system (Siemens Medical Solutions Diagnostics, United States).

Endocan detection

Serum endocan levels were measured by enzyme-linked immunosorbent assay using the PicokineTM ELISA Kit for human ESMI/Endocan (Boster Biological Technology Co., Ltd., CA, United States, cat# EK0752).

RT-PCR for HCV

Nucleic acids were extracted using the Qiagen viral RNA Mini Extraction Kit.

Expression assay for miRNA 9-3p

miRNA was isolated from plasma using the Qiagen™ RNA extraction Kit MiRNeasy Kit (QIAGEN). miRNA was purified and then its concentration and purity were quantified using a NanoDrop® N50 nanophotometer (Implant GmbH and Implen, Inc. Schatzbogen 52 81829 München, Germany). Purified miRNA was stored at -80 °C until reverse transcription, which was accomplished using the Qiagen®miScript II RT Kit (QIAGEN) following the manufacturer's instructions. Each 20-µl reaction tube contained 4 µL 5 × miScript HiSpec Buffer, 2 µL 10 × miScript Nuclease Mix, 2 µL RNase-free water, 2 µL miScript Reverse Transcriptase Mix, and 10 µL template RNA. Reverse transcription was carried out at 37 °C for 60 min and 95 °C for 5 min on an Applied Biosystems 2720 thermal cycler (Bioline, Singapore, United States). The cDNA product was diluted to 5 ng/ul before determining the transcript levels by real-time quantitative PCR. Real-time quantitative PCR was performed using the miScript SYBR Green PCR Kit (QIAGEN) according to the manufacturer's instructions. The reaction mixture contained 12.5 µL 2x QuantiTect SYBR Green PCR Master Mix, 2.5 µL 10x miScript Universal Primer based on mRNA sequences obtained from the miRBase database for miRNA 9-3p, 2.5 µL template cDNA and 3.5 µL RNase-free water. The Applied Biosystems®7500 real-time thermal cycler (Applied Biosystems, Foster City, CA, United States) was programmed to run 40 cycles of the following steps: 95 °C for 15 min (initial denaturation step), denaturation at 94 °C for 15 s, annealing for 30 s at 55 °C and extension for 30 s at 70 °C. U6 snRNA was used as an endogenous control. Relative quantification expression levels were calculated using the comparative $2^{-\Delta\Delta Ct}$ method with Applied Biosystems 7500 software version 2.0.1. Each run was completed using melting curve analysis to confirm the specificity of the amplification and absence of primer dimers.

All procedures involving human participants were performed according to the ethical standards of the institutional and/or national research committee and the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The study was approved by the Menoufia University Faculty of Medicine Research Ethics Committee. Every patient in this research provided their written consent to participate in the research, provided that they were not identified in the paper.

Statistical analysis

Data were analyzed using IBM SPSS statistics software version 20 (SPSS Inc. Released 2011. IBM SPSS statistics for windows, version 20.0, Armonk, NY, United States: IBM Corp). Quantitative data are presented as means, standard deviations, medians and interquartile ranges, while qualitative data are presented as frequencies and percentages. The relationship between qualitative variables was evaluated by the χ^2 test. Pairs of groups of non-normally distributed quantitative data were compared by the Mann–Whitney test, while three groups were compared by the Kruskal–Wallis test (non-parametric analysis of variance). Based on Kruskal–Wallis distribution, a post-hoc test was performed for pairwise comparisons. Correlations were assessed using the Spearman correlation test. The diagnostic values of serum miRNA 9-3p and endocan in HCC patients were evaluated by the receiver operating characteristic (ROC) curve analysis. The most independent factor associated with metastasis was identified by logistic regression analysis. P values of < 0.05 were considered statistically significant.

RESULTS

The patients in all three groups did not differ statistically in terms of age and gender. Biochemical analyses results (Table 1) showed that the HCC group and the chronic liver disease group differed in the following: ALT, direct bilirubin, international normalized ratio, endocan and miRNA 9-3p ($P = 0.005$, $P = 0.003$, $P = 0.002$, $P < 0.001$ and $P < 0.001$, respectively).

The HCC group differed significantly from the control group in terms of ALT, AST, platelet count, serum albumin, direct bilirubin, international normalized ratio, α-fetoprotein level, endocan and miRNA 9-3p ($P < 0.001$). The chronic liver disease group differed significantly from the control group in terms of ALT, AST, hemoglobin level, platelet count, international normalized ratio, α-fetoprotein level, endocan and miRNA 9-3p ($P = 0.003$, $P < 0.001$, $P = 0.019$, $P = 0.022$, $P = 0.001$, $P < 0.001$, $P < 0.001$ and $P < 0.001$ respectively).

Serum endocan levels in the HCC group were significantly higher than those in the chronic liver disease and control groups ($P < 0.001$). Furthermore, serum miRNA 9-3p expression levels in the HCC group were significantly lower than those in the chronic liver disease and control groups ($P < 0.001$), while levels in the chronic liver disease group were significantly lower than those in the control group ($P < 0.001$).

In the HCC group, 62.9% of patients (22 patients) were classified as grade A and (37.1%) of patients (13 patients) were classified as grade B according to Child-Pugh classifications; 4 (11.4%) patients were classified as grade A in BCLC stage, 18 (51.4 %) patients in stage B and 13 (37.1%) patients were in stage C. Detailed tumor characteristics of the HCC group are shown in Table 2.

The correlations between serum miRNA 9-3p levels and clinical data in HCC patients are shown in Table 3. miRNA 9-3p expression levels were significantly inversely correlated to vascular invasion, BCLC classification and metastatic site. Moreover, miRNA 9-3p expression levels were also significantly inversely correlated to serum endocan levels (Figure 1).

Univariate and multivariate logistic regression analyses on the HCC group indicated that miRNA 9-3p is an independent predictor factor of metastasis ($P = 0.041$; 95% confidence interval: 0.089-0.951) (Table 4).

ROC analysis of miRNA 9-3p and endocan levels indicated that at a cutoff point of 0.26, miRNA 9-3p can discriminate between patients with HCC and those with chronic liver disease with a sensitivity of 91.43%, a specificity of 87.88%, a positive predictive value of 88.90% and a negative predictive value of 90.60%. Meanwhile, at a cutoff point of 2370 pg/mL, endocan can discriminate between HCC and chronic liver disease patients with a sensitivity of 82.86%, a specificity of 84.85%, a positive predictive value of 85.30% and a negative predictive value of 82.40%. In comparison, α -fetoprotein was less sensitive and specific (60.00% and 33.30%, respectively).

At a cutoff point of 1.01, miRNA 9-3p can discriminate between HCC and control group patients with a sensitivity of 91.43%, a specificity of 87.50%, a positive predictive value of 88.90% and a negative predictive value of 90.30%. Meanwhile, at a cutoff point of 1510 pg/mL, endocan can discriminate between HCC and chronic liver disease patients with a sensitivity of 85.71%, a specificity of 87.50%, a positive predictive value of 88.20% and a negative predictive value of 84.80%. In comparison, α -fetoprotein was less sensitive and specific (80.00% and 71.87%, respectively). Diagnostically, both miRNA 9-3p and endocan performed better than α-fetoprotein at discriminating HCC patients from both chronic liver disease and healthy patients (Figures 2 and 3).

ROC analysis of miRNA 9-3p levels in the HCC group indicated that at a cutoff point of 0.02, miRNA 9-3p can discriminate between metastatic and non-metastatic HCC patients with a sensitivity of 91.67%, a specificity of 82.61%, a positive predictive value of 73.30% and a negative predictive value of 95.00%.

DISCUSSION

The increasing prevalence of HCC worldwide and its associated poor prognosis make it a global health problem. Studies in Egypt shows the increasing role of HCV infection in liver cancer etiology, and among all cancer deaths in Egypt, HCC is the primary cause[17,18].

HCC is often detected late, when it is no longer operable, which limits curative surgical treatment to only a few cases involving small HCC malignancies. Moreover, as a diagnostic tool, α-fetoprotein is limited in its accuracy[19]. In contrast, circulating

Table 1 Demographic and laboratory data of the study participants

Variables	Group I	Group II	Group III	*P* value	
	HCC	Chronic liver disease	Control		
	n = 35	*n* = 33	*n* = 32		
Gender					
Male, *n* (%)	30 (85.7)	23 (69.7)	27 (84.4)	NS	
Female, *n* (%)	5 (14.3)	10 (30.3)	5 (15.6)		
Age (yr)					
mean ± SD	55.2 ± 5.2	52.7 ± 5.3	52.8 ± 5.6	NS	
ALT (IU/L), median (IQR)	50.0 (35.0-55.0)	34.0 (28.0-50.0)	29.7 (23.5-31.7)	$P < 0.001$	[a]$P = 0.005$; [b]$P < 0.001$; [c]$P = 0.003$
AST (IU/L), median (IQR)	52.0 (39.0-70.0)	42.0 (32.0-57.0)	32.8 (30.0-36.0)	< 0.001	[a]$P = 0.060$; [b]$P < 0.001$; [c]$P < 0.001$
Hb (mg/dL), mean ± SD	13.1 ± 1.7	12.5 ± 1.6	13.5 ± 1.0	0.025	[a]$P = 0.260$; [b]$P = 0.438$; [c]$P = 0.019$
Platelets, (× 10^3/μL), median (IQR)	141.0 (104.5-193.5)	162.0 (134.0-213.0)	197.5 (180.5-246.0)	0.002	[a]$P = 0.225$; [b]$P < 0.001$; [c]$P = 0.022$
Serum ALB (g/dL), mean ± SD	3.6 ± 0.7	3.8 ± 0.6	4.1 ± 0.4	0.001	[a]$P = 0.382$; [b]$P = 0.001$; [c]$P = 0.050$
INR, mean ± SD	1.3 ± 0.2	1.1 ± 0.4	0.8 ± 0.2	< 0.001	[a]$P = 0.002$; [b]$P = 0.001$; [c]$P = 0.001$
α-fetoprotein (ng/mL), median (IQR)	240.0 (28.2-635.0)	124.0 (108.9-166.0)	17.4 (14.0-24.0)	< 0.001	[a]$P = 0.895$; [b]$P < 0.001$; [c]$P < 0.001$
Endocan (pg/mL), median (IQR)	3450.0 (3188.5-4135.0)	1934.0 (1450.0-2257.0)	878.5 (850.0-1188.0)	< 0.001	[a]$P < 0.001$; [b]$P < 0.001$; [c]$P = 0.001$
microRNA 9-3p, median (IQR)	0.03 (0.02-0.05)	0.42 (0.29-1.35)	1.70 (1.40-2.15)	< 0.001	[a]$P < 0.001$; [b]$P < 0.001$; [c]$P < 0.001$

[a]*P*: *P* value for comparing between HCC and chronic liver disease.
[b]*P*: *P* value for comparing between HCC and control.
[c]*P*: *P* value for comparing between chronic liver disease and control. Statistically significant at $P \leq 0.05$. SD: Standard deviation; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; Hb: Hemoglobin; ALB: Albumin; INR: International normalized ratio; HCC: Hepatocellular carcinoma; NS: Not significant; IQR: Interquartile range.

miRNAs may serve as biomarkers and a useful diagnostic approach for the early detection of HCC[20].

In the present study, clinical and laboratory data from the three different groups of patients revealed that serum α-fetoprotein levels of HCC and chronic liver disease patients was significantly different from those of control patients. α-fetoprotein is known to be overexpressed in HCC[21-23], and the severity of cirrhosis is a significant predictor of elevated serum α-fetoprotein levels; higher serum α-fetoprotein levels are significantly correlated with advanced cirrhosis in patients with chronic HCV[24].

We found that serum α-fetoprotein levels in the HCC group did not differ significantly from those of the chronic liver disease group. This agrees with the results of Massironi *et al*[25], who reported similar findings in HCC and liver cirrhosis subjects. In our study, at a cutoff value of 23 ng/dL, α-fetoprotein discriminates between HCC and control patients at a sensitivity of 80.00% and a specificity of 71.87%. These results are similar to those of Massironi *et al*[25] and Metwaly *et al*[26], who reported a sensitivity of 75% and a specificity of 80% at a cutoff value of 16.9 ng/dL.

Our findings show higher serum endocan levels in HCC patients than in chronic liver disease patients, which agrees with previous studies by Nault *et al*[27] and Ozaki *et al*[28].

Recent studies on HCC show that elevated serum endocan levels and endocan expression by stromal endothelial cells in HCC tissues are correlated with poor survival[29]. Endocan expression in tumors undergoing angiogenesis reflects the

Table 2 Clinical characteristics of tumors in the hepatocellular carcinoma group, *n* = 35

Number of the focal lesions	*n* (%)
Single	16 (45.7)
Multiple	19 (54.3)
Tumor size in cm	
Small < 3	7 (20.0)
Medium 3-5	15 (42.9)
Large > 5	13 (37.1)
Location of the focal lesions	
Rt. Lobe	19 (54.3)
Lt. Lobe	8 (22.9)
Both	7 (20.0)
Caudate lobe	1 (2.9)
BCLC stage	
A	4 (11.4)
B	18 (51.4)
C	13 (37.1)
Vascular invasion	
Negative	25 (71.4)
Positive	10 (28.6)
LN metastasis	
Negative	28 (80.0)
Positive	7 (20.0)
Ascites	
No	25 (73.5)
Mild	8 (23.5)
Moderate	1 (2.9)
Child Pugh classA	22 (62.9)
B	13 (37.1)
C	0 (0)
Distant metastasis	
No	23 (65.7)
Yes	12 (34.3)

Rt.: Right; Lt.: Left; BCLC: Barcelona Clinic Liver Cancer; LN: Lymph node.

processes of angiogenesis and tumor invasion. Structurally, the glycan form and phenylalanine-rich region of endocan are its key effective sections through the nuclear factor-κB/IκB pathway[30]. However, the involvement of endocan in HCC development remains unclear.

We found that plasma miRNA 9-3p levels are significantly lower in HCC patients compared to chronic liver disease and control patients. Overall, the order of miRNA 9-3p expression among the different groups is as follows: HCC < chronic liver disease < control.

This supports the concept of the antitumor function of miRNA 9-3p as reported by Higashi *et al*[12], Yang *et al*[31] and Tang *et al*[32]. In contrast, Sun *et al*[33] showed that miR-9 increases the levels of migration and invasion of HCC cell lines. It is possible that miR-9 (*i.e.* miR-9-5p) and miR-9* (miR-9-3p) are two different miRNAs that

Table 3 Correlations between microRNA 9-3p levels and clinical data in hepatocellular carcinoma group

	n	microRNA 9-3p	
		Median (IQR)	*P* value
Vascular invasion			
Negative	25	0.04 (0.02-0.26)	0.002
Positive	10	0.02 (0.02-0.02)	
LN metastasis			
Negative	28	0.04 (0.02-0.17)	0.072
Positive	7	0.02 (0.02-0.03)	
Distant metastasis			
No	23	0.04 (0.03-0.26)	< 0.001
Yes	12	0.02 (0.02-0.02)	
Child Pugh class			
A	22	0.03 (0.02-0.04)	0.389
B	13	0.04 (0.02-0.26)	
Tumor number			
Single	16	0.03 (0.02-0.17)	0.935
Multiple	19	0.03 (0.02-0.05)	
Tumor size in cm			
Small < 3	7	0.03 (0.02-0.15)	0.852
Medium 3-5	15	0.03 (0.02-0.06)	
Large > 5	13	0.04 (0.02-0.04)	
Tumor site			
Rt lobe	19	0.04 (0.02-0.06)	0.432
Lt lobe	8	0.04 (0.03-0.15)	
Both	7	0.02 (0.02-0.04)	
Caudate lobe	1		
BCLC stage			
A	4	0.26 (0.17-0.26)	< 0.001
B	18	0.04 (0.03-0.05)	
C	13	0.02 (0.02-0.02)	

Rt.: Right; Lt.: Left; BCLC: Barcelona Clinic Liver Cancer; IQR: Interquartile range; LN: Lymph node.

originate from the same precursor, and they can play either synergistic or opposite roles within one malignancy[34].

Interestingly, we observed significantly lower levels of miRNA 9-3p expression and vascular invasion at the advanced stage of BCLC and at the metastatic site of the HCC group.

In cervical adenocarcinoma, miRNA 9-3p is downregulated and acts as a tumor suppressor. Ectopic expression of miR-9-3p inhibits the JAK/STAT3 pathway by targeting interleukin 6, leading to the upregulation of vascular endothelial growth factor and increased angiogenesis. This results in decreased proliferation and migration and reduced tumor growth *in vivo*[35]. Moreover, Tang *et al*[32] reported that exosomal miRNA 9-3p suppresses the development and progression of HCC.

Cai *et al*[15] reported that increased exosomal miR-9-3p counteracts bladder cancer growth and metastasis and decreases endocan protein expression in nude mice. We similarly observed that miR-9-3p expression is inversely correlated to serum endocan levels in the HCC group.

Table 4 Univariate and multivariate regression analyses for the parameters affecting metastasis in hepatocellular carcinoma group

	Univariate		Multivariate	
	***P* value**	**OR (95%CI)**	***P* value**	**OR (95%CI)**
microRNA 9-3p	0.008	0.193 (0.057-0.653)	0.041	0.291 (0.089-0.951)
Endocan	0.023	1.002 (1.000-1.003)	0.358	1.001 (0.999-1.002)

Statistically significant at $P \leq 0.05$. OR: Odds ratio; CI: Confidence interval.

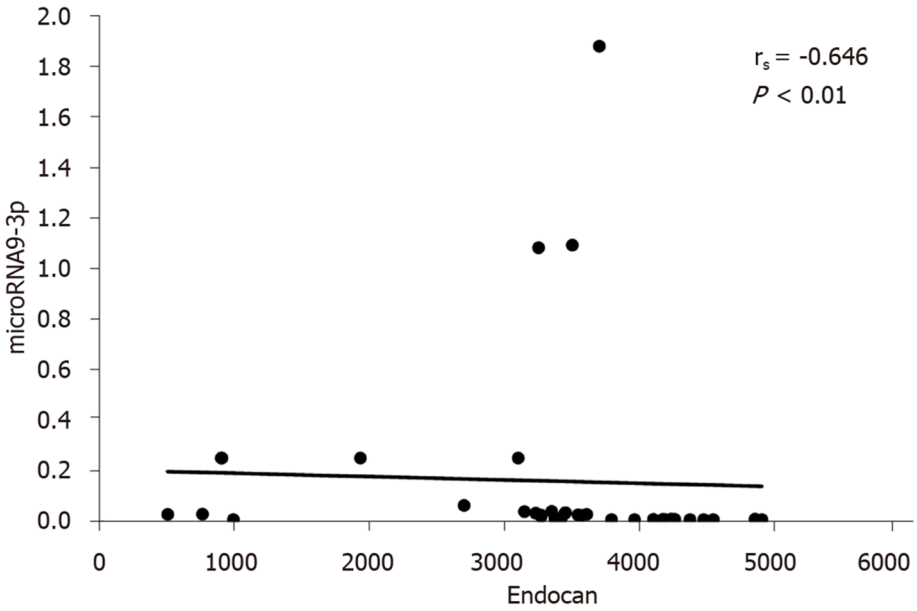

Figure 1 Correlation between microRNA 9-3p and endocan levels in the hepatocellular carcinoma group.

We performed ROC analysis to compare the diagnostic accuracies of miRNA 9-3p, endocan and the traditional HCC tumor marker, α-fetoprotein. Diagnostically, both miRNA 9-3p and endocan perform better than α-fetoprotein in discriminating patients with HCC from those with or without (*i.e.* healthy) chronic liver disease. Furthermore, ROC analysis revealed that miRNA 9-3p performed well at discriminating between metastatic and non-metastatic patients in the HCC group. Statistically, miRNA 9-3p is an independent predictor factor of metastasis. This study could be the nucleus of a larger study working on a larger number of patients that may include those with other causes of chronic liver disease like alcoholism as our study was limited to HCV-induced chronic liver disease as it is highly prevalent in Egypt.

CONCLUSION

Endocan and miRNA 9-3p could be biomarkers with potential use for the early diagnosis of HCV-related HCC. In this regard, they are more valuable than α-fetoprotein. Moreover, miRNA 9-3p is an independent predictor of metastasis in HCC patients.

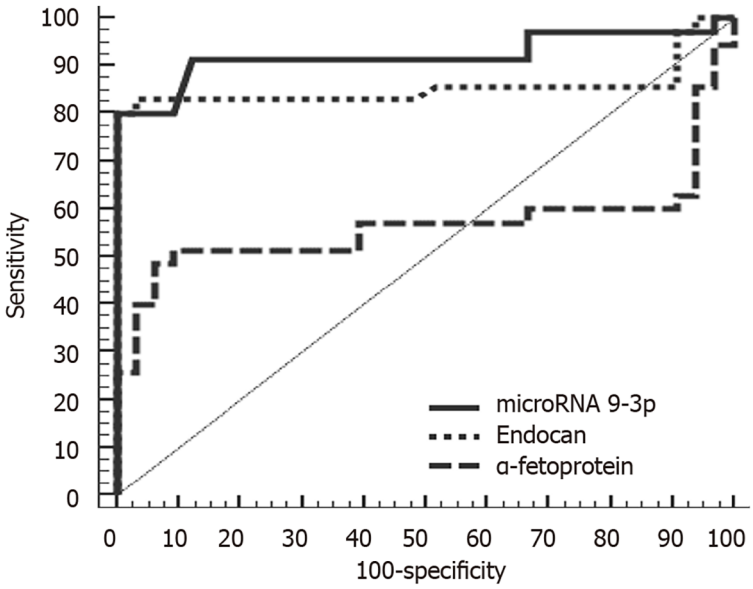

Figure 2 Receiver operating characteristic curve analysis of microRNA 9-3p, endocan and α-fetoprotein for discriminating between hepatocellular carcinoma and chronic liver disease.

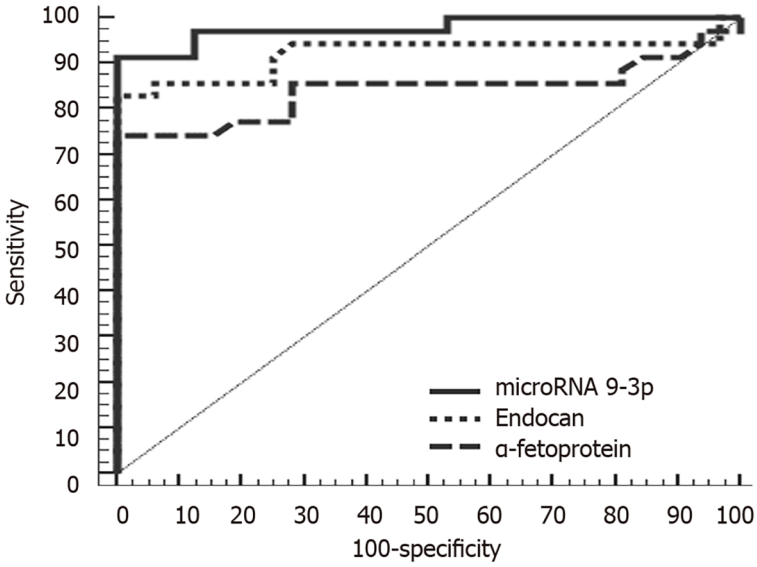

Figure 3 Receiver operating characteristic curve analysis of microRNA 9-3p, endocan and α-fetoprotein for discriminating between hepatocellular carcinoma and control.

ARTICLE HIGHLIGHTS

Research background

The high mortality rate of hepatocellular carcinoma (HCC) in Egypt is due mainly to the increasing prevalence of hepatitis C virus infection (HCV) and late diagnosis of the carcinoma.

Research motivation

MicroRNAs (miRNA), which regulate tumor proliferation and metastasis in HCC, may serve as a useful diagnostic approach for the early detection of HCC, thus decreasing its mortality. Meanwhile, endocan is a protein with angiogenic and inflammatory properties that are associated with tumor progression and poor outcomes.

Research objectives
To analyze the levels of miRNA 9-3p and endocan in HCV-infected HCC patients and correlate them with clinicopathological parameters.

Research methods
We compared levels of endocan and circulating miRNA 9-3p from 35 HCV-related HCC patients to 33 patients with HCV-induced chronic liver disease and 32 age and gender matched healthy controls.

Research results
The levels of circulating miRNA 9-3p were significantly lower in the HCC group compared to the chronic liver disease ($P < 0.001$) and control ($P < 0.001$) groups, while levels in the chronic liver disease were significantly lower than those in the control group ($P < 0.001$). While the levels of serum endocan were significantly higher in the HCC group compared to the chronic liver disease ($P < 0.001$) and control ($P < 0.001$) groups. Moreover, miRNA 9-3p and endocan performed better than α-fetoprotein in discriminating HCC patients from cirrhosis and healthy patients. The levels of miRNA 9-3p are significantly inversely correlated to vascular invasion ($P = 0.002$), stage of advancement of Barcelona Clinical Liver Cancer ($P < 0.001$ and the metastatic site ($P < 0.001$) of the HCC group.

Research conclusions
Endocan and miRNA 9-3p could be biomarkers with potential use for the early diagnosis of HCV-related HCC. In this regard, they are more valuable than α-fetoprotein. Moreover, miRNA 9-3p is an independent predictor of metastasis in HCC patients.

Research perspectives
The findings of this study warrant additional investigation in prospective trials with larger cohorts and longer follow-up for confirming our results and validating the potential clinical use of these markers in early HCC detection.

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REFERENCES
1 **Bray F**, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* 2018; **68**: 394-424 [PMID: 30207593 DOI: 10.3322/caac.21492]
2 **Ferlay J**, Colombet M, Soerjomataram I, Mathers C, Parkin DM, Piñeros M, Znaor A, Bray F. Estimating the global cancer incidence and mortality in 2018: GLOBOCAN sources and methods. *Int J Cancer* 2019; **144**: 1941-1953 [PMID: 30350310 DOI: 10.1002/ijc.31937]
3 **Rashed WM**, Kandeil MAM, Mahmoud MO, Ezzat S. Hepatocellular Carcinoma (HCC) in Egypt: A comprehensive overview. *J Egypt Natl Canc Inst* 2020; **32**: 5 [PMID: 32372179 DOI: 10.1186/s43046-020-0016-x]
4 **El Kassas M**, Elbaz T, Elsharkawy A, Omar H, Esmat G. HCV in Egypt, prevention, treatment and key barriers to elimination. *Expert Rev Anti Infect Ther* 2018; **16**: 345-350 [PMID: 29506418 DOI: 10.1080/14787210.2018.1448709]
5 **Zucman-Rossi J**, Villanueva A, Nault JC, Llovet JM. Genetic Landscape and Biomarkers of Hepatocellular Carcinoma. *Gastroenterology* 2015; **149**: 1226-1239.e4 [PMID: 26099527 DOI: 10.1053/j.gastro.2015.05.061]
6 **Cancer Genome Atlas Research Network**; Comprehensive and Integrative Genomic Characterization of Hepatocellular Carcinoma. *Cell* 2017; **169**: 1327-1341.e23 [PMID: 28622513 DOI: 10.1016/j.cell.2017.05.046]
7 **El-Gebaly F**, Abou-Saif S, Elkadeem M, Helmy A, Abd-Elsalam S, Yousef M, Elkhouly RA, Amer IF, El-Demerdash T. Study of Serum Soluble Programmed Death Ligand 1 as a Prognostic Factor in Hepatocellular Carcinoma in Egyptian Patients. *Curr Cancer Drug Targets* 2019; **19**: 896-905 [PMID: 31538897 DOI: 10.2174/1568009619666190718141647]
8 **Catalanotto C**, Cogoni C, Zardo G. MicroRNA in Control of Gene Expression: An Overview of Nuclear Functions. *Int J Mol Sci* 2016; **17** [PMID: 27754357 DOI: 10.3390/ijms17101712]

9 **Hu Q**, Du K, Mao X, Ning S. miR-197 is downregulated in cervical carcinogenesis and suppresses cell proliferation and invasion through targeting forkhead box M1. *Oncol Lett* 2018; **15**: 10063-10069 [PMID: 29928375 DOI: 10.3892/ol.2018.8565]

10 **Coolen M**, Katz S, Bally-Cuif L. miR-9: a versatile regulator of neurogenesis. *Front Cell Neurosci* 2013; **7**: 220 [PMID: 24312010 DOI: 10.3389/fncel.2013.00220]

11 **Ma L**, Young J, Prabhala H, Pan E, Mestdagh P, Muth D, Teruya-Feldstein J, Reinhardt F, Onder TT, Valastyan S, Westermann F, Speleman F, Vandesompele J, Weinberg RA. miR-9, a MYC/MYCN-activated microRNA, regulates E-cadherin and cancer metastasis. *Nat Cell Biol* 2010; **12**: 247-256 [PMID: 20173740 DOI: 10.1038/ncb2024]

12 **Higashi T**, Hayashi H, Ishimoto T, Takeyama H, Kaida T, Arima K, Taki K, Sakamoto K, Kuroki H, Okabe H, Nitta H, Hashimoto D, Chikamoto A, Beppu T, Baba H. miR-9-3p plays a tumour-suppressor role by targeting TAZ (WWTR1) in hepatocellular carcinoma cells. *Br J Cancer* 2015; **113**: 252-258 [PMID: 26125451 DOI: 10.1038/bjc.2015.170]

13 **Sarrazin S**, Adam E, Lyon M, Depontieu F, Motte V, Landolfi C, Lortat-Jacob H, Bechard D, Lassalle P, Delehedde M. Endocan or endothelial cell specific molecule-1 (ESM-1): a potential novel endothelial cell marker and a new target for cancer therapy. *Biochim Biophys Acta* 2006; **1765**: 25-37 [PMID: 16168566 DOI: 10.1016/j.bbcan.2005.08.004]

14 **Leroy X**, Aubert S, Zini L, Franquet H, Kervoaze G, Villers A, Delehedde M, Copin MC, Lassalle P. Vascular endocan (ESM-1) is markedly overexpressed in clear cell renal cell carcinoma. *Histopathology* 2010; **56**: 180-187 [PMID: 20102396 DOI: 10.1111/j.1365-2559.2009.03458.x]

15 **Cai H**, Yang X, Gao Y, Xu Z, Yu B, Xu T, Li X, Xu W, Wang X, Hua L. Exosomal MicroRNA-9-3p Secreted from BMSCs Downregulates ESM1 to Suppress the Development of Bladder Cancer. *Mol Ther Nucleic Acids* 2019; **18**: 787-800 [PMID: 31734559 DOI: 10.1016/j.omtn.2019.09.023]

16 **Llovet JM**, Brú C, Bruix J. Prognosis of hepatocellular carcinoma: the BCLC staging classification. *Semin Liver Dis* 1999; **19**: 329-338 [PMID: 10518312 DOI: 10.1055/s-2007-1007122]

17 **Patel M**, Shariff MI, Ladep NG, Thillainayagam AV, Thomas HC, Khan SA, Taylor-Robinson SD. Hepatocellular carcinoma: diagnostics and screening. *J Eval Clin Pract* 2012; **18**: 335-342 [PMID: 21114800 DOI: 10.1111/j.1365-2753.2010.01599.x]

18 **Abudeif A**. Epidemiology and Risk Factors of Hepatocellular Carcinoma in Egypt. *Sohag Medical Journal* 2019; **23**: 8-12 [DOI: 10.21608/SMJ.2019.13376.1019]

19 **Sun S**, Poon RT, Lee NP, Yeung C, Chan KL, Ng IO, Day PJ, Luk JM. Proteomics of hepatocellular carcinoma: serum vimentin as a surrogate marker for small tumors (<or=2 cm). *J Proteome Res* 2010; **9**: 1923-1930 [PMID: 20121168 DOI: 10.1021/pr901085z]

20 **Chen X**, Ba Y, Ma L, Cai X, Yin Y, Wang K, Guo J, Zhang Y, Chen J, Guo X, Li Q, Li X, Wang W, Wang J, Jiang X, Xiang Y, Xu C, Zheng P, Zhang J, Li R, Zhang H, Shang X, Gong T, Ning G, Zen K, Zhang CY. Characterization of microRNAs in serum: a novel class of biomarkers for diagnosis of cancer and other diseases. *Cell Res* 2008; **18**: 997-1006 [PMID: 18766170 DOI: 10.1038/cr.2008.282]

21 **Köberle V**, Kronenberger B, Pleli T, Trojan J, Imelmann E, Peveling-Oberhag J, Welker MW, Elhendawy M, Zeuzem S, Piiper A, Waidmann O. Serum microRNA-1 and microRNA-122 are prognostic markers in patients with hepatocellular carcinoma. *Eur J Cancer* 2013; **49**: 3442-3449 [PMID: 23810247 DOI: 10.1016/j.ejca.2013.06.002]

22 **El-Abd NE**, Fawzy NA, El-Sheikh SM, Soliman ME. Circulating miRNA-122, miRNA-199a, and miRNA-16 as Biomarkers for Early Detection of Hepatocellular Carcinoma in Egyptian Patients with Chronic Hepatitis C Virus Infection. *Mol Diagn Ther* 2015; **19**: 213-220 [PMID: 26133725 DOI: 10.1007/s40291-015-0148-1]

23 **Naz Z**, Usman S, Saleem K, Ahmed S, Bashir H, Bilal M, Sumrin A. Alpha-fetoprotein: A fabulous biomarker in hepatocellular, gastric and rectal cancer diagnosis. *Biomed Res* 2018; **29**: 2478-2483 [DOI: 10.4066/biomedicalresearch.29-17-1550]

24 **Chu CW**, Hwang SJ, Luo JC, Lai CR, Tsay SH, Li CP, Wu JC, Chang FY, Lee SD. Clinical, virologic, and pathologic significance of elevated serum alpha-fetoprotein levels in patients with chronic hepatitis C. *J Clin Gastroenterol* 2001; **32**: 240-244 [PMID: 11246354 DOI: 10.1097/00004836-200103000-00014]

25 **Massironi S**, Fraquelli M, Paggi S, Sangiovanni A, Conte D, Sciola V, Ciafardini C, Colombo M, Peracchi M. Chromogranin A levels in chronic liver disease and hepatocellular carcinoma. *Dig Liver Dis* 2009; **41**: 31-35 [PMID: 18762462 DOI: 10.1016/j.dld.2008.05.002]

26 **Metwaly K**, Sameea EA, El-Azab G, Assem M, Abbas M, Zakareya T, Abo Raia G. Mean platelet volume and mean platelet volume/platelet count ratio as markers for hepatocellular carcinoma in patients with chronic hepatitis C virus related cirrhosis. *J Cancer Res Clin Oncol* 2016; **8**: 33-40 [DOI: 10.5897/JCREO2016.0135]

27 **Nault JC**, Guyot E, Laguillier C, Chevret S, Ganne-Carrie N, N'Kontchou G, Beaugrand M, Seror O, Trinchet JC, Coelho J, Lasalle P, Charnaux N, Delehedde M, Sutton A, Nahon P. Serum proteoglycans as prognostic biomarkers of hepatocellular carcinoma in patients with alcoholic cirrhosis. *Cancer Epidemiol Biomarkers Prev* 2013; **22**: 1343-1352 [PMID: 23780836 DOI: 10.1158/1055-9965.EPI-13-0179]

28 **Ozaki K**, Toshikuni N, George J, Minato T, Matsue Y, Arisawa T, Tsutsumi M. Serum endocan as a novel prognostic biomarker in patients with hepatocellular carcinoma. *J Cancer* 2014; **5**: 221-230 [PMID: 24665346 DOI: 10.7150/jca.7691]

29 **Huang GW**, Tao YM, Ding X. Endocan expression correlated with poor survival in human hepatocellular carcinoma. *Dig Dis Sci* 2009; **54**: 389-394 [PMID: 18592377 DOI:

10.1007/s10620-008-0346-3]
30 **Chen LY**, Liu X, Wang SL, Qin CY. Over-expression of the Endocan gene in endothelial cells from hepatocellular carcinoma is associated with angiogenesis and tumour invasion. *J Int Med Res* 2010; **38**: 498-510 [PMID: 20515564 DOI: 10.1177/147323001003800213]
31 **Yang L**, Mu Y, Cui H, Liang Y, Su X. MiR-9-3p augments apoptosis induced by H2O2 through down regulation of Herpud1 in glioma. *PLoS One* 2017; **12**: e0174839 [PMID: 28430789 DOI: 10.1371/journal.pone.0174839]
32 **Tang J**, Li Y, Liu K, Zhu Q, Yang WH, Xiong LK, Guo DL. Exosomal miR-9-3p suppresses HBGF-5 expression and is a functional biomarker in hepatocellular carcinoma. *Minerva Med* 2018; **109**: 15-23 [PMID: 28750499 DOI: 10.23736/S0026-4806.17.05167-9]
33 **Sun Z**, Han Q, Zhou N, Wang S, Lu S, Bai C, Zhao RC. MicroRNA-9 enhances migration and invasion through KLF17 in hepatocellular carcinoma. *Mol Oncol* 2013; **7**: 884-894 [PMID: 23684102 DOI: 10.1016/j.molonc.2013.04.007]
34 **Schraivogel D**, Weinmann L, Beier D, Tabatabai G, Eichner A, Zhu JY, Anton M, Sixt M, Weller M, Beier CP, Meister G. CAMTA1 is a novel tumour suppressor regulated by miR-9/9* in glioblastoma stem cells. *EMBO J* 2011; **30**: 4309-4322 [PMID: 21857646 DOI: 10.1038/emboj.2011.301]
35 **Zhang J**, Jia J, Zhao L, Li X, Xie Q, Chen X, Wang J, Lu F. Down-regulation of microRNA-9 Leads to activation of IL-6/Jak/STAT3 pathway through directly targeting IL-6 in HeLa cell. *Mol Carcinog* 2016; **55**: 732-742 [PMID: 25809226 DOI: 10.1002/mc.22317]

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ORIGINAL ARTICLE

Retrospective Cohort Study

Do peripartum and postmenopausal women with primary liver cancer have a worse prognosis? A nationwide cohort in Taiwan

Guan-Woei Tseng, Mei-Chen Lin, Shih-Wei Lai, Cheng-Yuan Peng, Po-Heng Chuang, Wen-Pang Su, Jung-Ta Kao, Hsueh-Chou Lai

ORCID number: Guan-Woei Tseng 0000-0003-3897-5439; Mei-Chen Lin 0000-0003-0274-6108; Shih-Wei Lai 0000-0002-7420-1572; Cheng-Yuan Peng 0000-0001-9030-6086; Po-Heng Chuang 0000-0003-3711-5445; Wen-Pang Su 0000-0003-0311-3601; Jung-Ta Kao 0000-0002-3801-5342; Hsueh-Chou Lai 0000-0002-0126-6447.

Author contributions: Tseng GW did the study conception and design, and initial draft of the manuscript; Lin MC did the data analysis and interpretation, and initial draft of the manuscript; Lai SW, C Peng CY, Chuang PH, Su WP, Kao JT participated in the study conception; Lai HC did the data analysis and interpretation, manuscript drafting and revision. All authors have read and approved the final manuscript.

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Informed consent statement: For more information about the usage of National Health Insurance Research Database (NHIRD), please refer to https://nhird.nhri.org.tw/en/Data_Protection.html

Conflict-of-interest statement: All

Guan-Woei Tseng, Department of Medicine, China Medical University, Taichung 40402, Taiwan

Mei-Chen Lin, Management Office for Health Data, China Medical University Hospital, Taichung 404, Taiwan

Shih-Wei Lai, Department of Family Medicine, China Medical University Hospital, Taichung 404, Taiwan

Cheng-Yuan Peng, Po-Heng Chuang, Wen-Pang Su, Jung-Ta Kao, Hsueh-Chou Lai, Center for Digestive Disease Department of Internal Medicine, China Medical University Hospital, Taichung 404, Taiwan

Hsueh-Chou Lai, School of Chinese Medicine, China Medical University, Taichung 404, Taiwan

Corresponding author: Hsueh-Chou Lai, MD, PhD, Professor, School of Chinese Medicine, China Medical University, No. 91 Xueshi Road, North District, Taichung 404, Taiwan. t674233@ms54.hinet.net

Abstract

BACKGROUND

While primary liver cancer (PLC) is one of the most common cancers around the world, few large-scale population-based studies have been reported that evaluated the clinical survival outcomes among peripartum and postmenopausal women with PLC.

AIM

To investigate whether peripartum and postmenopausal women with PLC have lower overall survival rates compared with women who were not peripartum and postmenopausal.

METHODS

The Taiwan National Health Insurance claims data from 2000 to 2012 was used for this propensity-score-matched study. A cohort of 40 peripartum women with PLC and a reference cohort of 160 women without peripartum were enrolled. In the women with PLC with/without menopause study, a study cohort of 10752

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menopausal females with PLC and a comparison cohort of 2688 women without menopause were enrolled.

RESULTS

Patients with peripartum PLC had a non-significant risk of death compared with the non-peripartum cohort [adjusted hazard ratios (aHR) = 1.40, 95% confidence intervals (CI): 0.89-2.20, P = 0.149]. The survival rate at different follow-up durations between peripartum PLC patients and those in the non-peripartum cohort showed a non-significant difference. Patients who were diagnosed with PLC younger than 50 years old (without menopause) had a significant lower risk of death compared with patients diagnosed with PLC at or older than 50 years (postmenopausal) (aHR = 0.64, 95%CI: 0.61-0.68, P < 0.001). The survival rate of women < 50 years with PLC was significantly higher than older women with PLC when followed for 0.5 (72.44% *vs* 64.16%), 1 (60.57% *vs* 51.66%), 3 (42.92% *vs* 31.28%), and 5 year(s) (37.02% *vs* 21.83%), respectively (P < 0.001).

CONCLUSION

Peripartum females with PLC have no difference in survival rates compared with those patients without peripartum. Menopausal females with PLC have worse survival rates compared with those patients without menopause.

Key Words: Primary liver cancer; Peripartum and postmenopausal women; Prognosis; Nationwide cohort; Peripartum women; Postmenopausal women

Core Tip: This is the first nationwide study to evaluate the survival rate of peripartum and postmenopausal women with primary liver cancer (PLC) using the National Health Insurance Research Database in Taiwan. The results showed that patients with peripartum PLC had a non-significant risk of death compared with those in the non-peripartum cohort. Patients who were diagnosed with PLC younger than 50 years (without menopause) had a significantly lower risk of death compared with patients diagnosed with PLC at 50 years or older (after menopause). We believe that the results presented in this study provide important information on clinical applications.

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INTRODUCTION

Primary liver cancer (PLC), the sixth most common cancer, and the fourth leading cause of cancer-related death around the world in 2018, put a heavy burden on global health[1,2]. Hepatocellular carcinoma (HCC) and intrahepatic cholangiocarcinoma, account for 70%-75% and 15% of cases, respectively, and comprise most primary liver malignancies[3]. The common risk factors of PLC are male gender, excess body fat, type II diabetes mellitus, chronic infection with hepatitis B virus (HBV) and/or hepatitis C virus (HCV), cigarette smoking, aflatoxin, and heavy alcohol consumption [4,5]. Men appear to have a higher occurrence and worse outcomes, with two to three times higher incidence and mortality compared with women[1,6]. Thus, most studies have included too few women to draw accurate conclusions.

Animal studies indicated that the primary etiology behind the protective effect of the female sex hormone might involve the anti-inflammatory modulation of estrogen, as chronic inflammation was a major contributor to carcinogenic processes[7-9]. Nevertheless, controversial results were obtained in research targeting women of reproductive age. Despite the rarity, PLC diagnosed during pregnancy generally caused a shorter survival compared with non-pregnant patients with inoperable PLC

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[10-12]. Several early reports suggested that the adverse influence of pregnancy for the development of PLC was probably due to an alteration of the hormonal milieu[13,14]. In contrast, other recent papers attributed the consequence to delayed diagnosis[11, 15]. However, the latest cohort analysis needs further interpretation, as most of the published articles were case reports, with the largest including 48 cases published in 2011[12,16]. In addition, evidence implied that the downturn in ovarian function in menopause is related to the spontaneous elevation in pro-inflammatory cytokines[17-19], which may have an undesirable effect on PLC development and progression. While there were limited epidemiologic statistics with the survival outcome among females, the research indicated that there was a reduced risk. It increased overall survival times of PLCs in postmenopausal patients receiving hormone replacement therapy (HRT)[20]. It is estimated that 1.2 billion women worldwide will be menopausal or postmenopausal by the year 2030[21]. Therefore, there is a growing necessity to make a thorough exploration of the morbidity and mortality of PLCs among this sector of the population.

To date, few large-scale population-based studies have been conducted to elucidate the relationship between pregnancy, menopause, and survival outcomes among women with PLCs. Our primary aim was to determine if pregnant and postmenopausal female patients with PLCs have a lower survival rate relative to population-based controls using a nationwide database in Taiwan.

MATERIALS AND METHODS

Data source

Taiwan government built a nationwide health record-related database named the National Health Insurance Research Database (NHIRD) in 1995. The database contains comprehensive health information, representative study subjects, and long-term follow-up periods. This study was conducted using the population-based hospitalization file, including all hospitalization records of Taiwan citizens. The identification was encrypted before the database released the records for medical research to protect the privacy of each patient.

All previous diagnoses in the database were coded according to the International Classification of Disease, Ninth Revision, Clinical Modification (ICD-9-CM). The Research Ethics Committee of China Medical University and Hospital in Taiwan approved the study (CMUH-104-REC2-115-R3).

Study population

According to the study objective, we would like to confirm the association between peripartum PLC and survival. We selected patients with peripartum PLC (ICD-9-CM: 155) who were diagnosed between 10 mo before and six months after delivery, during 2000-2012, as the exposed cohort. We defined the date of newly diagnosed PLC as the index date. The unexposed group was defined as patients with PLC who were diagnosed outside of the pregnancy period and selected by 4:1 propensity score matching with the exposed cohort. The matching variables included age, index year, and comorbidities, such as HBV, unspecified chronic hepatitis, alcoholic liver disease, cirrhosis, biliary stones, cholecystitis, and cholangitis. To further realize the correlation between menopause and PLC prognosis, we defined women aged 50 and beyond as postmenopausal period. While natural menopause may occur from 45 to 55 of age[22], a recent cohort analysis including 36931 postmenopausal women indicated that the mean age at menopause is 50.2 years in Taiwan[23]. Propensity score matching and matching variables mentioned above were applied. Patients with PLC before the index date were excluded from the study. The study population was followed up until death, withdrawn from NHIRD, or until December 31, 2013.

The comorbidities of concern in this study were HBV (ICD-9-CM: 070.2, 070.3, and V02.61), unspecified chronic hepatitis (ICD-9-CM: 070.9, 571.4, 571.8, 571.9), alcoholic liver disease (ICD-9-CM: 571.0, 571.1, 571.2, 571.3), cirrhosis (ICD-9-CM: 571.5, 571.6), biliary stones (ICD-9-CM: 574), cholecystitis (ICD-9-CM: 575), and cholangitis (ICD-9-CM: 576). The comorbidities above were defined as at least one hospitalization before the index date.

Statistical analysis

This study included demographic and comorbidities variables. The continuous and the categorical variables were shown by mean ± SD and number (%), and to compare the difference of each variable in two groups, a *t*-test and chi-square test were used,

respectively. To calculate the risk of death in the exposed and the unexposed cohorts, Cox proportional hazard models were used and presented using hazard ratios, adjusted hazard ratios (aHR) and 95% confidence intervals (CIs). The survival rate of death in the two cohorts was presented by the Kaplan-Meier method. The log-rank test was used to compare the difference between two survival curves. All statistical analyses were performed with SAS statistical software version 9.4 (SAS Institute Inc., Cary, NC). The Figure of the cumulative incidence curve was plotted by R software. The significance criteria were set up as a two-sided test with a P value of less than 0.05.

RESULTS

Of 200 eligible subjects in this study (Table 1), 40 were diagnosed with peripartum PLC, and the other 160 were selected as the unexposed cohort. Among patients with peripartum PLC, the dominant age group was younger than 30 years old (47.5%), 11 (27.5%) with HBV, one (2.5%) with unspecified chronic hepatitis, one with alcoholic liver disease, three (7.5%) with cirrhosis, two (5%) with biliary stone, four (10%) with cholecystitis, and three (7.5%) with cholangitis. The mean age of the exposed and unexposed cohort was 30.9 and 31.3 years, respectively. The characteristics and comorbidities showed a non-significant difference between the two cohorts after propensity matching ($P > 0.05$).

Table 2 presents the risk factors of death associated with and without peripartum PLC. Patients with peripartum PLC had a non- significant risk of death compared with the unexposed cohort (aHR = 1.40, 95%CI: 0.89-2.20, $P = 0.149$). Considering their older age and comorbidities, patients with HBV (aHR = 0.48, 95%CI: 0.30-0.77, $P = 0.002$) and cholecystitis (aHR = 0.30, 95%CI: 0.12-0.75) showed a decreased risk of death; patients with cholangitis showed a significantly higher risk of death (aHR = 3.34, 95%CI: 1.49-7.47, $P = 0.003$). Figure 1 illustrates the non-significant difference in the survival curves between the two cohorts ($P = 0.1649$).

The survival rate at different follow-up durations between patients with peripartum PLC and the unexposed cohort (Table 3) revealed a non-significant difference. When followed for less than 0.5 years, 1 year, 3 years, or 5 years, the survival rate in patients with peripartum PLC was lower than that in the unexposed cohort (71.79% *vs* 78.94%; 60.84 *vs* 63.61%; 30.42 *vs* 44.85%; 27.38 *vs* 39.59%), but without a significant difference between the two cohorts ($P > 0.05$).

We enrolled 13440 study subjects to learn more about the influence of age and menopause on survival outcomes. Of these women, 2688 were diagnosed with PLC, younger than 50 years, and without menopause (Table 4). The other group comprised 10752 women who were PLC patients, aged 50 years and older, and with menopause (postmenopausal). The mean ages were 39.7 and 69.1 years, respectively. The percentage of comorbidities had no significant difference between the two cohorts after propensity score matching by age and comorbidities ($P > 0.05$), except alcoholic liver disease ($P = 0.041$).

Table 5 shows the risk factors for developing death. Patients who were diagnosed with PLC at less than 50 years old had a substantially lower risk of death compared with patients diagnosed with PLC at 50 years or older (aHR = 0.64, 95%CI: 0.61-0.68, $P < 0.001$). Patients with HBV (aHR = 0.76, 95%CI: 0.72–0.80, $P < 0.001$), HCV (aHR = 0.72, 95%CI: 0.67-0.78, $P < 0.001$) and cholecystitis (aHR = 0.71, 95%CI: 0.64-0.78, $P < 0.001$) showed a significantly lower risk of developing death. patients with comorbidities such as cirrhosis (aHR = 1.18, 95%CI: 1.13-1.24, $P < 0.001$), and cholangitis (aHR = 1.77, 95%CI: 1.63-1.92, $P < 0.001$) had a notably higher risk of death. Figure 2 shows that the survival rate was significantly higher in women younger than 50 years old with PLC than in the older cohort ($P < 0.001$).

Table 6 presents the survival rates at different follow-up durations. The survival rate in women < 50 years with PLC was significantly higher than in older women with PLC when followed for 0.5 year (72.44% *vs* 64.16%), 1 year (60.57% *vs* 51.66%), 3 years (42.92% *vs* 31.28%), and 5 years (37.02% *vs* 21.83%), respectively ($P < 0.001$).

DISCUSSION

To our knowledge, this large-scale, population-based, cohort study is one of the pioneering research investigations that focused on women under different conditions to determine the relationship between peripartum and postmenopause and the risk of death from liver cancer. Based on our results, despite no significant difference, overall

Table 1 Demographic characteristics and comorbidities of patients with newly diagnosed peripartum primary liver cancer in Taiwan during 1996-2012

Characteristics	Total, *N*	Peripartum primary liver cancer		*P* value
		No, *n* = 160	Yes, *n* = 40	
		n (%) / mean ± SD	*n* (%) / mean ± SD	
Age				0.788
< 30	88	69 (43.1)	19 (47.5)	
30-34	64	53 (33.1)	11 (27.5)	
35-49	48	38 (23.8)	10 (25)	
mean ± SD[1]		31.3 ± 5.1	30.9 ± 4.8	0.673
Baseline comorbidity				
HBV	58	47 (29.4)	11 (27.5)	0.815
Unspecified chronic hepatitis	2	1 (0.6)	1 (2.5)	0.286
Alcoholic liver disease	6	5 (3.1)	1 (2.5)	0.836
Cirrhosis	9	6 (3.8)	3 (7.5)	0.306
Biliary stone	6	4 (2.5)	2 (5)	0.407
Cholecystitis	12	8 (5)	4 (10)	0.234
Cholangitis	10	7 (4.4)	3 (7.5)	0.417

[1]*t*-test, Chi-square test.
HBV: Hepatitis B virus; SD: Standard deviation.

low survival was found in PLCs diagnosed either within or outside of the peripartum period among women of reproductive age (15-49 years old). Our data revealed that five-year survival rates in non-peripartum and peripartum PLCs were 39.59% and 27.38% (aHR = 1.40, 95%CI: 0.89-2.20, *P* = 0.149), respectively. However, postmenopausal women (> 50 years old) with PLCs have a considerable decrease in survival rates (five-year survival rates in fertile and postmenopausal women were 37.02% and 21.83%, respectively), compared with a significantly higher risk of death in premenopausal female patients (aHR = 0.64; 95%CI: 0.61-0.68). Although the molecular mechanisms underlying this protective effect are complicated, previous research suggested that the inhibitory role of estrogen was responsible for the gender disparity of PLCs partly *via* micro RNA, DNA repair, and obesity-associated pathways[7]. Moreover, the number of estrogen receptors (ERs) correlated with the risk of tumor occurrence and invasion. Some research proposed that ERs suppressed the proliferation and progression of liver cancer by decreasing the peroxisome proliferator-activated receptor γ and transcription of metastatic tumor antigen 1[24,25]. In the time of limited estrogen supply (*e.g.*, Postmenopause), sex hormone binding globulin (SHBG), a plasma protein that involved in the maintenance of a reservoir of sex steroid hormones, played a crucial role in potentiating estrogenic action[26].

We focused on women of childbearing age to gain a deeper understanding of the influence of reproductive hormones. Because of the elevation of estrogen and progesterone during pregnancy, the diagnosis of PLCs within this period is rare. Nevertheless, among the 62 cases reported to date worldwide, all ended with poor outcomes when compared with non-pregnant women with PLCs[10]. As early as in 1995, Lau and his colleague[27] concluded that pregnancy has an adverse effect on the prognosis of patients with HCC, and therefore measurement of AFP level is recommended for screening HCC in pregnant women at high risk. The largest retrospective review published by Choi *et al*[12] demonstrated poor yet improving survival rates over time (median survivals of the groups before and during/after 1995 were 18 and 25.5 mo, respectively) among all 48 HCC cases in pregnancy. Contrary to prior research, our analysis of the nationwide database revealed an overall unpleasant prognosis among women of childbearing age. There was no significant difference in survival rates between parous and non-parous women with PLCs. This could probably be explained by the limited number of cases and the nationwide coverage of health insurance. Since almost all women received check-ups during the prenatal and

Table 2 Cox model measured hazard ratios and 95% confidence intervals of death associated non-peripartum primary liver cancer and peripartum primary liver cancer patients

Characteristics	Event, *n* = 124	Person, yr	IR	Crude		Adjusted	
				HR (95%CI)	*P* value	HR (95%CI)	*P* value
Peripartum primary liver cancer							
No	97	587	16.53	Ref.		Ref.	
Yes	27	99	27.17	1.35 (0.88-2.08)	0.166	1.40 (0.89-2.20)	0.149
Age at baseline							
< 30	54	350	15.44	Ref.		Ref.	
30-34	39	169	23.08	1.21 (0.80-1.84)	0.359	1.47 (0.95-2.28)	0.083
35-49	31	167	18.52	1.29 (0.83-2.00)	0.266	1.13 (0.69-1.85)	0.617
Baseline comorbidity							
HBV	27	230	11.72	0.52 (0.34-0.80)	0.003	0.48 (0.30-0.77)	0.002
Unspecified chronic hepatitis	1	11	9.04	0.86 (0.12-6.17)	0.882	0.56 (0.08-4.10)	0.565
Alcoholic liver disease	6	8	73.37	2.85 (1.25-6.49)	0.013	2.15 (0.73-6.36)	0.165
Cirrhosis	7	40	17.55	1.12 (0.52-2.40)	0.773	1.49 (0.57-3.90)	0.411
Biliary stone	4	11	35.47	1.23 (0.45-3.33)	0.686	0.64 (0.17-2.35)	0.499
Cholecystitis	5	80	6.26	0.43 (0.18-1.06)	0.066	0.30 (0.12-0.75)	0.010
Cholangitis	7	4	179.30	3.76 (1.71-8.26)	< 0.001	3.34 (1.49-7.47)	0.003

Adjusted HR: Adjusted for gender, age, and all comorbidities in Cox proportional hazards regression; CI: Confidence interval; HR: Hazard ratios; HBV: Hepatitis B virus; IR: Incidence rate.

Table 3 Survival rates of different follow-up durations between non-peripartum primary liver cancer and peripartum primary liver cancer patients

Follow-up duration	Survival rate (%)		*P* value
	Non-peripartum primary liver cancer	Peripartum primary liver cancer	
≤ 0.5	78.94	71.79	0.254
≤ 1	63.61	60.84	0.611
≤ 3	44.85	30.42	0.111
≤ 5	39.59	27.38	0.117

postnatal period under the national health insurance program, proper management could be provided in time to improve outcomes.

Because menopause represents a state of gradual estrogen deficiency in the setting of physiologic aging, we also divided the study population into two groups by age, either younger or older than 50 years. According to Yang's research[28] investigating patients with HCC, women of 18 years old to 64 years old were noted as having longer survival than men of the same age, with the largest difference in survival among women aged 18 years to 44 years. Furthermore, Shimizu *et al*[29] reported that hepatic ER levels, which were inversely related to the progression of HCC, were significantly higher in premenopausal women compared with postmenopausal women. While El Mahdy Korah *et al*[30] stated that there was no clear relationship between sex hormone and HCC development or progression by analyzing total testosterone, estrogen, progesterone and prolactin levels among 40 selected HCC patients, Petrick's cohort study in 2019[31] indicated that higher levels of SHBG and circulating estradiol were associated with an increased risk of HCC and ICC, respectively, among women after menopause. These data suggest that climacteric status may adversely mediate the outcomes of PLCs. Our results are consistent with those of previous studies, that

Table 4 Demographic characteristics and comorbidities of female patients newly diagnosed with and without menopause primary liver cancer patients in Taiwan during 1996-2012

Characteristics	Total, *N* = 13440	Liver cancer		*P* value
		≥ 50 yr, *n* = 10752	< 50 yr, *n* = 2688	
		n (%)/mean ± SD	*n* (%)/mean ± SD	
Age				
mean ± SD[1]		69.1 ± 9.6	39.7 ± 10.5	< 0.001
Baseline comorbidity				
HBV	2971	2358 (21.9)	613 (22.8)	0.329
HCV	1168	931 (8.7)	237 (8.8)	0.795
Unspecified chronic hepatitis	780	619 (5.8)	161 (6)	0.645
Alcoholic liver disease	211	157 (1.5)	54 (2)	0.041
Cirrhosis	4142	3321 (30.9)	821 (30.5)	0.730
Biliary stone	1269	1012 (9.4)	257 (9.6)	0.813
Cholecystitis	626	489 (4.5)	137 (5.1)	0.227
Cholangitis	818	649 (6)	169 (6.3)	0.626

[1]*t*-test, Chi-square test.
HBV: Hepatitis B virus; HCV: Hepatitis C virus; SD: Standard deviation.

Table 5 Cox model measured hazard ratios and 95% confidence intervals of death associated with and without menopause primary liver cancer patients

Characteristics	Event, *N* = 9982	Person, yr	IR	Crude		Adjusted	
				HR (95%CI)	*P* value	HR (95%CI)	*P* value
Liver cancer							
≥ 50 yr	8279	23410	35.37	Ref.		Ref.	
< 50 yr	1703	9149	18.61	0.65 (0.61-0.68)	< 0.001	0.64 (0.61-0.68)	< 0.001
Baseline comorbidity							
HBV	2049	7552	27.13	0.81 (0.77-0.85)	< 0.001	0.76 (0.72-0.80)	< 0.001
HCV	831	3513	23.65	0.75 (0.70-0.81)	< 0.001	0.72 (0.67-0.78)	< 0.001
Unspecified chronic hepatitis	584	2224	26.25	0.90 (0.83-0.98)	0.015	0.96 (0.88-1.05)	0.349
Alcoholic liver disease	165	449	36.72	1.04 (0.89-1.21)	0.640	1.07 (0.91-1.25)	0.408
Cirrhosis	3186	9924	32.10	1.01 (0.97-1.05)	0.739	1.18 (1.13-1.24)	< 0.001
Biliary stone	955	2730	34.98	1.08 (1.01-1.15)	0.024	0.98 (0.91-1.05)	0.562
Cholecystitis	416	2162	19.25	0.70 (0.63-0.77)	< 0.001	0.71 (0.64-0.78)	< 0.001
Cholangitis	687	989	69.45	1.76 (1.63-1.91)	< 0.001	1.77 (1.63-1.92)	< 0.001

Adjusted HR: Adjusted for comorbidities in Cox proportional hazards regression; CI: Confidence interval; HR: Hazard ratios; HBV: Hepatitis B virus; HCV: Hepatitis C virus; IR: Incidence rate.

implied a negative interplay between age and hormonal factors in the disease course since women beyond reproductive age (> 50 years old) with PLCs were found to have lower half-year, one-year, three-year, and five-year survival rates. Although it is difficult to distinguish how the two factors account for the consequence individually, it is certain that they interact with each other. This interaction results in diminishing immunologic responses to injury, and the imbalance between antioxidant formation and oxidative stress.

Table 6 Survival rates of different follow-up durations between primary liver cancer patients with and without menopause

Follow-up duration	Survival rate (%)		*P* value
	≥ 50 yr	< 50 yr	
≤ 0.5	64.16	72.44	< 0.001
≤ 1	51.66	60.57	< 0.001
≤ 3	31.28	42.92	< 0.001
≤ 5	21.83	37.02	< 0.001

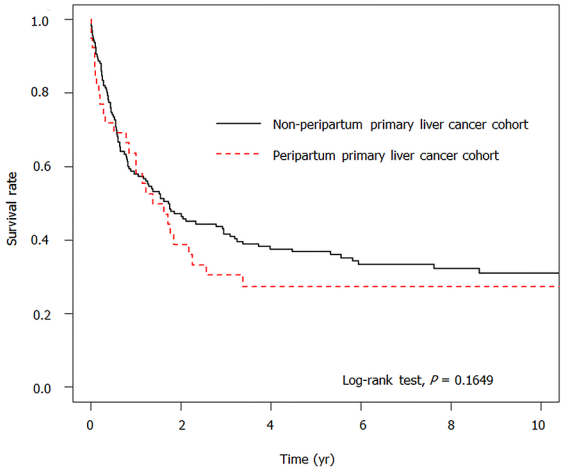

Figure 1 The estimated survival rates between non-peripartum primary liver cancer and peripartum primary liver cancer patients by Kaplan-Meier analysis.

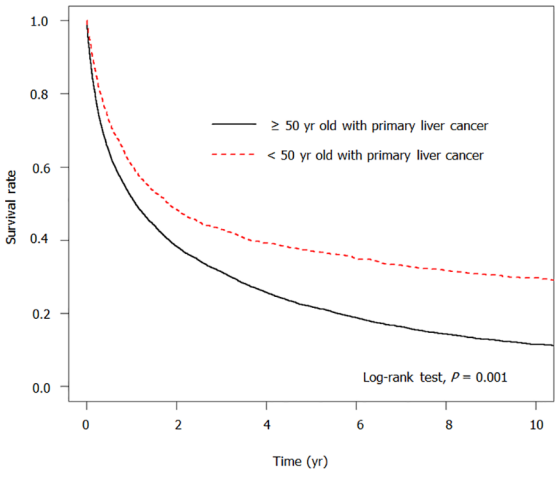

Figure 2 The estimated survival rates between patients younger than 50 years old with primary liver cancer (without menopause) and those older with primary liver cancer (with menopause) by Kaplan-Meier analysis.

The use of a broad, representative, nationwide, population-based sample to observe the survival outcome of PLC in reproductive and postmenopausal female patients increased the validity of the results. Nevertheless, these results should be interpreted with caution because of several limitations in this study. First, detailed information related to the risk of PLC is not available. This information includes data on body mass index, smoking and alcohol use, high-fat diet, lower physical activity lifestyle, history of receiving HRT, and family history of PLC. Second, tumor burden, staging, and management strategies of PLC are not accessible from the NHIRD and therefore cannot be analyzed. Third, defining menopause by age alone may not be comprehensive enough since it is hard to make an optimal covariate adjustment. Fourth, the

generalization of the findings to Western or non-Taiwanese populations is a concern. For instance, the high incidence of PLC warrants further follow-up in other populations. Fifth, the small number of cases during the peripartum period may lead to biased findings. Hence, future studies with an improved design, larger sample sizes, and better control of confounding factors are required to enable a more thorough understanding.

CONCLUSION

In summary, among female patients with PLC, we found a trend for older age to be associated with increased risk for both incidence and mortality of PLC. In contrast, no apparent relationship was noted between pregnancy and prognosis. Even though subsequent clinical studies are necessary for further validation, the present research demonstrates that age and hormonal factors have a protective influence on the occurrence and deterioration of PLCs. Moreover, patients with more risk factors are recommended to follow up regularly to achieve a better prognosis.

ARTICLE HIGHLIGHTS

Research background

Primary liver cancer (PLC), the sixth most common cancer, accounts for the fourth leading cause of cancer-related death worldwide. Given the continuous rise of the global burden, there are increasing concerns about PLC outcomes in different populations.

Research motivation

For a long time, most studies about PLC put their focus on men due to higher incidence and riskier morbidities compared to women. Even with growing evidence on the protective effects of female sex hormones in animal research, few clinical cohorts pay attention to women with PLCs. Therefore, we are interested in the issue of how female reproductive status is related to the prognosis of PLCs.

Research objectives

This study aimed to assess whether peripartum and postmenopausal women with PLC have lower overall survival rates in a large cohort of subjects in Taiwan.

Research methods

This is a retrospective cohort of the PLC prognosis among peripartum, non-peripartum, premenopausal, and postmenopausal women using the Taiwan National Health Insurance Research Database from 2000-2012. There were 200 eligible subjects enrolled in the study of peripartum PLC, whereas 13440 subjects enrolled in the research of menopausal PLC. 4:1 Propensity score matching was applied to adjust the covariates.

Research results

While the survival rate was overall lower in patients with peripartum PLC, there was no significant difference in the risk of death and the survival rate at different follow-up durations among patients with/without peripartum PLC. In the menopausal PLC cohort, significantly lower risk of death (aHR = 0.64, 95%CI: 0.61-0.68, $P < 0.001$) and higher survival rate when followed for 0.5 year (72.44% *vs* 64.16%), 1 year (60.57% *vs* 51.66%), 3 years (42.92% *vs* 31.28%), and 5 years were seen in patients diagnosed with PLC younger than 50 years old (without menopause) compared with patients diagnosed with PLC at or older than 50 years (with menopause).

Research conclusions

According to our dataset, it is concluded that younger age and female hormonal factors may reduce the occurrence and deterioration of PLCs. Females with paripartum PLC have no difference in survival rates compared with those patients without peripartum. Menopausal females with PLC have worse survival rates compared with those patients without menopause.

Research perspectives

To further clarify the association between sexual hormone and PLC outcome, future studies with more detailed information and better-controlled confounders are required.

REFERENCES

1 **Bray F**, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* 2018; **68**: 394-424 [PMID: 30207593 DOI: 10.3322/caac.21492]

2 **Lin L**, Yan L, Liu Y, Qu C, Ni J, Li H. The Burden and Trends of Primary Liver Cancer Caused by Specific Etiologies from 1990 to 2017 at the Global, Regional, National, Age, and Sex Level Results from the Global Burden of Disease Study 2017. *Liver Cancer* 2020; **9**: 563-582 [PMID: 33083281 DOI: 10.1159/000508568]

3 **Massarweh NN**, El-Serag HB. Epidemiology of Hepatocellular Carcinoma and Intrahepatic Cholangiocarcinoma. *Cancer Control* 2017; **24**: 1073274817729245 [PMID: 28975830 DOI: 10.1177/1073274817729245]

4 **Rawla P**, Sunkara T, Muralidharan P, Raj JP. Update in global trends and aetiology of hepatocellular carcinoma. *Contemp Oncol (Pozn)* 2018; **22**: 141-150 [PMID: 30455585 DOI: 10.5114/wo.2018.78941]

5 **Petrick JL**, Freedman ND, Demuth J, Yang B, Van Den Eeden SK, Engel LS, McGlynn KA. Obesity, diabetes, serum glucose, and risk of primary liver cancer by birth cohort, race/ethnicity, and sex: Multiphasic health checkup study. *Cancer Epidemiol* 2016; **42**: 140-146 [PMID: 27148890 DOI: 10.1016/j.canep.2016.04.009]

6 **Global Burden of Disease Liver Cancer Collaboration**, Akinyemiju T, Abera S, Ahmed M, Alam N, Alemayohu MA, Allen C, Al-Raddadi R, Alvis-Guzman N, Amoako Y, Artaman A, Ayele TA, Barac A, Bensenor I, Berhane A, Bhutta Z, Castillo-Rivas J, Chitheer A, Choi JY, Cowie B, Dandona L, Dandona R, Dey S, Dicker D, Phuc H, Ekwueme DU, Zaki MS, Fischer F, Fürst T, Hancock J, Hay SI, Hotez P, Jee SH, Kasaeian A, Khader Y, Khang YH, Kumar A, Kutz M, Larson H, Lopez A, Lunevicius R, Malekzadeh R, McAlinden C, Meier T, Mendoza W, Mokdad A, Moradi-Lakeh M, Nagel G, Nguyen Q, Nguyen G, Ogbo F, Patton G, Pereira DM, Pourmalek F, Qorbani M, Radfar A, Roshandel G, Salomon JA, Sanabria J, Sartorius B, Satpathy M, Sawhney M, Sepanlou S, Shackelford K, Shore H, Sun J, Mengistu DT, Topór-Mądry R, Tran B, Ukwaja KN, Vlassov V, Vollset SE, Vos T, Wakayo T, Weiderpass E, Werdecker A, Yonemoto N, Younis M, Yu C, Zaidi Z, Zhu L, Murray CJL, Naghavi M, Fitzmaurice C. The Burden of Primary Liver Cancer and Underlying Etiologies From 1990 to 2015 at the Global, Regional, and National Level: Results From the Global Burden of Disease Study 2015. *JAMA Oncol* 2017; **3**: 1683-1691 [PMID: 28983565 DOI: 10.1001/jamaoncol.2017.3055]

7 **Li Y**, Xu A, Jia S, Huang J. Recent advances in the molecular mechanism of sex disparity in hepatocellular carcinoma. *Oncol Lett* 2019; **17**: 4222-4228 [PMID: 30988804 DOI: 10.3892/ol.2019.10127]

8 **Sukocheva OA**. Estrogen, estrogen receptors, and hepatocellular carcinoma: Are we there yet? *World J Gastroenterol* 2018; **24**: 1-4 [PMID: 29358876 DOI: 10.3748/wjg.v24.i1.1]

9 **Ma WL**, Lai HC, Yeh S, Cai X, Chang C. Androgen receptor roles in hepatocellular carcinoma, fatty liver, cirrhosis and hepatitis. *Endocr Relat Cancer* 2014; **21**: R165-R182 [PMID: 24424503 DOI: 10.1530/ERC-13-0283]

10 **Matsuo M**, Furukawa K, Shimizu H, Yoshitomi H, Takayashiki T, Kuboki S, Takano S, Suzuki D, Sakai N, Kagawa S, Nojima H, Ohsuka M. Novel treatment strategy with radiofrequency ablation and surgery for pregnant patients with hepatocellular carcinoma: a case report. *Surg Case Rep* 2018; **4**: 43 [PMID: 29721779 DOI: 10.1186/s40792-018-0434-3]

11 **Li AJ**, Zhou WP, Lu JH, Cui LJ, Yang XY, Yin L, Wu MC. Surgery for pregnancy-associated primary hepatocellular carcinoma: Report of four cases. *Int J Surg Case Rep* 2014; **5**: 882-885 [PMID: 25462058 DOI: 10.1016/j.ijscr.2014.06.003]

12 **Choi KK**, Hong YJ, Choi SB, Park YN, Choi JS, Lee WJ, Kim KS. Hepatocellular carcinoma during pregnancy: is hepatocellular carcinoma more aggressive in pregnant patients? *J Hepatobiliary Pancreat Sci* 2011; **18**: 422-431 [PMID: 21116657 DOI: 10.1007/s00534-010-0345-6]

13 **Giannitrapani L**, Soresi M, La Spada E, Cervello M, D'Alessandro N, Montalto G. Sex hormones and risk of liver tumor. *Ann N Y Acad Sci* 2006; **1089**: 228-236 [PMID: 17261770 DOI: 10.1196/annals.1386.044]

14 **Hsing AW**, McLaughlin JK, Hoover RN, Co Chien HT, Blot WJ, Fraumeni JF Jr. Parity and primary liver cancer among young women. *J Natl Cancer Inst* 1992; **84**: 1118-1119 [PMID: 1619686 DOI: 10.1093/jnci/84.14.1118]

15 **Garko SB**, David OS, Mohammed T, Isah MS, Bakari AG, Oguntayo AO, Shehu MS, Aminu SM. Hepatocellular carcinoma in pregnancy. *Ann Afr Med* 2009; **8**: 284-286 [PMID: 20139556 DOI: 10.4103/1596-3519.59588]

16 **Russell P**, Sanjay P, Dirkzwager I, Chau K, Johnston P. Hepatocellular carcinoma during pregnancy: case report and review of the literature. *N Z Med J* 2012; **125**: 141-145 [PMID: 22522274]

17 **Shimizu I**. Impact of oestrogens on the progression of liver disease. *Liver Int* 2003; **23**: 63-69 [PMID: 12640729 DOI: 10.1034/j.1600-0676.2003.00811.x]

18 **Brady CW**. Liver disease in menopause. *World J Gastroenterol* 2015; **21**: 7613-7620 [PMID: 26167064 DOI: 10.3748/wjg.v21.i25.7613]

19 **Zhong GC**, Liu Y, Chen N, Hao FB, Wang K, Cheng JH, Gong JP, Ding X. Reproductive factors, menopausal hormone therapies and primary liver cancer risk: a systematic review and dose-response meta-analysis of observational studies. *Hum Reprod Update* 2016; **23**: 126-138 [PMID: 27655589 DOI: 10.1093/humupd/dmw037]

20 **Hassan MM**, Botrus G, Abdel-Wahab R, Wolff RA, Li D, Tweardy D, Phan AT, Hawk E, Javle M, Lee JS, Torres HA, Rashid A, Lenzi R, Hassabo HM, Abaza Y, Shalaby AS, Lacin S, Morris J, Patt YZ, Amos CI, Khaderi SA, Goss JA, Jalal PK, Kaseb AO. Estrogen Replacement Reduces Risk and Increases Survival Times of Women With Hepatocellular Carcinoma. *Clin Gastroenterol Hepatol* 2017; **15**: 1791-1799 [PMID: 28579181 DOI: 10.1016/j.cgh.2017.05.036]

21 Research on the menopause in the 1990s: report of a WHO scientific group. [cited 27 December 2020]. Available from: https://apps.who.int/iris/handle/10665/41841

22 **Feasey R**. Infertility and Non-Traditional Family Building: From Assisted Reproduction to Adoption in the Media, 1st ed.; Palgrave Macmillan: London, U.K., 2019 [DOI: 10.1007/978-3-030-17787-4]

23 **Shen TY**, Strong C, Yu T. Age at menopause and mortality in Taiwan: A cohort analysis. *Maturitas* 2020; **136**: 42-48 [PMID: 32386665 DOI: 10.1016/j.maturitas.2020.04.008]

24 **Lin YM**, Velmurugan BK, Yeh YL, Tu CC, Ho TJ, Lai TY, Tsai CH, Tsai FJ, Huang CY. Activation of estrogen receptors with E2 downregulates peroxisome proliferator-activated receptor γ in hepatocellular carcinoma. *Oncol Rep* 2013; **30**: 3027-3031 [PMID: 24126791 DOI: 10.3892/or.2013.2793]

25 **Deng L**, Yang H, Tang J, Lin Z, Yin A, Gao Y, Wang X, Jiang R, Sun B. Inhibition of MTA1 by ERα contributes to protection hepatocellular carcinoma from tumor proliferation and metastasis. *J Exp Clin Cancer Res* 2015; **34**: 128 [PMID: 26503703 DOI: 10.1186/s13046-015-0248-0]

26 **Lee SR**, Lee YH, Yang H, Lee HW, Lee GS, An BS, Jeung EB, Park BK, Hong EJ. Sex hormone-binding globulin suppresses NAFLD-triggered hepatocarcinogenesis after menopause. *Carcinogenesis* 2019; **40**: 1031-1041 [PMID: 31168625 DOI: 10.1093/carcin/bgz107]

27 **Lau WY**, Leung WT, Ho S, Lam SK, Li CY, Johnson PJ, Williams R, Li AK. Hepatocellular carcinoma during pregnancy and its comparison with other pregnancy-associated malignancies. *Cancer* 1995; **75**: 2669-2676 [PMID: 7743468 DOI: 10.1002/1097-0142(19950601)75:11<2669::aid-cncr2820751105>3.0.co;2-b]

28 **Yang D**, Hanna DL, Usher J, LoCoco J, Chaudhari P, Lenz HJ, Setiawan VW, El-Khoueiry A. Impact of sex on the survival of patients with hepatocellular carcinoma: a Surveillance, Epidemiology, and End Results analysis. *Cancer* 2014; **120**: 3707-3716 [PMID: 25081299 DOI: 10.1002/cncr.28912]

29 **Shimizu I**, Inoue H, Yano M, Shinomiya H, Wada S, Tsuji Y, Tsutsui A, Okamura S, Shibata H, Ito S. Estrogen receptor levels and lipid peroxidation in hepatocellular carcinoma with hepatitis C virus infection. *Liver* 2001; **21**: 342-349 [PMID: 11589771 DOI: 10.1034/j.1600-0676.2001.210507.x]

30 **El Mahdy Korah T**, Abd Elfatah Badr E, Mohamed Emara M, Ahmed Samy Kohla M, Gamal Saad Michael G. Relation between sex hormones and hepatocellular carcinoma. *Andrologia* 2016; **48**: 948-955 [PMID: 26791111 DOI: 10.1111/and.12536]

31 **Petrick JL**, Florio AA, Zhang X, Zeleniuch-Jacquotte A, Wactawski-Wende J, Van Den Eeden SK, Stanczyk FZ, Simon TG, Sinha R, Sesso HD, Schairer C, Rosenberg L, Rohan TE, Purdue MP, Palmer JR, Linet MS, Liao LM, Lee IM, Koshiol J, Kitahara CM, Kirsh VA, Hofmann JN, Guillemette C, Graubard BI, Giovannucci E, Gaziano JM, Gapster SM, Freedman ND, Engel LS, Chong DQ, Chen Y, Chan AT, Caron P, Buring JE, Bradwin G, Beane Freeman LE, Campbell PT, McGlynn KA. Associations Between Prediagnostic Concentrations of Circulating Sex Steroid Hormones and Liver Cancer Among Postmenopausal Women. *Hepatology* 2020; **72**: 535-547 [PMID: 31808181 DOI: 10.1002/hep.31057]

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ORIGINAL ARTICLE

Retrospective Cohort Study

Nonalcoholic fatty liver disease is associated with worse intestinal complications in patients hospitalized for *Clostridioides difficile* infection

Yi Jiang, Salil Chowdhury, Bing-Hong Xu, Mohamad Aghaie Meybodi, Konstantinos Damiris, Samanthika Devalaraju, Nikolaos Pyrsopoulos

ORCID number: Yi Jiang 0000-0001-5114-0183; Salil Chowdhury 0000-0002-4310-2328; Bing-Hong Xu 0000-0001-6660-3558; Mohamad Aghaie Meybodi 0000-0002-5321-688X; Konstantinos Damiris 0000-0001-9972-740X; Samanthika Devalaraju 0000-0002-1095-687X; Nikolaos Pyrsopoulos 0000-0002-6950-8174.

Author contributions: Jiang Y and Pyrsopoulos N planned and designed the study; Chowdhury S, Xu BH, Meybodi MA, Damiris K and Devalaraju S conducted the data collection and interpretation; Jiang Y, Chowdhury S, Xu BH, Damiris K and Devalaraju S contributed to the manuscript preparation; All authors contributed to the manuscript revisions, reviewed, and approved the final submitted manuscript.

Institutional review board statement: This retrospective cohort study did not directly involve any patients in the data collection process and the National Inpatient Sample (NIS) database is de-identified and available for the public. Therefore, Institutional Review Board approval was not required.

Yi Jiang, Salil Chowdhury, Mohamad Aghaie Meybodi, Konstantinos Damiris, Samanthika Devalaraju, Department of Medicine, Rutgers New Jersey Medical School, Newark, NJ 07101, United States

Bing-Hong Xu, Liver Center and Center for Asian Health, RWJBH-Saint Barnabas Medical Center, Florham Park, NJ 07932, United States

Nikolaos Pyrsopoulos, Department of Medicine, Gastroenterology and Hepatology, Rutgers New Jersey Medical School, Newark, NJ 07101, United States

Corresponding author: Nikolaos Pyrsopoulos, FAASLD, AGAF, FACG, MD, PhD, Director, Professor, Department of Medicine, Gastroenterology and Hepatology, Rutgers New Jersey Medical School, 185 S. Orange Avenue, Medical Science Building H-536, Newark, NJ 07101, United States. pyrsopni@njms.rutgers.edu

Abstract

BACKGROUND

Nonalcoholic fatty liver disease (NAFLD) has become the leading cause of chronic liver disease with increasing prevalence worldwide. *Clostridioides difficile* infection (CDI) remains the most common cause of nosocomial diarrhea in developed countries.

AIM

To assess the impact of NAFLD on the outcomes of hospitalized patients with CDI.

METHODS

This study was a retrospective cohort study. The Nationwide Inpatient Sample database was used to identify a total of 7239 adults admitted as inpatients with a primary diagnosis of CDI and coexisting NAFLD diagnosis from 2010 to 2014 using ICD-9 codes. Patients with CDI and coexisting NAFLD were compared to those with CDI and coexisting alcoholic liver disease (ALD) and viral liver disease (VLD), individually. Primary outcomes included mortality, length of stay, and total hospitalization charges. Secondary outcomes were in-hospital complications. Multivariate regression was used for outcome analysis after adjusting for possible

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confounders.

RESULTS
CDI with NAFLD was independently associated with lower rates of acute respiratory failure (2.7% *vs* 4.2%, $P < 0.01$; 2.7% *vs* 4.2%, $P < 0.05$), shorter length of stay (days) (5.75 ± 0.16 *vs* 6.77 ± 0.15, $P < 0.001$; 5.75 ± 0.16 *vs* 6.84 ± 0.23, $P < 0.001$), and lower hospitalization charges (dollars) (38150.34 ± 1757.01 *vs* 46326.72 ± 1809.82, $P < 0.001$; 38150.34 ± 1757.01 *vs* 44641.74 ± 1660.66, $P < 0.001$) when compared to CDI with VLD and CDI with ALD, respectively. CDI with NAFLD was associated with a lower rate of acute kidney injury (13.0% *vs* 17.2%, $P < 0.01$), but a higher rate of intestinal perforation ($P < 0.01$) when compared to VLD. A lower rate of mortality (0.8% *vs* 2.7%, $P < 0.05$) but a higher rate of intestinal obstruction (4.6% *vs* 2.2%, $P = 0.001$) was also observed when comparing CDI with NAFLD to ALD.

CONCLUSION
Hospitalized CDI patients with NAFLD had more intestinal complications compared to CDI patients with VLD and ALD. Gut microbiota dysbiosis may contribute to the pathogenesis of intestinal complications.

Key Words: Nonalcoholic fatty liver disease; *Clostridioides difficile* infection; Gut microbiota; Intestinal complications; Alcoholic liver disease; Viral liver disease

Core Tip: This study demonstrated that patients hospitalized with *Clostridioides difficile* infection (CDI) and coexisting nonalcoholic fatty liver disease (NAFLD) had more favorable overall outcomes but higher rates of intestinal complications when compared to those with alcoholic liver disease and viral liver disease individually, which suggests altering gut microbiota may play an essential role in the pathogenesis of both CDI and NAFLD. NAFLD-associated metabolic syndrome may contribute significantly to gut dysbiosis and increase risk for CDI and its complications. This study provides potential directions for future prospective clinical research to identify the clinical meaningfulness of interactions between the gut microbiota, gut immunity and systemic inflammation.

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INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is a heterogeneous disease with a spectrum from simple steatosis to nonalcoholic steatohepatitis (NASH), cirrhosis, and hepatocellular carcinoma[1,2]. With a prevalence of 10 to 46 percent in the United States and 6% to 35% worldwide[3,4], NAFLD has become the leading cause of chronic liver disease, and its prevalence continues to increase, paralleled by the increase of obesity and type 2 diabetes[5].

Clostridioides difficile (*C. difficile*) is a gram-positive, spore-forming bacterium, known as the most common pathogen causing nosocomial diarrhea in developed countries [6]. Symptoms of *C. difficile* infection (CDI) range from mild to severe diarrhea, which can progress to sepsis, fulminant colitis, and bowel perforation[7]. Severe colitis may also present as ileus and megacolon, which are characterized by symptoms of intestinal obstruction[8,9]. Gut microbiota dysbiosis due to the administration of antibiotics is the most prominent risk factor for the development of CDI. Advanced age, prolonged hospitalization and gastric acid suppression are some common additional risk factors for CDI[10,11].

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Recently, a number of animal and human studies have revealed the role of the gut microbiota in the pathophysiology of NAFLD. It is proposed that dysbiosis-induced dysregulation of the gut barrier function and translocation of the bacteria link the gut microbiome to NAFLD[12,13]. In addition, it has been well documented that patients with chronic liver disease are more susceptible to CDI due to frequent hospitalization and antibiotics use. Specifically, recent studies have observed that NAFLD is an independent risk factor for CDI by single-centered retrospective design[14,15].

Although a strong association between NAFLD and CDI has been observed, gut microbiota dysbiosis likely plays a vital role in the pathogenesis of both aforementioned diseases. However, the inpatient outcomes of CDI in the NAFLD population, have not been well studied in large populations. The aim of this nationwide study was to assess the impact of NAFLD on the outcomes of hospitalized patients with CDI.

MATERIALS AND METHODS

Data source and study population

The largest all-payer inpatient care database in the United States, the Nationwide Inpatient Sample (NIS) database was accessed. The NIS database represents approximately 20% of all inpatient hospitalizations. Weighted, it estimates more than 35 million hospitalizations nationally[16]. It includes demographic information (age, sex, race, income), hospital characteristics (*e.g.*, bed size, type), insurance status, discharge status, diagnoses and procedures (identified by The International Classification of Diseases-Ninth Edition Revision Clinical Modification (ICD-9 CM) codes), total hospitalization charges, length of stay (LOS), severity and other comorbidity measures. Yearly sampling weights are applied to generate national estimates.

This retrospective cohort study examined all adult (18-90 years old) patients hospitalized with CDI as the primary diagnosis from 2010 to 2014. Within this CDI population, patients with NAFLD were selected to compare to those with viral liver disease (VLD) (including hepatitis B infection and hepatitis C infection) and those with alcoholic liver disease (ALD). Notably, CDI was identified by ICD-9 CM code 008.45. NAFLD was identified by ICD-9 CM code 571.80 with the exclusion of all diagnostic codes for previous organ recipients and donors as well as other causes of chronic liver disease including hepatitis B and hepatitis C infection, ALD, hemochromatosis, primary biliary cholangitis, autoimmune hepatitis, and other unspecified liver diseases. The diagnosis of VLD was identified by the ICD-9 CM codes for hepatitis B and C caused liver diseases with the exclusion of previous organ recipients and donors, as well as other causes of chronic liver disease including NAFLD, ALD, hemochromatosis, primary biliary cholangitis, autoimmune hepatitis, and other unspecified liver diseases. Similarly, ALD was identified by the ICD-9 CM codes for ALD with the exclusion of previous organ recipients and donors as well as other causes of chronic liver disease including NAFLD, VLD, hemochromatosis, primary biliary cholangitis, autoimmune hepatitis, and other unspecified liver diseases (see Supplementary Table 1, supplemental digital content 1, which demonstrates ICD-9 diagnostic and procedure codes). VLD and ALD were assessed as separate groups which excluded patients with concomitant diagnoses of VLD and ALD. Information such as patients' demographics, comorbidities, disposition, selected outcomes and surgical interventions were extracted from the NIS database. Elixhauser Comorbidity Index (ECI)[17], which measures 29 general medical conditions, then assigns different weights to compile a longitudinal score, allowing for further description of comorbidity burden.

Primary outcomes included mortality, length of stay, and total hospitalization charges. Secondary outcomes were CDI related complications and interventions.

Statistical analysis

SAS Survey Procedures (SAS 9.4, SAS Institute Inc, Cary, NC, United States) was utilized for all statistical analyses. The national estimates were calculated after accounting for sample design elements (clusters, strata, and trend weights) provided by the NIS. Continuous variables were reported as weighted mean ± SE; categorical variables were reported as weighted numbers (*n*) and percentages (%). The SEs of weighted means were estimated using the Taylor linearization method that incorporated the sample design. Weighted Student's *t*-tests were used to analyze the normally distributed continuous variables, while Rao-Scott modified chi-square tests were used to test the difference of distribution for categorical variables. Wilcoxon Rank-Sum Tests were used to test the variables that are not normally distributed.

Multivariate linear regression was used to estimate the average change in LOS and total hospitalization charges after adjusting for patient demographics, hospital characteristics, insurance type, median household income, ECI score, obesity, diabetes, tobacco use disorder, hypertension, dyslipidemia, cirrhosis and its complications, numbers of cirrhosis complications, and hepatocellular carcinoma. Multivariate logistic regression was used to estimate the odds ratio (OR) of mortality, CDI complications and interventions after adjusting for the same confounding variables as noted above.

The statistical methods of this study were reviewed by Dr. Chunyi Wu, PhD of Epidemiology from University of Michigan Medical School.

RESULTS

Patient demographics and baseline characteristics

From 2010 to 2014, the numbers of patients hospitalized for CDI with coexisting NAFLD, VLD and ALD were 7239, 11857 and 5938, respectively. The CDI with NAFLD cohort in this study was predominantly Caucasian with an average age 56.3 years old. In the aforementioned cohort, 69.4% of the patients were female, 41.6% were admitted to southern hospitals, and 58.6% were admitted to large hospitals (Table 1). Compared to CDI with VLD or ALD individually, the CDI with NAFLD group had significantly more patients in the 18-39 and greater than 70-year-old age groups (P < 0.0001), were more likely to be female (P < 0.0001), from the southern hospital region (P < 0.0001), and less likely to be Medicaid insured (P < 0.0001). Additionally, the CDI with coexisting VLD group was associated with a higher percentage of African American patients and had less patients with a high household income (Q3 and Q4, median household income for ZIP code between 51th and 100th percentile) compared to the CDI with NAFLD group.

In regard to comorbidities (Table 2), when compared to the CDI with VLD or ALD groups individually, CDI patients with NAFLD had a greater prevalence of obesity (P < 0.0001, P < 0.0001), diabetes (P < 0.0001, P < 0.0001), hypertension (P = 0.0006, P < 0.0001) and dyslipidemia (P < 0.0001, P < 0.0001). CDI with NAFLD was also associated with a significantly lower rate of cirrhosis (P < 0.0001, P < 0.0001) when compared to the other two groups. None of the patients in the CDI with NAFLD group had cirrhosis-related ascites, esophageal varices bleeding, spontaneous bacterial peritonitis or hepatorenal syndrome. Moreover, a lower rate of hepatocellular carcinoma (P < 0.0001, P = 0.0217) was observed in the CDI with NAFLD group compared to the CDI with VLD or ALD groups individually.

Outcomes and regression analysis of CDI patients with NAFLD vs VLD

When compared to the CDI with NAFLD group, the CDI with VLD group was associated with higher rates of acute kidney injury (AKI) [adjusted OR (aOR) = 1.35, 95%CI: 1.10-1.67, P = 0.0041], respiratory failure (RF) (aOR = 1.83, 95%CI: 1.22-2.76, P = 0.0036), longer LOS (adjusted LOS ratio = 1.12, 95%CI: 1.06-1.18, P < 0.0001) and higher hospitalization charges (adjusted cost ratio = 1.13, 95%CI :1.06-1.2, P < 0.0001). However, a lower rate of intestinal perforation rate was observed in the CDI with VLD group (aOR = 0.12, 95%CI: 0.03-0.57, P = 0.0075). CDI with VLD was initially associated with higher rates of mortality, colectomy and ileostomy, however this difference no longer existed after adjusting for confounding factors (Table 3).

Outcomes and regression analysis of CDI patients with NAFLD vs ALD

When compared to CDI patients with NAFLD, CDI patients with ALD had higher rates of RF (aOR = 1.72, 95%CI: 1.09-2.72, P = 0.0201), mortality (aOR = 2.63, 95%CI: 1.25-5.51, P = 0.0107), longer LOS (adjusted LOS ratio = 1.18, 95%CI: 1.10-1.25, P < 0.0001) and higher hospitalization charges (adjusted cost ratio = 1.17, 95%CI: 1.09-1.26, P < 0.0001). However, a lower rate of intestinal obstruction (aOR = 0.45, 95%CI: 0.28-0.72, P = 0.0010) was found in the CDI with ALD group when compared to the CDI with NAFLD group. Higher rates of AKI and septic shock, and a lower rate of colectomy were initially observed in CDI with ALD group, but the difference no longer existed after adjusting for the aforementioned confounders (Table 4).

Table 1 Comparison of demographic data for patients hospitalized with *Clostridioides difficile* infection with coexisting nonalcoholic fatty liver disease, viral liver disease and alcoholic liver disease

Variables	CDI with NAFLD	CDI with VLD	CDI with ALD	*P* value	
***n* (weighted)**	**7239**	**11857**	**5938**	**CDI with NAFLD *vs* CDI with VLD**	**CDI with NAFLD *vs* CDI with ALD**
Age (yr)	56.32 ± 0.42	57 ± 0.26	56.13 ± 0.37	0.15	0.73
18-39	1133 (15.6%)	791 (6.7%)	557 (9.4%)	< 0.0001	< 0.0001
40-49	1290 (17.8%)	1811 (15.3%)	1051 (17.7%)		
50-59	1618 (22.4%)	4873 (41.1%)	2021 (34%)		
60-69	1620 (22.4%)	2791 (23.5%)	1439 (24.2%)		
≥ 70	1578 (21.8%)	1591 (13.4%)	870 (14.7%)		
Sex				< 0.0001	< 0.0001
Female	5023 (69.4%)	5795 (48.9%)	2300 (38.7%)		
Race				< 0.0001	0.17
Caucasian	5427 (75%)	6920 (58.4%)	4358 (73.4%)		
African American	482 (6.5%)	2773 (23.4%)	525 (8.8%)		
Hispanic	648 (9%)	1144 (9.6%)	515 (8.7%)		
Hospital bed size				0.033	0.9
Large	4241 (58.6%)	7414 (62.6%)	3461 (58.3%)		
Hospital region				< 0.0001	< 0.0001
Northeast	1091 (15.1%)	2618 (22.1%)	1243 (20.9%)		
Midwest	1618 (22.3%)	2514 (21.1%)	1584 (26.7%)		
South	3008 (41.6%)	4208 (35.5%)	1671 (28.1%)		
West	1522 (21%)	2517 (21.2%)	1440 (24.3%)		
Hospital type				< 0.0001	0.22
Urban teaching	3401 (47%)	7207 (60.8%)	3065 (51.6%)		
Insurance				< 0.0001	< 0.0001
Medicare	3086 (42.6%)	5493 (46.3%)	2239 (37.7%)		
Medicaid	914 (12.6%)	3329 (28.1%)	1261 (21.2%)		
Private	2526 (34.9%)	1835 (15.5%)	1391 (23.4%)		
Median household income for ZIP Code, %				< 0.0001	0.61
Q1	1790 (24.7%)	4205 (35.5%)	1592 (26.8%)		
Q2	1824 (25.2%)	3128 (26.4%)	1407 (23.7%)		
Q3	1926 (26.6%)	2353 (19.8%)	1503 (25.3%)		
Q4	1511 (20.9%)	1657 (14%)	1252 (21.1%)		

Values reported as weighted mean ± SE and weighted number [*n* (%)]. CDI: *Clostridioides difficile* infection; NAFLD: Nonalcoholic fatty liver disease; VLD: Viral liver disease; ALD: Alcoholic liver disease; Q1: Quartile 1, 0-25th percentile; Q2: Quartile 2, 26th-50th percentile; Q3: Quartile 3, 51th-75th percentile; Q4: Quartile 4, 76th-100th percentile.

DISCUSSION

This nationwide retrospective cohort study investigated the inpatient clinical characteristics and outcomes of CDI in hospitalized patients with coexisting liver diseases, with comparisons between NAFLD, VLD and ALD. We demonstrated that patients hospitalized with CDI and coexisting NAFLD had overall more favorable outcomes including a lower rate of RF, lower hospitalization charges and a shorter LOS when

Table 2 Comparison of comorbid conditions and complications for patients hospitalized with *Clostridioides difficile* infection with coexisting nonalcoholic fatty liver disease, viral liver disease and alcoholic liver disease

Variables	CDI with NAFLD	CDI with VLD	CDI with ALD	*P* value	
***n* (weighted)**	**7239**	**11857**	**5938**	**CDI with NAFLD *vs* CDI with VLD**	**CDI with NAFLD *vs* CDI with ALD**
Number of Elixhauser comorbidities				< 0.0001	< 0.0001
0	0 (0%)	114 (1%)	-		
1	244 (3.4%)	574 (4.8%)	116 (2%)		
2	656 (9.1%)	1409 (11.9%)	354 (6%)		
≥ 3	6338 (87.6%)	9760 (82.3%)	5463 (92%)		
Obesity	2012 (27.8%)	850 (7.2%)	372 (6.3%)	< 0.0001	< 0.0001
Diabetes	2750 (38%)	3451 (29.1%)	1170 (19.7%)	< 0.0001	< 0.0001
Hypertension	4300 (59.4%)	6347 (53.5%)	2980 (50.2%)	0.00058	< 0.0001
Dyslipidemia	2619 (36.2%)	1868 (15.8%)	905 (15.2%)	< 0.0001	< 0.0001
Hepatocellular carcinoma	-	253 (2.1%)	45 (0.8%)	< 0.0001	0.0217
Cirrhosis related comorbidities[1]					
Cirrhosis	401 (5.5%)	2508 (21.2%)	3407 (57.4%)	< 0.0001	< 0.0001
Number of cirrhosis complications				0.0013	< 0.0001
0	137 (34.2%)	1773 (70.7%)	2105 (61.8%)		
1	244 (60.8%)	688 (27.4%)	1104 (32.4%)		
v ≥ 2	20 (5.0%)	47 (1.9%)	198 (5.8%)		
Ascites	0 (0%)	0 (0%)	0 (0%)	NA	NA
Esophageal varices bleeding	0 (0%)	-	20 (0.6%)	NA	NA
Hepatic encephalopathy	110 (27.4%)	60 (2.4%)	569 (16.7%)	0.003338	< 0.0001
Hepatorenal syndrome	0 (0%)	15 (0.6%)	33 (1.0%)	NA	NA
Portal hypertension	175 (43.6%)	661 (26.4%)	843 (24.7%)	< 0.0001	< 0.0001
Spontaneous bacterial peritonitis	0 (0%)	38 (1.5%)	40 (1.2%)	NA	NA

[1]Value reported as percentage of all cirrhotic patients.

Values reported as weighted number [*n* (%)].-: Numbers were not displayed due to extremely small numbers were associated with increased risk for identification of persons; CDI: *Clostridioides difficile* infection; NAFLD: Nonalcoholic fatty liver disease; VLD: Viral liver disease; ALD: Alcoholic liver disease; NA: Not available.

compared to those with ALD and VLD individually. Interestingly, higher rates of intestinal complications were observed in the CDI with NAFLD group when compared to the CDI with ALD or VLD groups. Specifically, a significantly higher rate of intestinal obstruction was seen in the CDI with NAFLD group when compared to the CDI with ALD group, and a higher rate of intestinal perforation was seen when compared to CDI patients with concomitant VLD.

Our findings of worse intestinal complications in patients hospitalized with CDI and coexisting NAFLD compared to CDI patients with VLD and ALD, linked the gut pathology to the liver. The crosstalk between the gut and liver is increasingly recognized as the gut-liver axis[18]. Receiving more than 70% of the blood supply from the intestinal venous outflow, the liver represents the first line of defense against gut derived antigens with a broad array of immune cells[19]. The liver also releases many bioactive mediators into the systemic circulation, allowing for communication with the intestine. In the intestine, the endogenous and exogenous products from host and microbial metabolism translocate to the liver through the portal venous system, ultimately influencing liver function[20].

Table 3 Multivariate regression analysis of outcomes for patients hospitalized for *Clostridioides difficile* infection with coexisting nonalcoholic fatty liver disease *vs* viral liver disease

Outcomes	CDI with NAFLD	CDI with VLD	Unadjusted ratio (95%CI)	*P* value	Adjusted ratio[1] (95%CI)	*P* value
***n* (weighted)**	**7239**	**11857**				
Hospital mortality	59 (0.8%)	186 (1.6%)	1.94 (1.44, 2.6)	< 0.0001	1.87 (0.95, 3.7)	0.071
Acute kidney injury	938 (13%)	2035 (17.2%)	1.39 (1.28, 1.51)	< 0.0001	1.35 (1.1, 1.67)	0.0041
Respiratory failure	192 (2.7%)	504 (4.2%)	1.63 (1.37, 1.92)	< 0.0001	1.83 (1.22, 2.76)	0.0036
Septic shock	39 (0.5%)	115 (1%)	1.8 (1.25, 2.59)	0.0015	1.64 (0.67, 4.02)	0.27
Intestinal perforation	-	-	0.3 (0.1, 0.89)	0.03	0.12 (0.03, 0.57)	0.0075
Intestinal obstruction	331 (4.6%)	527 (4.4%)	0.97 (0.84, 1.12)	0.67	0.94 (0.66, 1.33)	0.725
Peritonitis	61 (0.8%)	106 (0.9%)	1.06 (0.77, 1.45)	0.71	0.72 (0.35, 1.52)	0.39
Colectomy	45 (0.6%)	105 (0.9%)	1.43 (1.01, 2.03)	0.044	1.38 (0.6, 3.15)	0.44
Ileostomy	-	41 (0.3%)	2.47 (1.24, 4.92)	0.01	2.62 (0.66, 10.41)	0.17
LOS (days)	5.75 ± 0.16	6.77 ± 0.15	1.11 (1.06, 1.16)	< 0.0001	1.12 (1.06, 1.18)	< 0.0001
Total hospitalizationcharges (dollars)	38150.34 ± 1757.01	46326.72 ± 1809.82	1.14 (1.07, 1.2)	< 0.0001	1.13 (1.06, 1.2)	< 0.0001

[1]Adjusted for age, sex, race, primary insurance payer, hospital type, hospital bed size, hospital region, income quartile, Elixhauser Comorbidity Index score, obesity, diabetes, tobacco use disorder, hypertension, dyslipidemia, cirrhosis and its complications, numbers of cirrhosis complications, and hepatocellular carcinoma.

-: Numbers were not displayed due to extremely small numbers were associated with increased risk for identification of persons. Values reported as weighted mean ± SE and weighted numbers [*n* (%)]; CDI: *Clostridioides difficile* infection; NAFLD: Nonalcoholic fatty liver disease; VLD: Viral liver disease; CI: Confidence interval; LOS: Length of stay.

How does NAFLD influence the intestinal complications of CDI through the gut-liver axis? Convincing evidence has shown that NAFLD is associated with significantly increased gut permeability and inflammation in both animal[21] and human models. Miele *et al*[22] found that NAFLD patients had significantly increased gut permeability measured by urine radiolabeled markers and immunohistochemical analysis of zona occludens -1 expression in intestinal biopsy specimens, compared with healthy volunteers. They also discovered that both gut permeability and the prevalence of small intestinal bacterial overgrowth are correlated with the severity of steatosis. Verdam *et al*[23] found that plasma immunoglobulin G levels against endotoxin were increased in NASH patients, which positively correlated with the severity of inflammation. Furthermore, transmission electron microscopy observed irregular microvilli and widened tight junctions in the gut mucosa of the NAFLD patients[24]. In addition, decreased numbers of CD4+ and CD8+ T lymphocytes and increased levels of TNF-α, IL-6 and IFN-γ were detected in the NAFLD patient group compared to healthy control. All of these results suggested impaired gut permeability and increased levels of inflammation at both the tissue and cellular levels in NAFLD disease models.

The gut microbiota-mediated inflammation, the related disturbance of the intestinal integrity and the impairment in mucosal immune function have been reported to play important roles, not only in the pathophysiology of CDI[25] but also in the pathogenesis of NAFLD[13,24,26]. The gut microbiota normally exerts significant influence on intestinal epithelial cell health, nutrient metabolism and mucosal defense [19,27]. Early evidence in animal studies demonstrated that altered gut microbiota composition[28] independently contributed to the development of NAFLD in mice. In addition, altered interaction between the gut and the host (produced by defective inflammasome sensing in inflammasome-deficient mouse models) may govern the rate of progression of multiple metabolic syndrome-associated abnormalities[29]. With the recent developments in genome sequencing technologies, bioinformatics, and culturomics; it has been recognized that NAFLD and NASH are associated with decreased richness of the gut flora and increased risk of pathogenic flora in pediatric and adult patients[30-34], which are both well known risk factors for CDI. Although it is still unclear which specific microorganisms are harmful given conflicting results in

Table 4 Multivariate regression analysis of outcomes for patients hospitalized for Clostridioides difficile infection with coexisting nonalcoholic fatty liver disease *vs* alcoholic liver disease

Outcomes	CDI with NAFLD	CDI with ALD	Unadjusted ratio (95%CI)	*P* value	Adjusted ratio[1] (95%CI)	*P* value
n (weighted)	7239	5938				
Hospital mortality	59 (0.8%)	159 (2.7%)	3.34 (2.48, 4.52)	< 0.0001	2.63 (1.25, 5.51)	0.0107
Acute kidney injury	938 (13%)	935 (15.8%)	1.26 (1.14, 1.39)	< 0.0001	1.2 (0.93, 1.54)	0.15
Respiratory failure	192 (2.7%)	249 (4.2%)	1.61 (1.33, 1.94)	< 0.0001	1.72 (1.09, 2.72)	0.0201
Septic shock	39 (0.5%)	79 (1.3%)	2.48 (1.69, 3.64)	< 0.0001	2.14 (0.84, 5.46)	0.109
Intestinal perforation	-	0 (0%)	NA	NA	NA	NA
Intestinal obstruction	331 (4.6%)	133 (2.2%)	0.48 (0.39, 0.59)	< 0.0001	0.45 (0.28, 0.72)	0.0010
Peritonitis	61 (0.8%)	69 (1.2%)	1.38 (0.97, 1.95)	0.071	0.54 (0.25, 1.18)	0.12
Colectomy	45 (0.6%)	15 (0.3%)	0.42 (0.23, 0.74)	0.003	0.44 (0.14, 1.39)	0.16
Ileostomy	-	-	0.65 (0.23, 1.85)	0.42	0.99 (0.15, 6.61)	0.98
LOS (days)	5.75 ± 0.16	6.84 ± 0.23	1.14 (1.08, 1.21)	< 0.0001	1.18 (1.1, 1.25)	< 0.0001
Totalhospitalizationcharges (dollars)	38150.34 ± 1757.01	44641.74 ± 1660.66	1.14 (1.07, 1.22)	< 0.0001	1.17 (1.09, 1.26)	< 0.0001

[1]Adjusted for age, sex, race, primary insurance payer, hospital type, hospital bed size, hospital region, income quartile, Elixhauser Comorbidity Index score, obesity, diabetes, tobacco use disorder, hypertension, dyslipidemia, cirrhosis and its complications, numbers of cirrhosis complications, and hepatocellular carcinoma.

Values reported as weighted mean ± SE and weighted numbers [*n* (%)]. -: Numbers were not displayed due to extremely small numbers were associated with increased risk for identification of persons. CDI: *Clostridioides difficile* infection; NAFLD: Nonalcoholic fatty liver disease; ALD: Alcoholic liver disease; LOS: Length of stay.

human and animal studies[35], it is believed that gut microbiota-derived signatures extracted by whole-genome shotgun sequencing of DNA can be used for diagnosis of advanced fibrosis in NAFLD[36], and modification of gut microbiota analyzed by 16S ribosomal RNA pyrosequencing can be used for therapeutic purposes in NASH patients[37]. Additionally, increased pathogenic flora in NAFLD and NASH further disturb the immune balance and cause worsened dysbiosis through various mechanisms involving short-chain fatty acids[38], lipopolysaccharide[21], choline metabolism[39], bile acid metabolism[40] and bacteria-derived ethanol[41]. Collectively, NAFLD and NASH related alterations of gut microbiota and its downstream dysbiosis pathways may contribute to CDI risk and worse intestinal complications.

On the other end, we sought to identify the characteristics of gut microbiota changes in ALD and VLD. Compared to NAFLD, ALD is remarkably similar histologically[42] and initiated directly from the gut by alcohol intake or binges. It has been well documented that alcohol intake can lead to changes in gut microbiota composition[43] and gut permeability[44] early on, even before the development of liver disease. These alterations involve multiple physical and biochemical layers of defense in the intestinal barrier[19]. In VLD, the gut microbiome works as an effective tool early on for immunity against the hepatitis virus, and helps with viral clearance[45]. In chronic VLD, large translocations of intestinal microbiota were observed and thought to contribute to not only dysregulation of immune cells and dysfunction of the intestinal barrier, but also viral replication[27]. Comparison analysis revealed that, compared to other cirrhosis etiologies, alcoholic cirrhosis is associated with worse gut dysbiosis after adjusting for Model For End-Stage Liver Disease score and body mass index[46]. In two other studies[47,48], which primarily compared the gut microbiota composition in HBV/HCV related and alcoholic cirrhosis, no difference was observed at the phylum and class level.

Intriguingly, in our study, the majority (94.5%) of patients in CDI with NAFLD group were non-cirrhotic; the percentage of cirrhotic patients in CDI with NAFLD group was significantly less than those in CDI with ALD or VLD group. CDI with NAFLD group was associated with a higher rate of intestinal complications after adjusting for cirrhosis and its complications. These results suggested that NAFLD is

associated with altered gut microbiota that is predisposed to CDI and its complications, likely independent from the liver disease severity. In fact, NAFLD has been reported as an independent risk factor for CDI[14]. Although ALD and VLD cirrhosis was previously found to be associated with worse gut dysbiosis than NAFLD cirrhosis, this finding should be treated cautiously for non-cirrhotic patients, because the alteration of the gut microbiome is associated with the severity of liver disease, as significant differences in gut microbiota have been found between non-cirrhotic, compensated and decompensated cirrhotic patients[49,50]. Importantly, the standard of care therapies in cirrhotic patients such as lactulose, rifaximin, antibiotics and acid-suppressants that can affect the gut microbiota, may be playing a critical role[51]. In summary, our study suggested that NAFLD may be associated with worse dysbiosis in early liver disease stages and therefore a higher risk for CDI and its complications compared to ALD and VLD.

Aside from aforementioned gut microbiota changes that directly link NAFLD to CDI and intestinal complications, NAFLD related metabolic syndrome and systemic inflammation also play crucial roles in intestinal pathology. Recently, metabolic dysfunction-associated fatty liver disease has been proposed as a more appropriate name to replace NAFLD by an international panel of experts, with emphasis on the underlying metabolic dysfunction[52,53]. Clinical evidence has demonstrated that NAFLD, along with other components of metabolic syndrome, such as diabetes and obesity, are associated with an increased prevalence of small intestinal bacterial overgrowth (SIBO)[54,55] by insulin resistance, oxidative stress and chronic low grade inflammation[56]. Subsequently, the dysmotility induced by SIBO can further promote SIBO in NAFLD patients, causing a vicious cycle[57]. In fact, dysmotility itself is associated with NAFLD and may be a potential therapeutic target for NAFLD from a Japanese study[58,59]. Moreover, diabetes, a component of metabolic syndrome which may cause vasculopathies and neuropathies in the intestines, also contributes to dysmotility[60]. Additionally, diverticular disease, irritable bowel disease[61] and inflammatory bowel disease[62], together with SIBO and dysmotility have all been shown to have increased prevalence in NAFLD patients. Not surprisingly, the structural and functional abnormalities in the gut associated with NAFLD and metabolic dysfunction further increase the risk of CDI and its complications.

The strengths of this study include the utilization of the NIS database to provide a unique opportunity to investigate a nationwide population hospitalized for CDI. To the best of our knowledge, this study is a leading clinical research analysis that provided a comprehensive nationwide comparison of outcomes between NAFLD and other common chronic liver diseases, ALD and VLD, in hospitalized CDI patients. There are also limitations in this study. Particularly, NIS data acquisition relies on the accuracy ICD-9-CM codes for medical diagnoses and no lab results, biopsy or image studies were available for NAFLD diagnosis and severity stratification. It is also difficult to determine which cases of CDI were hospital acquired or community acquired because ICD-9 codes are assigned at discharge. To strengthen the validity of ICD-9 codes for NAFLD, VLD and ALD, we used not only diagnostic codes but also excluded the codes for all other chronic liver diseases (Supplementary Table 1)[63]. The ICD-9 codes for CDI were validated previously with good diagnostic accuracy[64, 65].

CONCLUSION

In conclusion, this study found more favorable overall outcomes but higher rates of intestinal complications in patients hospitalized with CDI and coexisting NAFLD, compared to CDI with coexisting ALD and VLD, individually. These results suggested that NAFLD may be associated with a higher risk of CDI associated intestinal complications through alteration of gut microbiota. Our study also suggested that NAFLD associated metabolic syndrome may contribute significantly to the gut dysbiosis even in the early liver disease stages and cause increased risk for CDI and its complications. During the last few years, the novel and rapidly evolving research technologies for the gut microbiome have been opening up an exciting era in the microbiota therapeutics for different disease models[66]. Tremendous progress has been observed in the treatment of NAFLD and CDI through gut microbiome manipulation. Our study may help increase awareness and diagnose intestinal complications in patients with two common diseases: CDI and NAFLD. Unraveling the significance of interactions between gut microbiota, gut immunity and systemic metabolic impact of NAFLD with prospective studies will provide more insights into the future microbiota therapeutics

for CDI and NAFLD.

ARTICLE HIGHLIGHTS

Research background

The ongoing exploration of liver-gut axis has discovered strong association between gut dysbiosis and nonalcoholic fatty liver disease (NAFLD) in both basic science and clinical research. Small-scaled studies have observed that NAFLD is an independent risk factor for *Clostridioides difficile* infection (CDI).

Research motivation

CDI, as the most common cause of nosocomial diarrhea in developed countries, carries high hospitalization burden. NAFLD, as the leading cause of chronic liver disease, is commonly seen in hospitalized patients with CDI. So far the inpatient outcomes of CDI in the NAFLD population have not been well studied.

Research objectives

The authors aimed to examine the impact of NAFLD on the inpatient outcomes of hospitalized patients with CDI, by comparing the effect of NAFLD with alcoholic liver disease (ALD) and viral liver disease (VLD) individually.

Research methods

This nationwide retrospective cohort study was conducted according to STROBE statement using the National Inpatient Sample database. Inpatient CDI with coexisting NAFLD cases were selected using ICD-9 codes. Multivariate regression analysis was used with adjustment for a large group of possible confounders. Elixhauser Comorbidity Index (ECI) was used for a full description of comorbidity burden.

Research results

CDI with NAFLD was independently associated with lower rates of acute respiratory failure, shorter length of stay and lower hospitalization charges when compared to CDI with VLD and CDI with ALD. However, CDI with NAFLD was associated with a higher rate of intestinal perforation when compared to VLD, and a higher rate of intestinal obstruction when compared to ALD.

Research conclusions

CDI and coexisting NAFLD is associated with favorable overall outcomes, but higher rates of intestinal complications compared to CDI with coexisting ALD and VLD, individually.

Research perspectives

This finding suggests that alteration of gut microbiota may play an important role in the pathogenesis of both CDI and NAFLD. NAFLD associated metabolic syndrome may contribute significantly to the gut dysbiosis and cause increased risk for CDI and its complications. This study provides potential directions for future prospective clinical research to identify the clinical meaningfulness of interactions between gut microbiota, gut immunity and systemic inflammation. The study may open the door for potential microbiota therapeutic targets and manipulation as future treatment options for chronic liver diseases.

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REFERENCES

1 **Younossi ZM**, Koenig AB, Abdelatif D, Fazel Y, Henry L, Wymer M. Global epidemiology of nonalcoholic fatty liver disease-Meta-analytic assessment of prevalence, incidence, and outcomes.

Hepatology 2016; **64**: 73-84 [PMID: 26707365 DOI: 10.1002/hep.28431]
2 **Huang DQ**, El-Serag HB, Loomba R. Global epidemiology of NAFLD-related HCC: trends, predictions, risk factors and prevention. *Nat Rev Gastroenterol Hepatol* 2021; **18**: 223-238 [PMID: 33349658 DOI: 10.1038/s41575-020-00381-6]
3 **Williams CD**, Stengel J, Asike MI, Torres DM, Shaw J, Contreras M, Landt CL, Harrison SA. Prevalence of nonalcoholic fatty liver disease and nonalcoholic steatohepatitis among a largely middle-aged population utilizing ultrasound and liver biopsy: a prospective study. *Gastroenterology* 2011; **140**: 124-131 [PMID: 20858492 DOI: 10.1053/j.gastro.2010.09.038]
4 **Vernon G**, Baranova A, Younossi ZM. Systematic review: the epidemiology and natural history of non-alcoholic fatty liver disease and non-alcoholic steatohepatitis in adults. *Aliment Pharmacol Ther* 2011; **34**: 274-285 [PMID: 21623852 DOI: 10.1111/j.1365-2036.2011.04724.x]
5 **Younossi ZM**. Non-alcoholic fatty liver disease - A global public health perspective. *J Hepatol* 2019; **70**: 531-544 [PMID: 30414863 DOI: 10.1016/j.jhep.2018.10.033]
6 **Evans CT**, Safdar N. Current Trends in the Epidemiology and Outcomes of Clostridium difficile Infection. *Clin Infect Dis* 2015; **60** Suppl 2: S66-S71 [PMID: 25922403 DOI: 10.1093/cid/civ140]
7 **Rupnik M**, Wilcox MH, Gerding DN. Clostridium difficile infection: new developments in epidemiology and pathogenesis. *Nat Rev Microbiol* 2009; **7**: 526-536 [PMID: 19528959 DOI: 10.1038/nrmicro2164]
8 **McDonald LC**, Gerding DN, Johnson S, Bakken JS, Carroll KC, Coffin SE, Dubberke ER, Garey KW, Gould CV, Kelly C, Loo V, Shaklee Sammons J, Sandora TJ, Wilcox MH. Clinical Practice Guidelines for Clostridium difficile Infection in Adults and Children: 2017 Update by the Infectious Diseases Society of America (IDSA) and Society for Healthcare Epidemiology of America (SHEA). *Clin Infect Dis* 2018; **66**: e1-e48 [PMID: 29462280 DOI: 10.1093/cid/cix1085]
9 **Nwachuku E**, Shan Y, Senthil-Kumar P, Braun T, Shadis R, Kirton O, Vu TQ. Toxic Clostridioides (formerly Clostridium) difficile colitis: No longer a diarrhea associated infection. *Am J Surg* 2021; **221**: 240-242 [PMID: 32680621 DOI: 10.1016/j.amjsurg.2020.06.026]
10 **Kistangari G**, Lopez R, Shen B. Frequency and Risk Factors of Clostridium difficile Infection in Hospitalized Patients With Pouchitis: A Population-based Study. *Inflamm Bowel Dis* 2017; **23**: 661-671 [PMID: 28296825 DOI: 10.1097/MIB.0000000000001057]
11 **Czepiel J**, Dróżdż M, Pituch H, Kuijper EJ, Perucki W, Mielimonka A, Goldman S, Wultańska D, Garlicki A, Biesiada G. Clostridium difficile infection: review. *Eur J Clin Microbiol Infect Dis* 2019; **38**: 1211-1221 [PMID: 30945014 DOI: 10.1007/s10096-019-03539-6]
12 **Safari Z**, Gérard P. The links between the gut microbiome and non-alcoholic fatty liver disease (NAFLD). *Cell Mol Life Sci* 2019; **76**: 1541-1558 [PMID: 30683985 DOI: 10.1007/s00018-019-03011-w]
13 **Aron-Wisnewsky J**, Vigliotti C, Witjes J, Le P, Holleboom AG, Verheij J, Nieuwdorp M, Clément K. Gut microbiota and human NAFLD: disentangling microbial signatures from metabolic disorders. *Nat Rev Gastroenterol Hepatol* 2020; **17**: 279-297 [PMID: 32152478 DOI: 10.1038/s41575-020-0269-9]
14 **Nseir WB**, Hussein SHH, Farah R, Mahamid MN, Khatib HH, Mograbi JM, Peretz A, Amara AE. Nonalcoholic fatty liver disease as a risk factor for Clostridium difficile-associated diarrhea. *QJM* 2020; **113**: 320-323 [PMID: 31688897 DOI: 10.1093/qjmed/hcz283]
15 **Papić N**, Jelovčić F, Karlović M, Marić LS, Vince A. Nonalcoholic fatty liver disease as a risk factor for Clostridioides difficile infection. *Eur J Clin Microbiol Infect Dis* 2020; **39**: 569-574 [PMID: 31782025 DOI: 10.1007/s10096-019-03759-w]
16 **Agency for Healthcare Research and Quality**. Healthcare Cost and Utilization Project (HCUP). 2019. Available from: https://www.ahrq.gov/data/hcup/index.html
17 **Moore BJ**, White S, Washington R, Coenen N, Elixhauser A. Identifying Increased Risk of Readmission and In-hospital Mortality Using Hospital Administrative Data: The AHRQ Elixhauser Comorbidity Index. *Med Care* 2017; **55**: 698-705 [PMID: 28498196 DOI: 10.1097/MLR.0000000000000735]
18 **Tripathi A**, Debelius J, Brenner DA, Karin M, Loomba R, Schnabl B, Knight R. The gut-liver axis and the intersection with the microbiome. *Nat Rev Gastroenterol Hepatol* 2018; **15**: 397-411 [PMID: 29748586 DOI: 10.1038/s41575-018-0011-z]
19 **Son G**, Kremer M, Hines IN. Contribution of gut bacteria to liver pathobiology. *Gastroenterol Res Pract* 2010; **2010** [PMID: 20706692 DOI: 10.1155/2010/453563]
20 **Stärkel P**, Schnabl B. Bidirectional Communication between Liver and Gut during Alcoholic Liver Disease. *Semin Liver Dis* 2016; **36**: 331-339 [PMID: 27997973 DOI: 10.1055/s-0036-1593882]
21 **Mao JW**, Tang HY, Zhao T, Tan XY, Bi J, Wang BY, Wang YD. Intestinal mucosal barrier dysfunction participates in the progress of nonalcoholic fatty liver disease. *Int J Clin Exp Pathol* 2015; **8**: 3648-3658 [PMID: 26097546]
22 **Miele L**, Valenza V, La Torre G, Montalto M, Cammarota G, Ricci R, Mascianà R, Forgione A, Gabrieli ML, Perotti G, Vecchio FM, Rapaccini G, Gasbarrini G, Day CP, Grieco A. Increased intestinal permeability and tight junction alterations in nonalcoholic fatty liver disease. *Hepatology* 2009; **49**: 1877-1887 [PMID: 19291785 DOI: 10.1002/hep.22848]
23 **Verdam FJ**, Rensen SS, Driessen A, Greve JW, Buurman WA. Novel evidence for chronic exposure to endotoxin in human nonalcoholic steatohepatitis. *J Clin Gastroenterol* 2011; **45**: 149-152 [PMID: 20661154 DOI: 10.1097/MCG.0b013e3181e12c24]
24 **Jiang W**, Wu N, Wang X, Chi Y, Zhang Y, Qiu X, Hu Y, Li J, Liu Y. Dysbiosis gut microbiota

associated with inflammation and impaired mucosal immune function in intestine of humans with non-alcoholic fatty liver disease. *Sci Rep* 2015; **5**: 8096 [PMID: 25644696 DOI: 10.1038/srep08096]

25 **Theriot CM**, Koenigsknecht MJ, Carlson PE Jr, Hatton GE, Nelson AM, Li B, Huffnagle GB, Z Li J, Young VB. Antibiotic-induced shifts in the mouse gut microbiome and metabolome increase susceptibility to Clostridium difficile infection. *Nat Commun* 2014; **5**: 3114 [PMID: 24445449 DOI: 10.1038/ncomms4114]

26 **Campo L**, Eiseler S, Apfel T, Pyrsopoulos N. Fatty Liver Disease and Gut Microbiota: A Comprehensive Update. *J Clin Transl Hepatol* 2019; **7**: 56-60 [PMID: 30944821 DOI: 10.14218/JCTH.2018.00008]

27 **Sehgal R**, Bedi O, Trehanpati N. Role of Microbiota in Pathogenesis and Management of Viral Hepatitis. *Front Cell Infect Microbiol* 2020; **10**: 341 [PMID: 32850467 DOI: 10.3389/fcimb.2020.00341]

28 **Le Roy T**, Llopis M, Lepage P, Bruneau A, Rabot S, Bevilacqua C, Martin P, Philippe C, Walker F, Bado A, Perlemuter G, Cassard-Doulcier AM, Gérard P. Intestinal microbiota determines development of non-alcoholic fatty liver disease in mice. *Gut* 2013; **62**: 1787-1794 [PMID: 23197411 DOI: 10.1136/gutjnl-2012-303816]

29 **Henao-Mejia J**, Elinav E, Jin C, Hao L, Mehal WZ, Strowig T, Thaiss CA, Kau AL, Eisenbarth SC, Jurczak MJ, Camporez JP, Shulman GI, Gordon JI, Hoffman HM, Flavell RA. Inflammasome-mediated dysbiosis regulates progression of NAFLD and obesity. *Nature* 2012; **482**: 179-185 [PMID: 22297845 DOI: 10.1038/nature10809]

30 **Tsai MC**, Liu YY, Lin CC, Wang CC, Wu YJ, Yong CC, Chen KD, Chuah SK, Yao CC, Huang PY, Chen CH, Hu TH, Chen CL. Gut Microbiota Dysbiosis in Patients with Biopsy-Proven Nonalcoholic Fatty Liver Disease: A Cross-Sectional Study in Taiwan. *Nutrients* 2020; **12** [PMID: 32204538 DOI: 10.3390/nu12030820]

31 **Schwimmer JB**, Johnson JS, Angeles JE, Behling C, Belt PH, Borecki I, Bross C, Durelle J, Goyal NP, Hamilton G, Holtz ML, Lavine JE, Mitreva M, Newton KP, Pan A, Simpson PM, Sirlin CB, Sodergren E, Tyagi R, Yates KP, Weinstock GM, Salzman NH. Microbiome Signatures Associated With Steatohepatitis and Moderate to Severe Fibrosis in Children With Nonalcoholic Fatty Liver Disease. *Gastroenterology* 2019; **157**: 1109-1122 [PMID: 31255652 DOI: 10.1053/j.gastro.2019.06.028]

32 **Del Chierico F**, Nobili V, Vernocchi P, Russo A, De Stefanis C, Gnani D, Furlanello C, Zandonà A, Paci P, Capuani G, Dallapiccola B, Miccheli A, Alisi A, Putignani L. Gut microbiota profiling of pediatric nonalcoholic fatty liver disease and obese patients unveiled by an integrated meta-omics-based approach. *Hepatology* 2017; **65**: 451-464 [PMID: 27028797 DOI: 10.1002/hep.28572]

33 **Shen F**, Zheng RD, Sun XQ, Ding WJ, Wang XY, Fan JG. Gut microbiota dysbiosis in patients with non-alcoholic fatty liver disease. *Hepatobiliary Pancreat Dis Int* 2017; **16**: 375-381 [PMID: 28823367 DOI: 10.1016/S1499-3872(17)60019-5]

34 **Wang B**, Jiang X, Cao M, Ge J, Bao Q, Tang L, Chen Y, Li L. Altered Fecal Microbiota Correlates with Liver Biochemistry in Nonobese Patients with Non-alcoholic Fatty Liver Disease. *Sci Rep* 2016; **6**: 32002 [PMID: 27550547 DOI: 10.1038/srep32002]

35 **Jennison E**, Byrne CD. The role of the gut microbiome and diet in the pathogenesis of non-alcoholic fatty liver disease. *Clin Mol Hepatol* 2021; **27**: 22-43 [PMID: 33291863 DOI: 10.3350/cmh.2020.0129]

36 **Loomba R**, Seguritan V, Li W, Long T, Klitgord N, Bhatt A, Dulai PS, Caussy C, Bettencourt R, Highlander SK, Jones MB, Sirlin CB, Schnabl B, Brinkac L, Schork N, Chen CH, Brenner DA, Biggs W, Yooseph S, Venter JC, Nelson KE. Gut Microbiome-Based Metagenomic Signature for Non-invasive Detection of Advanced Fibrosis in Human Nonalcoholic Fatty Liver Disease. *Cell Metab* 2017; **25**: 1054-1062.e5 [PMID: 28467925 DOI: 10.1016/j.cmet.2017.04.001]

37 **Wong VW**, Tse CH, Lam TT, Wong GL, Chim AM, Chu WC, Yeung DK, Law PT, Kwan HS, Yu J, Sung JJ, Chan HL. Molecular characterization of the fecal microbiota in patients with nonalcoholic steatohepatitis--a longitudinal study. *PLoS One* 2013; **8**: e62885 [PMID: 23638162 DOI: 10.1371/journal.pone.0062885]

38 **Cani PD**, Possemiers S, Van de Wiele T, Guiot Y, Everard A, Rottier O, Geurts L, Naslain D, Neyrinck A, Lambert DM, Muccioli GG, Delzenne NM. Changes in gut microbiota control inflammation in obese mice through a mechanism involving GLP-2-driven improvement of gut permeability. *Gut* 2009; **58**: 1091-1103 [PMID: 19240062 DOI: 10.1136/gut.2008.165886]

39 **Dumas ME**, Barton RH, Toye A, Cloarec O, Blancher C, Rothwell A, Fearnside J, Tatoud R, Blanc V, Lindon JC, Mitchell SC, Holmes E, McCarthy MI, Scott J, Gauguier D, Nicholson JK. Metabolic profiling reveals a contribution of gut microbiota to fatty liver phenotype in insulin-resistant mice. *Proc Natl Acad Sci U S A* 2006; **103**: 12511-12516 [PMID: 16895997 DOI: 10.1073/pnas.0601056103]

40 **Parséus A**, Sommer N, Sommer F, Caesar R, Molinaro A, Ståhlman M, Greiner TU, Perkins R, Bäckhed F. Microbiota-induced obesity requires farnesoid X receptor. *Gut* 2017; **66**: 429-437 [PMID: 26740296 DOI: 10.1136/gutjnl-2015-310283]

41 **Aragonès G**, González-García S, Aguilar C, Richart C, Auguet T. Gut Microbiota-Derived Mediators as Potential Markers in Nonalcoholic Fatty Liver Disease. *Biomed Res Int* 2019; **2019**: 8507583 [PMID: 30719448 DOI: 10.1155/2019/8507583]

42 **Brunt EM**, Tiniakos DG. Histopathology of nonalcoholic fatty liver disease. *World J Gastroenterol* 2010; **16**: 5286-5296 [PMID: 21072891 DOI: 10.3748/wjg.v16.i42.5286]

43 **Bajaj JS**. Alcohol, liver disease and the gut microbiota. *Nat Rev Gastroenterol Hepatol* 2019; **16**: 235-246 [PMID: 30643227 DOI: 10.1038/s41575-018-0099-1]

44 **Parlesak A**, Schäfer C, Schütz T, Bode JC, Bode C. Increased intestinal permeability to macromolecules and endotoxemia in patients with chronic alcohol abuse in different stages of alcohol-induced liver disease. *J Hepatol* 2000; **32**: 742-747 [PMID: 10845660 DOI: 10.1016/S0168-8278(00)80242-1]

45 **Xu D**, Huang Y, Wang J. Gut microbiota modulate the immune effect against hepatitis B virus infection. *Eur J Clin Microbiol Infect Dis* 2015; **34**: 2139-2147 [PMID: 26272175 DOI: 10.1007/s10096-015-2464-0]

46 **Bajaj JS**, Heuman DM, Hylemon PB, Sanyal AJ, White MB, Monteith P, Noble NA, Unser AB, Daita K, Fisher AR, Sikaroodi M, Gillevet PM. Altered profile of human gut microbiome is associated with cirrhosis and its complications. *J Hepatol* 2014; **60**: 940-947 [PMID: 24374295 DOI: 10.1016/j.jhep.2013.12.019]

47 **Chen Y**, Yang F, Lu H, Wang B, Chen Y, Lei D, Wang Y, Zhu B, Li L. Characterization of fecal microbial communities in patients with liver cirrhosis. *Hepatology* 2011; **54**: 562-572 [PMID: 21574172 DOI: 10.1002/hep.24423]

48 **Kakiyama G**, Pandak WM, Gillevet PM, Hylemon PB, Heuman DM, Daita K, Takei H, Muto A, Nittono H, Ridlon JM, White MB, Noble NA, Monteith P, Fuchs M, Thacker LR, Sikaroodi M, Bajaj JS. Modulation of the fecal bile acid profile by gut microbiota in cirrhosis. *J Hepatol* 2013; **58**: 949-955 [PMID: 23333527 DOI: 10.1016/j.jhep.2013.01.003]

49 **Qin N**, Yang F, Li A, Prifti E, Chen Y, Shao L, Guo J, Le Chatelier E, Yao J, Wu L, Zhou J, Ni S, Liu L, Pons N, Batto JM, Kennedy SP, Leonard P, Yuan C, Ding W, Hu X, Zheng B, Qian G, Xu W, Ehrlich SD, Zheng S, Li L. Alterations of the human gut microbiome in liver cirrhosis. *Nature* 2014; **513**: 59-64 [PMID: 25079328 DOI: 10.1038/nature13568]

50 **Qin N**, Le Chatelier E, Guo J, Prifti E, Li L, Ehrlich SD. Qin *et al*. reply. *Nature* 2015; **525**: E2-E3 [PMID: 26381989 DOI: 10.1038/nature14852]

51 **Bajaj JS**, Betrapally NS, Gillevet PM. Decompensated cirrhosis and microbiome interpretation. *Nature* 2015; **525**: E1-E2 [PMID: 26381988 DOI: 10.1038/nature14851]

52 **Eslam M**, Newsome PN, Sarin SK, Anstee QM, Targher G, Romero-Gomez M, Zelber-Sagi S, Wai-Sun Wong V, Dufour JF, Schattenberg JM, Kawaguchi T, Arrese M, Valenti L, Shiha G, Tiribelli C, Yki-Järvinen H, Fan JG, Grønbæk H, Yilmaz Y, Cortez-Pinto H, Oliveira CP, Bedossa P, Adams LA, Zheng MH, Fouad Y, Chan WK, Mendez-Sanchez N, Ahn SH, Castera L, Bugianesi E, Ratziu V, George J. A new definition for metabolic dysfunction-associated fatty liver disease: An international expert consensus statement. *J Hepatol* 2020; **73**: 202-209 [PMID: 32278004 DOI: 10.1016/j.jhep.2020.03.039]

53 **Eslam M**, Sanyal AJ, George J; International Consensus Panel. MAFLD: A Consensus-Driven Proposed Nomenclature for Metabolic Associated Fatty Liver Disease. *Gastroenterology* 2020; **158**: 1999-2014.e1 [PMID: 32044314 DOI: 10.1053/j.gastro.2019.11.312]

54 **Ferolla SM**, Armiliato GN, Couto CA, Ferrari TC. The role of intestinal bacteria overgrowth in obesity-related nonalcoholic fatty liver disease. *Nutrients* 2014; **6**: 5583-5599 [PMID: 25479248 DOI: 10.3390/nu6125583]

55 **Ghoshal UC**, Goel A, Quigley EMM. Gut microbiota abnormalities, small intestinal bacterial overgrowth, and non-alcoholic fatty liver disease: An emerging paradigm. *Indian J Gastroenterol* 2020; **39**: 9-21 [PMID: 32291578 DOI: 10.1007/s12664-020-01027-w]

56 **Augustyn M**, Grys I, Kukla M. Small intestinal bacterial overgrowth and nonalcoholic fatty liver disease. *Clin Exp Hepatol* 2019; **5**: 1-10 [PMID: 30915401 DOI: 10.5114/ceh.2019.83151]

57 **Wu WC**, Zhao W, Li S. Small intestinal bacteria overgrowth decreases small intestinal motility in the NASH rats. *World J Gastroenterol* 2008; **14**: 313-317 [PMID: 18186574 DOI: 10.3748/wjg.14.313]

58 **Kessoku T**, Imajo K, Kobayashi T, Ozaki A, Iwaki M, Honda Y, Kato T, Ogawa Y, Tomeno W, Kato S, Higurashi T, Yoneda M, Kirikoshi H, Kubota K, Taguri M, Yamanaka T, Usuda H, Wada K, Kobayashi N, Saito S, Nakajima A. Lubiprostone in patients with non-alcoholic fatty liver disease: a randomised, double-blind, placebo-controlled, phase 2a trial. *Lancet Gastroenterol Hepatol* 2020; **5**: 996-1007 [PMID: 32805205 DOI: 10.1016/S2468-1253(20)30216-8]

59 **Schattenberg JM**. Intestinal motility: a therapeutic target for NAFLD? *Lancet Gastroenterol Hepatol* 2020; **5**: 957-958 [PMID: 32805206 DOI: 10.1016/S2468-1253(20)30204-1]

60 **Gotfried J**, Priest S, Schey R. Diabetes and the Small Intestine. *Curr Treat Options Gastroenterol* 2017; **15**: 490-507 [PMID: 28913777 DOI: 10.1007/s11938-017-0155-x]

61 **Weaver MJ**, McHenry SA, Sayuk GS, Gyawali CP, Davidson NO. Bile Acid Diarrhea and NAFLD: Shared Pathways for Distinct Phenotypes. *Hepatol Commun* 2020; **4**: 493-503 [PMID: 32258945 DOI: 10.1002/hep4.1485]

62 **Reddy SK**, Zhan M, Alexander HR, El-Kamary SS. Nonalcoholic fatty liver disease is associated with benign gastrointestinal disorders. *World J Gastroenterol* 2013; **19**: 8301-8311 [PMID: 24363521 DOI: 10.3748/wjg.v19.i45.8301]

63 **Bush H**, Golabi P, Otgonsuren M, Rafiq N, Venkatesan C, Younossi ZM. Nonalcoholic Fatty Liver is Contributing to the Increase in Cases of Liver Disease in US Emergency Departments. *J Clin Gastroenterol* 2019; **53**: 58-64 [PMID: 29608451 DOI: 10.1097/MCG.0000000000001026]

64 **Dubberke ER**, Reske KA, McDonald LC, Fraser VJ. ICD-9 codes and surveillance for Clostridium difficile-associated disease. *Emerg Infect Dis* 2006; **12**: 1576-1579 [PMID: 17176576 DOI: 10.3201/eid1210.060016]

65 **Scheurer DB**, Hicks LS, Cook EF, Schnipper JL. Accuracy of ICD-9 coding for Clostridium difficile infections: a retrospective cohort. *Epidemiol Infect* 2007; **135**: 1010-1013 [PMID: 17156501 DOI: 10.1017/S0950268806007655]

66 **Khoruts A**, Staley C, Sadowsky MJ. Faecal microbiota transplantation for Clostridioides difficile: mechanisms and pharmacology. *Nat Rev Gastroenterol Hepatol* 2021; **18**: 67-80 [PMID: 32843743 DOI: 10.1038/s41575-020-0350-4]

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ORIGINAL ARTICLE

Observational Study

Six-minute walking test performance is associated with survival in cirrhotic patients

Carolina Frade M G Pimentel, Ana Cristina de Castro Amaral, Adriano Miziara Gonzalez, Michelle Lai, Daniel de Oliveira Mota, Maria Lucia Gomes Ferraz, Wilson Mathias Junior, Mario Kondo

ORCID number: Carolina Frade M G Pimentel 0000-0001-8092-1106; Ana Cristina de Castro Amaral 0000-0002-8290-7073; Adriano Miziara Gonzalez 0000-0002-5425-7886; Michelle Lai 0000-0002-1035-0659; Daniel de Oliveira Mota 0000-0002-9629-8512; Maria Lucia Gomes Ferraz 0000-0001-8992-8494; Wilson Mathias Junior 0000-0003-0201-6754; Mario Kondo 0000-0002-0079-2955.

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Carolina Frade M G Pimentel, Department of Medicine, Federal University of Sao Paulo, Sao Paulo 04026090, Brazil

Ana Cristina de Castro Amaral, Maria Lucia Gomes Ferraz, Mario Kondo, Department of Gastroenterology, Federal University of Sao Paulo, Sao Paulo 04023062, Brazil

Adriano Miziara Gonzalez, Department of Surgery, Liver Transplantation Service, Federal University of Sao Paulo, Sao Paulo 04026090, Brazil

Michelle Lai, Department of Medicine, Division of Gastroenterology and Hepatology, Beth Israel Deaconess Medical Center, Boston, MA 02215, United States

Daniel de Oliveira Mota, Department of Industrial Engineering, University of Sao Paulo, Sao Paulo 05508010, Brazil

Wilson Mathias Junior, Department of Cardiology, Heart Institute, University of Sao Paulo, Sao Paulo 05403900, Brazil

Corresponding author: Carolina Frade M G Pimentel, MD, Professor, Department of Medicine, Federal University of Sao Paulo, Botucatu Street n 740, Sao Paulo 04026090, Brazil.
carolinapimentel.gastro@gmail.com

Abstract

BACKGROUND

Patients with cirrhosis are at risk of cirrhotic cardiomyopathy, with resulting cardiac dysfunction and exercise limitations. Six minute walking test (6MWT) assesses functional status and predicts morbidity and mortality in cardiopulmonary diseases.

AIM

To determine if it associates with mortality by analyzing 6MWT performance in patients with liver cirrhosis.

METHODS

A cohort of 106 cirrhotic patients was evaluated in the outpatient setting with echocardiogram and 6MWT and follow up for one year to document hepatic decompensation and mortality. The distance in meters was recorded at the end of

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6 min (6MWD).

RESULTS
This cohort had a mean age of 51 years and 56% male; patients were staged as Child A in 21.7%, B 66% and C 12.3%. Walk distance inversely correlated with Child scores, and was significantly reduced as Child stages progresses. Patients who died (10.4%) showed shorter mean 6MWD (P = 0.006). Low 6MWD was an independent predictor of mortality (P = 0.01).

CONCLUSION
6MWT is a noninvasive inexpensive test whose result is related to Child scores and mortality. It is useful to identify patients with liver cirrhosis at high risk of mortality for closer monitoring and potential early intervention.

Key Words: Six-minute walking test; Liver cirrhosis; Hospital admission and mortality; Child score

Core Tip: Our study proposes that six-minute walking test, a simple exercise test, can be applicable in the evaluation of cirrhotic patients. This is a well-none routine assessment in patients with cardiopulmonary diseases, where it is used to predict mortality in this population. Its use in liver cirrhosis is limited. Patients with chronic hepatic insufficient are at risk of progressively muscle loss, frailty, and exercise limitation, all factors directly associated with poor survival. We propose by using six-minute walk test a practical and simple manner of assess this risks and provide a better understanding of how exercise limitation can directly affect survival.

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INTRODUCTION

Liver cirrhosis is related to functional impairment leading to reduction in physical fitness[1,2]. Some possible factors implicated in this process are profound muscle wasting (or cirrhotic myopathy)[3], cardiac dysfunction (cirrhotic cardiomyopathy)[4], autonomic dysfunction (chronotropic incompetence) and concurrent pulmonary disease (portopulmonary hypertension and hepatopulmonary syndrome). Recently studies reinforce the importance of frailty scores as a prediction of mortality in liver transplantation list[5,6], giving emphasis in sarcopenia and physical fitness as important factors associated with mortality[7].

The six-minute walk test (6MWT) is a practical simple inexpensive test that provides a global assessment of all systems involved during exercise[8]. Although it does not give information about specific organ impairment, it evaluates overall exercise capacity and has been shown, in patients with cardiac disease, to correlate with the maximal oxygen consumption (VO_2) and survival[9].

Some studies demonstrated that short distance during 6MWT (6MWD) predicted poorer prognosis and disease outcome in patients with heart failure[10] and chronic obstructive pulmonary disease[11]. In addition, this test can be used to assess the overall functional status and quantify response to a certain intervention[8] in a variety of other chronic diseases and in the elderly population[9-12].

Previous studies highlight the importance of 6MWD in predicting survival in cirrhotic and non-cirrhotic patients[13-16]. There are also evidences suggesting an association between exercise performance and increase risk of death on the waiting liver transplantation list[15-18]. Despite its role in long term survival in different chronic diseases, the impact in mortality prediction in cirrhotic patients is underes-

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timated over years.

The aim of this study was to analyze the association between 6MWT and long-term mortality in a cohort of cirrhotic patients.

MATERIALS AND METHODS

A total of 106 outpatients with liver cirrhosis (57 male, mean age 51.2 ± 12.9 years) was included in the present study. Cirrhosis was defined by clinical history, physical examination, laboratory analysis and at least one imaging data. Disease prognosis and severity were established based on Child and MELD scores, according to original scores definitions[19,20]. Exclusion criteria were any previous or current cardiovascular or pulmonary disease, heart failure or diagnosis of hemochromatosis (when cardiac involvement was documented). Patients who had a history of alcohol abuse (more than 20 g and 60 g of ethanol per day for women and men, respectively) [21] were included if they had abstained from alcohol use for at least 6 mo prior to enrollment. Patients with non-sinus rhythm, decompensated arterial hypertension, low peripheral oxygen saturation (SpO_2 < 90%), recent history (less than 3 mo) of new liver related decompensation or hospitalizations were also excluded (patients with previous ascites or encephalopathy were included, those characterized with chronic decompensated patients). Patients with neuromuscular diseases, myopathy, balance deficits or orthopedic disorders were also excluded. Patients who have previously received a liver transplant were not included. No paracentesis was performed within at least one week prior to exercise, avoiding volume depletion or electrolyte imbalances.

One hundred and sixty-four patients were consecutively screened from two liver transplantation centers between October 2014 and December 2014, 58 out of 164 were excluded according to previous criteria, most of the due to cardiovascular disorders (26%) or active alcohol consumption (19%). On the day of enrollment, patients provided written informed consent and had blood samples collected and 6MWT done. Electrocardiogram and transthoracic bubble echocardiogram were performed within 1 mo of enrollment.

Patients were followed-up by clinical visits, hospital records or telephone calls to patients to capture deaths and their causes. Patients were stratified according to their ability to complete 6MWT, whether they achieved or not predicted distance according to gender and age, and pattern of symptom secondary to physical effort due to the test. Patients included were follow-up to one year, main outcomes were defined as death or liver transplantation.

The study has been performed in accordance with the Declaration of Helsinki (2000) and approved by the Ethics Committee of our institution.

6MWT

The 6MWT was conducted according to American Thoracic Society guidelines[8] and supervised by a qualified physician. The test was performed indoors, along a 30 m flat, straight corridor with a hard surface and free of any type of obstacles. Before starting the test, all patients were provided instructions by the evaluator, encouraged to walk as far as possible within 6 min, and instructed to stop if pain, dyspnea, or other symptoms. The distance in meters was recorded at the end of the six minutes (6MWD). Predicted distances were computed according to specific equations for gender, weight, height and age[22]. Predicted distance achieved percentage (%6MWD) is then derived by dividing the actual 6MWD divided by the predicted distance.

Statistical analysis

Data were analyzed using a statistical software program (IBM® SPSS® Statistics, version 22.0). Logistic regressions were performed to evaluate the independent association between 6MWD and death. Receiver operating curves (ROC) and the area under ROC (AUROC) were computed to estimate sensitivity, specificity and cut-off points for 6MWD used in regression models, selected by Youden's index. COX regression analysis and Kaplan-Meier curves were performed and significant differences between the later were assessed by means of the log-rank test. We performed subgroup analysis according achievement of liver transplantation in order to evaluated 6MWT distance as a predictor of death.

RESULTS

Patient characteristics

The main demographic, clinical, and laboratory characteristics of the patients are presented in Table 1. One hundred and six patients were selected from two liver transplantation centers in Sao Paulo, Brazil. The majority was male (56%), and non-alcoholic etiology of the liver disease was the most common (69.8%). The mean MELD was 11.1, Child B more common (66%), and 74% of patients presented a history of at least one liver related decompensation. Ascites was identified in 32.1% and hepatic encephalopathy in 10.4% of patients on the day of the test.

All patients were followed until death, time of transplantation or end of study follow-up (12 mo). During the study period, 11 patients died and 3 underwent liver transplantation. All deaths were related to hepatic decompensation.

The majority of this cohort (71.7%) did not achieve the predicted distance adjusted for age and gender according to standardized equations[22] (678 ± 131m, 402-890 m) (see Figure 1). 6MWT performance is demonstrated in Table 2. The mean 6MWD of this cohort was 515 ± 138 m, 180-960 m. Not surprisingly, older patients with higher Child score, worse hepatic synthetic function (lower albumin) and anemia performed worse. It was found to be inversely correlated with age (r = -0.391, P < 0.001) and Child score (r = -0.228, P = 0.019), and positively correlated with albumin (r = 0.242, P = 0.012), creatinine (r = 0.242, P = 0.018) and hemoglobin (r = 0.192, P = 0.048). Patients with a history of at least one hepatic decompensation in the past (74.5%) presented with significant shorter 6MWD (496 ± 141 m *vs* 571 ± 115 m, P = 0.015).

The mean 6MWD was progressively shorter among Child classes (A = 570 ± 144 m, B = 504 ± 137 m and C = 471 ± 115 m) and statistical significance was demonstrated between Child A and C (P = 0.04) and when Child A was compared with more advanced stages (B and C), P = 0.02. 6MWD was different among compensated (Child Pugh A) and decompensated (Child Pugh B and C) patients (P = 0.031) (see Figure 2). Patients decompensated with ascites or hepatic encephalopathy on the day of the test achieved shorter distances than those who did not have ascites or hepatic encephalopathy (472 *vs* 534m, P = 0.03; 440 *vs* 525m, P = 0.04, respectively). All patients previously included were submitted to 6MWT, even those with hepatic decompensation at the moment of evaluation, ascites or encephalopathy. 6MWD did not differ according to the etiologies of cirrhosis (P = 0.08), past history of alcohol abuse (P = 0.58), use of beta-blocker (P = 0.19), tobacco (P = 0.97) and presence of anemia (P = 0.84).

None of the patient presented with liver related decompensation within 2 wk following the exercise, meaning no detectable clinically significant portal hypertension increase induced by exercise. All patients were able to perform exercise adequately, without help, interruptions, or any significantly adverse effect.

To emphasize the role of 6MWD and %6MWD in the prediction mortality, as an additional factor besides liver disease severity, logistic regression models were designed to evaluate if the inclusion of 6MWT parameters improves the model performance and increases the AUROC computed using regression models. MELD and Child score were used to quantify the severity of liver disease. When 6MWT parameters were added to the models designed to predicted mortality using MELD or Child score, we observed an improvement in model performance, defined as a significant difference according to Omnibus Chi-square test (P = 0.01) and higher AUROCs in combining models (see Figure 3).

Cutoff points associated with mortality was 387 m for 6MWD (sensibility 90.9 and specificity 88.4) and 0.82 for %6MWD (sensibility 100 and specificity 83.2). After exclusion of patients who were submitted to liver transplantation, patients who died (11, 10.4%) had a shorter mean 6MWD (423 m *vs* 526 m, P = 0.006) and lower %6MWD (0.72 *vs* 0.92, P = 0.004). Just one of them achieved the predicted distance during 6MWT. 6MWD and %6MWD were independent predictors of mortality, after adjusted for Child scores, according to multivariate regression model analysis (Table 3). Patients who achieved distances shorter than 387 m or %6MWD < 0.82 presented higher mortality, and statistical difference according to Kaplan-Meier and log-rank analysis (P = 0.004 and P = 0.006, respectively) (Figure 4).

DISCUSSION

6MWT is a safe, easy-to-administer, and inexpensive test to determine the functional capacity of cirrhotic patients and also has prognostic value. We found that a decreased

Table 1 Patients' characteristics (*n* = 106), *n* (%)

Characteristic	*n* (%) or means ± SD
Gender M/F	59/47 (56/44)
Age (yr)	51 ± 13
BMI (kg/m^2)	25.7 ± 4.7
PASP (mmHg)	25.4 ± 8.0
Cirrhosis etiology	
Virus	36 (33.9)
Alcohol	32 (30.2)
NASH	8 (7.5)
Others	30 (28.4)
Child-Pugh class *n*	7.1 ± 1.8
A	23 (21.7)
B	70 (66)
C	13 (12.3)
MELD	11.1 ± 3.1
Previous history of liver related decompensation	76 (73.8)
Hypertension	19 (17.9)
Diabetes	26 (24.5)
Tobacco smoking	12 (11.4)
Beta-blocker use	32 (30.2)
Hepatic decompensation on the day of the test	
Ascites	34 (32.1)
(Grade 1, 2, and 3)	(11.3, 17, 5)
Peripheral edema	13 (12.3)
Hepatic encephalopathy	13 (12.3)
(Grade 1, 2, 3, and 4)	(10.4, 1.9, 0, 0)
Hepatocellular carcinoma	5 (4.7)
Patient on the liver transplantation waiting list	35 (33)
Baseline laboratory[1]	
Hemoglobin (mg/dL)	13.1 ± 1.9
Hematocrit (%)	39.3 ± 5.4
Albumin (g/dL)Bilirubin (mg/dL)INR	3.5 ± 0.62.0 ± 1.51.2 ± 0.2
Creatinine (mg/dL)	0.8 ± 0.3
Na (mmol/L)	137.8 ± 2.1
K (mmol/L)	4.1 ± 0.5
Mg (mg/dL)	1.8 ± 0.2
Ca (mmol/L)	1.2 ± 0.1

[1]Continuous variables are shown as means ± SD.
Reference range values: Na (136-145); K (3.5-5.0); Mg (1.6-2.6) and Ca (1.15-1.29).
M: Male; F: Female; PASP: Pulmonary arterial systolic pressure.

Table 2 Six minute walking test performance in 106 patients with liver cirrhosis

Variable	6MWD (m)	*P* (*t*-test when applicable)	6MWD (%)	*P* (*t*-test when applicable)
Mean 6MWD (m)	515 ± 138			
Mean 6MWD (%)			0.91 ± 2.3	
6MWD according to Child classes				
A	570 ± 144		0.97 ± 0.22	
B	504 ± 137		0.88 ± 0.21	
C	471 ± 115		0.82 ± 0.25	
6MWD according to				
Liver decompensation				
Ascites (w vs wo)	473 ± 20 *vs* 535 ± 17	0.03	0.86 ± 0.22 *vs* 0.95 ± 0.21	0.028
Hepatic encephalopathy (w *vs* wo)	435 ± 34 *vs* 525 ± 14	0.04	0.87 ± 0.25 *vs* 0.91 ± 0.21	0.87
History of previous hepatic decompensation (w *vs* wo)	496 ± 141 *vs* 571 ± 115	0.02	0.86 ± 0.22 *vs* 1.02 ± 0.17	0.004
Hospital admission during follow-up (w *vs* wo)	444 ± 172 *vs* 531 ± 125	0.01	0.77 ± 0.25 *vs* 0.92 ± 0.20	0.004
Survival (died *vs* survived)	423 ± 122 *vs* 526 ± 137	0.02	0.72 ± 0.21 *vs* 0.93 ± 0.21	

6MWT: Six-minute walking test; 6MWD: Six-minute walking distance; 6MW (%): Predicted distance achieved percentage; w: With; wo: Without.

6MWD, as a marker of impaired exercise capacity, is associated with hepatic dysfunction. In addition, 6MWD and %6MWD performed as independent predictors of mortality, becoming an important tool during risk evaluation of severe complications and death in liver cirrhosis. Also, this study reinforces the key importance of physical evaluation during cirrhotic patients, especially those referred to liver transplantation team.

Basal exercise capacity was significantly impaired in our patients, as only 28.3% achieved the pre-test predicted distance. The 6MWD results in our cohort of patients was similar to previous studies in patients with cirrhosis which found a significantly lower 6MWD values than expected for healthy population[22]. Our cohort had a mean 89.7% (34.8%-149%) of predicted 6MWD (*vs* 63% found by Román *et al*[18], and a mean 6MWD of 515 m (180-960 m), compared to 306 m in Alameri *et al*[14]'s cohort of 98 patients with cirrhosis. The poor performance during 6MWT meets with the current knowledge about the abnormal exercise capacity in cirrhotic patients. Future studies should verify those findings and evaluate if 6MWD can be used as a more general tool able to evaluate outcomes and quality of life in this group[15].

We reported a weak inverse correlation between 6MWD and Child scores (r = -0.228, P = 0.019), although it was clear the tendency in walk distance reduction along Child classes. Carey *et al*[15], studying 121 cirrhotic patients, showed a strong correlation with MELD. In this particular study, all patients were listed for liver transplant, denoting a population with more advanced disease, making us understand that this stronger correlation reflects a major prevalence of their patient's overall disability when comparing to our study group. In the same way, by comparing subjects with advanced disease (Child B and C) and those without it (Child A), we detected a significant difference between these groups (P = 0.02), supporting the previous interpretation. Furthermore, patients with a history of at least one hepatic decompensation in the past, presented shorter 6MWD (P = 0.015) and subjects presenting with ascites or encephalopathy at the moment of evaluation performed worse, these facts highlight the relationship between shorter distances and severity of liver disease in our study. Similarly, Wong *et al*[23] reported that patients with decompensated cirrhosis with ascites performed worse during cycle ergometer evaluation when compared to well compensated patients, however, no specific data is

Table 3 Association between six-minute walking test parameters and unfavorable clinical outcomes (hospital admissions and mortality) using logistic regression models

	Hospital Admission						Mortality					
Predictors	**Univariate**			**Multivariate**			**Univariate**			**Multivariate**		
	b	p	OR	b	p	OR	b	p	OR	b	p	OR
Child score	0.74	< 0.01	2.1	0.72	< 0.01	2.05	1.01	< 0.01	2.75	1.03	< 0.01	2.8
6MWD	-0.005	< 0.01	0.99	-0.005	0.24	0.99	-0.007	0.01	0.99	-0.007	0.04	0.99
%6MWD	-0.04	0.01	0.96	-0.03	0.03	0.96	-0.05	0.02	0.95	-0.05	0.03	0.95
6MWD ≤ 444 m	-1.395	0.007	0.3	-1.462	0.01	0.2	-	-	-	-	-	-
6MWD ≤ 387 m	-	-	-	-	-	-	1.659	0.004	5.25	-1.17	0.2	0.3[1]

[1]Confidential intervals for odds ratio are not represented but consider adequate for all analysis except forodds ratio.
OR: Odds ratio.

available regarding 6MWT.

Although the gold standard measurement of exercise capacity is maximal VO_2[24] measurement during treadmill or cycle ergometer tests, 6MWT is a cheap and simple test found to correlate with oxygen consumption that can be administered without special equipment or skilled staff that you can perform in clinic to give an immediate result. Noticeable that all patients in our study completed the full test, independently of the presence of ascites or encephalopathy, demonstrating one great advantage above other exercise tests, that sometimes require a more complex adaptation and comprehension about the technique. Cahalin *et al*[9] performed 6MWT and symptom-limited cardiopulmonary exercise testing in patients with heart failure during cardiac transplant evaluation. The authors described a significant correlation between 6MWD and peak VO_2 ($r = 0.64$, $P < 0.001$), concluding that 6MWT is a valuable tool to predict VO_2 and short-term survival. These results should be validated in cirrhotic population, but represent a good evidence that 6MWT could be introduced in routine practice without loss of diagnostic accuracy in exercise capacity estimation. While our study did not evaluate the association between VO_2 and 6MWD, it did show the safety and practicality of this procedure. García-Pagàn *et al*[25] reported that moderate exercise (30% of the maximum) significantly increases portal pressure in patients with portal hypertension, and, therefore, could increase the risk of variceal bleeding, ascites and encephalopathy. Although 6MWT is a submaximal exercise, we did not identify any clinical event directly associated with it during the period following the test. Recent studies do not mention the prevalence of adverse events induced by exercise, and more studies designed to respond this issue should be carried out.

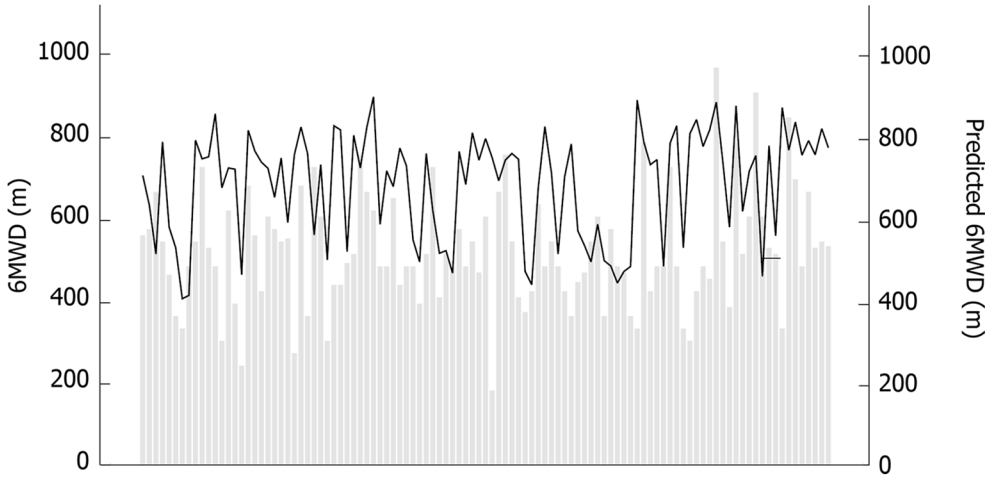

Figure 1 Relationship between predicted (line) and performed (bars) walking distance during six minute walking test. 6MWD: Six minute walking distance.

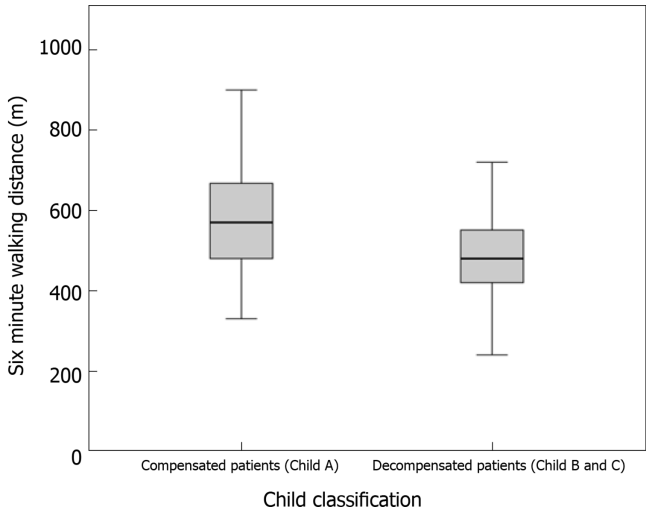

Figure 2 Distance in meters was recorded at the end of the six minutes among compensated (Child Pugh A) and decompensated (Child Pugh B and C) patients

Previously studies who reported the relationship between 6MWT and mortality were conducted with small populations and during a short period of followup[11,12]. Poor performance during 6MWT may warrant that the at-risk patients should be followed more closely due to the risk of adverse events. Notwithstanding, 6MWT has been proposed as a tool during frailty status evaluation, giving emphasis in this role as a practical and cheap method for this proposal. This study reinforces this importance, adding more powerful results due to our long period of follow-up, demonstrating how physical exercise evaluation may be an interesting long predictor of prognosis in cirrhotic patients.

In our study, 6MWD was an independent predictor of death, consistent with findings from previous studies by Alameri *et al*[14], and Carey *et al*[15]. In the first study, mortality was evaluated in the whole group, including patients with non-cirrhotic chronic hepatitis, which may bias the interpretation about causality between 6MWD and cirrhosis. Also, Carey *et al*[15] studied a population with more advanced disease, all of them on the liver transplant waiting list with a high frequency of liver transplantation (50.4%) performed in a short period of time (5-6 mo). The statistical power of 6MWT in predicting mortality could be affected by pulling out so many patients after transplant from this cohort.

The role of 6MWD and %6MWD in the prediction of mortality were independently of Child scores as demonstrated by multivariate logistic regression analysis. These facts highlight the association of 6MWT parameters with disease progression and adverse outcomes, despite the severity of liver disease.

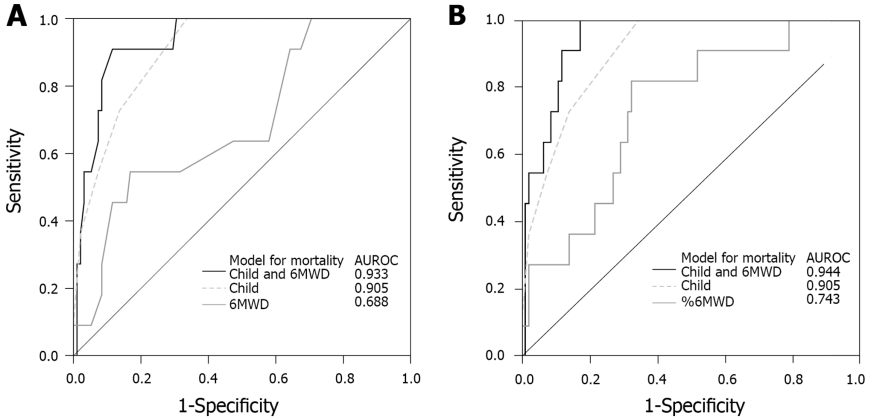

Figure 3 Progressive improvement in prediction of mortality using models combing six minute walking test parameters and Child scores.
6MWD: Six minute walking distance.

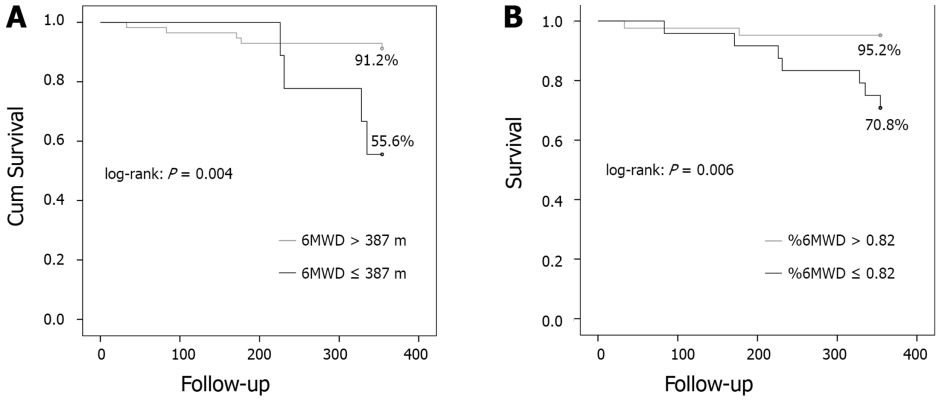

Figure 4 Kaplan Meier analysis for overall survival.

There are several limitations to our study. First, we did not proceed an external validation of 6MWD cutoffs used in our study, although our main objectives were focused in the transversal and descriptive characterization of study population. Second, we did neither evaluated nutritional status nor calculate the Frailty score of our patients. When study was designed there were no clear parameters specific settle for this diagnosis and a retrospective evaluation was not possible due to lack of complete data. Although recent studies suggest a close relationship between malnourished patients and physical capacity, in order to better evaluate this relationship, another specific protocol must be designed, which was not in accordance with our main objectives. Finally, we did not submit this cohort to a second phase 6MWT to evaluate the relationship between test performance and disease progression. Maybe this analysis could enhance the comprehension about the association of shorter 6MWD and severity of liver disease and its role as a marker of liver decompensation episodes. As we proposed a sectional evaluation of cirrhotic patients with 6MWT, future prospective studies should be able to better answer the previous questions.

CONCLUSION

In summary, 6MWT is a very simple, inexpensive, well tolerated, noninvasive test to assess exercise capacity and the result of which is related to MELD and Child scores. The present study showed that 6MWD is an independent predictor of mortality in this population. 6MWT is a promising prognostic marker in patients with liver cirrhosis and should be considered as part of liver transplantation evaluation especially in those referred for the liver transplantation team.

ARTICLE HIGHLIGHTS

Research background

Patients with cirrhosis are at risk of exercise limitations due to progressive limitations related to liver dysfunction. Sarcopenia and cirrhotic cardiomyopathy may be possible related factors. The six-minute walking test (6MWT) is a known simple and practical tool used to evaluate patients with cardiopulmonary disease.

Research motivation

In face of limited diagnosis tools focused on exercise capacity, we purposed to evaluate the role of 6MWT in this population.

Research objectives

The aim of our study was to analyzed 6MWT performance in patients with liver cirrhosis to determine if it associates with mortality.

Research methods

We analyzed 6MWT performance in 106 cirrhotic patients. They were evaluated in the outpatient setting with 6MWT and follow up for one year. Hepatic decompensation and mortality were documented.

Research results

This cohort had a mean age of 51 years and 56% male; patients were staged as Child A in 21.7%, B 66%, and C 12.3%. Walk distance inversely correlated with Child scores, and was significantly reduced as Child stages progress. Patients who died (10.4%) showed a shorter mean 6MWD (P = 0.006). Low 6MWD was an independent predictor of mortality (P = 0.01).

Research conclusions

6MWT is a noninvasive inexpensive test whose result is related to Child scores and mortality.

Research perspectives

It is a useful, simple, practical test that can be incorporated into cirrhotic evaluation due to its relation with mortality for closer monitoring and potential early intervention.

ACKNOWLEDGEMENTS

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REFERENCES

1 **Jones JC**, Coombes JS, Macdonald GA. Exercise capacity and muscle strength in patients with cirrhosis. *Liver Transpl* 2012; **18**: 146-151 [PMID: 22139897 DOI: 10.1002/lt.22472]
2 **Campillo B**, Fouet P, Bonnet JC, Atlan G. Submaximal oxygen consumption in liver cirrhosis. Evidence of severe functional aerobic impairment. *J Hepatol* 1990; **10**: 163-167 [PMID: 2332586 DOI: 10.1016/0168-8278(90)90046-t]
3 **Bunchorntavakul C**, Reddy KR. Review article: malnutrition/sarcopenia and frailty in patients with cirrhosis. *Aliment Pharmacol Ther* 2020; **51**: 64-77 [PMID: 31701570 DOI: 10.1111/apt.15571]
4 **Izzy M**, VanWagner LB, Lin G, Altieri M, Findlay JY, Oh JK, Watt KD, Lee SS; Cirrhotic Cardiomyopathy Consortium. Redefining Cirrhotic Cardiomyopathy for the Modern Era. *Hepatology* 2020; **71**: 334-345 [PMID: 31342529 DOI: 10.1002/hep.30875]
5 **Montano-Loza AJ**. Muscle wasting: a nutritional criterion to prioritize patients for liver transplantation. *Curr Opin Clin Nutr Metab Care* 2014; **17**: 219-225 [PMID: 24613858 DOI: 10.1097/MCO.0000000000000046]
6 **Lai JC**, Volk ML, Strasburg D, Alexander N. Performance-Based Measures Associate With Frailty in Patients With End-Stage Liver Disease. *Transplantation* 2016; **100**: 2656-2660 [PMID: 27495749 DOI: 10.1097/TP.0000000000001433]
7 **Bhanji RA**, Montano-Loza AJ, Watt KD. Sarcopenia in Cirrhosis: Looking Beyond the Skeletal

Muscle Loss to See the Systemic Disease. *Hepatology* 2019; **70**: 2193-2203 [PMID: 31034656 DOI: 10.1002/hep.30686]

8 **ATS Committee on Proficiency Standards for Clinical Pulmonary Function Laboratories**. ATS statement: guidelines for the six-minute walk test. *Am J Respir Crit Care Med* 2002; **166**: 111-117 [PMID: 12091180 DOI: 10.1164/ajrccm.166.1.at1102]

9 **Cahalin LP**, Mathier MA, Semigran MJ, Dec GW, DiSalvo TG. The six-minute walk test predicts peak oxygen uptake and survival in patients with advanced heart failure. *Chest* 1996; **110**: 325-332 [PMID: 8697828 DOI: 10.1378/chest.110.2.325]

10 **Roul G**, Germain P, Bareiss P. Does the 6-minute walk test predict the prognosis in patients with NYHA class II or III chronic heart failure? *Am Heart J* 1998; **136**: 449-457 [PMID: 9736136 DOI: 10.1016/S0002-8703(98)70219-4]

11 **Celli BR**, Cote CG, Marin JM, Casanova C, Montes de Oca M, Mendez RA, Pinto Plata V, Cabral HJ. The body-mass index, airflow obstruction, dyspnea, and exercise capacity index in chronic obstructive pulmonary disease. *N Engl J Med* 2004; **350**: 1005-1012 [PMID: 14999112 DOI: 10.1056/NEJMoa021322]

12 **Troosters T**, Gosselink R, Decramer M. Six minute walking distance in healthy elderly subjects. *Eur Respir J* 1999; **14**: 270-274 [PMID: 10515400 DOI: 10.1034/j.1399-3003.1999.14b06.x]

13 **Enright PL**, McBurnie MA, Bittner V, Tracy RP, McNamara R, Arnold A, Newman AB; Cardiovascular Health Study. The 6-min walk test: a quick measure of functional status in elderly adults. *Chest* 2003; **123**: 387-398 [PMID: 12576356 DOI: 10.1378/chest.123.2.387]

14 **Alameri HF**, Sanai FM, Al Dukhayil M, Azzam NA, Al-Swat KA, Hersi AS, Abdo AA. Six Minute Walk Test to assess functional capacity in chronic liver disease patients. *World J Gastroenterol* 2007; **13**: 3996-4001 [PMID: 17663517 DOI: 10.3748/wjg.v13.i29.3996]

15 **Carey EJ**, Steidley DE, Aqel BA, Byrne TJ, Mekeel KL, Rakela J, Vargas HE, Douglas DD. Six-minute walk distance predicts mortality in liver transplant candidates. *Liver Transpl* 2010; **16**: 1373-1378 [PMID: 21117246 DOI: 10.1002/lt.22167]

16 **Dharancy S**, Lemyze M, Boleslawski E, Neviere R, Declerck N, Canva V, Wallaert B, Mathurin P, Pruvot FR. Impact of impaired aerobic capacity on liver transplant candidates. *Transplantation* 2008; **86**: 1077-1083 [PMID: 18946345 DOI: 10.1097/TP.0b013e318187758b]

17 **Veloso-Guedes CA**, Rosalen ST, Thobias CM, Andreotti RM, Galhardo FD, Oliveira da Silva AM, Araujo O, Boin IF. Validation of 20-meter corridor for the 6-minute walk test in men on liver transplantation waiting list. *Transplant Proc* 2011; **43**: 1322-1324 [PMID: 21620120 DOI: 10.1016/j.transproceed.2011.03.057]

18 **Román E**, Torrades MT, Nadal MJ, Cárdenas G, Nieto JC, Vidal S, Bascuñana H, Juárez C, Guarner C, Córdoba J, Soriano G. Randomized pilot study: effects of an exercise programme and leucine supplementation in patients with cirrhosis. *Dig Dis Sci* 2014; **59**: 1966-1975 [PMID: 24599772 DOI: 10.1007/s10620-014-3086-6]

19 **Child CG**, Turcotte JG. Surgery and portal hypertension. *Major Probl Clin Surg* 1964; **1**: 1-85 [PMID: 4950264]

20 **Malinchoc M**, Kamath PS, Gordon FD, Peine CJ, Rank J, ter Borg PC. A model to predict poor survival in patients undergoing transjugular intrahepatic portosystemic shunts. *Hepatology* 2000; **31**: 864-871 [PMID: 10733541 DOI: 10.1053/he.2000.5852]

21 **O'Shea RS**, Dasarathy S, McCullough AJ; Practice Guideline Committee of the American Association for the Study of Liver Diseases; Practice Parameters Committee of the American College of Gastroenterology. Alcoholic liver disease. *Hepatology* 2010; **51**: 307-328 [PMID: 20034030 DOI: 10.1002/hep.23258]

22 **Enright PL**, Sherrill DL. Reference equations for the six-minute walk in healthy adults. *Am J Respir Crit Care Med* 1998; **158**: 1384-1387 [PMID: 9817683 DOI: 10.1164/ajrccm.158.5.9710086]

23 **Wong F**, Girgrah N, Graba J, Allidina Y, Liu P, Blendis L. The cardiac response to exercise in cirrhosis. *Gut* 2001; **49**: 268-275 [PMID: 11454805 DOI: 10.1136/gut.49.2.268]

24 **McArdle W**, Katch F, Katch V. Exercise Physiology. 7th ed. The Point, editor. Lippincott Williams & Wilkins; 2010: 192–247

25 **García-Pagàn JC**, Santos C, Barberá JA, Luca A, Roca J, Rodriguez-Roisin R, Bosch J, Rodés J. Physical exercise increases portal pressure in patients with cirrhosis and portal hypertension. *Gastroenterology* 1996; **111**: 1300-1306 [PMID: 8898644 DOI: 10.1053/gast.1996.v111.pm8898644]

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SYSTEMATIC REVIEWS

Incidence of umbilical vein catheter-associated thrombosis of the portal system: A systematic review and meta-analysis

Iliana Bersani, Fiammetta Piersigilli, Giulia Iacona, Immacolata Savarese, Francesca Campi, Andrea Dotta, Cinzia Auriti, Enrico Di Stasio, Matteo Garcovich

ORCID number: Iliana Bersani 0000-0002-3020-2274; Fiammetta Piersigilli 0000-0002-6581-2822; Giulia Iacona 0000-0002-9360-385X; Immacolata Savarese 0000-0002-1675-8081; Francesca Campi 0000-0001-7389-9000; Andrea Dotta 0000-0002-1260-6427; Cinzia Auriti 0000-0001-9820-6557; Enrico Di Stasio 0000-0003-1047-4261; Matteo Garcovich 0000-0002-5805-7953.

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Country/Territory of origin: Italy

Iliana Bersani, Immacolata Savarese, Francesca Campi, Andrea Dotta, Cinzia Auriti, Medical and Surgical Neonatology, Bambino Gesù Children's Hospital, Rome 00165, Italy

Fiammetta Piersigilli, Department of Neonatology, Cliniques Universitaires Saint Luc, Universit è Catholique de Louvain, Bruxelles 1200, Belgium

Giulia Iacona, Faculty of Medicine, Imperial College London, London SW7 2AZ, United Kingdom

Enrico Di Stasio, Department of Biochemistry and Clinical Biochemistry, Fondazione Policlinico Universitario Agostino Gemelli IRCCS, Università Cattolica del Sacro Cuore, Roma 00168, Italy

Matteo Garcovich, CEMAD Digestive Disease Center, Fondazione Policlinico Universitario Agostino Gemelli IRCCS, Rome 00168, Italy

Matteo Garcovich, CEMAD Digestive Disease Center, Università Cattolica del Sacro Cuore, Rome 00168, Italy

Corresponding author: Enrico Di Stasio, MD, PhD, Academic Research, Doctor, Department of Biochemistry and Clinical Biochemistry, Fondazione Policlinico Universitario Agostino Gemelli IRCCS, Università Cattolica del Sacro Cuore, Largo gemelli 8, Roma 00168, Italy. enrico.distasio@unicatt.it

Abstract

BACKGROUND

The use of umbilical venous catheters (UVCs) in the perinatal period may be associated with severe complications, including the occurrence of portal vein thrombosis (PVT).

AIM

To assess the incidence of UVC-related PVT in infants with postnatal age up to three months.

METHODS

A systematic and comprehensive database searching (PubMed, Cochrane Library, Scopus, Web of Science) was performed for studies from 1980 to 2020 (the search was last updated on November 28, 2020). We included in the final analyses all

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Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review report's scientific quality classification
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Grade B (Very good): B
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Grade D (Fair): 0
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peer-reviewed prospective cohort studies, retrospective cohort studies and case-control studies. The reference lists of included articles were hand-searched to identify additional studies of interest. Studies were considered eligible when they included infants with postnatal age up to three months with UVC-associated PVT. Incidence estimates were pooled by using random effects meta-analyses. The quality of included studies was assessed using the Newcastle-Ottawa scale. The systematic review was performed according to the Preferred Reporting Items for Systematic reviews and Meta-Analysis (PRISMA) guidelines.

RESULTS
Overall, 16 studies were considered eligible and included in the final analyses. The data confirmed the relevant risk of UVC-related thrombosis. The mean pooled incidence of such condition was 12%, although it varied across studies (0%-49%). In 15/16 studies (94%), diagnosis of thrombosis was made accidentally during routine screening controls, whilst in 1/16 study (6%) targeted imaging assessments were carried out in neonates with clinical concerns for a thrombus. Tip position was investigated by abdominal ultrasound (US) alone in 1/16 (6%) studies, by a combination of radiography and abdominal US in 14/16 (88%) studies and by a combination of radiography, abdominal US and echocardiography in 1/16 (6%) studies.

CONCLUSION
To the best of our knowledge, this is the first systematic review specifically investigating the incidence of UVC-related PVT. The use of UVCs requires a high index of suspicion, because its use is significantly associated with PVT. Well-designed prospective studies are required to assess the optimal approach to prevent UVC-related thrombosis of the portal system.

Key Words: Portal vein thrombosis; Umbilical venous catheter; Portal system thrombosis; Hepatic thrombosis; Neonate; Incidence

Core Tip: Portal vein thrombosis (PVT) is a dreadful complication that can occur after umbilical vein catheterization in neonates. Although previous observational studies have provided a general overview about the risk of this complication, the present systematic review specifically investigates the incidence catheter-related PVT and identifies relevant gaps in knowledge about the optimal diagnostic approach highlighting the need for prospective randomized studies and updated guidelines.

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INTRODUCTION

The placement of an umbilical venous catheter (UVC) is a common procedure in neonatology and has multiple clinical indications driven by the need for quick and secure access for medication administration[1]. During placement, the UVC should run through the umbilical vein, pass the medial portion of the left portal vein at the umbilico-portal confluence, join the direct communication existing between the umbilical vein and the ductus venosus and, through it, bypass the liver and join the inferior vena cava[2,3]. The UVC has to be placed in a central position, ideally at the junction between the inferior vena cava and the right atrium. If a central position is not achieved, then the tip of the catheter can be left below the liver, *i.e.*, below the level of umbilical-portal confluence (peripheral position). The UVC in peripheral position can be used as an emergency access, but it has to be replaced as soon as possible by a

central venous catheter. To prevent UVC-related complications, a proper assessment of catheter tip position is mandatory before its use. In fact, if the tip of the catheter is too deep, it can cause complications such as thrombo-embolic disorders, arrhythmias, and pericardial effusion. On the other hand, if the tip of the UVC is too low, then it can be associated with necrotizing enterocolitis, colon perforation, hepatic abscess, and portal vein thrombosis (PVT)[1,4-9]. Furthermore, if the ductus venosus is not perfectly aligned to the umbilical vein, the UVC may unintentionally enter the portal system through the left portal vein during placement and possibly lead to severe complications involving both the hepatic vasculature and parenchyma[1,2,5-8,10-16]. Such liver complications may arise from multiple mechanisms including thrombosis of the portal system vasculature, infusion of irritating drugs and/or hypertonic solutions within the UVC leading to hepatic necrotizing direct mechanical injury[3,17-19]. Besides individual hereditary or acquired predisposing factors (such as prematurity, hereditary prothrombotic disorders, sepsis, the need of transfusions, hyper-viscosity syndrome, dehydration, asphyxia, congenital malformations *etc*.), whose actual role is still debated[3,10,19-26], umbilical venous catheterization itself represents a risk factor for the development of PVT[18]. In fact, multiple factors may explain the association between UVC and PVT: The introduction of a foreign surface with thrombogenic properties in a small diameter vessel, endothelial damage, and the well-known prothrombotic predisposition typical of the neonatal period[27-29]. Symptoms/signs suggestive of PVT may include unexplained thrombocytopenia, catheter-obstructed fluid delivery, increased UVC in-line pressure, impaired lower body/extremity perfusion, although PVT may remain completely asymptomatic[30,31]. When persisting, PVT may inflict substantial damage to the liver leading to portal hypertension, mainly related to the increased vascular resistance in the portal venous system, and to liver atrophy[11,19,32].

In the present systematic review, we specifically focused our search attention on the risk of UVC-related PVT. Although multiple observational studies have provided an overview about the risk of PVT after UVC positioning, to the best of our knowledge no reviews explored systematically this issue. Our aim was to investigate the most accurate information about the actual incidence of UVC-related PVT in the neonatal setting, and to assess if any particular risk factor was systematically associated with the development of such complication.

MATERIALS AND METHODS

The review followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) reporting guidelines[33].

The PICOS strategy was used, which comprised the following (PRISMA): Population: Infants with less than three months of postnatal age; Intervention (or exposure): Umbilical venous catheter; Comparison: No catheter; Outcome (primary): Incidence of PVT; Outcome (secondary): Association with a specific risk factor; Study type: Peer-reviewed observational, cohort and case-control studies.

There was no funding agency for this study. The systematic review did not require ethical approval/informed consent since there was no direct contact with individual patients, and only previously published data were included in the analyses.

Outcomes

The primary outcome was the incidence of PVT related to the use of UVCs (UVC only/attempted UVC/UVC + umbilical artery catheters) in infants with postnatal age up to three months. The secondary outcome was the identification of any risk factor associated with the development of UVC-related PVT.

Search strategy and selection criteria

The following search strategy was used: (portal OR vein OR system OR hepatic) AND (thrombosis) AND (neonat* OR newborn OR pediatric*) AND (catheter* OR umbilical). For reliability, three review authors (Bersani I, Iacona G and Piersigilli F) independently analyzed the currently available literature through systematic and comprehensive database searching (PubMed, Cochrane Library, Scopus, Web of Science) from 1980 to 2020 (the search was last updated on November 28, 2020). Reviews, *in vitro* studies, animal studies, autopsy studies and conference abstracts were excluded. The reference lists of the included articles were hand-searched to identify additional studies of interest. We obtained the full texts of all the potentially eligible studies.

Eligibility criteria

Three review authors independently undertook eligibility assessment (Bersani I, Iacona G and Piersigilli F). Any disagreement about study eligibility was resolved by discussion with a fourth review author (Garcovich M) until consensus. We considered the studies eligible if they investigated the incidence of UVC-related PVT in infants with postnatal age up to three months. For articles resulting eligible based on the title or abstract, the full paper was retrieved. Case reports were considered not eligible for the final analyses being the calculation of an incidence not possible for such study design. Non-English studies were considered not eligible for the final analyses. We finally included all peer-reviewed, English-language, prospective/retrospective cohort studies and case-control studies.

Study quality assessment

To assess the risk of bias, two authors (Bersani I and Garcovich M) independently used the Newcastle-Ottawa Scale for comparative nonrandomized studies corresponding to each study's design (cohort/cross-sectional)[34]. Such scale is a validated quality assessment instrument for non-randomized trials which evaluates three parameters of study quality: selection, comparability and exposure assessment. The scale assigns a maximum score of 4 for selection, 2 for comparability, and 3 for exposure, for a maximum total score of 9. Studies with a total score of ≥ 5 or ≥ 7 were considered to be of moderate or high quality, whereas those with a score of less than 5 were considered low-quality studies with high risk of bias. The scale results were tabulated in Table 1.

Data extraction

Three review authors independently performed data extraction (Bersani I, Iacona G and Piersigilli F). Disagreements about data extraction were resolved by discussion with a fourth review author (Garcovich M) until consensus. Pertinent findings from the included studies were tabulated in Table 2 and assessed according to pre-specified subgroups analyses: (1) Year of publication: 1980-2000 or 2001-2020; (2) Indication for thrombosis assessment: Abdominal US as systematic screening or abdominal ultrasound (US) only in case of a clinical concern for thrombosis; (3) Type of diagnostic technique to detect tip position: Radiography or/and (US) evaluation; (4) UVC model: UVC material, size (French), single or double lumen; (5) Thrombosis localization and type: Exact localization within the portal system, complete or partial; (6) Dwell time: Mean UVC in situ persistence (in days); and (7) Prophylaxis: None or heparin infusion or other.

Statistical analysis

Because of high heterogeneity, pooled data on the incidence of UVC-related PVT were analyzed using a random effects (DerSimonian and Laird method) model approach. Statistical heterogeneity among studies was assessed with Cochran's *Q* and quantified with Higgins I^2 statistic[35,36]. We considered an I^2 of < 25% as low heterogeneity, I^2 of 25% to 75% as moderate heterogeneity and I^2 > 75% as high heterogeneity. Publication bias was assessed graphically using funnel plots and qualitatively using Egger's regression and Begg rank correlation method. Statistical analysis was performed by using the Statistical Package for Social Science (SPSS 22.0; SPSS Inc, Chicago, IL, United States) and Microsoft Excel (Version 16.45).

RESULTS

The searches identified 2460 potentially relevant papers, 1835 after duplicates were removed. After title and abstract screening, 53 full-text studies were considered potentially eligible for inclusion and 37 studies were then excluded for the following reasons: (1) Not relevant comparators (n = 23); (2) Non-English language (n = 3); and (3) Wrong study design (n = 11) (Figure 1). Since the design/methodologies varied among different studies, information was not uniformly available for all analyses. For example, some studies could not be considered eligible, although pertinent, since the exact incidence UVC-associated PVT and/or the exact site of a catheter-related thrombosis and/or the exact age of patients with PVT could not be clearly extrapolated from the results.

According to the Newcastle-Ottawa Scale assessing the risk of bias, all the included studies were of moderate-high quality (Table 1). The characteristics and most relevant findings of the included studies are summarized in Table 2[5,21,30-32,37-45]. Of the 16

Table 1 Risk of bias assessment (Newcastle-Ottawa scale for non-randomized studies)

Ref.	Selection	Comparability	Outcome	Total score
Levit *et al*[42], 2020	4	2	3	9
Dubbink-Verheij *et al*[31], 2020	4	2	3	9
Chen *et al*[15], 2020	4	0	3	7
Hwang *et al*[46], 2020	4	2	3	9
Çakır *et al*[38], 2020	4	0	3	7
Cabannes *et al*[32], 2018	4	2	3	9
Derinkuyu *et al*[5], 2018	4	0	3	7
Chandrashekhar *et al*[45], 2015	4	0	3	7
Michel *et al*[37], 2012	4	2	3	9
Gharehbaghi *et al*[39], 2011	4	2	3	9
Sakha *et al*[41], 2007	4	2	3	9
Turebylu *et al*[21], 2007	4	2	3	9
Kim *et al*[30], 2001	4	2	3	9
Boo *et al*[44], 1999	4	2	3	9
Schwartz *et al*[40], 1997	4	0	3	7
Yadav *et al*[43], 1993	4	0	2	6

included studies, 14 were prospective and 2 were retrospective[15,46]. In some cases, the information about the clinical features of the included population was generically related to the overall cohort rather than specifically to neonates with UVC-related PVT and could not be extrapolated.

In the present review a total pooled sample of 4509 of neonates aged less than three months with UVC was included, 195 of whom experienced UVC-related PVT. The sample sizes ranged widely across studies (median, 83 patients; range, 22-2017). Mean gestational age and birth weight were 30.9 wk and 1738 g respectively, but it was not possible to extrapolate these data from each study, since neonates with PVT sometimes only represented a subgroup, whilst the available data mostly referred to the overall cohort. Figure 2 presents the results of overall meta-analysis with a random effects overall pooled-estimated incidence of UVC-related PVT of 12% [95% confidence interval (CI): 5.91-20.16], with high heterogeneity [I^2 = 97.5% (95%CI: 97.1%-97.9%)]. Figure 3 shows evidence of publication bias, as indicated by visual inspection of the funnel plot and by the Egger test for small study effects for the primary outcome [bias coefficient for the main analysis, 3.5309 (95%CI: 1.983176-5.078624); P = 0.0002].

When investigating the pre-specified subgroups analyses, we found the following data (Table 2): (1) Year of publication: Overall, 3/16 (19%) studies were published between 1980 and 2000, whereas 13/16 (81%) between 2001 and 2020; (2) Indication for thrombosis assessment: In 15/16 studies (94%), the diagnosis of thrombosis was made accidentally during routine screening controls, whilst in 1/16 study (6%) targeted imaging assessments were carried out in neonates with clinical concerns for a thrombus. In most studies it was not possible to extrapolate mean age at the time of PVT diagnosis (Table 2); (3) Type of diagnostic technique used to assess tip position: Tip position was never assessed exclusively by radiography or echocardiography alone, while it was investigated by abdominal US alone in 1/16 (6%) studies, by a combination of radiography and abdominal US in 14/16 (88%) studies and by a combination of radiography, abdominal US and echocardiography in 1/16 (6%) studies. Only a minority of studies (3/16 studies, with a total number of 39/195 neonates) explicitly specified wrong tip position at the first imaging assessment, in UVC-related PVT cases[32,37,39]. However, most of the studies did not provide such information specifically for neonates who developed PVT, but rather for the overall population. Follow-up imaging controls were scheduled differently across studies; (4) UVC model: Information about UVC material, size and lumen number was only specified by a minority of studies. When the information was available, the studies reported the use of polyvinyl UVCs (n = 3/16) or polyurethane (n = 3/16) UVCs. When described, UVC size varied from 2.5 French to 5 French; (5) Thrombosis

Table 2 Characteristics of included studies

Ref.	Study design	UVC with PVT	UVC without PVT	Dwel time UVC with PVT	Dwel time UVC without PVT	Indication to UVC control	Type of imaging	Country/territory
Levit *et al*[42], 2020	Prospective	1	2016	N/A	N/A	Clinical Suspicion	X-ray + US	United States
Dubbink-Verheij *et al*[31], 2020	Prospective	13	27	N/A	N/A	Screening	X-ray + US	The Netherlands
Chen *et al*[15], 2020	Retrospective	7	1320	N/A	N/A	Screening	X-ray + US	Taiwan
Hwang *et al*[46], 2020	Retrospective	15	54	N/A	N/A	Screening	X-ray + US	South Korea
Çakır *et al*[38], 2020	Prospective	13	83	10.5 ± 4.3[1]	12.2 ± 4.1[1]	Screening	X-ray + US	Turkey
Cabannes *et al*[32], 2018	Prospective	51	53	N/A	N/A	Screening	X-ray + US	France
Derinkuyu *et al*[5], 2018	Prospective	15	229	N/A	N/A	Screening	X-ray + US	Turkey
Chandrashekhar *et al*[45], 2015	Prospective	3	27	N/A	N/A	Screening	X-ray + US	India
Michel *et al*[37], 2012	Prospective	2	59	N/A	N/A	Screening	X-ray + US + Echocardiography	France
Gharehbaghi *et al*[39], 2011	Prospective	5	159	N/A	N/A	Screening	X-ray + US	Iran
Sakha *et al*[41], 2007	Prospective	17	33	2 ± 1.12[1]	N/A	Screening	US	Iran
Turebylu *et al*[21], 2007	Prospective	2	26	N/A	6	Screening	X-ray + US	United States
Kim *et al*[30], 2001	Prospective	43	57	> 6 d in 23/43	> 6 d in 6/57	Screening	X-ray + US	South Korea
Boo *et al*[44], 1999	Prospective	0	57	N/A	N/A	Screening	X-ray + US	Malaysia
Schwartz *et al*[40], 1997	Prospective	1	99	3	4 (0-12)[2]	Screening	X-ray + US	United States
Yadav *et al*[43], 1993	Prospective	7	15	N/A	N/A	Screening	X-ray + US	India

[1]Results are expressed as mean ± SD, if reported.

[2]Results are expressed as median (range), if reported.

UVC: umbilical venous catheter; PVT: portal vein thrombosis; N/A: Not applicable; US: Ultrasound (abdominal).

localization and type: Only a minority of studies specified PVT exact localization within the portal system. When reported, the left portal vein was the most frequently involved. Similarly, only a minority of studies (in a total number of 84/195 neonates) specified if PVT was complete or partial[5,30,38-41]. According to the available data, PVT was complete in 27/84 (32%) cases and partial in 57/84 (68%) cases; (6) Dwell time: Only a minority of studies reported explicitly the mean UVC dwelling time in neonates with PVT (since most of the studies provided mean dwelling time for the overall population); and (7) Prophylaxis: Only 6/16 (37%) studies reported a prophylactic administration of heparin[21,38,39,42,44,46].

DISCUSSION

To the best of our knowledge, this is the first systematic review specifically investigating the issue of UVC-related PVT. One of the most important limitations that emerged when reviewing the scientific literature was the extreme heterogeneity of study designs across the investigated studies (Table 2 and Figure 3).

As a whole, the data achieved by our systematic review confirmed the relevant risk of PVT associated with umbilical catheterization. The mean reported pooled incidence of neonatal UVC-related PVT among studies was 12%, with a range which varied from 0% to 49% from study to study (Figure 2). Such large difference might be attributed to

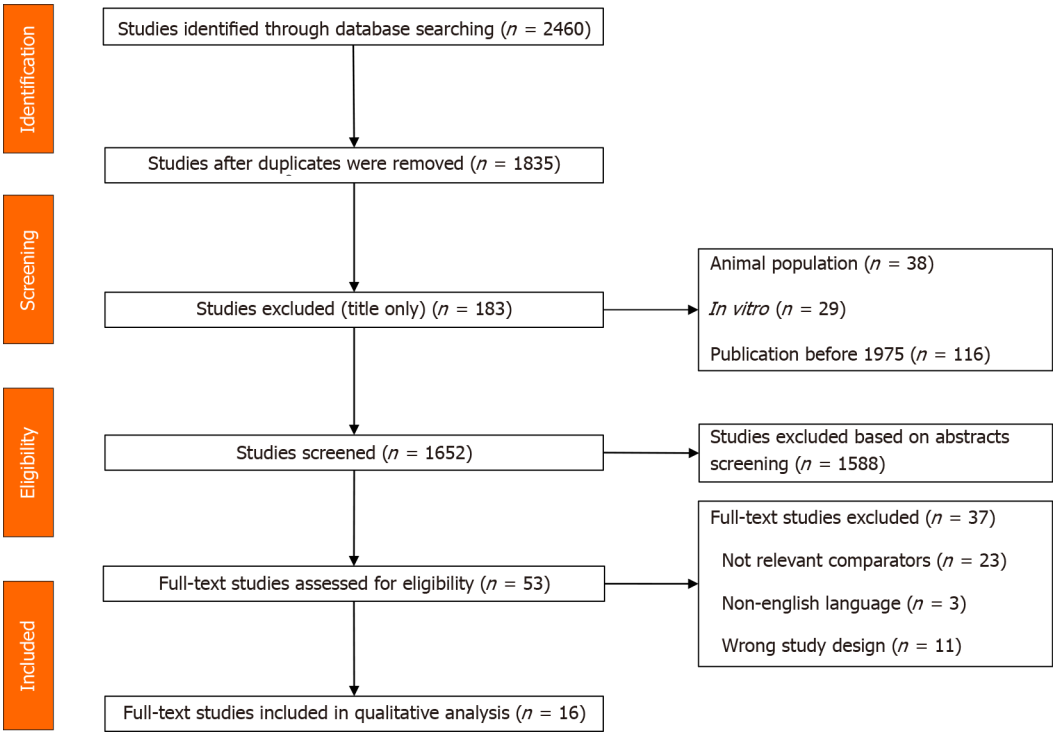

Figure 1 Flow-chart of study selection process.

multiple factors, including the different indication to imaging diagnostics, the different imaging time schedules, the heterogeneous UVC size/position/duration, and the proportion of preterm/term neonates[30,40,43]. Moreover, the time frame of research and publication may have influenced the incidence of UVC-related PVT as well. In fact, across literature, PVT was more frequently reported in the most recent studies. For example, a large multicenter registry assessing all thrombotic events occurring between 1989 and 1992 in 22 Canadian and 42 international centers from Europe, Australia and United States, recorded only 97 thrombotic events but did not explicitly report any case of PVT at all[47]. In contrast, a more recent large multicenter survey which included 187 children with a diagnosis of PVT (mean age at diagnosis: 4 years) reported a history of neonatal UVC placement in 65% of cases[19]. The higher incidence of PVT in recent years might be explained by the fact that clinicians are more aware of the thrombotic risk associated with the use of UVC and are more attentive to its detection. Furthermore, advances in US techniques make the detection of PVT easier.

The scientific literature emphasizes that UVC-related PVT is mostly related to improper tip position. Considering the small distance required for an UVC to become dislodged, UVC may migrate into the portal vein even following an initial proper positioning[2,15,16,42,48-52]. Therefore, tip location must be verified with accuracy not only soon after placement but also at regular intervals throughout time[30,31]. For this purpose, US is the ideal tool to check the position of the tip, since it is easy to perform for clinicians, it can be done at bedside and is not invasive for the patient.

When reviewing the literature, we found differences regarding the indication for US assessment, *i.e.*, systematic surveillance in asymptomatic neonates with history of UVCs *vs* targeted diagnostic test in neonates with clinical concerns for a thrombus. However, in the studies which were finally included in the analyses, UVC-related PVT was mostly asymptomatic and only detected thanks to systematic imaging surveillance. Levit *et al*[42] found that in their neonatal unit, where routine US screening for PVT was not conducted, the rate of clinically identified thrombi was only 0.15% of all UVCs placed and 1.1% of all UVC-associated complications. On the other hand, Kim *et al*[30] found clinically silent PVT after UVC placement in 43% of critically ill neonates undergoing systematic US assessment. This indicates that UVC-related PVT might be largely underestimated if not properly investigated[42], once more confirming the need for routine imaging screenings in all neonates with UVC to exactly determine the incidence of UVC related PVT. Notably, PVT might also be associated with short- and long-term severe complications, deserving meticulous clinical evaluation[5,15].

Study	PVT	no PVT	Weight (random)	IV, random [95% CI]
Levit *et al*[42], 2020	1	2017	6.7%	0.000 [0.000-0.003]
Dubbink-Verheij *et al*[31], 2020	13	40	6.0%	0.325 [0.190-0.490]
Chen *et al*[15], 2020	7	1327	6.7%	0.005 [0.002-0.011]
Hwang *et al*[46], 2020	15	69	6.3%	0.217 [0.130-0.330]
Cakir *et al*[38], 2020	13	96	6.4%	0.135 [0.074-0.220]
Cabannes *et al*[32], 2018	51	104	6.4%	0.490 [0.391-0.590]
Derinkuyu *et al*[5], 2017	15	244	6.6%	0.061 [0.035-0.099]
Chandrashekhar *et al*[45], 2015	3	30	5.8%	0.100 [0.021-0.265]
Michel *et al*[37], 2012	2	61	6.2%	0.033 [0.004-0.113]
Gharehbaghi *et al*[39], 2011	5	164	6.5%	0.030 [0.001-0.070]
Sakha *et al*[41], 2007	17	50	6.1%	0.340 [0.212-0.488]
Turebylu *et al*[21], 2007	2	28	5.7%	0.071 [0.009-0.235]
Kim *et al*[30], 2001	43	100	6.4%	0.430 [0.331-0.533]
Boo *et al*[44], 1999	0	57	6.2%	0.000 [0.000-0.063]
Schwartz *et al*[40], 1997	1	100	6.4%	0.010 [0.000-0.054]
Yadav *et al*[43], 1993	7	22	5.5%	0.318 [0.139-0.549]
Total	**195**	**4314**	**100%**	**0.121 [0.059-0.202]**

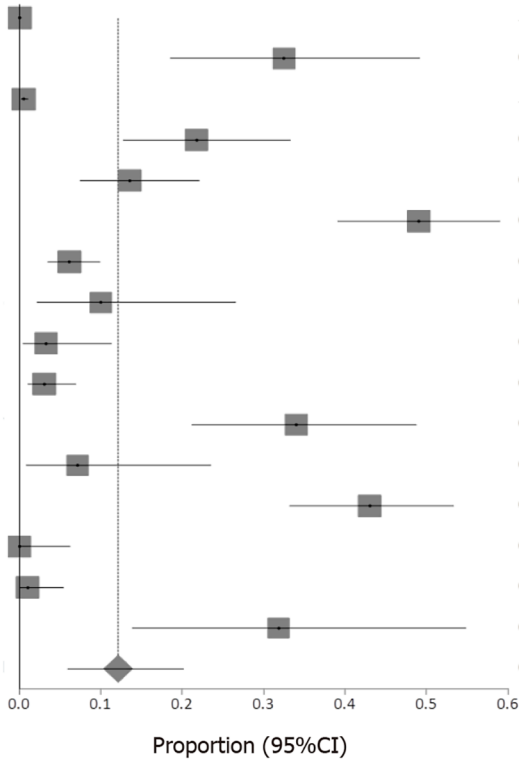

Figure 2 Forest plot showing the incidence of umbilical venous catheter-related portal vein thrombosis. PVT: Portal vein thrombosis; CI: Confidence interval.

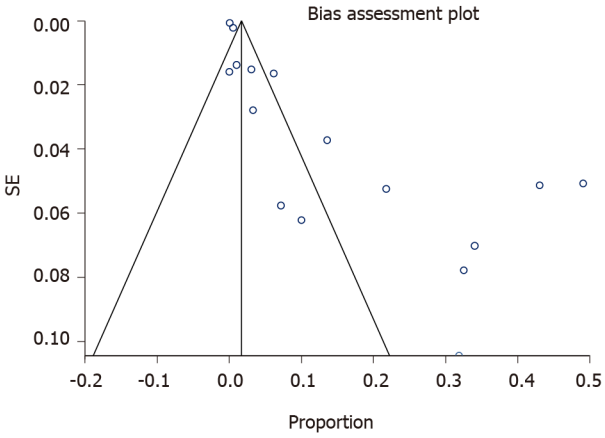

Figure 3 Funnel plot.

According to the results of our systematic review, UVC-related PVT was reliably investigated by US assessment. Nevertheless, we found large discrepancies across studies concerning data presentation. As described above in the text, only a minority of studies reported the exact thrombus position/extension within the portal system and if the occlusion was partial/complete. After PVT detection, imaging follow-up controls were performed with heterogeneous time schedules across studies. As a whole, however, the data confirmed that US is a valid, non-invasive, bed-side diagnostic technique for PVT detection. But whereas assessment of tip position is easy, requires a minimal training, and can be performed by the neonatologist bedside, detection of PVT at an early stage usually warrants a higher degree of US expertise. Besides the skill level of the radiologist/neonatologist, correct US examination might also depend on further technical factors (neonatal cooperation, abdominal gas distension, clinical instability, small-sized anatomical structures *etc*.) which may

influence the assessment.

A meticulous assessment of UVC tip position is needed to decrease catheter-related complications. Radiography is the most widely used technique to assess and follow-up UVC tip location[53,54]. However, most of the studies used only the anteroposterior view to assess tip location, although such view alone is not able to safely define the correct UVC tip position[54]. In case of wrong tip position within the portal system, radiography may show: (1) The tip below the diaphragm (below the vertebral body T10), overlying the liver; (2) Portal venous gas; and (3) Hypodiaphan lesions in the liver if fluid extravasation into liver parenchyma occurred[2,9,10,12,13]. However, radiographic assessments expose neonates to repeated ionizing radiations. US evaluation can be used in daily practice to check UVC tip position as well as the possible occurrence of UVC-associated hepatic complications. In fact, point-of-care US is able to assess in real-time UVC navigation and tip position during catheter placement[55]. Once UVC is correctly in place, US is the technique of choice to detect the development of UVC-related liver complications[5,30,31,53,56,57]. US and Doppler findings demonstrating hepatic complications include: (1) Detection of air in the portal venous system; (2) Portal venous thrombosis with impaired vascular patency; and (3) Liver parenchymal lesions presenting as nodular echogenic lesions/branched echogenic lesions/wide irregular heterogeneous lesions with laceration and the presence of peri-hepatic fluid[2,5,9,10,32]. Data exist comparing the ability of radiography and sonography to assess UVC positioning. A recent study found that US testing of UVC placement was able to identify catheter location in 100% of cases when compared to radiographic assessment[57]. Moreover, US is more accurate in the assessment of tip position compared to an estimation of catheter position achieved by its relationship to external structures on a radiograph[9,37,54,58]. Echocardiographic evaluation of UVC tip position was also assessed with success in recent years, although most studies focused on its ability to detect intra-cardiac abnormal tip position or atrial/inferior vena cava thrombosis, considering its limited ability to detect thrombi outside of the thoracic great vessels[24,59-62].

To date, the latest guidelines recommend the removal of UVCs after 7-10 d, although some authors reported an UVC *in situ* duration up to 28 d, once more proving how the management of UVCs is highly heterogeneous[4,22,24,38,42,61,63,64]. Unfortunately, the mean UVC dwell time in neonates with PVT was explicitly reported only by a minority of the included studies. Some authors found comparable UVC duration both in neonates with or without PVT[38-40], whilst in a large prospective study Kim *et al*[30] found an increased risk of PVT with a dwell time longer than 6 d. Noteworthy, PVT occurrence may develop soon after UVC position, as demonstrated by studies describing its detection already 12 h after placement[37]. It could be put forward that the presence of an UVC may itself represent a trigger for PVT development, presumably by raising vascular pressure in the ductus venosus and slowing down blood flow[18], and that such risk may eventually increase if catheterization persists. Such hypothesis deserves proper validation and large randomized controlled trials are warranted to achieve conclusive data about the benefits of early UVC removal.

Only a minority of studies described the occurrence of difficult or failed umbilical catheterization[30,65]. Considering that traumatic catheterization and/or failed insertion may induce vasculature injury and predispose to PVT by damaging the endothelial wall and decreasing portal flow[8], also the occurrence and number of failed attempts to UVC placement may play a role in PVT development and should be therefore considered either when programming diagnostic/follow-up controls for PVT or in the design of future studies.

The studies included in the final analyses reported the use of different models of UVCs, but unfortunately several studies did not specify the UVC model at all. Today, the most used UVC are dedicated catheters in polyurethane or in polyvinyl chloride but in the past several units used nasogastric tubes for venous umbilical catheterization. Furthermore, most of the studies did not specify the size and the number of lumens of the catheters that have been used. The use of different UVC models/materials may have influenced the incidence of UVC-related PVT in each study.

Concerning the presence of hereditary risk factors, the literature is, once more, quite vague and inconclusive. Turebylu *et al*[21] evaluated prospectively the prevalence of hereditary prothrombotic mutations in neonates with umbilical catheterization developing thrombotic lesions (including two cases of PVT). Interestingly, the authors found no increase in the risk of catheter-related thrombosis in patients carrying such prothrombotic mutations. In contrast, Heller *et al*[25] found that among 65 neonates, 24 of whom had PVT, the rate of genetic prothrombotic risk factors was higher than

healthy, age-/sex-matched controls.

Sepsis was suggested as possible risk factor for pediatric PVT development[3,66,67]. However, only a minority of patients affected by PVT presented with infection[3]. Furthermore, as for the studies included in the present review, only a minority of authors explicitly reported the presence of sepsis in case of PVT.

Recently, Hwang *et al*[46] reported for the first time significantly higher serum calcium concentrations in infants with umbilical catheter-related thrombosis. The authors assessed that such finding may reflect a possible role of calcium as a clotting factor leading to a hypercoagulable state. Further evidence is however required to confirm these results.

Only a minority of the studies included in our review reported a prophylactic treatment with heparin which, moreover, varied in terms of dosage[21,38,39,42,46]. After UVC-related PVT development, spontaneous resolution may often occur in UVC-related PVT, but this warrants close monitoring to determine either progression or resolution of the thrombus[21,30,32,40,46,64,68-70]. However, in case of thrombus extension with occlusion of the portal venous tract or clinical deterioration, antithrombotic therapy with unfractionated or low molecular weight heparin can be considered[64,68,70,71]. Kim *et al*[30] investigated prospectively the occurrence of UVC-related PVT in 100 neonates by subsequent US assessment. The authors found that 43% of neonates had a clinically silent PVT and reported complete resolution in 56% of neonates at follow-up controls, with recanalization being more frequent in neonates with partial rather than occlusive thrombi. Cabannes *et al*[32] investigated prospectively the occurrence of PVT in a cohort of patients including preterm neonates. PVT occurred in 53/123 of which 51 had an UVC. In these cases, the authors reported a spontaneous favorable evolution of left PVT in 95% of cases. In a prospective observational study, Dubbink-Verheij *et al*[31] investigated by serial US evaluations the incidence of catheter-related thrombosis in neonates with UVCs compared to a control group of neonates without UVC. The authors found the presence of thrombotic lesions in the UVC route in 30/40 cases (75%), of which 13 in the portal vein system. Most of the thrombotic lesions were asymptomatic and regressed spontaneously, whilst a minority required treatment with heparin. In contrast, Derinkuyu *et al*[5] treated with low-molecular-weight heparin all neonates with a diagnosis of UVC-related PVT (all described as asymptomatic). This heterogeneous approach may reflect the absence of solid evidence about safety/efficacy of antithrombotic therapy specifically addressing the neonatal period.

Our systematic review has multiple limitations, mostly attributable to the heterogeneity across studies. First, the intrinsic limitation of having included either retrospective studies or "old" studies (from 1980 onwards), *i.e.*, performed at timepoints during which clinical approach to patients and awareness about PVT was presumably different compared to more recent studies. Second, the lack of correlation between PVT and UVC tip position in most studies. Third, the different study designs regarding the indication and time schedule for imaging assessment. Fourth, the different approach of clinicians about the use of prophylactic/therapeutic treatment in neonates with indwelling UVCs.

CONCLUSION

In conclusion, the use of umbilical lines requires a high index of suspicion for PVT development, especially if considering that the need for an UVC obviously preselects ill newborns in whom multiple risk factors for the development of thrombotic disorders may coexist. To avoid or minimize the risk of PVT, some crucial key-points have to be followed, as checking the correct position before infusing in the catheter, checking again the correct tip position every 48 h, and removing the UVC after a maximum of 7 d.

As a whole, this systematic review revealed relevant gaps also in knowledge about the optimal diagnostic approach and treatment for UVC-related PVT, maybe related to the lack of updated, evidence-based guidelines addressing step-by-step all the aspects of what the best approach to the management of this complication should be. According to our opinion, this represents a call to action addressed to researchers and clinicians to design large prospective randomized studies and to draft specific, concrete and updated guidelines.

ARTICLE HIGHLIGHTS

Research background

The use of umbilical venous catheters (UVCs) in the perinatal period may be associated with severe complications, including the occurrence of portal vein thrombosis (PVT).

Research motivation

Although multiple observational studies have provided an overview about the risk of PVT after UVC positioning, no studies/reviews explored systematically this issue.

Research objectives

The main goal was to investigate the most accurate information about the actual incidence of UVC-related PVT in the neonatal setting, and to assess if any particular risk factor was systematically associated with the development of such complication.

Research methods

A systematic and comprehensive database searching (PubMed, Cochrane Library, Scopus, Web of Science) was performed for prospective cohort studies, retrospective cohort studies and case-control studies from 1980 to 2020. Incidence estimates were pooled by using random effects meta-analyses. The quality of included studies was assessed using the Newcastle-Ottawa scale.

Research results

Sixteen studies were considered eligible and included in the final analyses. The data confirmed the relevant risk of UVC-related thrombosis with a mean pooled incidence of 12%, although it varied across studies (0%-49%).

Research conclusions

This is the first systematic review specifically investigating the incidence of UVC-related PVT. The use of UVCs requires a high index of suspicion, because its use is significantly associated with PVT.

Research perspectives

Large prospective randomized studies and updated guidelines are warranted in order to define the best management of this dreaded complication.

REFERENCES

1 **El Ters N**, Claassen C, Lancaster T, Barnette A, Eldridge W, Yazigi F, Brar K, Herco M, Rogowski L, Strand M, Vachharajani A. Central *vs* Low-Lying Umbilical Venous Catheters: A Multicenter Study of Practices and Complications. *Am J Perinatol* 2019; **36**: 1198-1204 [PMID: 30566998 DOI: 10.1055/s-0038-1676482]

2 **Hagerott HE**, Kulkarni S, Restrepo R, Reeves-Garcia J. Clinical-radiologic features and treatment of hepatic lesions caused by inadvertent infusion of parenteral nutrition in liver parenchyma due to malposition of umbilical vein catheters. *Pediatr Radiol* 2014; **44**: 810-815 [PMID: 24557484 DOI: 10.1007/s00247-014-2895-2]

3 **Williams S**, Chan AK. Neonatal portal vein thrombosis: diagnosis and management. *Semin Fetal Neonatal Med* 2011; **16**: 329-339 [PMID: 21925985 DOI: 10.1016/j.siny.2011.08.005]

4 **Gordon A**, Greenhalgh M, McGuire W. Early planned removal of umbilical venous catheters to prevent infection in newborn infants. *Cochrane Database Syst Rev* 2017; **10**: CD012142 [PMID: 29017005 DOI: 10.1002/14651858.CD012142.pub2]

5 **Derinkuyu BE**, Boyunaga OL, Damar C, Unal S, Ergenekon E, Alimli AG, Oztunali C, Turkyilmaz C. Hepatic Complications of Umbilical Venous Catheters in the Neonatal Period: The Ultrasound Spectrum. *J Ultrasound Med* 2018; **37**: 1335-1344 [PMID: 29034490 DOI: 10.1002/jum.14443]

6 **Goh SSM**, Kan SY, Bharadwaj S, Poon WB. A review of umbilical venous catheter-related complications at a tertiary neonatal unit in Singapore. *Singapore Med J* 2019 [PMID: 31680179 DOI: 10.11622/smedj.2019140]

7 **Mutlu M**, Aslan Y, Kul S, Yılmaz G. Umbilical venous catheter complications in newborns: a 6-year single-center experience. *J Matern Fetal Neonatal Med* 2016; **29**: 2817-2822 [PMID: 26452458 DOI: 10.3109/14767058.2015.1105952]

8 **Sulemanji M**, Vakili K, Zurakowski D, Tworetzky W, Fishman SJ, Kim HB. Umbilical Venous Catheter Malposition Is Associated with Necrotizing Enterocolitis in Premature Infants. *Neonatology* 2017; **111**: 337-343 [PMID: 28092913 DOI: 10.1159/000451022]

9 **Selvam S**, Humphrey T, Woodley H, English S, Kraft JK. Sonographic features of umbilical catheter-related complications. *Pediatr Radiol* 2018; **48**: 1964-1970 [PMID: 30078110 DOI: 10.1007/s00247-018-4214-9]

10 **Schlesinger AE**, Braverman RM, DiPietro MA. Pictorial essay. Neonates and umbilical venous catheters: normal appearance, anomalous positions, complications, and potential aid to diagnosis. *AJR Am J Roentgenol* 2003; **180**: 1147-1153 [PMID: 12646473 DOI: 10.2214/ajr.180.4.1801147]

11 **Morag I**, Epelman M, Daneman A, Moineddin R, Parvez B, Shechter T, Hellmann J. Portal vein thrombosis in the neonate: risk factors, course, and outcome. *J Pediatr* 2006; **148**: 735-739 [PMID: 16769378 DOI: 10.1016/j.jpeds.2006.01.051]

12 **Grizelj R**, Vukovic J, Bojanic K, Loncarevic D, Stern-Padovan R, Filipovic-Grcic B, Weingarten TN, Sprung J. Severe liver injury while using umbilical venous catheter: case series and literature review. *Am J Perinatol* 2014; **31**: 965-974 [PMID: 24590868 DOI: 10.1055/s-0034-1370346]

13 **Auriti C**, Ronchetti MP, Bersani I, Gennari F, Piersigilli F. Intrahepatic Administration of Liposomal Amphotericin B (Ambisome) for the Management of a Liver Abscess from *Candida albicans* in a Preterm Infant. *Antimicrob Agents Chemother* 2018; **62** [PMID: 30224526 DOI: 10.1128/AAC.01239-18]

14 **Türk E**, Soylar R, Akca T, Serter S, Karaca İ. Venobiliary fistula related to umbilical venous catheter in a newborn. *Pediatr Int* 2015; **57**: 478-480 [PMID: 26011554 DOI: 10.1111/ped.12608]

15 **Chen HJ**, Chao HC, Chiang MC, Chu SM. Hepatic extravasation complicated by umbilical venous catheterization in neonates: A 5-year, single-center experience. *Pediatr Neonatol* 2020; **61**: 16-24 [PMID: 31186169 DOI: 10.1016/j.pedneo.2019.05.004]

16 **Yiğiter M**, Arda IS, Hiçsönmez A. Hepatic laceration because of malpositioning of the umbilical vein catheter: case report and literature review. *J Pediatr Surg* 2008; **43**: E39-E41 [PMID: 18485935 DOI: 10.1016/j.jpedsurg.2008.01.018]

17 **Macartney CA**, Chan AK. Thrombosis in children. *Semin Thromb Hemost* 2011; **37**: 763-761 [PMID: 22187399 DOI: 10.1055/s-0031-1297167]

18 **Sulemanji MN**, Azpurua H, Suh M, Potanos K, Cauley R, Kunisaki SM, Modi B, Zurakowski D, Fishman SJ, Kim HB. Ductus venosus closure results in transient portal hypertension--is this the silent trigger for necrotizing enterocolitis? *J Pediatr Surg* 2013; **48**: 2067-2074 [PMID: 24094959 DOI: 10.1016/j.jpedsurg.2013.01.022]

19 **Di Giorgio A**, De Angelis P, Cheli M, Vajro P, Iorio R, Cananzi M, Riva S, Maggiore G, Indolfi G, Calvo PL, Nicastro E, D'Antiga L. Etiology, presenting features and outcome of children with non-cirrhotic portal vein thrombosis: A multicentre national study. *Dig Liver Dis* 2019; **51**: 1179-1184 [PMID: 30928422 DOI: 10.1016/j.dld.2019.02.014]

20 **Ferri PM**, Rodrigues Ferreira A, Fagundes ED, Xavier SG, Dias Ribeiro D, Fernandes AP, Borges KB, Liu SM, de Melo Mdo C, Roquete ML, Penna FJ. Evaluation of the presence of hereditary and acquired thrombophilias in Brazilian children and adolescents with diagnoses of portal vein thrombosis. *J Pediatr Gastroenterol Nutr* 2012; **55**: 599-604 [PMID: 22684349 DOI: 10.1097/MPG.0b013e318261814d]

21 **Turebylu R**, Salis R, Erbe R, Martin D, Lakshminrusimha S, Ryan RM. Genetic prothrombotic mutations are common in neonates but are not associated with umbilical catheter-associated thrombosis. *J Perinatol* 2007; **27**: 490-495 [PMID: 17625574 DOI: 10.1038/sj.jp.7211786]

22 **Revel-Vilk S**, Ergaz Z. Diagnosis and management of central-line-associated thrombosis in newborns and infants. *Semin Fetal Neonatal Med* 2011; **16**: 340-344 [PMID: 21807572 DOI: 10.1016/j.siny.2011.07.003]

23 **Albisetti M**, Moeller A, Waldvogel K, Bernet-Buettiker V, Cannizzaro V, Anagnostopoulos A, Balmer C, Schmugge M. Congenital prothrombotic disorders in children with peripheral venous and arterial thromboses. *Acta Haematol* 2007; **117**: 149-155 [PMID: 17159337 DOI: 10.1159/000097462]

24 **Narang S**, Roy J, Stevens TP, Butler-O'Hara M, Mullen CA, D'Angio CT. Risk factors for umbilical venous catheter-associated thrombosis in very low birth weight infants. *Pediatr Blood Cancer* 2009; **52**: 75-79 [PMID: 18680150 DOI: 10.1002/pbc.21714]

25 **Heller C**, Schobess R, Kurnik K, Junker R, Günther G, Kreuz W, Nowak-Göttl U. Abdominal venous thrombosis in neonates and infants: role of prothrombotic risk factors - a multicentre case-control study. For the Childhood Thrombophilia Study Group. *Br J Haematol* 2000; **111**: 534-539 [PMID: 11122096 DOI: 10.1046/j.1365-2141.2000.02349.x]

26 **Avila ML**, Amiri N, Stanojevic S, Vu TT, Barron K, Krol P, Yue N, Williams S, Brandão LR. Can thrombophilia predict recurrent catheter-related deep vein thrombosis in children? *Blood* 2018; **131**: 2712-2719 [PMID: 29724900 DOI: 10.1182/blood-2017-10-811216]

27 **Sobczak A**, Kruczek P, Homa M, Kwinta P. A new microscopic insight into the thrombogenicity of umbilical catheters. *Thromb Res* 2018; **168**: 80-82 [PMID: 29936402 DOI: 10.1016/j.thromres.2018.06.007]

28 **Pottecher T**, Forrler M, Picardat P, Krause D, Bellocq JP, Otteni JC. Thrombogenicity of central venous catheters: prospective study of polyethylene, silicone and polyurethane catheters with phlebography or post-mortem examination. *Eur J Anaesthesiol* 1984; **1**: 361-365 [PMID: 6536520]

29 **Thornburg C**, Pipe S. Neonatal thromboembolic emergencies. *Semin Fetal Neonatal Med* 2006; **11**: 198-206 [PMID: 16520103 DOI: 10.1016/j.siny.2006.01.005]

30 **Kim JH**, Lee YS, Kim SH, Lee SK, Lim MK, Kim HS. Does umbilical vein catheterization lead to portal venous thrombosis? *Radiology* 2001; **219**: 645-650 [PMID: 11376248 DOI: 10.1148/radiology.219.3.r01jn17645]

31 **Dubbink-Verheij GH**, Visser R, Roest AA, van Ommen CH, Te Pas AB, Lopriore E. Thrombosis after umbilical venous catheterisation: prospective study with serial ultrasound. *Arch Dis Child Fetal Neonatal Ed* 2020; **105**: 299-303 [PMID: 31391204 DOI: 10.1136/archdischild-2018-316762]

32 **Cabannes M**, Bouissou A, Favrais G, Sembély-Taveau C, Morales L, Favreau A, Bertrand P, Saliba E, Sirinelli D, Morel B. Systematic ultrasound examinations in neonates admitted to NICU: evolution of portal vein thrombosis. *J Perinatol* 2018; **38**: 1359-1364 [PMID: 30082773 DOI: 10.1038/s41372-018-0132-9]

33 **Moher D**, Liberati A, Tetzlaff J, Altman DG; PRISMA Group. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *PLoS Med* 2009; **6**: e1000097 [PMID: 19621072 DOI: 10.1371/journal.pmed.1000097]

34 **Wells GA**, Shea B, O'Connell D, Peterson J, Welch V, Losos M, Tugwell P. The Newcastle-Ottawa Scale (NOS) for assessing the quality of nonrandomized studies in meta-analyses. [cited 28 November 2020]. In: The Ottawa Hospital Research Institute [Internet]. Available from: http://www.ohri.ca/programs/clinical_epidemiology/oxford.asp

35 **Higgins JP**, Thompson SG. Quantifying heterogeneity in a meta-analysis. *Stat Med* 2002; **21**: 1539-1558 [PMID: 12111919 DOI: 10.1002/sim.1186]

36 **Higgins JP**, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. *BMJ* 2003; **327**: 557-560 [PMID: 12958120 DOI: 10.1136/bmj.327.7414.557]

37 **Michel F**, Brevaut-Malaty V, Pasquali R, Thomachot L, Vialet R, Hassid S, Nicaise C, Martin C, Panuel M. Comparison of ultrasound and X-ray in determining the position of umbilical venous catheters. *Resuscitation* 2012; **83**: 705-709 [PMID: 22155219 DOI: 10.1016/j.resuscitation.2011.11.026]

38 **Çakır SÇ**, Özkan H, Dorum BA, Köksal N, Kudretoğlu P, Baytan B, Sezgin M, Güneş AM. The danger awaiting premature babies: Portal vein thrombosis. *Turk Pediatri Ars* 2020; **55**: 257-262 [PMID: 33061753 DOI: 10.14744/TurkPediatriArs.2020.65289]

39 **Gharehbaghi MM**, Nemati M, Hosseinpour SS, Taei R, Ghargharechi R. Umbilical vascular catheter associated portal vein thrombosis detected by ultrasound. *Indian J Pediatr* 2011; **78**: 161-164 [PMID: 21063811 DOI: 10.1007/s12098-010-0223-x]

40 **Schwartz DS**, Gettner PA, Konstantino MM, Bartley CL, Keller MS, Ehrenkranz RA, Jacobs HC. Umbilical venous catheterization and the risk of portal vein thrombosis. *J Pediatr* 1997; **131**: 760-762 [PMID: 9403662 DOI: 10.1016/s0022-3476(97)70109-4]

41 **Sakha SH**, Rafeey M, Tarzamani MK. Portal venous thrombosis after umbilical vein catheterization. *Indian J Gastroenterol* 2007; **26**: 283-284 [PMID: 18431012]

42 **Levit OL**, Shabanova V, Bizzarro MJ. Umbilical catheter-associated complications in a level IV neonatal intensive care unit. *J Perinatol* 2020; **40**: 573-580 [PMID: 31911645 DOI: 10.1038/s41372-019-0579-3]

43 **Yadav S**, Dutta AK, Sarin SK. Do umbilical vein catheterization and sepsis lead to portal vein thrombosis? *J Pediatr Gastroenterol Nutr* 1993; **17**: 392-396 [PMID: 8145094 DOI: 10.1097/00005176-199311000-00010]

44 **Boo NY**, Wong NC, Zulkifli SS, Lye MS. Risk factors associated with umbilical vascular catheter-associated thrombosis in newborn infants. *J Paediatr Child Health* 1999; **35**: 460-465 [PMID: 10571759 DOI: 10.1046/j.1440-1754.1999.355392.x]

45 **Chandrashekhar C**, Krishnegowda S, Vikas VM, Bhaktavatsala HR. Portal vein thrombosis following umbilical vein catheterization in neonates. *Pediatr Rev: Int J Pediatr Res* 2015; **2**: 135-137 [DOI: 10.17511/ijpr.2015.i04.18]

46 **Hwang JH**, Chung ML, Lim YJ. Incidence and risk factors of subclinical umbilical catheter-related thrombosis in neonates. *Thromb Res* 2020; **194**: 21-25 [PMID: 32563060 DOI: 10.1016/j.thromres.2020.05.034]

47 **Schmidt B**, Andrew M. Neonatal thrombosis: report of a prospective Canadian and international registry. *Pediatrics* 1995; **96**: 939-943 [PMID: 7478839]

48 **Hoellering A**, Tshamala D, Davies MW. Study of movement of umbilical venous catheters over time. *J Paediatr Child Health* 2018; **54**: 1329-1335 [PMID: 29806878 DOI: 10.1111/jpc.14073]

49 **Abiramalatha T**, Kumar M, Shabeer MP, Thomas N. Advantages of being diligent: lessons learnt from umbilical venous catheterisation in neonates. *BMJ Case Rep* 2016; **2016** [PMID: 26843419]

50 **Plooij-Lusthusz AM**, van Vreeswijk N, van Stuijvenberg M, Bos AF, Kooi EMW. Migration of Umbilical Venous Catheters. *Am J Perinatol* 2019; **36**: 1377-1381 [PMID: 30620943 DOI: 10.1055/s-0038-1677016]

51 **Dubbink-Verheij GH**, Visser R, Tan RNGB, Roest AAW, Lopriore E, Te Pas AB. Inadvertent Migration of Umbilical Venous Catheters Often Leads to Malposition. *Neonatology* 2019; **115**: 205-210 [PMID: 30645997 DOI: 10.1159/000494369]

52 **Franta J**, Harabor A, Soraisham AS. Ultrasound assessment of umbilical venous catheter migration in preterm infants: a prospective study. *Arch Dis Child Fetal Neonatal Ed* 2017; **102**: F251-F255 [PMID: 28424358 DOI: 10.1136/archdischild-2016-311202]

53 **Sharma D**, Farahbakhsh N, Tabatabaii SA. Role of ultrasound for central catheter tip localization in neonates: a review of the current evidence. *J Matern Fetal Neonatal Med* 2019; **32**: 2429-2437 [PMID: 29397784 DOI: 10.1080/14767058.2018.1437135]

54 **Guimarães AF**, Souza AA, Bouzada MC, Meira ZM. Accuracy of chest radiography for positioning of the umbilical venous catheter. *J Pediatr (Rio J)* 2017; **93**: 172-178 [PMID: 27424226 DOI: 10.1016/j.jped.2016.05.004]

55 **Kishigami M**, Shimokaze T, Enomoto M, Shibasaki J, Toyoshima K. Ultrasound-Guided Umbilical Venous Catheter Insertion With Alignment of the Umbilical Vein and Ductus Venosus. *J Ultrasound Med* 2020; **39**: 379-383 [PMID: 31400014 DOI: 10.1002/jum.15106]

56 **Simanovsky N**, Ofek-Shlomai N, Rozovsky K, Ergaz-Shaltiel Z, Hiller N, Bar-Oz B. Umbilical venous catheter position: evaluation by ultrasound. *Eur Radiol* 2011; **21**: 1882-1886 [PMID: 21533866 DOI: 10.1007/s00330-011-2129-z]

57 **Saul D**, Ajayi S, Schutzman DL, Horrow MM. Sonography for Complete Evaluation of Neonatal Intensive Care Unit Central Support Devices: A Pilot Study. *J Ultrasound Med* 2016; **35**: 1465-1473 [PMID: 27229130 DOI: 10.7863/ultra.15.06104]

58 **Greenberg M**, Movahed H, Peterson B, Bejar R. Placement of umbilical venous catheters with use of bedside real-time ultrasonography. *J Pediatr* 1995; **126**: 633-635 [PMID: 7699547 DOI: 10.1016/s0022-3476(95)70366-7]

59 **Ades A**, Sable C, Cummings S, Cross R, Markle B, Martin G. Echocardiographic evaluation of umbilical venous catheter placement. *J Perinatol* 2003; **23**: 24-28 [PMID: 12556923 DOI: 10.1038/sj.jp.7210851]

60 **Unal S**, Ekici F, Cetin İİ, Bilgin L. Heparin infusion to prevent umbilical venous catheter related thrombosis in neonates. *Thromb Res* 2012; **130**: 725-728 [PMID: 22901699 DOI: 10.1016/j.thromres.2012.07.018]

61 **Butler-O'Hara M**, Buzzard CJ, Reubens L, McDermott MP, DiGrazio W, D'Angio CT. A randomized trial comparing long-term and short-term use of umbilical venous catheters in premature infants with birth weights of less than 1251 g. *Pediatrics* 2006; **118**: e25-e35 [PMID: 16785289 DOI: 10.1542/peds.2005-1880]

62 **Wever ML**, Liem KD, Geven WB, Tanke RB. Urokinase therapy in neonates with catheter related central venous thrombosis. *Thromb Haemost* 1995; **73**: 180-185 [PMID: 7792727]

63 **O'Grady NP**, Alexander M, Burns LA, Dellinger EP, Garland J, Heard SO, Lipsett PA, Masur H, Mermel LA, Pearson ML, Raad II, Randolph AG, Rupp ME, Saint S; Healthcare Infection Control Practices Advisory Committee. Guidelines for the prevention of intravascular catheter-related infections. *Am J Infect Control* 2011; **39**: S1-34 [PMID: 21511081 DOI: 10.1016/j.ajic.2011.01.003]

64 **Park CK**, Paes BA, Nagel K, Chan AK, Murthy P; Thrombosis and Hemostasis in Newborns (THiN) Group. Neonatal central venous catheter thrombosis: diagnosis, management and outcome. *Blood Coagul Fibrinolysis* 2014; **25**: 97-106 [PMID: 24477225 DOI: 10.1097/MBC.0b013e328364f9b0]

65 **Aiyagari R**, Song JY, Donohue JE, Yu S, Gaies MG. Central venous catheter-associated complications in infants with single ventricle: comparison of umbilical and femoral venous access routes. *Pediatr Crit Care Med* 2012; **13**: 549-553 [PMID: 22805159 DOI: 10.1097/PCC.0b013e31824fbdb4]

66 **Sethi SK**, Dewan P, Faridi MM, Aggarwal A, Upreti L. Liver abscess, portal vein thrombosis and cavernoma formation following umbilical vein catherisation in two neonates. *Trop Gastroenterol* 2007; **28**: 79-80 [PMID: 18050847]

67 **Shah I**, Bhatnagar S. Liver abscess in a newborn leading to portal vein thrombosis. *Indian J Pediatr* 2009; **76**: 1268-1269 [PMID: 20012786 DOI: 10.1007/s12098-009-0244-5]

68 **Monagle P**, Chan AKC, Goldenberg NA, Ichord RN, Journeycake JM, Nowak-Göttl U, Vesely SK. Antithrombotic therapy in neonates and children: Antithrombotic Therapy and Prevention of Thrombosis, 9th ed: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines. *Chest* 2012; **141**: e737S-e801S [PMID: 22315277 DOI: 10.1378/chest.11-2308]

69 **Bhatt MD**, Patel V, Butt ML, Chan AKC, Paes B; Thrombosis and Hemostasis in Newborns (THiN) Group. Outcomes following neonatal portal vein thrombosis: A descriptive, single-center study and review of anticoagulant therapy. *Pediatr Blood Cancer* 2019; **66**: e27572 [PMID: 30520242 DOI: 10.1002/pbc.27572]

70 **Chalmers E**, Ganesen V, Liesner R, Maroo S, Nokes T, Saunders D, Williams M; British Committee for Standards in Haematology. Guideline on the investigation, management and prevention of venous thrombosis in children. *Br J Haematol* 2011; **154**: 196-207 [PMID: 21595646 DOI: 10.1111/j.1365-2141.2010.08543.x]

71 **Demirel N**, Aydin M, Zenciroglu A, Bas AY, Yarali N, Okumus N, Cinar G, Ipek MS. Neonatal thrombo-embolism: risk factors, clinical features and outcome. *Ann Trop Paediatr* 2009; **29**: 271-279 [PMID: 19941750 DOI: 10.1179/027249309X12547917868961]

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Editorial Board Member of *World Journal of Hepatology*, Manuel Luis Rodríguez-Perálvarez, MD, PhD, Consultant Hepatologist and Associate Professor of Medicine, Department of Hepatology and Liver Transplantation, Reina Sofía University Hospital, Córdoba 14014, Spain. ropeml@hotmail.com

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OPINION REVIEW

Non-alcoholic fatty liver disease in irritable bowel syndrome: More than a coincidence?

Huw Purssell, Peter J Whorwell, Varinder S Athwal, Dipesh H Vasant

ORCID number: Huw Purssell 0000-0003-1647-9629; Peter J Whorwell 0000-0002-5220-8474; Varinder S Athwal 0000-0002-1684-721X; Dipesh H Vasant 0000-0002-2329-0616.

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Huw Purssell, Varinder S Athwal, Hepatology, Manchester University NHS Foundation Trust, Manchester M23 9LT, United Kingdom

Huw Purssell, Peter J Whorwell, Varinder S Athwal, Dipesh H Vasant, Division of Diabetes, Endocrinology and Gastroenterology, University of Manchester, Manchester M23 9LT, United Kingdom

Peter J Whorwell, Neurogastroenterology Unit, Wythenshawe Hospital, Manchester University NHS Foundation Trust, Manchester M23 9LT, United Kingdom

Dipesh H Vasant, Neurogastroenterology Unit, Department of Gastroenterology, Manchester University NHS Foundation Trust, Wythenshawe Hospital, Manchester M23 9LT, United Kingdom

Corresponding author: Dipesh H Vasant, MBChB, MRCP, PhD, Senior Lecturer, Neurogastroenterology Unit, Department of Gastroenterology, Manchester University NHS Foundation Trust, Wythenshawe Hospital, Southmoor Road, Manchester M23 9LT, United Kingdom. dipesh.vasant@manchester.ac.uk

Abstract

Irritable bowel syndrome (IBS) and non-alcoholic fatty liver disease (NAFLD) are amongst the most common gastrointestinal and liver conditions encountered in primary and secondary care. Recently, there has been interest in the apparent co-incidence of NAFLD in patients with IBS mainly driven by improved understanding of their shared risk factors and pathophysiology. In this paper we summarize the shared risk factors which include; overlapping nutritional and dietary factors as well as shared putative mechanisms of pathophysiology. These include changes in the gut microbiome, gut permeability, immunity, small bowel bacterial overgrowth and bile acid metabolism. This paper describes how these shared risk factors and etiological factors may have practical clinical implications for these highly prevalent conditions. It also highlights some of the limitations of current epidemiological data relating to estimates of the overlapping prevalence of the two conditions which have resulted in inconsistent results and, therefore the need for further research. Early recognition and management of the overlap could potentially have impacts on treatment outcomes, compliance and morbidity of both conditions. Patients with known IBS who have abnormal liver function tests or significant risk factors for NAFLD should be investigated appropriately for this possibility. Similarly, IBS should be considered in patients with NAFLD

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and symptoms of abdominal pain associated with defecation, an altered bowel habit and bloating.

Key Words: Irritable bowel syndrome; Non-alcoholic fatty liver disease; Metabolic syndrome; Obesity; Prevalence; Pathophysiology

Core Tip: Irritable bowel syndrome (IBS) and non-alcoholic fatty liver disease (NAFLD) are amongst the most common gastrointestinal and liver conditions encountered in primary and secondary care. There has been interest in the apparent co-incidence of NAFLD in patients with IBS mainly driven by improved understanding of their shared risk factors and pathophysiology. In this paper we summarize the shared risk factors which include; overlapping nutritional and dietary factors as well as shared putative mechanisms of pathophysiology. Physicians should be aware of the possibility of co-existence of IBS and NAFLD and consider investigating patients with IBS or NAFLD with clinical features of the other condition.

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INTRODUCTION

Irritable Bowel syndrome (IBS) is a disorder of gut-brain interaction (DGBI) resulting in recurrent abdominal pain associated with defecation and an altered bowel habit. Patients are considered to have IBS when they fulfill the Rome IV diagnostic criteria which include an altered bowel habit (constipation, diarrhea or a mix of both), associated with frequent abdominal pain and abdominal bloating or distension for at least 6 mo prior to diagnosis[1]. A recent systematic review and meta-analysis has shown a worldwide prevalence of IBS of 9.2% with significant regional variability[2]. In the United Kingdom, DGBIs such as IBS are very common, and account for around a third of gastroenterology outpatient referrals[3]. IBS can be debilitating often resulting in an increasing risk of anxiety or depression[4] with symptoms such as fecal incontinence that can be difficult to manage leading to poor quality of life and distress [5]. There is often significant clinician prejudice and frustration towards patients with IBS[6] resulting in unfair public perceptions and significant stigmatization[7].

Non-alcoholic fatty liver disease (NAFLD) is characterized by the accumulation of more than 5% of fat in the liver in the absence of a secondary cause. It is one of the major causes of liver disease worldwide and its pathogenesis is linked to metabolic syndrome, obesity and Type 2 diabetes. The population based prevalence of NAFLD is between 25%-44% but rises to 70% in patients with Type 2 diabetes[8,9]. NAFLD is recognized as a heterogeneous condition with variable rates of progression. In certain patients isolated steatosis leads to steatohepatitis and fibrosis, progressing ultimately to cirrhosis, decompensated liver disease and sometimes hepatocellular carcinoma. Population based screening studies have shown a prevalence of advanced fibrosis in 8% of patients rising to 27% in those with risk factors[10,11]. Unfortunately, the majority of patients are only diagnosed with liver disease when they present with advanced disease and many are of working age. Consequently, liver disease is responsible for the loss of 38000 and 22000 working life years, in men and women, respectively. NAFLD has been increasing in incidence in the western world with a predictable commensurate increase in liver transplant in both the United States and Europe[12-14].

There is increasing recognition that both IBS and NAFLD share a number of over-lapping risk and aetiological factors leading to growing interest in the possibility of an association between the two conditions. However, there is limited high quality data on the concomitance of IBS and NAFLD. As a result, IBS symptoms may not be routinely screened for in hepatology clinics and vice versa. Therefore, the aims of this article are

to summarize the current understanding of relevant overlapping patho-physiological and aetiological factors, and to highlight areas for future research and their clinical implications.

THE PREVALENCE OF CO-EXISTING IBS AND NAFLD

Table 1 summarizes the literature on the co-existing prevalence of IBS and NAFLD to date. Most studies have examined the incidence of NAFLD in previously diagnosed IBS. Unfortunately, a review of the literature of concomitant IBS and NAFLD revealed a very high variability in estimates of the prevalence from 12.9% to 74%, with significant differences in methodology in the diagnostic approaches for both conditions and the populations studied[15-17]. Amongst the reasons for this heterogeneity and variability include the change in the Rome criteria for IBS from Rome III, to the current Rome IV iteration, which is known to be more restrictive[18]. From a hepatology perspective, it is notable that all the studies to date have used raised liver transaminases, with a negative viral hepatitis screen, in the absence of excessive alcohol consumption, and abdominal ultrasound to diagnose NAFLD, which in the absence of objective liver fibrosis assessment could be considered sub-optimal.

Shin *et al*[16] found that the prevalence of presumed NAFLD was 12.9% in patients with diarrhoea predominant IBS (IBS-D) compared to 9.0% in patients with constipation predominant IBS (IBS-C), although the reasons for this apparent difference are unclear and merit further investigation. In an interesting study by Lee *et al*[19], rather than evaluating patients with a formal diagnosis of NAFLD, the authors assessed the incidence of elevated liver transaminases and the metabolic syndrome in patients with IBS, compared to an age and sex matched control group. Those with IBS were found to have a significantly higher alanine aminotransferase (ALT) (16.9% *vs* 7.7%; $P = 0.015$) and Gamma-glutamyl transferase (GGT) (24.1% *vs* 11.5%; $P = 0.037$) compared to the control group, and there was a significantly higher prevalence of metabolic syndrome in the IBS group (32.5% *vs* 12.7%; $P < 0.001$).

To our knowledge, there have only been three previous reports on the incidence of functional bowel symptoms in patients with NAFLD. Appleby *et al*[20] found that in 127 patients with NAFLD, 25% had chronic diarrhea, and 12% had features of bile acid diarrhoea with both being associated with a raised NAFLD fibrosis score. Furthermore, Singh *et al*[21] studied 632 patients in India diagnosed with fatty liver disease and found that 29.4% had co-existing clinical features of IBS. Similar findings were reported by Jones-Pauley *et al*[22] in a cross sectional study looking at IBS diagnosed by Rome IV criteria in 130 NAFLD patients and as many as 38 (29.2%) patients had IBS based on Rome IV criteria. Interestingly, depression and anxiety were found to be more prevalent in the IBS cohort, compared to the non-IBS cohort, indicating the detrimental effect of co-existing bowel symptoms may have on quality of life, and the resulting need for a multi-systems approach in NAFLD patients with IBS symptoms.

In summary, regardless of the iteration of the Rome IBS diagnostic criteria used and the highlighted limitations of the previous studies, the data summarized in Table 1 on the co-existing prevalence of IBS in patients with NAFLD consistently report a much higher prevalence of IBS than that reported in global prevalence studies using either Rome III or Rome IV diagnostic criteria[2].

OVERLAPPING ETIOLOGICAL FACTORS IBS AND NAFLD

Multiple etiological factors overlap between IBS and NAFLD leading to interest in possible associations including obesity, gut microbiome, dietary factors and immune mediated causes as illustrated in Figure 1.

OBESITY

NAFLD is intrinsically linked with obesity, diabetes and the metabolic syndrome. In obese populations, NAFLD has a prevalence of up to 95%[23]. Excess adipose tissue exhausting peripheral storage capacity resulting in deposition in the liver and increased insulin resistance is thought to be the main culprit for NAFLD pathogenesis [24]. Weight loss through diet and exercise reduces hepatic steatosis and fibrosis, and in 109 obese patients[25]. Lassailly *et al*[26] showed that bariatric surgery resolved non-

Table 1 Summarizes the literature on the co-existing prevalence of irritable bowel syndrome and non-alcoholic fatty liver disease to date

Author	Population studied	Study design	No. patients	Criteria for IBS diagnosis	IBS subtypes	Criteria for NAFLD diagnosis	Prevalence of NAFLD in IBS/ IBS in NAFLD	Outcomes
Hasanain *et al*[15]	IBS	Cross sectional study	100 patients with IBS	Rome III	IBS-C: 45%; IBS-D: 23%; IBS-M: 32%,	Ultrasound; No history of alcohol exposure; No exposure to steatogenic medications; Negative viral screen	74% of those with IBS had co-existing NAFLD	Moderate/severe NAFLD significantly associated with moderate/severe IBS (OR: 2.4, 95%CI: 1.3-62.7, P = 0.026)
Shin *et al* [16]	Healthy individuals *via* NHANES	Cross sectional study	2345 patients with IBS	Rome IV	IBS-C: 1023; IBS-D: 1322	Raised ALT or AST; Absence of excessive alcohol; Negative viral hepatitis screen	Prevalence of NAFLD in IBS-D: 12.9% (95%CI: 9.8-15.9); IBS-C: 9.0% (95%CI: 7.0-11.0)	NAFLD associated with diarrhoea *vs* normal bowel pattern (OR: 1.340, 95%CI: 1.007-1.784) and constipation (OR: 1.445, 95%CI: 1.028-2.031)
Arasteh *et al*[17]	IBS	Cohort study	1067 patients with IBS	Rome IV	IBS-D: 57 (5.3%); IBS-C: 380 (35.6%); IBS-U: 630 (59%)	Not documented	3.7%	Liver disease not associated with IBS (Coefficient: 0.26, OR: 1.30, 95%CI: 0.92-1.82)
Lee *et al*[19]	IBS *vs* control	Retrospective, cross sectional, case control study	83 IBS patients; 260 age and sex matched control	Rome III	IBS-C: 14.8%; IBS-D: 49.4%; IBS-M: 31.3%; IBS-U: 4.5%	Investigated raised ALT, GGT, AST and features of metabolic syndrome	16.9% of IBS patients had raised ALT; 24.1% had raised GGT	Significantly higher ALT in patients with IBS (16.9% *vs* 7.7%; P = 0.015); Significantly higher GGT in patients with IBS (24.1% *vs* 11.5%; P = 0.037); Significantly higher prevalence of metabolic syndrome in patients with IBS (32.5% *vs* 12.7%; P < 0.001)
Sarmini *et al*[73]	IBS *vs* control	Observational study	637942	Clinical diagnosis	Not documented	Not documented	Not available	Patients with IBS significantly more likely to develop NAFLD compared to non-IBS group (OR: 3.204, 95%CI: 3.130-3.279, P < 0.001)
Singh *et al* [24]	NAFLD	Retrospective analysis	632	Clinical diagnosis	Not documented	Ultrasound; Alcohol consumption < 20 g/d; Normal aetiological liver screen	186 (29.4%) patients with NAFLD had clinical diagnosis of IBS	IBS symptoms are highly prevalent in those with NAFLD
Jones-Pauley *et al* [22]	NAFLD	Cross-sectional study	130	Rome IV	Not documented	Not documented	38 (29.2%) patients with NAFLD met Rome IV IBS criteria	High prevalence of IBS in patients with NAFLD; Significant increase in prevalence of depression (18.4% *vs* 5.4%, P = 0.01) and anxiety (31.6% *vs* 9.8%, P = 0.002) in those with co-existing IBS compared to those with NAFLD without IBS

IBS: Irritable bowel syndrome; NAFLD: Non-alcoholic fatty liver disease; IBS-C: Constipation predominant IBS; IBS-D: Diarrhoea predominant IBS; IBS-M: Mixed IBS; IBS-U: Unsubtyped IBS; OR: Odds ratio; CI: Cumulative incidence; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; GGT: Gamma-glutamyl transferase.

alcoholic steatohepatitis (NASH) within a year.

The association between IBS and obesity is more unclear[27]. Aro *et al*[28] found a significant association between the obesity and IBS symptoms such as abdominal pain and diarrhoea using the Abdominal Symptom Questionnaire as well as a positive association between obesity and a formal diagnosis of IBS. However, these have not been confirmed in several other studies[29-31]. Interestingly, Lee *et al*[30] found visceral abdominal adiposity was associated with increased risk of IBS-D. There is evidence that IBS is more prevalent in patients who are obese[32]. Schneck *et al*[33]

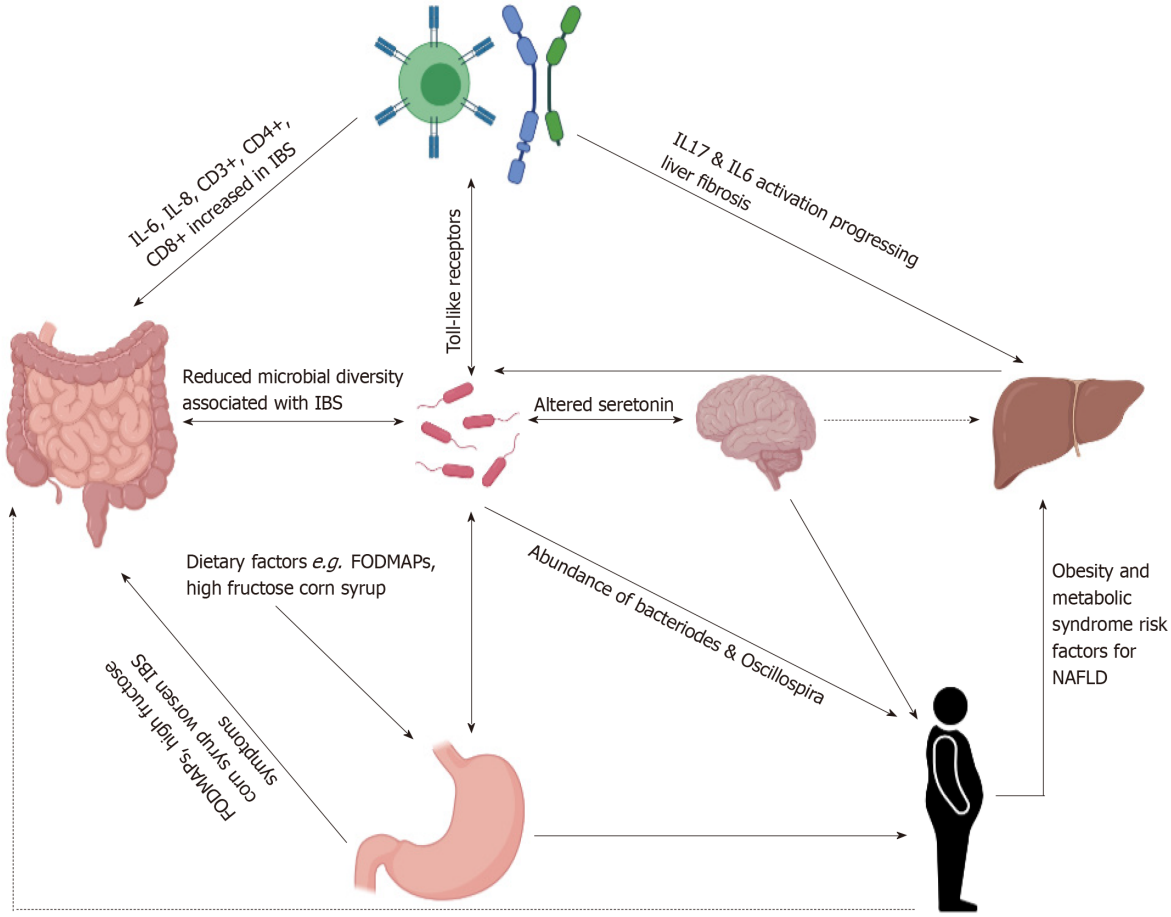

Figure 1 Schematic illustration summarizing associations and co-existing etiologies of irritable bowel syndrome and non-alcoholic fatty liver disease. IBS: Irritable bowel syndrome; NAFLD: Non-alcoholic fatty liver disease; IL: Interleukin.

described a cohort of patients with obesity undergoing bariatric surgery of which 30% fulfilled Rome III criteria for IBS. Further evidence for role of obesity in IBS is supported by the observation that increased visceral adiposity enhances perception of luminal stimuli, dysmotility and abdominal pain[34]. Higher body mass indexes have been associated with accelerated colonic and rectosigmoid transit and increased stool frequency[35]. Furthermore, weight loss through diet or bariatric surgery has been shown to improve symptoms[32,36]. Aasbrenn *et al*[37] prospectively analyzed the effect of a weight loss program on bowel symptoms using the IBS severity scoring system (IBS-SSS) and Gastrointestinal Symptom Rating Scale and found that there were significant improvements in the IBS-SSS in patients with IBS compared to those without.

MICROBIOME

The gut microbiota plays a vital role in the intestinal barrier function, metabolism of nutrients and development of immune tolerance and response. Dysregulation of the microbiome has been shown to be a component for the development of both NAFLD and IBS[38].

Long-term perturbation of the gut microbiota has been shown to contribute to metabolic syndrome and fatty liver disease[39]. Several mechanisms have been proposed on how the gut microbiota results in NAFLD development. This includes increased intestinal permeability leading greater lipopolysaccharide exposure to the host. This, in turn, results in toll like receptor (predominantly TLR4) activation of the innate immune system, causing liver inflammation as they are transported from the gut to the liver. Additionally, microbially produced metabolites, such as lactate and ethanol, can directly activate inflammatory cascades within the liver. Enterohepatic bile acid homeostasis is important for multiple processes, including fat absorption,

inflammation, immunity and microbial diversity. Significant differences have been noted in bile acid composition in metabolic diseases associating with progression of NAFLD[38,40].

Patients with hepatic steatosis and NASH have been shown to have increased *Proteobacteria, Enterobacteriaceae, Escherichia* and *Citrobacter* with reductions in abundance of *Rikenellaceae, Ruminococcaceae, Anaerosporobacter* and *Coprococcus*[39,40] Reductions in *Bifidobacteria* have also been observed and *Bifidobacteria* possibly reduce gut wall permeability to lipopolysaccherides, suggesting a relationship with the development of disease[39]. Interestingly, Frost *et al*[39] followed up patients who had incidental findings of fatty liver or diabetes and found changes in *Clostridium XIVa* as a result of dysbiosis with a strong association for increasing fatty acid biosynthesis. Type 2 diabetes is also noted to result in increased gut permeability. Aron-Wisnewsky *et al* [40] found significant overlap in microbial signatures between patients with NAFLD and NASH with obesity and diabetes, finding changes in abundance of *Oscillospira* and *Bacteriodes.* Further evidence on the importance of the gut microbiome in metabolic syndrome, is shown by fecal microbiota transplant being associated with a temporary improvement in peripheral insulin resistance[41].

Changes in intestinal microbial diversity is also thought to contribute to the development of IBS as the microbiota impacts on intestinal motility and sensitivity. Some patients with IBS have been shown to have changes in the *Fermicutes*-to-*Bacteriodes* ratio, reduced *lactobacilli* and *bifidobacterial* as well as reduced microbial diversity[38,42].

The gut-brain-microbiome axis is known to have an important role in glucose regulation. Gut microbiota modulation produces changes in the immune, neurotransmitter and monoaminergic activity of this axis. Serotonin secretion affects motility, pain perception but also plays a role in mood control[43]. NAFLD and the gut-brain axis may also be inter-related. There is evidence that depression is associated with NAFLD. However, disentangling the multiple contributors to depression in multifactorial disease states (as often seen in patients with metabolic syndrome) can be exceptionally difficult[44,45].

Dysregulation of the microbiome itself can lead to poor glycaemic control, acting through nitric oxide formation which affects the neuronal response to gut hormone Glucagon-like peptide-1 (GLP-1)[46]. The GLP-1 receptor antagonist, Semaglutide, has been shown to reduce liver fat and NASH resolution in patients with NAFLD[47]. It has also been used to treat weight loss and type-2 diabetes mellitus[48]. Given the known functions of GLP-1 on the gut microbiota, the effect seen in these studies may well be related to beneficial alterations in microbiome composition[49].

DIETARY FACTORS

Dietary factors have been shown to be integral to the man-agement of both IBS and NAFLD. Weight loss through diet and exercise is the mainstay of NAFLD management. Adherence to a Mediterranean diet reduces hepatic steatosis and achieves a greater weight loss in patients with NAFLD[50]. By contrast, patients with IBS have been shown to have a poorer adherence to a Mediterranean diet than healthy controls[50], a dietary factor which may therefore be relevant in the development of NAFLD in those with IBS. There is also some evidence that conservative weight loss can help IBS symptoms. Aasbrenn *et al*[37] found that a weight loss program resulted in a significant improvement in IBS symptoms as assessed by IBS-SSS questionnaires and Gastrointestinal Symptom Rating Scale[37].

Certain food groups appear to worsen IBS symptoms and contribute to NAFLD development. High fructose corn syrup (HFCS) is a disaccharide which is frequently used in artificial sweeteners, processed, canned and baked goods worldwide. HFCS has been shown to induce IBS symptoms through increased osmotic pressure and bacterial fermentation resulting in gas production, abdominal bloating and pain[51]. HFCS has also been shown to downregulate the insulin signaling pathway which would contribute to the pathogenesis of NAFLD[52]. Fructose consumption has also been shown to increase intestinal permeability potentially leading to the development of both NAFLD and IBS through the processes already outlined[53].

Certainly more research into the dietary implications on NAFLD and IBS is needed. Many patients with IBS notice that 'healthy' foods such as fruit and vegetables can make their symptoms worse and this results in some of them adopting a more 'unhealthy' diet which may lead to weight gain. There is evidence that a low FODMAP diet with excludes some fruits and vegeatables improves IBS symptoms

however to the authors' knowledge, there is a paucity of data on the effects of a low FODMAP diet on the progression of NAFLD.

IMMUNE MEDIATED FACTORS

Chronic inflammation is a critical driver of progressive disease in NAFLD and significant advances have been made to understand the role of inflammation[54,55]. The role of toll-like receptors (TLRs) and macrophage activation has already been discussed. Additionally, Natural killer cells and natural killer T cells contribute to inflammation by releasing cytokines and reactive oxygen species[56]. Tumor necrosis factor (TNF)-α, alongside other cytokines and growth factors, have also been shown to possible have a role in the development of NAFLD and NASH, in both animals and humans[38]. TNF-α in combination with interleukin (IL)-6 stimulates the production of leptin activating neutrophils and the innate immune system[38]. In addition, adaptive immune responses drive NASH as hepatic infiltration of B cells and CD4 and CD8 T cells exacerbate parenchymal injury and inflammation[56]. B cells play a profibrogenic role involving the stimulation of hepatic stellate cells and liver macrophages[57]. CD4+ T cells differentiate to type-17 T helper cells, producing IL-17 which has been implicated in the progression of NAFLD[58]. The balance of the adaptive immune cellular compartment within the liver can transition from a pro-resolution composition to pro-inflammatory subset, driving disease and fibrosis.

In IBS, a similar chronic low-grade inflammatory picture has also been described. The innate immune system is implicated with an increased number of mast cells throughout the intestines in some patients[59]. The adaptive immune response is also important with CD3+, CD4+ and CD8+ T cells increased in intestines and blood of patients with IBS[38]. Interestingly, an increase in IL-6 and IL-8 with reduced anti-inflammatory cytokines has been seen in serum of IBS patients[59]. The role of TLRs is also felt to be important with IL-6 and other cytokines acting through this mechanism [38]. TNF-α can act on the nervous system to cause hypersensitivity, gastric hypomotility and nausea[59].

SMALL INTESTINAL BACTERIAL OVERGROWTH

Small intestinal bacterial overgrowth (SIBO) can cause abdominal pain, bloating and chronic diarrhea. Although an area of controversy due to conflicting evidence, a number of previous studies have suggested that some patients with IBS have a relatively high prevalence of SIBO[60,61]. A recent metanalysis has shown that patients with IBS were more likely to test positive for SIBO than healthy controls[61]. Further circumstantial evidence for the gut-brain-microbiome-liver axis can be drawn from the effects of the non-absorbable antibiotic Rifaximin in both IBS and in liver disease. Whilst the mechanism is unclear, improvement in IBS symptoms have been demonstrated in patients in randomized controlled trials of Rifaximin[62,63]. Rifaximin is also often used to treat SIBO[64], a condition which has been shown to affect cognitive function in a subset of patients who present with brain fog[65]. Interestingly, treatment with Rifaximin has recently been shown in brain imaging studies to alter neuronal connectivity and increase cognitive flexibility through its effect on the gut microbiome particularly in beta and theta frequencies with a particular focus on the insular cortex, a region known to be affected in patients with IBS [66]. Furthermore, Rifaximin has an immunomodulatory action counteracting the pro-inflammatory response seen in gut microbiota dysbiosis[67]. In liver disease, Rifaximin is an established treatment for hepatic encephalopathy, with its effects attributed to alterations in the gut microbiome and resultant positive effects on cognitive function. Specifically in patients with biopsy proven NASH, Rifaximin has also been shown to reduce insulin resistance, inflammation and NAFLD fat scores[68]. Therefore, the effects of Rifaximin are multifactorial including reduced endotoxemia, modulation of inflammatory cytokines, and intestinal permeability as well as changing functional brain connectivity[62,66].

Further overlapping evidence for SIBO in this context comes from the obesity literature. There is evidence that obesity reduces gut motility, which may predispose to SIBO due to stasis, and plausibly this is thought to damage barrier function, which can result in bacterial translocation and altered gut-liver axis[53]. Furthermore, changes in the gut-liver axis may well a result of increased intestinal permeability. A high prevalence of SIBO has been observed in obese subjects however the association

between NAFLD and SIBO is less clear[53]. Studies have found the prevalence of SIBO in NAFLD to range from 39%-60% albeit in small numbers of patients. However, more recently, some research found 8% of NAFLD patients in their cohort had SIBO and there was no evidence that SIBO was associated with a higher risk of fibrosis[69-71].

BILE ACID DIARRHOEA

Bile acid malabsorption is a cause of chronic diarrhea and has been shown to be associated with an increased NAFLD fibrosis score. Hepatic bile acid production is regulated by Fibroblast growth factor 19 (FGF19) and Farnesoid-X-receptor (FXR) and obeticholic acid (a FXR agonist) has shown therapeutic potential in both bile acid related diarrhea and NAFLD[20]. Appleby *et al*[20] found that increased hepatic bile acid production and diarrhea were associated with an increased NAFLD score. Of further relevance to the link with NAFLD, bile acid diarrhoea has also been shown to be associated with raised body mass index[72]. This is therefore an important point to be considered in clinical practice when evaluating patients with suspected overlapping IBS and NAFLD, as up to a third of patients meeting the criteria for IBS-D have been shown to have bile salt malabsorption when investigated[72], and this condition should therefore be excluded in the context of watery diarrhea.

APPLICABILITY TO CLINICAL PRACTICE

Pulling this together, there is consistent evidence to show that IBS and NAFLD have a similar pathogenesis and therefore applying this to clinical practice, physicians should be aware that NAFLD may co-exist silently in patients with IBS and vice versa. Patients with IBS and incidental findings of elevated liver enzymes or with risk factors for NAFLD should be considered for non-invasive liver screening through ultrasound and appropriately available non-invasive fibrosis assessment using FIB-4 scoring, enhanced liver fibrosis testing or mechanical liver stiffness measurement.

Conversely, patients with NAFLD may not admit to the debilitating symptoms of IBS due to stigma or feeling that their symptoms are not relevant to their liver consultation. Screening for positive clinical features of IBS and targeted treatment for both conditions in unison may aid compliance with treatment, improve quality of life and ultimately improve morbidity.

However, as highlighted in this review, there is a lack of large, high quality cross-sectional data on the incidence of IBS in NAFLD patients and vice versa. To date, studies have been limited to the use of ultrasound and blood tests to diagnose NAFLD, however there is a lack of data that quantifies a fibrosis score which may be useful to correlate with IBS severity. From the currently available data (summarized in Table 1), whilst there is a suggestion that the IBS-D sub-type may be more common than IBS-C in patients with NAFLD, whether this is a genuine finding merits further evaluation in studies which have excluded bile salt malabsorption with appropriate investigations given its apparent independent association with NAFLD.

CONCLUSION

IBS and NAFLD are common conditions that can have significant effects on both physical and mental health[73], as well as significant healthcare and socioeconomic implications. There is some evidence that patients with IBS are more likely to develop NAFLD, and there are multiple different pathophysiological mechanisms that could contribute to both conditions, however more data is needed. Until such data clarifies this picture, the possibility of these conditions existing concomitantly should be considered proactively and investigated appropriately.

REFERENCES

1 **Mearin F**, Lacy BE, Chang L, Chey WD, Lembo AJ, Simren M, Spiller R. Bowel Disorders. *Gastroenterology* 2016 [PMID: 27144627 DOI: 10.1053/j.gastro.2016.02.031]

2 **Oka P**, Parr H, Barberio B, Black CJ, Savarino EV, Ford AC. Global prevalence of irritable bowel syndrome according to Rome III or IV criteria: a systematic review and meta-analysis. *Lancet*

Gastroenterol Hepatol 2020; **5**: 908-917 [PMID: 32702295 DOI: 10.1016/S2468-1253(20)30217-X]
3 **Shivaji UN**, Ford AC. Prevalence of functional gastrointestinal disorders among consecutive new patient referrals to a gastroenterology clinic. *Frontline Gastroenterol* 2014; **5**: 266-271 [PMID: 28839783 DOI: 10.1136/flgastro-2013-100426]
4 **Zamani M**, Alizadeh-Tabari S, Zamani V. Systematic review with meta-analysis: the prevalence of anxiety and depression in patients with irritable bowel syndrome. *Aliment Pharmacol Ther* 2019; **50**: 132-143 [PMID: 31157418 DOI: 10.1111/apt.15325]
5 **Atarodi S**, Rafieian S, Whorwell PJ. Faecal incontinence-the hidden scourge of irritable bowel syndrome: a cross-sectional study. *BMJ Open Gastroenterol* 2014; **1**: e000002 [PMID: 26462260 DOI: 10.1136/bmjgast-2014-000002]
6 **Dixon-Woods M**, Critchley S. Medical and lay views of irritable bowel syndrome. *Fam Pract* 2000; **17**: 108-113 [PMID: 10758070 DOI: 10.1093/fampra/17.2.108]
7 **Hearn M**, Whorwell PJ, Vasant DH. Stigma and irritable bowel syndrome: a taboo subject? *Lancet Gastroenterol Hepatol* 2020; **5**: 607-615 [PMID: 31924568 DOI: 10.1016/S2468-1253(19)30348-6]
8 **Blachier M**, Leleu H, Peck-Radosavljevic M, Valla DC, Roudot-Thoraval F. The burden of liver disease in Europe: a review of available epidemiological data. *J Hepatol* 2013; **58**: 593-608 [PMID: 23419824 DOI: 10.1016/j.jhep.2012.12.005]
9 **Targher G**, Byrne CD, Tilg H. NAFLD and increased risk of cardiovascular disease: clinical associations, pathophysiological mechanisms and pharmacological implications. *Gut* 2020; **69**: 1691-1705 [PMID: 32321858 DOI: 10.1136/gutjnl-2020-320622]
10 **Roulot D**. Screening for Liver Fibrosis in General or At-Risk populations using Transient Elastrography. Liver Elastrography. Springer Nature, 2020: 545-550 [DOI: 10.1007/978-3-030-40542-7_47]
11 **Harman DJ**, Ryder SD, James MW, Jelpke M, Ottey DS, Wilkes EA, Card TR, Aithal GP, Guha IN. Direct targeting of risk factors significantly increases the detection of liver cirrhosis in primary care: a cross-sectional diagnostic study utilising transient elastography. *BMJ Open* 2015; **5**: e007516 [PMID: 25941185 DOI: 10.1136/bmjopen-2014-007516]
12 **Public Health England**. Liver disease: applying All Our Health. [cited 23 March 2021]. Available from: https://www.gov.uk/government/publications/liver-disease-applying-all-our-health/liverdisease-applying-all-our-health
13 **Cotter TG**, Charlton M. Nonalcoholic Steatohepatitis After Liver Transplantation. *Liver Transpl* 2020; **26**: 141-159 [PMID: 31610081 DOI: 10.1002/lt.25657]
14 **Haldar D**, Kern B, Hodson J, Armstrong MJ, Adam R, Berlakovich G, Fritz J, Feurstein B, Popp W, Karam V, Muiesan P, O'Grady J, Jamieson N, Wigmore SJ, Pirenne J, Malek-Hosseini SA, Hidalgo E, Tokat Y, Paul A, Pratschke J, Bartels M, Trunecka P, Settmacher U, Pinzani M, Duvoux C, Newsome PN, Schneeberger S; European Liver and Intestine Transplant Association (ELITA). Outcomes of liver transplantation for non-alcoholic steatohepatitis: A European Liver Transplant Registry study. *J Hepatol* 2019; **71**: 313-322 [PMID: 31071367 DOI: 10.1016/j.jhep.2019.04.011]
15 **Hasanain AF**, Abdel-Rahman ME, Ali AM, Abdel-Aal SM. Nonalcoholic fatty liver disease among patients with irritable bowel syndrome: prevalence and contribution to disease severity. *Gastroenterol Hepatol Endoscopy* 2018; **3** (3): 1-4 [DOI: 10.15761/GHE.1000163]
16 **Shin A**, Xu H, Imperiale TF. Associations of chronic diarrhoea with non-alcoholic fatty liver disease and obesity-related disorders among US adults. *BMJ Open Gastroenterol* 2019; **6**: e000322 [PMID: 31523443 DOI: 10.1136/bmjgast-2019-000322]
17 **Arasteh P**, Maharlouei N, Eghbali SS, Amini M, Lankarani KB, Malekzadeh R. A Comprehensive Look at Irritable Bowel Syndrome and its Associated Factors Considering the Rome IV Criteria: A Penalized Smoothly Clipped Absolute Deviation Regression Approach in the Pars Cohort Study. *Middle East J Dig Dis* 2018; **10**: 149-159 [PMID: 30186578 DOI: 10.15171/mejdd.2018.104]
18 **Vasant DH**. Global prevalence of irritable bowel syndrome: time to consider factors beyond diagnostic criteria? *Lancet Gastroenterol Hepatol* 2020; **5**: 879-880 [PMID: 32702294 DOI: 10.1016/S2468-1253(20)30211-9]
19 **Lee SH**, Kim KN, Kim KM, Joo NS. Irritable Bowel Syndrome May Be Associated with Elevated Alanine Aminotransferase and Metabolic Syndrome. *Yonsei Med J* 2016; **57**: 146-152 [PMID: 26632395 DOI: 10.3349/ymj.2016.57.1.146]
20 **Appleby RN**, Moghul I, Khan S, Yee M, Manousou P, Neal TD, Walters JRF. Non-alcoholic fatty liver disease is associated with dysregulated bile acid synthesis and diarrhea: A prospective observational study. *PLoS One* 2019; **14**: e0211348 [PMID: 30682184 DOI: 10.1371/journal.pone.0211348]
21 **Singh SP**, Kar SK, Panigrahi MK, Misra B, Pattnaik K, Bhuyan P, Meher C, Agrawal O, Rout N, Swain M. Profile of patients with incidentally detected nonalcoholic fatty liver disease (IDNAFLD) in coastal eastern India. *Trop Gastroenterol* 2013; **34**: 144-152 [PMID: 24851523 DOI: 10.7869/tg.118]
22 **Jones-Pauley M**, Franco L, Tamimi O, Nashatian L, Victor D, Quigley EM. Mo1503 Irritable bowel syndrome symptoms in non-alcoholic fatty liver disease patients are an indicator of depression and anxiety. *Gastroenterology* 2020; **158** (6): S-1429 [DOI: 10.1016/S0016-5085(20)34239-6]
23 **Pallayova M**, Taheri S. Non-alcoholic fatty liver disease in obese adults: clinical aspects and current management strategies. *Clin Obes* 2014; **4**: 243-253 [PMID: 25825857 DOI: 10.1111/cob.12068]
24 **Godoy-Matos AF**, Silva Júnior WS, Valerio CM. NAFLD as a continuum: from obesity to metabolic syndrome and diabetes. *Diabetol Metab Syndr* 2020; **12**: 60 [PMID: 32684985 DOI: 10.1186/s13098-020-00570-y]

25 **Marchesini G**, Petta S, Dalle Grave R. Diet, weight loss, and liver health in nonalcoholic fatty liver disease: Pathophysiology, evidence, and practice. *Hepatology* 2016; **63**: 2032-2043 [PMID: 26663351 DOI: 10.1002/hep.28392]

26 **Lassailly G**, Caiazzo R, Buob D, Pigeyre M, Verkindt H, Labreuche J, Raverdy V, Leteurtre E, Dharancy S, Louvet A, Romon M, Duhamel A, Pattou F, Mathurin P. Bariatric Surgery Reduces Features of Nonalcoholic Steatohepatitis in Morbidly Obese Patients. *Gastroenterology* 2015; **149**: 379-88; quiz e15 [PMID: 25917783 DOI: 10.1053/j.gastro.2015.04.014]

27 **Pickett-Blakely O**. Obesity and irritable bowel syndrome: a comprehensive review. *Gastroenterol Hepatol (N Y)* 2014; **10**: 411-416 [PMID: 25904828]

28 **Aro P**, Ronkainen J, Talley NJ, Storskrubb T, Bolling-Sternevald E, Agréus L. Body mass index and chronic unexplained gastrointestinal symptoms: an adult endoscopic population based study. *Gut* 2005; **54**: 1377-1383 [PMID: 15917313 DOI: 10.1136/gut.2004.057497]

29 **Akhondi N**, Memar Montazerin S, Soltani S, Saneei P, Hassanzadeh Keshteli A, Esmaillzadeh A, Adibi P. General and abdominal obesity in relation to the prevalence of irritable bowel syndrome. *Neurogastroenterol Motil* 2019; **31**: e13549 [PMID: 30657237 DOI: 10.1111/nmo.13549]

30 **Lee CG**, Lee JK, Kang YS, Shin S, Kim JH, Lim YJ, Koh MS, Lee JH, Kang HW. Visceral abdominal obesity is associated with an increased risk of irritable bowel syndrome. *Am J Gastroenterol* 2015; **110**: 310-319 [PMID: 25583325 DOI: 10.1038/ajg.2014.422]

31 **Talley NJ**, Howell S, Poulton R. Obesity and chronic gastrointestinal tract symptoms in young adults: a birth cohort study. *Am J Gastroenterol* 2004; **99**: 1807-1814 [PMID: 15330923 DOI: 10.1111/j.1572-0241.2004.30388.x]

32 **Pugliese G**, Muscogiuri G, Barrea L, Laudisio D, Savastano S, Colao A. Irritable bowel syndrome: a new therapeutic target when treating obesity? *Hormones (Athens)* 2019; **18**: 395-399 [PMID: 31228102 DOI: 10.1007/s42000-019-00113-9]

33 **Schneck AS**, Anty R, Tran A, Hastier A, Amor IB, Gugenheim J, Iannelli A, Piche T. Increased Prevalence of Irritable Bowel Syndrome in a Cohort of French Morbidly Obese Patients Candidate for Bariatric Surgery. *Obes Surg* 2016; **26**: 1525-1530 [PMID: 26424705 DOI: 10.1007/s11695-015-1907-0]

34 **Emerenziani S**, Guarino MPL, Trillo Asensio LM, Altomare A, Ribolsi M, Balestrieri P, Cicala M. Role of Overweight and Obesity in Gastrointestinal Disease. *Nutrients* 2019; **12** [PMID: 31906216 DOI: 10.3390/nu12010111]

35 **Sadik R**, Björnsson E, Simrén M. The relationship between symptoms, body mass index, gastrointestinal transit and stool frequency in patients with irritable bowel syndrome. *Eur J Gastroenterol Hepatol* 2010; **22**: 102-108 [PMID: 19701093 DOI: 10.1097/MEG.0b013e32832ffd9b]

36 **Clements RH**, Gonzalez QH, Foster A, Richards WO, McDowell J, Bondora A, Laws HL. Gastrointestinal symptoms are more intense in morbidly obese patients and are improved with laparoscopic Roux-en-Y gastric bypass. *Obes Surg* 2003; **13**: 610-614 [PMID: 12935364 DOI: 10.1381/096089203322190835]

37 **Aasbrenn M**, Lydersen S, Farup PG. A Conservative Weight Loss Intervention Relieves Bowel Symptoms in Morbidly Obese Subjects with Irritable Bowel Syndrome: A Prospective Cohort Study. *J Obes* 2018; **2018**: 3732753 [PMID: 29686892 DOI: 10.1155/2018/3732753]

38 **Scalera A**, Di Minno MN, Tarantino G. What does irritable bowel syndrome share with non-alcoholic fatty liver disease? *World J Gastroenterol* 2013; **19**: 5402-5420 [PMID: 24023483 DOI: 10.3748/wjg.v19.i33.5402]

39 **Frost F**, Kacprowski T, Rühlemann M, Pietzner M, Bang C, Franke A, Nauck M, Völker U, Völzke H, Dörr M, Baumbach J, Sendler M, Schulz C, Mayerle J, Weiss FU, Homuth G, Lerch MM. Long-term instability of the intestinal microbiome is associated with metabolic liver disease, low microbiota diversity, diabetes mellitus and impaired exocrine pancreatic function. *Gut* 2021; **70**: 522-530 [PMID: 33168600 DOI: 10.1136/gutjnl-2020-322753]

40 **Aron-Wisnewsky J**, Vigliotti C, Witjes J, Le P, Holleboom AG, Verheij J, Nieuwdorp M, Clément K. Gut microbiota and human NAFLD: disentangling microbial signatures from metabolic disorders. *Nat Rev Gastroenterol Hepatol* 2020; **17**: 279-297 [PMID: 32152478 DOI: 10.1038/s41575-020-0269-9]

41 **Zhang Z**, Mocanu V, Cai C, Dang J, Slater L, Deehan EC, Walter J, Madsen KL. Impact of Fecal Microbiota Transplantation on Obesity and Metabolic Syndrome-A Systematic Review. *Nutrients* 2019; **11** [PMID: 31557953 DOI: 10.3390/nu11102291]

42 **Bhattarai Y**, Muniz Pedrogo DA, Kashyap PC. Irritable bowel syndrome: a gut microbiota-related disorder? *Am J Physiol Gastrointest Liver Physiol* 2017; **312**: G52-G62 [PMID: 27881403 DOI: 10.1152/ajpgi.00338.2016]

43 **Agustí A**, García-Pardo MP, López-Almela I, Campillo I, Maes M, Romaní-Pérez M, Sanz Y. Interplay Between the Gut-Brain Axis, Obesity and Cognitive Function. *Front Neurosci* 2018; **12**: 155 [PMID: 29615850 DOI: 10.3389/fnins.2018.00155]

44 **Tomeno W**, Kawashima K, Yoneda M, Saito S, Ogawa Y, Honda Y, Kessoku T, Imajo K, Mawatari H, Fujita K, Hirayasu Y, Nakajima A. Non-alcoholic fatty liver disease comorbid with major depressive disorder: The pathological features and poor therapeutic efficacy. *J Gastroenterol Hepatol* 2015; **30**: 1009-1014 [PMID: 25619308 DOI: 10.1111/jgh.12897]

45 **Kim D**, Yoo ER, Li AA, Tighe SP, Cholankeril G, Harrison SA, Ahmed A. Depression is associated with non-alcoholic fatty liver disease among adults in the United States. *Aliment Pharmacol Ther* 2019; **50**: 590-598 [PMID: 31328300 DOI: 10.1111/apt.15395]

46 **Grasset E**, Burcelin R. The gut microbiota to the brain axis in the metabolic control. *Rev Endocr Metab Disord* 2019; **20**: 427-438 [PMID: 31656993 DOI: 10.1007/s11154-019-09511-1]

47 **Newsome PN**, Buchholtz K, Cusi K, Linder M, Okanoue T, Ratziu V, Sanyal AJ, Sejling AS, Harrison SA; NN9931-4296 Investigators. A Placebo-Controlled Trial of Subcutaneous Semaglutide in Nonalcoholic Steatohepatitis. *N Engl J Med* 2021; **384**: 1113-1124 [PMID: 33185364 DOI: 10.1056/NEJMoa2028395]

48 **Nauck MA**, Meier JJ. MANAGEMENT OF ENDOCRINE DISEASE: Are all GLP-1 agonists equal in the treatment of type 2 diabetes? *Eur J Endocrinol* 2019; **181**: R211-R234 [PMID: 31600725 DOI: 10.1530/EJE-19-0566]

49 **Everard A**, Cani PD. Gut microbiota and GLP-1. *Rev Endocr Metab Disord* 2014; **15**: 189-196 [PMID: 24789701 DOI: 10.1007/s11154-014-9288-6]

50 **Kataria R**, Pickett-Blakely O. The Mediterranean Diet in Gastrointestinal and liver diseases. *Curr treatm opt gastroenterol* 2020; **18**: 718-728 [DOI: 10.1007/s11938-020-00318-7]

51 **Ikechi R**, Fischer BD, DeSipio J, Phadtare S. Irritable Bowel Syndrome: Clinical Manifestations, Dietary Influences, and Management. *Healthcare (Basel)* 2017; **5** [PMID: 28445436 DOI: 10.3390/healthcare5020021]

52 **Collison KS**, Saleh SM, Bakheet RH, Al-Rabiah RK, Inglis AL, Makhoul NJ, Maqbool ZM, Zaidi MZ, Al-Johi MA, Al-Mohanna FA. Diabetes of the liver: the link between nonalcoholic fatty liver disease and HFCS-55. *Obesity (Silver Spring)* 2009; **17**: 2003-2013 [PMID: 19282820 DOI: 10.1038/oby.2009.58]

53 **Paolella G**, Mandato C, Pierri L, Poeta M, Di Stasi M, Vajro P. Gut-liver axis and probiotics: their role in non-alcoholic fatty liver disease. *World J Gastroenterol* 2014; **20**: 15518-15531 [PMID: 25400436 DOI: 10.3748/wjg.v20.i42.15518]

54 **Gao B**, Tsukamoto H. Inflammation in Alcoholic and Nonalcoholic Fatty Liver Disease: Friend or Foe? *Gastroenterology* 2016; **150**: 1704-1709 [PMID: 26826669 DOI: 10.1053/j.gastro.2016.01.025]

55 **Pellicoro A**, Ramachandran P, Iredale JP, Fallowfield JA. Liver fibrosis and repair: immune regulation of wound healing in a solid organ. *Nat Rev Immunol* 2014; **14**: 181-194 [PMID: 24566915 DOI: 10.1038/nri3623]

56 **Sutti S**, Albano E. Adaptive immunity: an emerging player in the progression of NAFLD. *Nat Rev Gastroenterol Hepatol* 2020; **17**: 81-92 [PMID: 31605031 DOI: 10.1038/s41575-019-0210-2]

57 **Thapa M**, Chinnadurai R, Velazquez VM, Tedesco D, Elrod E, Han JH, Sharma P, Ibegbu C, Gewirtz A, Anania F, Pulendran B, Suthar MS, Grakoui A. Liver fibrosis occurs through dysregulation of MyD88-dependent innate B-cell activity. *Hepatology* 2015; **61**: 2067-2079 [PMID: 25711908 DOI: 10.1002/hep.27761]

58 **Tang Y**, Bian Z, Zhao L, Liu Y, Liang S, Wang Q, Han X, Peng Y, Chen X, Shen L, Qiu D, Li Z, Ma X. Interleukin-17 exacerbates hepatic steatosis and inflammation in non-alcoholic fatty liver disease. *Clin Exp Immunol* 2011; **166**: 281-290 [PMID: 21985374 DOI: 10.1111/j.1365-2249.2011.04471.x]

59 **Ghoshal UC**. Marshall and Warren Lecture 2019: A paradigm shift in pathophysiological basis of irritable bowel syndrome and its implication on treatment. *J Gastroenterol Hepatol* 2020; **35**: 712-721 [PMID: 32162356 DOI: 10.1111/jgh.15032]

60 **Ford AC**, Spiegel BM, Talley NJ, Moayyedi P. Small intestinal bacterial overgrowth in irritable bowel syndrome: systematic review and meta-analysis. *Clin Gastroenterol Hepatol* 2009; **7**: 1279-1286 [PMID: 19602448 DOI: 10.1016/j.cgh.2009.06.031]

61 **Ghoshal UC**, Nehra A, Mathur A, Rai S. A meta-analysis on small intestinal bacterial overgrowth in patients with different subtypes of irritable bowel syndrome. *J Gastroenterol Hepatol* 2020; **35**: 922-931 [PMID: 31750966 DOI: 10.1111/jgh.14938]

62 **Pimentel M**, Lembo A, Chey WD, Zakko S, Ringel Y, Yu J, Mareya SM, Shaw AL, Bortey E, Forbes WP; TARGET Study Group. Rifaximin therapy for patients with irritable bowel syndrome without constipation. *N Engl J Med* 2011; **364**: 22-32 [PMID: 21208106 DOI: 10.1056/NEJMoa1004409]

63 **Meyrat P**, Safroneeva E, Schoepfer AM. Rifaximin treatment for the irritable bowel syndrome with a positive lactulose hydrogen breath test improves symptoms for at least 3 months. *Aliment Pharmacol Ther* 2012; **36**: 1084-1093 [PMID: 23066911 DOI: 10.1111/apt.12087]

64 **Pimentel M**, Saad RJ, Long MD, Rao SSC. ACG Clinical Guideline: Small Intestinal Bacterial Overgrowth. *Am J Gastroenterol* 2020; **115**: 165-178 [PMID: 32023228 DOI: 10.14309/ajg.0000000000000501]

65 **Rao SSC**, Rehman A, Yu S, Andino NM. Brain fogginess, gas and bloating: a link between SIBO, probiotics and metabolic acidosis. *Clin Transl Gastroenterol* 2018; **9**: 162 [PMID: 29915215 DOI: 10.1038/s41424-018-0030-7]

66 **Sometti D**, Ballan C, Wang H, Braun C, Enck P. Effects of the antibiotic rifaximin on cortical functional connectivity are mediated through insular cortex. *Sci Rep* 2021; **11**: 4479 [PMID: 33627763 DOI: 10.1038/s41598-021-83994-4]

67 **Ponziani FR**, Gerardi V, Pecere S, D'Aversa F, Lopetuso L, Zocco MA, Pompili M, Gasbarrini A. Effect of rifaximin on gut microbiota composition in advanced liver disease and its complications. *World J Gastroenterol* 2015; **21**: 12322-12333 [PMID: 26604640 DOI: 10.3748/wjg.v21.i43.12322]

68 **Abdel-Razik A**, Mousa N, Shabana W, Refaey M, Elzehery R, Elhelaly R, Zalata K, Abdelsalam M, Eldeeb AA, Awad M, Elgamal A, Attia A, El-Wakeel N, Eldars W. Rifaximin in nonalcoholic fatty liver disease: hit multiple targets with a single shot. *Eur J Gastroenterol Hepatol* 2018; **30**: 1237-1246 [PMID: 30096092 DOI: 10.1097/MEG.0000000000001232]

69 **Miele L**, Valenza V, La Torre G, Montalto M, Cammarota G, Ricci R, Mascianà R, Forgione A,

Gabrieli ML, Perotti G, Vecchio FM, Rapaccini G, Gasbarrini G, Day CP, Grieco A. Increased intestinal permeability and tight junction alterations in nonalcoholic fatty liver disease. *Hepatology* 2009; **49**: 1877-1887 [PMID: 19291785 DOI: 10.1002/hep.22848]

70 **Rafiei R**, Bemanian M, Rafiei F, Bahrami M, Fooladi L, Ebrahimi G, Hemmat A, Torabi Z. Liver disease symptoms in non-alcoholic fatty liver disease and small intestinal bacterial overgrowth. *Rom J Intern Med* 2018; **56**: 85-89 [PMID: 29101772 DOI: 10.1515/rjim-2017-0042]

71 **Lira MMP**, de Medeiros Filho JEM, Baccin Martins VJ, da Silva G, de Oliveira Junior FA, de Almeida Filho ÉJB, Silva AS, Henrique da Costa-Silva J, de Brito Alves JL. Association of worsening of nonalcoholic fatty liver disease with cardiometabolic function and intestinal bacterial overgrowth: A cross-sectional study. *PLoS One* 2020; **15**: e0237360 [PMID: 32845887 DOI: 10.1371/journal.pone.0237360]

72 **Aziz I**, Mumtaz S, Bholah H, Chowdhury FU, Sanders DS, Ford AC. High Prevalence of Idiopathic Bile Acid Diarrhea Among Patients With Diarrhea-Predominant Irritable Bowel Syndrome Based on Rome III Criteria. *Clin Gastroenterol Hepatol* 2015; **13**: 1650-5.e2 [PMID: 25769413 DOI: 10.1016/j.cgh.2015.03.002]

73 **Sarmini MT**, Asfari MM, Alomari M, Al-Khadra Y, Khoudari G, Dasarathy S, McCullough AJ. Assessing the relation between Irritable bowel syndrome and nonalcoholic liver disease. *Am J Gastroenterol* 2019; **114**: S277-S278 [DOI: 10.14309/01.ajg.0000591444.20831.c0]

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REVIEW

Liver-side of inflammatory bowel diseases: Hepatobiliary and drug-induced disorders

Stefano Mazza, Sara Soro, Maria Chiara Verga, Biagio Elvo, Francesca Ferretti, Fabrizio Cereatti, Andrea Drago, Roberto Grassia

ORCID number: Stefano Mazza 0000-0002-9068-3209; Sara Soro 0000-0002-4802-8403; Maria Chiara Verga 0000-0001-6871-1229; Biagio Elvo 0000-0001-5695-0310; Francesca Ferretti 0000-0002-6445-0954; Fabrizio Cereatti 0000-0003-0628-4473; Andrea Drago 0000-0002-9777-8665; Roberto Grassia 0000-0003-4491-4050.

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Stefano Mazza, Sara Soro, Maria Chiara Verga, Biagio Elvo, Fabrizio Cereatti, Andrea Drago, Roberto Grassia, Gastroenterology and Digestive Endoscopy Unit, ASST Cremona, Cremona 26100, Italy

Francesca Ferretti, Gastroenterology Unit, ASST Fatebenefratelli-Sacco, Department of Biomedical and Clinical Sciences (DIBIC), Università degli Studi di Milano, Milan 20157, Italy

Corresponding author: Stefano Mazza, MD, Doctor, Gastroenterology and Digestive Endoscopy Unit, ASST Cremona, Viale Concordia 1, Cremona 26100, Italy. stem311089@gmail.com

Abstract

Hepatobiliary disorders are among the most common extraintestinal manifestations in inflammatory bowel diseases (IBD), both in Crohn's disease and ulcerative colitis (UC), and therefore represent a diagnostic challenge. Immune-mediated conditions include primary sclerosing cholangitis (PSC) as the main form, variant forms of PSC (namely small-duct PSC, PSC-autoimmune hepatitis overlap syndrome and IgG4-related sclerosing cholangitis) and granulomatous hepatitis. PSC is by far the most common, presenting in up to 8% of IBD patients, more frequently in UC. Several genetic foci have been identified, but environmental factors are preponderant on disease pathogenesis. The course of the two diseases is typically independent. PSC diagnosis is based mostly on typical radiological findings and exclusion of secondary cholangiopathies. Risk of cholangiocarcinoma is significantly increased in PSC, as well as the risk of colorectal cancer in patients with PSC and IBD-related colitis. No disease-modifying drugs are approved to date. Thus, PSC management is directed against symptoms and complications and includes medical therapies for pruritus, endoscopic treatment of biliary stenosis and liver transplant for end-stage liver disease. Other non-immune-mediated hepatobiliary disorders are gallstone disease, whose incidence is higher in IBD and reported in up to one third of IBD patients, non-alcoholic fatty liver disease, pyogenic liver abscess and portal vein thrombosis. Drug-induced liver injury (DILI) is an important issue in IBD, since most IBD therapies may cause liver toxicity; however, the incidence of serious adverse events is low. Thiopurines and methotrexate are the most associated with DILI, while the risk related to anti-tumor necrosis factor-α and anti-integrins is low. Data on hepatotoxicity of newer drugs approved for IBD, like anti-interleukin 12/23 and tofacitinib, are still scarce, but the evidence from other rheumatic diseases is

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reassuring. Hepatitis B reactivation during immunosuppressive therapy is a major concern in IBD, and adequate screening and vaccination is warranted. On the other hand, hepatitis C reactivation does not seem to be a real risk, and hepatitis C antiviral treatment does not influence IBD natural history. The approach to an IBD patient with abnormal liver function tests is complex due to the wide range of differential diagnosis, but it is of paramount importance to make a quick and accurate diagnosis, as it may influence the therapeutic management.

Key Words: Inflammatory bowel diseases; Hepatobiliary disorders; Primary sclerosing cholangitis; Drug-induced liver injury; Biological drugs; Viral hepatitis

Core Tip: Hepatobiliary disorders are commonly associated with inflammatory bowel diseases (IBD) and represent a management challenge. They include (1) Immune-mediated diseases that can coexist with IBD, mainly primary sclerosing cholangitis; (2) Other non-immune-mediated disorders like gallstone disease; (3) Liver injury induced by drugs used in IBD; and (4) Risks related to concomitant viral hepatitis B and C. All these conditions are summarized in this review, according to the latest literature evidence and the current clinical practice guidelines.

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INTRODUCTION

Hepatobiliary disorders are common extraintestinal manifestations of inflammatory bowel diseases (IBD) and may occur in both Crohn's disease (CD) and ulcerative colitis (UC). The range of IBD-associated hepatobiliary disorders is wide and can underlie different pathogenetic mechanisms. They include diseases with immune-mediated pathogenesis, which typically have a course independent of intestinal activity, the most common being primary sclerosing cholangitis (PSC); variant form of PSC, like small-duct PSC, must also be considered. Other non-immune-mediated conditions include gallstone disease, whose incidence is increased in IBD patients, non-alcoholic fatty liver disease (NAFLD), pyogenic liver abscess and portal vein thrombosis. Drug-induced liver diseases is another important chapter, since several drugs used in IBD, mainly thiopurines, methotrexate and anti-tumor necrosis factor-α (anti-TNF) may induce liver toxicity. Concomitant viral hepatitis B and C in IBD is also a relevant issue, particularly hepatitis B reactivation under immunosuppressive therapy; however, the recent introduction of potent antiviral drugs for both the infections and the spread of the anti-hepatitis B virus vaccine (HBV) contributed to significantly lower the risk. The diagnosis of such hepatobiliary conditions is of great importance, since they may influence the management and therapeutic approach to IBD, contraindicate the use of some therapies, or prevent the evolution towards the end stage of liver disease. The main hepatobiliary disorders, which are discussed in this review, are summarized in Table 1. A proposed practical approach to abnormal liver function tests (LFT) in a patient with IBD is presented in Figure 1.

IMMUNE-MEDIATED CONDITIONS

PSC

PSC is the most common hepatobiliary manifestation associated with IBD. It is a rare, idiopathic, chronic cholestatic syndrome characterized by chronic inflammation, fibrosis and finally destruction of intra- and/or extra-hepatic bile ducts. PSC is a

Table 1 Main features of hepatobiliary manifestations associated with inflammatory bowel diseases

Hepatobiliary manifestation	Main features
Immune-mediated	
PSC	The most frequent (50%-80% of PSC patients have IBD, and 2%-8% of IBD patients have PSC)
	No medical treatment approved. Therapies directed towards PSC complications
	Increased risk of cholangiocarcinoma and colorectal cancer (surveillance needed)
Small duct PSC	Histological evidence of PSC, but normal cholangiogram
	More benign disease course than classic PSC (cholangiocarcinoma risk not increased)
PSC-AIH overlap syndrome	Coexistence of biochemical and histological features of AIH and PSC-associated biliary tract alterations
	Better response to steroids and immunosuppressants than PSC
IgG4-related sclerosing cholangitis	Part of the IgG4-related systemic disease
	Characterized by histological evidence of IgG4+ plasma cells infiltrate
	Good response to steroids
Granulomatous hepatitis	Rare, generally in Crohn's disease
	Autoimmune or drug-induced pathogenesis
	Good response to steroids
Non-immune-mediated	
Gallstone disease	Incidence increased in IBD, more in Crohn's disease
	Bile salts malabsorption underlying the pathogenesis
NAFLD	Not strictly associated with IBD; similar risk factors in the general population
	Higher NAFLD prevalence in patients with severe IBD activity
Pyogenic liver abscess	Rare, mainly in Crohn's disease
	Penetrating disease, steroid treatment and malnutrition are risk factors
Portal vein thrombosis	Increased risk in IBD, especially during severe disease flare and after surgery. Prophylactic treatment indicated in these settings
DILI	
Aminosalicylates	Low risk of DILI
	LFT monitoring not necessary
Thiopurines	DILI quite frequent (prevalence of about 3%); both dose-independent and dose-dependent toxicities are possible
	Regular LFT monitoring indicated
Methotrexate	DILI quite frequent, with a prevalent dose-dependent mechanism
	Regular LFT monitoring indicated
	Folic acid supplementation indicated during treatment
Anti-tumour necrosis factor-α	Low risk of DILI, mainly with infliximab
	LFT monitoring not necessary
Anti-integrins	Low risk of DILI
	LFT monitoring not necessary
Anti-interleukin 12/23	Low risk of DILI
	LFT monitoring not necessary
Tofacitinib	Data in IBD still scarce
	Alanine aminotransferase elevation quite frequent in rheumatoid arthritis, but generally mild
Hepatitis B reactivation	A relevant concern
	Antiviral therapy indicated in HBsAg positive patients

	LFT monitoring indicated in HBsAg negative/anti-HBc positive patients
	Vaccination indicated in naïve patients
Hepatitis C reactivation	Not a relevant concern

IBD: Inflammatory bowel diseases; PSC: Primary sclerosing cholangitis; LFT: Liver function tests; HBsAg: Hepatitis B surface antigen; DILI: Drug-induced liver injury; NAFLD: Non-alcoholic fatty liver disease; AIH: Autoimmune hepatitis.

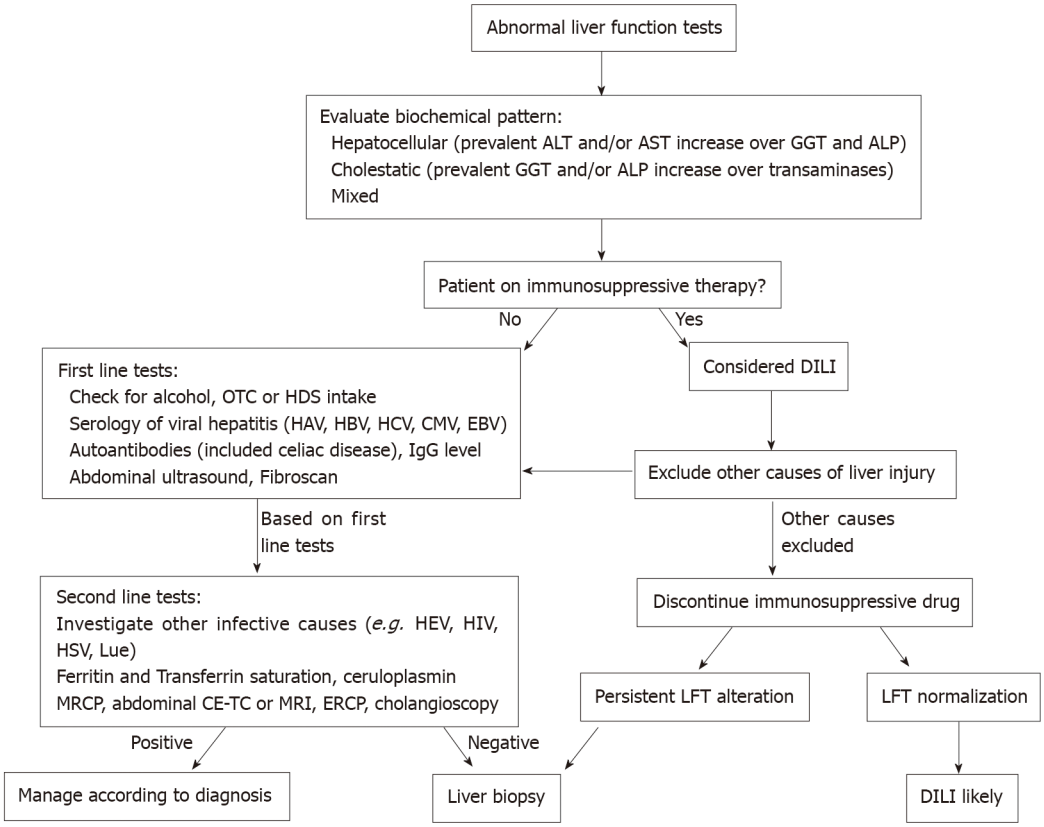

Figure 1 Mind map describing a practical approach to the inflammatory bowel disease patient with abnormal liver function tests. ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; ALP: Alkaline phosphatase; CE-CT: Contrast-enhancement computed tomography; CMV: Cytomegalovirus; DILI: Drug-induced liver injury; EBV: Epstein-Barr virus; GGT: Gamma-glutamyl transpeptidase; HAV: Hepatitis A virus; HBV: Hepatitis B virus; HCV: Hepatitis C virus; HDS: Herbal and dietary supplements; HEV: Hepatitis E virus; HIV: Human immunodeficiency virus; HSV: Herpes simplex virus; MRCP: Magnetic resonance cholangiopancreatography; MRI: Magnetic resonance imaging; OTC: Over-the-counter drugs.

progressive disease, leading to liver biliary cirrhosis and portal hypertension.

Epidemiology: According to a recent systematic review, the incidence and prevalence rates of PSC range from 0 to 1.3 *per* 100.000 inhabitants/year and from 0 to 16.2 *per* 100.000 inhabitants, respectively. There is a 2:1 male predominance and a peak of incidence between 30 to 40 years old[1]. PSC is commonly associated with IBD, with about 50%-80% of patients with PSC having concomitant IBD, more frequently UC[2], and about 2%-8% of patients with IBD having PSC[3]. PSC diagnosis usually precedes that of IBD, although PSC may be diagnosed many years after proctocolectomy for colitis[4].

Etiology: The exact etiology of PSC is unknown. A multifactorial pathogenesis has been proposed, in which genetic, immunological, and environmental factors contribute to the development of the disease. The increased risk of PSC in first-degree relatives suggests a genetic predisposition. Multiple human leukocyte antigen (HLA) haplotypes related to PSC susceptibility have been reported: HLA-B8, HLA-DRB1*0301 (DR3), HLA-DRB3*0101 (DRw52a) and HLA-DRB1*0401 (DR4)[5]. Interestingly, three UC susceptibility loci, harboring the genes *REL*, *IL2*, and *CARD9*, have been linked to PSC, supporting the association UC-PSC as a separate disease entity. However, genetic factors are implicated in a minority of PSC cases, clearly

emphasizing the predominant role of environmental risk factors in the overall disease liability[6,7]; colonic toxins, gut microbiota, portal bacteria and viral infections[6], are some of the main environmental determinants, which are discussed below. Based on the association between certain HLA haplotypes, the acute and chronic inflammatory infiltrate at histology, and given the association with several other autoimmune conditions, PSC has been classically considered an autoimmune disease[8]. Several autoantibodies may be present, including antinuclear antibodies in 24%-53%, smooth muscle antibodies in 13%-20%, and anti-perinuclear cytoplasmic antibodies (pANCA) in 65%-88% of patients[9]. However, none of these autoantibodies are reliable for diagnosis and there is no significant response of the disease to immunosuppressants. Chronic portal bacteremia is another important mechanism postulated: the bacterial translocation from the gut into the portal system can lead to biliary inflammation and recurrent cholangitis, probably through activation of the innate immune response in susceptible individuals[10]. Growing evidence suggests a relevant role of the gut microbiome in the pathogenesis of PSC, independently of IBD. Patients with PSC are characterized by a fecal overrepresentation of *Escherichia*, *Lactobacillus*, *Fusobacterium*, *Enterococcus* and *Ruminococcus*, and decreased populations of *Clostridium* cluster II, *Prevotella* and Bacteroides, compared to healthy individuals and patients with IBD alone[11-13]. Gut dysbiosis has been linked to an increase Gut dysbiosis has been linked to an increase in gut permeability and bacterial translocation that enter the enterohepatic circulation[14]. Other etiologic mechanisms such as ischemia and chronic viral infections have been postulated, but more evidence is needed.

Clinical presentation and diagnosis: Since most patients with PSC are asymptomatic at diagnosis, the disease is frequently suspected after routine liver biochemical tests. When the disease is symptomatic, the most common symptoms are pruritus, fatigue, right upper abdominal pain, and weight loss. Acute cholangitis is the first clinical manifestation of PSC in about 15% of cases[15]. Biochemical tests typically show a cholestatic pattern: An increased alkaline phosphatase (ALP) is the most frequent alteration, usually together with a raise of gamma-glutamyl transpeptidase. Notably, although an elevated ALP is a sensitive diagnostic marker, a normal level does not exclude PSC[6]. A high level of serum bilirubin is observed in an advanced stage of disease and is a marker of poor prognosis. Aminotransferases are often normal or mildly raised. As mentioned above, multiple autoantibodies, most frequently pANCA, have been associated with PSC, but they are not specific nor related to disease activity and prognosis[16]. Diagnosis is confirmed if the typical morphological alterations of biliary ducts are identified and causes of secondary sclerosing cholangitis are excluded. Magnetic resonance cholangiopancreatography (MRCP) should be the technique of choice for the investigation of suspected PSC, with a sensitivity and specificity for diagnosis of 0.86 and 0.94, respectively[17]. MRCP demonstrates diffuse, multifocal strictures and dilations of the intra- and extra-hepatic bile ducts. In about 40% of cases, the gallbladder and cystic duct are also involved[18]. Endoscopic retrograde cholangiopancreatography (ERCP) should be reserved for patients with biliary strictures requiring tissue acquisition (*e.g.* cytological brushing) or when therapeutic intervention is indicated (*e.g.* jaundice or acute cholangitis)[6]. In recent years, peroral cholangioscopy has emerged as a useful endoscopic tool in PSC management. It can provide a direct intraductal visualization, which allows guided biliary biopsies and can be helpful in distinguishing between benign and malignant strictures. A recent meta-analysis found a sensitivity and specificity of cholangioscopy-directed biopsies for all indications (*i.e.*, not limited to PSC) of 71.9% and 99.1%, respectively[19,20]; however, data on patients with PSC are still limited. Moreover, cholangioscopy has been recently used in the treatment of biliary stones in patients with PSC, with promising results[19]. Liver biopsy is not required to establish a diagnosis of a "classic" form of PSC. However, it is essential in presence of abnormal liver tests and normal cholangiogram to investigate small duct PSC, or in PSC patients with disproportionately elevated serum aminotransferase values to exclude PSC–autoimmune hepatitis (AIH) overlap syndrome. The most specific histological finding of PSC is periductal fibrosis with an "onion skin" pattern. In clinical practice, however, histological assessment is often non-specific, demonstrating general features of cholestasis that are similar to those find in primary biliary cirrhosis. Liver biopsy can also play a role in staging the disease and in defining the prognosis[6].

Complications and prognosis: PSC is a progressive disease that leads to severe complications involving liver, biliary tree and intestine. Fibrotic obliteration of intra-hepatic bile ducts finally evolves into liver cirrhosis, hepatic failure and portal hypertension. Disease progression towards end-stage liver disease is unavoidable in

most patients, and liver transplantation (LT) is considered the only curative treatment option[21]. In the literature, the median time from diagnosis to death or LT range from 7 to 22 years, with higher survival rates observed in overall PSC populations respected to cohorts of patients from liver transplant centers, which suffer from referral bias[22, 23]. In IBD patients, performing colectomy before PSC diagnosis was associated with lower risk of LT and death in a large cohort study in Sweden[14]. Portal hypertension is a frequent complication of PSC, and the presence of esophageal varices at diagnosis or history of variceal hemorrhage are considered predictors of worse prognosis[24]. PSC patients are at increased risk of cholangiocarcinoma (CCA), gallbladder carcinoma, hepatocellular carcinoma (HCC), and colorectal carcinoma (CCR). The estimated annual incidence of CCA in patients with PSC range from 0.5% to 1.5%[25, 26], with 20%-30% of CCA found synchronously at PSC diagnosis, and 50% of CCA occurring within 1 year[25]. According to a large international, multicentre, PSC cohort study (7121 patients from 37 countries), 10.9% of PSC patients developed a hepatopancreatobiliary malignancy, which was CCA in about 80% of cases[27]. Importantly, concomitant UC was a risk factor for future development of hepatopancreatobiliary malignancies[27]. Gallbladder cancer and HCC are less frequent complication of PSC, with a lifetime incidence of 3%-14% and 0.3%-2.8%, respectively[28]. An increased risk of CCR has been clearly demonstrated in patients with PSC-IBD, compared to patients with IBD or PSC alone. According to a recent meta-analysis of observational studies, patients with IBD and PSC were at increased risk of colorectal cancer compared with patients with IBD alone, with an odds ratio of 3.41 (95%CI: 2.13-5.48). Interestingly, stratification by IBD type revealed that PSC was a risk factor for colorectal cancer in patients with UC, but not in CD patients[29]. In addition, unlike in patients with UC alone, CCR risk in PSC-UC seems to manifest soon after the combined diagnosis, with a peak of incidence within the first 2 years of diagnosis[30]; thus, cancer surveillance is strongly recommended in PSC-UC, even in patients with ileal pouch-anal anastomosis (IPAA) after colectomy[31]. Finally, IBD patients with IPAA and concomitant PSC are at increased risk of pouchitis, with an almost double incidence at 10 years as compared to patients without PSC[32].

Treatment: Treatment of PSC associated with IBD does not differ from PSC without IBD. To date, no medical treatments have been demonstrated to modify the course of "classic" PSC. In particular, ursodeoxycholic acid (UDCA) has shown to improve LFT in several studies, but two meta-analyses and a large multicentre study failed to show benefit from UDCA towards important clinical outcomes (*e.g.* complications and death) in patients with PSC[33,34]. Despite previous studies suggested a role of UDCA in prevention of cancer (CCR or CCA) in PSC, more recent meta-analyses and a randomized control trial did not confirm this effect[35,36]. UDCA is not currently recommended by PSC guidelines for either the treatment or cancer prevention[6,37]. Despite the presumed immune-mediated pathogenesis of the disease, corticosteroids and immunosuppressants are not recommended as well[6]. Thus, treatments goals in PSC are directed to the control of symptoms and management of complications, such as varices, liver decompensation, cholangitis, jaundice, pruritus, and malignancies. Endoscopic interventions, mainly ERCP, are a mainstay of PSC management, and specific guidelines have been published from collaboration of European Society of Gastrointestinal Endoscopy and European Association for the Study of the Liver (EASL)[38]. Main indications of ERCP in PSC are acute cholangitis, treatment of dominant strictures and suspicion of CCA. LT is a potential resolutive therapy in PSC patients with end-stage liver disease. Other disease-specific indications are intractable pruritus, recurrent cholangitis, and limited cases of very early stage of CCA[3]. A single-center experience from the Mayo Clinic reported survival rates after LT for PSC-related end-stage liver disease of 86% at 5 years and 70% at 10 years[39]. Recurrence of PSC after LT is a concern, occurring in 12%-37% of cases and causing significant impact on long term graft and recipient survival[40].

Variant forms of PSC

Small duct PSC: A minority of patients with cholestatic biochemistry and typical liver histology with concentric 'onion skin' fibrosis around the bile ducts, but with entirely normal cholangiogram, was first described by Wee and Ludwig[41] in 1985; they coined the term "small duct PSC". In a large multicentre study, 81% of patients with small-duct PSC had IBD, predominantly UC (78%) compared to CD (21%). In this study, none of the patients developed CCA or other intestinal malignancies during a median follow-up of 13 years, but 28% of them shown evidence of progression to large duct PSC at repeated cholangiography[42]. In a large bicentric study from United

Kingdom and Norway, only 12% of small duct PSC patients either required LT or died, compared to 47% of patients with "classic" PSC[43].

Overlap between PSC and AIH: PSC/AIH overlap syndrome is a rare disorder characterized by concomitant occurrence of the biochemical and histological features of AIH and the cholangiography abnormalities found in PSC. In a cohort of 211 PSC patients from United States, according to the International AIH group scoring system, AIH was diagnosed as "definite" in 1.4% and "probable" in 6% of patients[44]. An Italian cohort of PSC/AIH patients showed a lower mean age at presentation and higher alanine aminotransferase (ALT) and aspartate aminotransferase (AST) values compared to "classic" PSC[45]. There is also a strong association between PSC/AIH and IBD; according to a recent systematic review, IBD was present in 44% of PSC/AIH patients, that was UC in 68% of cases[46]. Patients with an established diagnosis of AIH who also have IBD should be evaluated for concomitant PSC. Patients with PSC/AIH seem to benefit from treatment with immunosuppressive medications and have a better prognosis compared to patients with PSC alone[45].

IgG4-related sclerosing cholangitis: IgG4-related sclerosing cholangitis (IgG4-SC) is the biliary manifestation of the multi-organ inflammatory IgG4-related disease. Diagnosis requires histological evidence of IgG4+ plasma cells infiltrate (> 10 *per* high-power field), imaging of biliary tract involvement (which may be indistinguishable from the "classic" PSC), elevated serum IgG4 levels (> 135 mg/dL), evidence of other organ involvement and response to steroid treatment[47]. Autoimmune pancreatitis is the most frequent organ involvement associated with IgG4-SC, being present in > 90% of cases[48]. An increase in serum IgG4 is reported in 9%-22% of patients with PSC overall[48], making it difficult to distinguish a PSC with high serum IgG4 levels from a "true" IgG4-SC. EASL Cholestatic Liver Disease Guidelines recommends measurement of serum IgG4 in all patients with large-duct PSC at diagnosis[37]. While association with IBD is prevalent in PSC, this is rarely seen in IgG4-SC; high serum IgG4 levels have been observed in about 5% of IBD patients[49]. Unlike in PSC, response to steroid treatment in IgG4-SC is excellent. However, relapse after steroid withdrawal is common[50]; in these cases, second-line treatments include immunomodulators and rituximab[50,51].

AIH

AIH is an immune-mediated chronic liver disease characterized by hepatocellular inflammation, necrosis and progression to cirrhosis. The clinical presentation varies from persistent mild elevation of AST and ALT to fulminant forms of acute hepatitis. Mean age at presentation shows a bimodal pattern with one peak during childhood/teenage years and another between the 4th and 6th decade of life. The diagnosis of AIH must be suspected in presence of autoantibodies (mainly antinuclear, smooth muscle, soluble liver antigen/liver pancreas and liver/kidney microsomal type 1 antibodies), IgG elevation, consistent liver histology and exclusion of other forms of hepatitis[52]. Despite most of the data about AIH/IBD coexistence comes from studies focusing on PSC and AIH/PSC overlap syndrome, a higher prevalence of AIH has been found in patients with IBD, compared to subjects without IBD. In the cross-sectional study by Halling *et al*[53], AIH was more frequent in males and females with IBD compared with matched controls without IBD, with an odds ratio of 7.8 and 17.9, respectively[53]. Another study by Perdigoto *et al*[54] found a 16% prevalence of UC in patients with AIH, 42% of whom had also PSC features at cholangiography[54]. In this study, patients with colitis failed treatment for AIH more commonly and progressed to cirrhosis more frequently; similar results emerged from the study by Perdigoto *et al*[54].

Granulomatous hepatitis

Granulomatous hepatitis is a rare complication of IBD, with only a few cases of IBD-associated granulomatous hepatitis reported in literature[55-57]. It occurs more frequently in CD and can underlie an autoimmune pathogenesis or be induced by mesalamine or sulfasalazine therapy[58]. Clinical manifestations include fever, hepatomegaly and increase in cholestatic enzymes, although patients can be completely asymptomatic[59]. Response to corticosteroid therapy is generally good; methotrexate may be considered as second-line therapy in patients relapsing after steroids[60]. Prognosis is usually benign[61].

NON-IMMUNE-MEDIATED DISORDERS

Gallstone disease

Several studies and a meta-analysis showed a prevalence of cholelithiasis in CD ranging from 8% to 34%, with a 2- to 5-fold increased risk compared to the general population[62-68]. Three studies also evaluated UC patients, reporting a prevalence of gallstone disease of 4%-10%; only one of these found a significantly higher risk compared to a population without UC[62], while the other two studies, including the aforementioned meta-analysis, did not demonstrate this increased risk[64-66]. Most studies relied on abdominal ultrasound to diagnose the lithiasis. A recent case-cohort study on a large cohort of IBD patients reported an incidence of cholelithiasis of 5.21/1000 persons/year, compared to a 3.49/1000 persons/year incidence of a matched non-IBD cohort ($P < 0.001$); the significance was also maintained by differentiating CD and UC[69]. Another case-control study reported an incidence of gallstone disease in CD and UC of 14.35/1000 persons/year and 7.48/1000 persons/year, respectively, that were significantly higher than those of the matched control populations[70]. In all studies assessing both CD and UC, prevalence of gallstone disease was higher in CD compared to UC. Among the risk factors, ileal disease location, previous ileal resection and long-standing disease were the most frequently associated with gallstone disease in IBD[62-64,67,68,70]. The pathogenesis of cholelithiasis in IBD patients is usually attributed to bile salts malabsorption at the terminal ileum; this leads to a decrease in the total bile acid pool, leading to supersaturated bile in gallbladder, which predispose to stone formation[71,72]. Lapidus and Einarsson[71] reported that patients with ileal resection due to CD are characterized by lower cholesterol saturation, but increased bilirubin concentration in fasting duodenal bile, compared to healthy controls; therefore, these patients seem not predisposed to the formation of cholesterol stones, but rather at risk of developing pigment stones[71].

NAFLD

NAFLD refers to a clinical and pathological syndrome that includes a spectrum of histological findings ranging from benign steatosis to non-alcoholic steatohepatitis. Non-alcoholic steatohepatitis is defined by histological evidence of hepatic steatosis associated with inflammation, and can progress to hepatic fibrosis and cirrhosis. A recent meta-analysis reported a worldwide prevalence of NAFLD of 25% in the general population[73], a prevalence that seems to be worryingly increasing over time [74]. In the literature, the prevalence of NAFLD in patients with IBD is variable. Two recent meta-analyses reported a pooled prevalence of NAFLD in IBD of 27.5%[75] and 32%[76]; older age, obesity, type 2 diabetes, longer IBD duration and previous surgery were the main risk factors associated with the development of NAFLD[77]. A further meta-analysis specifically addressing the role of IBD treatment on the risk of NAFLD found no significant association between medications of all types (*i.e.*, steroids, biological agents, immunomodulators, methotrexate) and the risk of developing NAFLD[78]. Several studies also reported a higher prevalence of NAFLD among IBD patients with severe disease activity at the time of liver evaluation, compared to mild-moderate IBD cases[77,79,80].

Pyogenic liver abscess

Pyogenic liver abscesses are rarely seen in IBD, with only a few cases reported in literature, mainly in CD. A nationwide case-cohort study from Taiwan reported an incidence of pyogenic liver abscess in IBD patients of 6.7 cases/10000 persons/year, which was significantly higher compared to controls without IBD[81]. Clinical manifestations include fever, chills, anorexia, weight loss and abdominal pain with right upper quadrant tenderness, which can mimic an IBD flare and lead to a diagnostic delay. Moreover, hepatic abscesses have been reported as the initial presentation of CD in several cases[82,83]. Risk factors predisposing to liver abscesses in IBD include abdominal surgery, fistulizing disease, intra-abdominal abscess, malnutrition, and corticosteroid treatment[84]. Dissemination from intra-abdominal abscesses and portal bacteremia secondary to impaired intestinal permeability are the most involved pathogenic mechanism[84].

Portal vein thrombosis

IBD patients are at increased risk of venous thromboembolism (VTE)[85]. In two studies on large cohorts of IBD patients with a follow-up time over 10 years, thromboembolic complications were reported in about 1% of patients, with an incidence of VTE of 2.6/1000 persons/year[85,86]. Porto-mesenteric venous system is a

frequent site of thrombosis in IBD and is a potentially catastrophic complication, which may lead to bowel ischemia or infarction and to acute or chronic portal hypertension; the mortality rate range between 3%-25%[86,87]. Incidence is higher during disease flares and after surgical procedures[88-90], and prophylactic treatment with low-molecular-weight heparin in severely active disease is indicated by guidelines to reduce the risk of thromboembolism[91]. However, about 30%-50% of thrombosis occurs in remission phases of the disease[92-94], indicating that factors other than inflammatory status can be involved in the pathogenesis of the thrombotic event. Immobilization, extensive colonic disease, central catheters, corticosteroids, and smoking are other known prothrombotic risk factors[90,95]. A hematologic prothrombotic condition can be found in up to 40% of portal vein thrombotic events in IBD, hyperhomocysteinemia being the most frequently found[95]. Thrombocytosis is frequently seen during IBD flares and may result from systemic inflammatory activity and/or iron-deficiency anemia[96]; however, no data on a possible association between thrombocytosis and VTE in IBD is available to date, since large clinical studies addressing this association are still lacking[97]. Moreover, IBD are associated with significant changes in circulating levels of various coagulation factors, as result of an imbalance between procoagulant and anti-coagulant pathways. Specifically, higher levels of prothrombin fragment 1 and 2, fibrinogen, factors V and VIII, thrombin-antithrombin complex, plasmin-α2-antiplasmin complex, and an impairment of the protein C pathway have been described in IBD[97-99]. Specific mutations in clotting factors, *e.g.* Factor V Leiden, are rare, but important to be identified as they may indicate long-term anticoagulant treatment[100]. European Crohn's and Colitis Organization (ECCO) guidelines recommend appropriate screening for prothrombotic condition after IBD diagnosis and anticoagulant treatment in accordance with international guidelines[95].

DRUG-INDUCED LIVER DISEASE IN IBD

The therapeutic armamentarium for the treatment of IBD is gradually expanding. This certainly offers greater potential for therapeutic benefit, but the risk of hepatotoxicity is a concern. Although the overall risk of serious adverse events is low, cases of drug-induced liver injury (DILI) have been reported for most drugs used in IBD, and some therapies carry a significant risk of liver toxicity. DILI induced by IBD drugs can be allergic/idiosyncratic (dose-independent) or related to hepatotoxins (typically dose-dependent). In addition, some drugs can cause hepatotoxicity with more than one pathogenic mechanism. According to EASL guidelines, the exclusion of other causes of hepatotoxicity is necessary for the diagnosis of DILI, and recovery after drug discontinuation is an important criterion for the causality assessment[101] (Figure 1). The following paragraphs will describe the association between the main drugs used in IBD and the risk and type of DILI.

Aminosalicylates

Sulfasalazine was the first aminosalicylate approved for the induction and maintenance of remission in mild-to-moderate UC. Within the bowel, sulfasalazine is cleaved into sulfapyridine and 5-aminosalicylic acid, most called mesalamine. Sulfapyridine, a sulfa-containing antibacterial agent, is then absorbed from the colon into the bloodstream, transported to the liver, and acetylated; acetylation was reported to be genetically programmed, with slow acetylators having higher levels of free sulfasalazine and more drug-induced adverse events[102]. Mesalamine is minimally absorbed and largely excreted in the stools and is primarily responsible for the anti-inflammatory effect on the colon. The introduction of the various mesalamine formulations has almost completely supplanted the use of sulfasalazine in UC, while the utility of aminosalicylates in CD remains unclear[91,103]. Both sulfapyridine and mesalamine are rarely associated with liver injury. According to the United Kingdom's Committee on the Safety of Medicines, from 1991 to 1998 the incidence of hepatitis in patients treated with mesalamine was 3.1 cases *per* million, compared to 6 cases *per* million in patients treated with sulfasalazine[104]. A French pharmacovigilance study on mesalamine microgranules (Pentasa®) reported 0.79 cases of LFT elevations *per* million treatment days over a 2-year period[105]. The toxic effect almost always occurs within the first 2 mo of treatment, and LFT normalize in most cases after drug discontinuation[105]. For sulfasalazine, sporadic cases of granulomatous hepatitis or fulminant hepatitis have been reported[106-108]. Due to this low risk of hepatotoxicity, a close monitoring of liver chemistries is not necessary in patients treated with

aminosalicylates.

Thiopurines

Azathioprine (AZA) and its metabolite 6-mercaptopurine (6-MP) are two thiopurine analogues widely used for the treatment of IBD. Main indication of AZA and 6-MP is the maintenance of remission in steroid-dependent CD and UC[91,103]. Purine analogues act as DNA synthesis inhibitors by antagonizing endogenous purines, and lead to both cytotoxic and immunosuppressive effects[109]. Overall, adverse events due to thiopurines are frequent and occur in 15%-40% of patients, leading to dose reduction or drug withdrawal[110]. Thiopurine-related adverse events are classified into dose-independent (or allergic/idiosyncratic) and dose-dependent. The former are thought to be immune-mediated and include rash, fever, arthralgia, and pancreatitis; the latter include myelotoxicity as the main manifestation. Thiopurine-induced hepatotoxicity can be both dose-dependent and independent, based on the pathogenetic mechanism involved[111,112]. Dose-independent liver toxicity usually occurs within 3 mo of therapy and includes hypersensitivity and idiosyncratic reactions[111]; type of hepatotoxicity can be described as acute hepatocellular hepatitis, with prevalent increase of aminotransferase levels, acute cholestatic hepatitis, with prevalent increase of serum ALP, or mixed[113,114]. Other less frequent findings include peliosis hepatis, hepatic sinusoidal dilatation, veno-occlusive disease, perisinusoidal and portal fibrosis, and nodular regenerative hyperplasia[113]. Thiopurine-related DILI has been related to thiopurine metabolites. After absorption, AZA is metabolized in the liver to 6-MP, which undergo a complex metabolization by three enzymes; one of them is the thiopurine S-methyltransferase (TPMT), that lead to 6-methylmercaptopurine (6-MMP) formation. 6-MMP is a non-effective metabolite which is important in hepatotoxicity development[109]. Approximately 15%–20% of IBD patients treated with thiopurines demonstrate hypermethylation (or shunting), a phenomenon due to a high TPMT activity that leads to preferential methylation of 6-MP to 6-MMP over bioactivation to thioguanine nucleotides (TGNs); the usual definition of hypermethylation is a ratio of 6-MMP to TGNs of > 11. Subtherapeutic TGNs level results in a poor response to therapy, while a high 6-MMP level (> 5700 pmol/8 × 10^8 erythrocytes) has been correlated with a 3-fold increased risk of liver toxicity[115]. Allopurinol is a xanthine oxidase inhibitor that prevents the breakdown of thiopurines into thiouric acid (TUA), thus increasing the bioavailability of 6-MP. Several studies have demonstrated that the combination of low dose thiopurine, *i.e.* 25%–50% of the standard dose, with 100 mg of allopurinol corrects hypermethylation in patients who have experienced thiopurines-induced hepatotoxicity or who have had a poor response to thiopurines treatment[116,117]. However, Shaye *et al*[118] showed that about 90% of patients with 6-MMP > 5700 pmol/8 × 10^8 erythrocytes have no hepatotoxicity and almost 40% of subjects with hepatotoxicity had 6-MMP levels below this cut-off[118]. Moreover, a recent case-control study and a meta-analysis failed to demonstrate any correlations between *TPMT* gene polymorphisms and hepatic adverse events in IBD patients[119,120]. The reported frequency of thiopurine-related hepatotoxicity varies widely among studies, ranging from to 3% to 17%[108,115,121,122]; a systematic review by Gisbert *et al*[113] reported a mean prevalence of thiopurine-induced liver injury of 3%, with a mean annual rate of 1.4%[113]. In a prospective cohort study, abnormal liver function (defined by ALT or ALP levels > 50% the upper normal limit) occurred in 13% of patients, while hepatotoxicity (defined by ALT or ALP levels greater than twice the upper normal limit) developed in 10% [111]. CD, liver steatosis and concomitant steroid therapy are reported risk factors for liver injury during thiopurine therapy[108,111,123]. It has been shown that most cases of thiopurine-induced liver injury completely resolved after dose reduction, while the need to discontinue therapy only occurred in about 3%-4% of cases[111,118,124]. Switching from AZA to 6-MP in the case of AZA-induced DILI is a possible strategy, which is effective in resolving the liver toxicity in 71%-87% of cases[114,125]. Despite an optimal frequency has not yet been established, regular monitoring of blood tests should be performed for the entire duration of thiopurine treatment, more frequently in the first 3 mo of therapy[113,126,127]. British Society of Gastroenterology (BSG) guidelines on IBD recommend the monitoring of full blood count and LFT at 2, 4, 8 and 12 wk of thiopurine therapy, and every 12 wk thereafter[127].

Methotrexate

Methotrexate (MTX) is a folic acid analogue with inhibitory activity against many enzymes in the metabolic pathway of folic acid. MTX inhibits production of thymidylate, purines, and methionine and leads to accumulation of adenosine, which has a potent anti-inflammatory activity. These actions inhibit cellular proliferation and

tissue migration, and decrease production of inflammatory mediators[128]. MTX is currently indicated for the maintenance of remission in steroid-dependent CD[129], while its role in UC is still controversial[130]. The hepatotoxic potential of MTX is well known. A meta-analysis of clinical trials on IBD patients treated with MTX reported a pooled incidence rate of abnormal hepatic aminotransferase levels, which the author defined as up to a 2-fold increase over the upper limit of the normal, of 1.4 *per* 100 person-months. The rate of hepatotoxicity, defined as aminotransferase levels greater than a 2-fold over the upper normal limit, was 0.9 *per* 100 person-months. The rate of withdrawal of MTX due to these abnormalities was 0.8 *per* 100 person-months[112,131]. Alcohol intake is a main risk factor for MTX-induced hepatotoxicity and should be strictly avoided. Other potential risk factors are obesity, diabetes mellitus and chronic viral hepatitis[112,131,132]. Folic acid supplementation has been correlated with reduction of methotrexate-induced hepatic adverse events and is therefore recommended[133]. Regular liver chemistry tests are recommended for the monitoring of hepatotoxicity, every 2 wk for the first 2 mo and then every 2-3 mo[134]; the drug should be stopped if transaminases exceed twice the upper normal limit[127]. Although liver biopsy was previously indicated after an MTX-treatment cumulative dose ≥ 1.5 g, this practice is no longer recommended by current rheumatologic guidelines[134], this is based on recent evidence that show a low incidence of liver injury in patients receiving a chronic low dose of MTX[112]. In a retrospective study on 87 IBD patients with a mean MTX cumulative dose of 1813 mg, 76% of patients maintained normal liver chemistry tests throughout MTX therapy; a liver biopsy was performed in 11 patients after a cumulative dose ≥ 1.5 g and found no case of moderate or severe fibrosis[112]. Another study evaluating 20 liver biopsies after a cumulative methotrexate dose of ≥ 1.5 g (mean dose 2.6 g) found mild histological abnormalities in 95% of patients; abnormal liver chemistry tests were present in 30% of patients and did not correlate with histological toxicity[135]. However, liver biopsy should be performed in cases of persistent alteration of transaminases, especially in case of no reduction after lowering the drug dose. Transient elastography is a promising tool for the monitoring of liver fibrosis in MTX-treated patients and can be useful in selecting patients for liver biopsy[136].

Biological agents

Anti-TNF-α: Since its introduction in the 1990s, anti-TNF-α antibody therapy has revolutionized the treatment of IBD. Anti-TNFs, which include infliximab, adalimumab, golimumab and certolizumab pegol, are approved for the treatment of moderate-to-severe CD and UC and demonstrated high efficacy in the induction and maintenance of both clinical and endoscopic remission[91,103]. Several types of anti-TNF-related adverse events have been reported, mostly of infectious, auto-immune and tumoral types. DILI caused by anti-TNF is uncommon, mostly mild and related to infliximab. However, cases of liver failure requiring transplantation has rarely been reported[137-139]. Shelton *et al*[140] evaluated the incidence of liver enzyme elevation in a large cohort of IBD patients treated with anti-TNF: Only 102 out of 1753 patients (6%) developed ALT elevation, and in about half of cases this could clearly be linked to an alternative etiology. Infliximab was the involved anti-TNF in 96% cases. Compared to a control population of anti-TNF-treated patients without liver enzyme elevation, no difference in concomitant immunomodulator therapy, body max index, age and gender was found. The majority of patients with ALT elevation continued anti-TNF, most of them normalizing the liver enzyme during the follow-up. In 10 patients switching to a second anti-TNF was performed, without recurrence of liver injury [140]. Ghabril *et al*[141] identified 34 cases of DILI related to anti-TNF used for a variety of auto-immune conditions from a review of the United States DILI Network database and PubMed research. The drug presumed to have caused DILI was infliximab in 76% of cases. The liver injury was scored as mild-to-moderate in 93% of cases. Fifteen of the 17 patients undergoing liver biopsy showed clear features of autoimmunity. All patients improved after discontinuation of the anti-TNF[141]. The mechanism underlying liver toxicity remains to be elucidated. Infliximab-related hepatitis seems to be sustained by an immune-mediated mechanism, mimicking the characteristics of AIH type I, although a direct liver damage cannot be ruled out[112]. Currently, Food and Drug Administration classifies infliximab as a Most-DILI-concern drug, adalimumab as a Less-DILI-concern drug, and golimumab and certolizumab as Ambiguous-DILI-concern drug[142]. The current consensus recommends the use of infliximab in selected cases of patients with significant liver disease, and that treatment should be discontinued or avoided in patients with transaminases above three times the upper limit of normal[143].

Anti-integrins: Natalizumab is a monoclonal antibody that antagonizes both the integrins α4β1 and α4β7, which are necessary for the homing of lymphocytes to brain and gut, respectively. Natalizumab is therefore approved for the treatment of multiple sclerosis, and has been tested with good results in CD; however, the risk of JC virus-associated progressive multifocal leukoencephalopathy (PML) has limited its use in IBD[144-146]. Vedolizumab is a humanized monoclonal antibody which, unlike natalizumab, specifically inhibits α4β7 integrin, thus eliminating the risk of PML[147]. Vedolizumab is approved for the treatment of both moderate to severely active CD and UC since 2014[91,103]. To date, only sporadic cases of liver injury during vedolizumab therapy have been reported[148,149]. In the prelicensure trials, three patients developed hepatitis, although it is unclear whether the increase in transaminases indicated drug-induced or autoimmune etiology[150]. In the GEMINI-1 and GEMINI-2 phase III trials, no differences in LFT were found compared to placebo[151,152]. Therefore, vedolizumab is considered almost free from liver toxicity.

Anti-interleukin 12/23: Ustekinumab is a human monoclonal antibody directed against the p40 subunit, which is a component of both interleukin (IL)-12 and IL-23, allowing this drug to simultaneously inhibit both these cytokines. Ustekinumab has been recently approved as a second line therapy for moderate-to-severe CD and UC since 2016 and 2019, respectively[153,154]. Although current data are limited, liver injury related to ustekinumab seems to be very uncommon. In the phase III trial on CD, a similar rate of adverse events compared to placebo was reported, with no mention of hepatotoxicity[153]. According to the Clinical and Research Information on DILI database, mild-to-moderate serum aminotransferase elevation was reported in 0.5% to 1.4% of patients during ustekinumab therapy. However, this event was no more frequent than placebo and resolved without discontinuing the drug[155]. Risankizumab is a monoclonal antibody directed against p19 subunit of IL-23 and therefore selectively inhibit this cytokine. Phase II and III trials in IBD are ongoing and safety data are still limited[156].

Tofacitinib

Tofacitinib is an oral Janus kinase inhibitor and is the first drug of this class approved for the treatment of IBD, specifically UC since May 2018[157], while others are currently being tested in phase II and III trials[158]. Tofacitinib is indicated for the treatment of adult patients with moderately to severely active UC, who have had an inadequate response or who are intolerant to anti-TNF[127,158]. Data about hepatotoxic effects of tofacitinib mainly derive from rheumatoid arthritis, where a slight ALT elevation was reported in about 30% of patients, but elevation above 3 times the upper normal limit occurred in 1%-2% of patients[159-161]. Data regarding tofacitinib-induced liver toxicity in IBD are still limited. However, no increased incidence of liver injury has been reported either in the pivotal trial or in subsequent real-life studies[162-165].

IBD AND VIRAL HEPATITIS B AND C

In literature, the reported prevalence of hepatitis B surface antigen (HBsAg) and anti-HBc positivity in IBD patients ranges from 0.6%-5.7% and from 1.6%-41.6%, respectively, depending on the geographic area considered[166]. Despite previous studies reported a higher prevalence of HBV positivity in IBD patients compared to the general population, more recent studies indicated an equal or lower prevalence which tends to decrease over time, suggesting that preventive measures like vaccination, use of disposable materials and implementation of transfusion safety programs are effective[166,167]. The risk of viral reactivation is a major concern in HBV patients treated with immunosuppressants. This event is closely related both to the stage of the infection and the type of immunosuppressive drug used. HBV reactivation, defined as the increase in HBV viremia of more than 1 Log10 IU/mL, is characterized by a broad spectrum of clinical manifestations, that range from viremia without clinically relevant manifestations to fulminant life-threatening hepatitis[168]. For this reason, both ECCO and BSG guidelines recommend hepatitis B screening immediately after diagnosis of IBD, checking for HBsAg, anti-HBs, and anti-HBc[127,169]. If screening was not performed at the time of diagnosis, it should be performed before immunosuppressive therapy initiation[127,169]. HBsAg-positive/anti-HBc–positive patients carry the higher risk of reactivation, and should receive potent anti-viral agents (nucleoside/nucleotide analogues with high barrier to resistance)

Table 2 Management of patients with inflammatory bowel disease undergoing immunosuppressive therapy according to hepatitis B status

Hepatitis B status	Indications
HBsAg positive/anti-HBc positive (chronic hepatitis B)	Antiviral treatment (start 3-4 wk before and continue at least 12 mo after the immunosuppressive treatment)
HBsAg negative/anti-HBc positive (occult hepatitis B)	Liver function tests monitoring every 2-3 mo
HBsAg negative/anti-HBc negative/anti-HBs negative (naïve for hepatitis B)	Vaccination (indicated at diagnosis)
HBsAg negative/anti-HBc negative/anti-HBs positive	Check previous hepatitis B vaccination. Dose hepatitis B virus-DNA if uncertainty

HBsAg: Hepatitis B surface antigen.

such as tenofovir and entecavir. Prophylactic treatment should be started 3-4 wk before immunosuppressive therapy and continued until at least 12 mo after the end of treatment[169]. HBsAg-negative/anti-HBc–positive patients are considered to have occult infection and viral reactivation is rare in this group with types of immunosuppressants used in IBD; in this case, HBV viremia (HBV-DNA) should be checked every 2-3 mo during the treatment and antiviral treatment started when HBV-DNA is detected[169]. Hepatitis B vaccination in all seronegative patients at IBD diagnosis is recommended by ECCO guidelines[169], while BSG guidelines indicate vaccination in high-risk groups[127]. Anti-HBs level should be measured after vaccination to confirm response; however, a reduction in vaccination during immunosuppressive therapy (mainly immunomodulators and anti-TNF) has emerged from several studies[170] and a recent meta-analysis[171]. Indications for the management of the IBD patient undergoing immunosuppressive therapy according to HBV status are summarized in Table 2.

Hepatitis C prevalence in IBD is similar to the general population[168]. The risk of HCV reactivation under immunosuppressive therapies used in IBD is low[172,173]. Small case series reported successful treatment of hepatitis C with direct-acting antiviral (DAA) in patients on anti-TNF therapy[170] and no drug-drug interaction between DAA and anti-TNF has emerged[174]; thus, concomitant treatment with DAA and anti-TNF seems to be safe, although more studies specifically addressing this setting are needed.

CONCLUSION

Hepatobiliary disorders are frequently seen in IBD, and PSC represents the most common of them. A broad spectrum of pathogenic mechanisms may underlie the disorders, ranging from autoimmune conditions, metabolic diseases, infections up to drug toxicity, and two or more diseases can co-exist in the same patient. Moreover, liver disease severity can range from mild, which only requires monitoring over time, to liver failure, that may require LT. A step-by-step approach to the IBD patient with abnormal LFTs is extremely important to make the correct diagnosis, prevent complications, and identify those cases that warrant early and aggressive treatment. Finally, the diagnostic complexity often requires a multidisciplinary management involving gastroenterologist and hepatologist.

REFERENCES

1 **Moon AM**, Singal AG, Tapper EB. Contemporary Epidemiology of Chronic Liver Disease and Cirrhosis. *Clin Gastroenterol Hepatol* 2020; **18**: 2650-2666 [PMID: 31401364 DOI: 10.1016/j.cgh.2019.07.060]

2 **de Vries AB**, Janse M, Blokzijl H, Weersma RK. Distinctive inflammatory bowel disease phenotype in primary sclerosing cholangitis. *World J Gastroenterol* 2015; **21**: 1956-1971 [PMID: 25684965 DOI: 10.3748/wjg.v21.i6.1956]

3 **Chapman R**, Fevery J, Kalloo A, Nagorney DM, Boberg KM, Shneider B, Gores GJ; American Association for the Study of Liver Diseases. Diagnosis and management of primary sclerosing cholangitis. *Hepatology* 2010; **51**: 660-678 [PMID: 20101749 DOI: 10.1002/hep.23294]

4 **Schaeffer DF**, Win LL, Hafezi-Bakhtiari S, Cino M, Hirschfield GM, El-Zimaity H. The phenotypic

expression of inflammatory bowel disease in patients with primary sclerosing cholangitis differs in the distribution of colitis. *Dig Dis Sci* 2013; **58**: 2608-2614 [PMID: 23670229 DOI: 10.1007/s10620-013-2697-7]

5 **Karlsen TH**, Schrumpf E, Boberg KM. Genetic epidemiology of primary sclerosing cholangitis. *World J Gastroenterol* 2007; **13**: 5421-5431 [PMID: 17907284 DOI: 10.3748/wjg.v13.i41.5421]

6 **Chapman MH**, Thorburn D, Hirschfield GM, Webster GGJ, Rushbrook SM, Alexander G, Collier J, Dyson JK, Jones DE, Patanwala I, Thain C, Walmsley M, Pereira SP. British Society of Gastroenterology and UK-PSC guidelines for the diagnosis and management of primary sclerosing cholangitis. *Gut* 2019; **68**: 1356-1378 [PMID: 31154395 DOI: 10.1136/gutjnl-2018-317993]

7 **Jiang X**, Karlsen TH. Genetics of primary sclerosing cholangitis and pathophysiological implications. *Nat Rev Gastroenterol Hepatol* 2017; **14**: 279-295 [PMID: 28293027 DOI: 10.1038/nrgastro.2016.154]

8 **Farh KK**, Marson A, Zhu J, Kleinewietfeld M, Housley WJ, Beik S, Shoresh N, Whitton H, Ryan RJ, Shishkin AA, Hatan M, Carrasco-Alfonso MJ, Mayer D, Luckey CJ, Patsopoulos NA, De Jager PL, Kuchroo VK, Epstein CB, Daly MJ, Hafler DA, Bernstein BE. Genetic and epigenetic fine mapping of causal autoimmune disease variants. *Nature* 2015; **518**: 337-343 [PMID: 25363779 DOI: 10.1038/nature13835]

9 **Terjung B**, Spengler U. Role of auto-antibodies for the diagnosis of chronic cholestatic liver diseases. *Clin Rev Allergy Immunol* 2005; **28**: 115-133 [PMID: 15879618 DOI: 10.1385/CRIAI:28:2:115]

10 **Tabibian JH**, Varghese C, LaRusso NF, O'Hara SP. The enteric microbiome in hepatobiliary health and disease. *Liver Int* 2016; **36**: 480-487 [PMID: 26561779 DOI: 10.1111/liv.13009]

11 **Bajer L**, Kverka M, Kostovcik M, Macinga P, Dvorak J, Stehlikova Z, Brezina J, Wohl P, Spicak J, Drastich P. Distinct gut microbiota profiles in patients with primary sclerosing cholangitis and ulcerative colitis. *World J Gastroenterol* 2017; **23**: 4548-4558 [PMID: 28740343 DOI: 10.3748/wjg.v23.i25.4548]

12 **Milosevic I**, Russo E, Vujovic A, Barac A, Stevanovic O, Gitto S, Amedei A. Microbiota and viral hepatitis: State of the art of a complex matter. *World J Gastroenterol* 2021; **27**: 5488-5501 [PMID: 34588747 DOI: 10.3748/wjg.v27.i33.5488]

13 **Little R**, Wine E, Kamath BM, Griffiths AM, Ricciuto A. Gut microbiome in primary sclerosing cholangitis: A review. *World J Gastroenterol* 2020; **26**: 2768-2780 [PMID: 32550753 DOI: 10.3748/wjg.v26.i21.2768]

14 **Mertz A**, Nguyen NA, Katsanos KH, Kwok RM. Primary sclerosing cholangitis and inflammatory bowel disease comorbidity: an update of the evidence. *Ann Gastroenterol* 2019; **32**: 124-133 [PMID: 30837784 DOI: 10.20524/aog.2019.0344]

15 **Prokopič M**, Beuers U. Management of primary sclerosing cholangitis and its complications: an algorithmic approach. *Hepatol Int* 2021; **15**: 6-20 [PMID: 33377990 DOI: 10.1007/s12072-020-10118-x]

16 **Hov JR**, Boberg KM, Karlsen TH. Autoantibodies in primary sclerosing cholangitis. *World J Gastroenterol* 2008; **14**: 3781-3791 [PMID: 18609700 DOI: 10.3748/wjg.14.3781]

17 **Dave M**, Elmunzer BJ, Dwamena BA, Higgins PD. Primary sclerosing cholangitis: meta-analysis of diagnostic performance of MR cholangiopancreatography. *Radiology* 2010; **256**: 387-396 [PMID: 20656832 DOI: 10.1148/radiol.10091953]

18 **Vázquez-Elizondo G**, Muciño-Bermejo J, Méndez-Sánchez N. Gallbladder disease in patients with primary sclerosing cholangitis. *Ann Hepatol* 2008; **7**: 182-183 [PMID: 18626441]

19 **Fung BM**, Fejleh MP, Tejaswi S, Tabibian JH. Cholangioscopy and its Role in Primary Sclerosing Cholangitis. *Eur Med J Hepatol* 2020; **8**: 42-53 [PMID: 32714560]

20 **Badshah MB**, Vanar V, Kandula M, Kalva N, Badshah MB, Revenur V, Bechtold ML, Forcione DG, Donthireddy K, Puli SR. Peroral cholangioscopy with cholangioscopy-directed biopsies in the diagnosis of biliary malignancies: a systemic review and meta-analysis. *Eur J Gastroenterol Hepatol* 2019; **31**: 935-940 [PMID: 30896553 DOI: 10.1097/MEG.0000000000001402]

21 **Aadland E**, Schrumpf E, Fausa O, Elgjo K, Heilo A, Aakhus T, Gjone E. Primary sclerosing cholangitis: a long-term follow-up study. *Scand J Gastroenterol* 1987; **22**: 655-664 [PMID: 3659828 DOI: 10.3109/00365528709011139]

22 **Ponsioen CY**, Vrouenraets SM, Prawirodirdjo W, Rajaram R, Rauws EA, Mulder CJ, Reitsma JB, Heisterkamp SH, Tytgat GN. Natural history of primary sclerosing cholangitis and prognostic value of cholangiography in a Dutch population. *Gut* 2002; **51**: 562-566 [PMID: 12235081 DOI: 10.1136/gut.51.4.562]

23 **Barner-Rasmussen N**, Pukkala E, Jussila A, Färkkilä M. Epidemiology, risk of malignancy and patient survival in primary sclerosing cholangitis: a population-based study in Finland. *Scand J Gastroenterol* 2020; **55**: 74-81 [PMID: 31902255 DOI: 10.1080/00365521.2019.1707277]

24 **Goode EC**, Clark AB, Mells GF, Srivastava B, Spiess K, Gelson WTH, Trivedi PJ, Lynch KD, Castren E, Vesterhus MN, Karlsen TH, Ji SG, Anderson CA, Thorburn D, Hudson M, Heneghan MA, Aldersley MA, Bathgate A, Sandford RN, Alexander GJ, Chapman RW, Walmsley M; UK-PSC Consortium, Hirschfield GM, Rushbrook SM. Factors Associated With Outcomes of Patients With Primary Sclerosing Cholangitis and Development and Validation of a Risk Scoring System. *Hepatology* 2019; **69**: 2120-2135 [PMID: 30566748 DOI: 10.1002/hep.30479]

25 **Fevery J**, Verslype C, Lai G, Aerts R, Van Steenbergen W. Incidence, diagnosis, and therapy of cholangiocarcinoma in patients with primary sclerosing cholangitis. *Dig Dis Sci* 2007; **52**: 3123-

3135 [PMID: 17431781 DOI: 10.1007/s10620-006-9681-4]
26 **Lleo A**, de Boer YS, Liberal R, Colombo M. The risk of liver cancer in autoimmune liver diseases. *Ther Adv Med Oncol* 2019; **11**: 1758835919861914 [PMID: 31320937 DOI: 10.1177/1758835919861914]
27 **Weismüller TJ**, Trivedi PJ, Bergquist A, Imam M, Lenzen H, Ponsioen CY, Holm K, Gotthardt D, Färkkilä MA, Marschall HU, Thorburn D, Weersma RK, Fevery J, Mueller T, Chazouillères O, Schulze K, Lazaridis KN, Almer S, Pereira SP, Levy C, Mason A, Naess S, Bowlus CL, Floreani A, Halilbasic E, Yimam KK, Milkiewicz P, Beuers U, Huynh DK, Pares A, Manser CN, Dalekos GN, Eksteen B, Invernizzi P, Berg CP, Kirchner GI, Sarrazin C, Zimmer V, Fabris L, Braun F, Marzioni M, Juran BD, Said K, Rupp C, Jokelainen K, Benito de Valle M, Saffioti F, Cheung A, Trauner M, Schramm C, Chapman RW, Karlsen TH, Schrumpf E, Strassburg CP, Manns MP, Lindor KD, Hirschfield GM, Hansen BE, Boberg KM; International PSC Study Group. Patient Age, Sex, and Inflammatory Bowel Disease Phenotype Associate With Course of Primary Sclerosing Cholangitis. *Gastroenterology* 2017; **152**: 1975-1984.e8 [PMID: 28274849 DOI: 10.1053/j.gastro.2017.02.038]
28 **Tabibian JH**, Ali AH, Lindor KD. Primary Sclerosing Cholangitis, Part 2: Cancer Risk, Prevention, and Surveillance. *Gastroenterol Hepatol (N Y)* 2018; **14**: 427-432 [PMID: 30166959]
29 **Clarke WT**, Feuerstein JD. Colorectal cancer surveillance in inflammatory bowel disease: Practice guidelines and recent developments. *World J Gastroenterol* 2019; **25**: 4148-4157 [PMID: 31435169 DOI: 10.3748/wjg.v25.i30.4148]
30 **Navaneethan U**, Kochhar G, Venkatesh PG, Lewis B, Lashner BA, Remzi FH, Shen B, Kiran RP. Duration and severity of primary sclerosing cholangitis is not associated with risk of neoplastic changes in the colon in patients with ulcerative colitis. *Gastrointest Endosc* 2012; **75**: 1045-1054.e1 [PMID: 22405258 DOI: 10.1016/j.gie.2012.01.015]
31 **Rabbenou W**, Ullman TA. Risk of Colon Cancer and Recommended Surveillance Strategies in Patients with Ulcerative Colitis. *Gastroenterol Clin North Am* 2020; **49**: 791-807 [PMID: 33121696 DOI: 10.1016/j.gtc.2020.08.005]
32 **Gionchetti P**, Calabrese C, Laureti S, Poggioli G, Rizzello F. Pouchitis: Clinical Features, Diagnosis, and Treatment. *Int J Gen Med* 2021; **14**: 3871-3879 [PMID: 34335051 DOI: 10.2147/IJGM.S306039]
33 **Shi J**, Li Z, Zeng X, Lin Y, Xie WF. Ursodeoxycholic acid in primary sclerosing cholangitis: meta-analysis of randomized controlled trials. *Hepatol Res* 2009; **39**: 865-873 [PMID: 19467021 DOI: 10.1111/j.1872-034X.2009.00527.x]
34 **Triantos CK**, Koukias NM, Nikolopoulou VN, Burroughs AK. Meta-analysis: ursodeoxycholic acid for primary sclerosing cholangitis. *Aliment Pharmacol Ther* 2011; **34**: 901-910 [PMID: 21883323 DOI: 10.1111/j.1365-2036.2011.04822.x]
35 **Hansen JD**, Kumar S, Lo WK, Poulsen DM, Halai UA, Tater KC. Ursodiol and colorectal cancer or dysplasia risk in primary sclerosing cholangitis and inflammatory bowel disease: a meta-analysis. *Dig Dis Sci* 2013; **58**: 3079-3087 [PMID: 23896754 DOI: 10.1007/s10620-013-2772-0]
36 **Palmela C**, Peerani F, Castaneda D, Torres J, Itzkowitz SH. Inflammatory Bowel Disease and Primary Sclerosing Cholangitis: A Review of the Phenotype and Associated Specific Features. *Gut Liver* 2018; **12**: 17-29 [PMID: 28376583 DOI: 10.5009/gnl16510]
37 **European Association for the Study of the Liver**. EASL Clinical Practice Guidelines: management of cholestatic liver diseases. *J Hepatol* 2009; **51**: 237-267 [PMID: 19501929 DOI: 10.1016/j.jhep.2009.04.009]
38 **Aabakken L**, Karlsen TH, Albert J, Arvanitakis M, Chazouilleres O, Dumonceau JM, Färkkilä M, Fickert P, Hirschfield GM, Laghi A, Marzioni M, Fernandez M, Pereira SP, Pohl J, Poley JW, Ponsioen CY, Schramm C, Swahn F, Tringali A, Hassan C. Role of endoscopy in primary sclerosing cholangitis: European Society of Gastrointestinal Endoscopy (ESGE) and European Association for the Study of the Liver (EASL) Clinical Guideline. *Endoscopy* 2017; **49**: 588-608 [PMID: 28420030 DOI: 10.1055/s-0043-107029]
39 **Gidwaney NG**, Pawa S, Das KM. Pathogenesis and clinical spectrum of primary sclerosing cholangitis. *World J Gastroenterol* 2017; **23**: 2459-2469 [PMID: 28465630 DOI: 10.3748/wjg.v23.i14.2459]
40 **Hildebrand T**, Pannicke N, Dechene A, Gotthardt DN, Kirchner G, Reiter FP, Sterneck M, Herzer K, Lenzen H, Rupp C, Barg-Hock H, de Leuw P, Teufel A, Zimmer V, Lammert F, Sarrazin C, Spengler U, Rust C, Manns MP, Strassburg CP, Schramm C, Weismüller TJ; German PSC Study Group. Biliary strictures and recurrence after liver transplantation for primary sclerosing cholangitis: A retrospective multicenter analysis. *Liver Transpl* 2016; **22**: 42-52 [PMID: 26438008 DOI: 10.1002/lt.24350]
41 **Wee A**, Ludwig J. Pericholangitis in chronic ulcerative colitis: primary sclerosing cholangitis of the small bile ducts? *Ann Intern Med* 1985; **102**: 581-587 [PMID: 3985511 DOI: 10.7326/0003-4819-102-5-581]
42 **Björnsson E**, Olsson R, Bergquist A, Lindgren S, Braden B, Chapman RW, Boberg KM, Angulo P. The natural history of small-duct primary sclerosing cholangitis. *Gastroenterology* 2008; **134**: 975-980 [PMID: 18395078 DOI: 10.1053/j.gastro.2008.01.042]
43 **Ricciuto A**, Kamath BM, Griffiths AM. The IBD and PSC Phenotypes of PSC-IBD. *Curr Gastroenterol Rep* 2018; **20**: 16 [PMID: 29594739 DOI: 10.1007/s11894-018-0620-2]
44 **Rust C**, Beuers U. Overlap syndromes among autoimmune liver diseases. *World J Gastroenterol* 2008; **14**: 3368-3373 [PMID: 18528934 DOI: 10.3748/wjg.14.3368]

45 **Floreani A**, Rizzotto ER, Ferrara F, Carderi I, Caroli D, Blasone L, Baldo V. Clinical course and outcome of autoimmune hepatitis/primary sclerosing cholangitis overlap syndrome. *Am J Gastroenterol* 2005; **100**: 1516-1522 [PMID: 15984974 DOI: 10.1111/j.1572-0241.2005.41841.x]

46 **Ballotin VR**, Bigarella LG, Riva F, Onzi G, Balbinot RA, Balbinot SS, Soldera J. Primary sclerosing cholangitis and autoimmune hepatitis overlap syndrome associated with inflammatory bowel disease: A case report and systematic review. *World J Clin Cases* 2020; **8**: 4075-4093 [PMID: 33024765 DOI: 10.12998/wjcc.v8.i18.4075]

47 **Ohara H**, Okazaki K, Tsubouchi H, Inui K, Kawa S, Kamisawa T, Tazuma S, Uchida K, Hirano K, Yoshida H, Nishino T, Ko SB, Mizuno N, Hamano H, Kanno A, Notohara K, Hasebe O, Nakazawa T, Nakanuma Y, Takikawa H; Research Committee of IgG4-related Diseases; Research Committee of Intractable Diseases of Liver and Biliary Tract; Ministry of Health, Labor and Welfare, Japan; Japan Biliary Association. Clinical diagnostic criteria of IgG4-related sclerosing cholangitis 2012. *J Hepatobiliary Pancreat Sci* 2012; **19**: 536-542 [PMID: 22717980 DOI: 10.1007/s00534-012-0521-y]

48 **Manganis CD**, Chapman RW, Culver EL. Review of primary sclerosing cholangitis with increased IgG4 Levels. *World J Gastroenterol* 2020; **26**: 3126-3144 [PMID: 32684731 DOI: 10.3748/wjg.v26.i23.3126]

49 **Song DJ**, Shen J, Chen MH, Liu ZJ, Cao Q, Hu PJ, Gao X, Qian JM, Wu KC, Lai LJ, Ran ZH. Association of Serum Immunoglobulins Levels With Specific Disease Phenotypes of Crohn's Disease: A Multicenter Analysis in China. *Front Med (Lausanne)* 2021; **8**: 621337 [PMID: 33996846 DOI: 10.3389/fmed.2021.621337]

50 **Löhr JM**, Beuers U, Vujasinovic M, Alvaro D, Frøkjær JB, Buttgereit F, Capurso G, Culver EL, de-Madaria E, Della-Torre E, Detlefsen S, Dominguez-Muñoz E, Czubkowski P, Ewald N, Frulloni L, Gubergrits N, Duman DG, Hackert T, Iglesias-Garcia J, Kartalis N, Laghi A, Lammert F, Lindgren F, Okhlobystin A, Oracz G, Parniczky A, Mucelli RMP, Rebours V, Rosendahl J, Schleinitz N, Schneider A, van Bommel EF, Verbeke CS, Vullierme MP, Witt H; UEG guideline working group. European Guideline on IgG4-related digestive disease - UEG and SGF evidence-based recommendations. *United European Gastroenterol J* 2020; **8**: 637-666 [PMID: 32552502 DOI: 10.1177/2050640620934911]

51 **Kamisawa T**, Nakazawa T, Tazuma S, Zen Y, Tanaka A, Ohara H, Muraki T, Inui K, Inoue D, Nishino T, Naitoh I, Itoi T, Notohara K, Kanno A, Kubota K, Hirano K, Isayama H, Shimizu K, Tsuyuguchi T, Shimosegawa T, Kawa S, Chiba T, Okazaki K, Takikawa H, Kimura W, Unno M, Yoshida M. Clinical practice guidelines for IgG4-related sclerosing cholangitis. *J Hepatobiliary Pancreat Sci* 2019; **26**: 9-42 [PMID: 30575336 DOI: 10.1002/jhbp.596]

52 **Tanaka A**. Autoimmune Hepatitis: 2019 Update. *Gut Liver* 2020; **14**: 430-438 [PMID: 32301319 DOI: 10.5009/gnl19261]

53 **Halling ML**, Kjeldsen J, Knudsen T, Nielsen J, Hansen LK. Patients with inflammatory bowel disease have increased risk of autoimmune and inflammatory diseases. *World J Gastroenterol* 2017; **23**: 6137-6146 [PMID: 28970729 DOI: 10.3748/wjg.v23.i33.6137]

54 **Perdigoto R**, Carpenter HA, Czaja AJ. Frequency and significance of chronic ulcerative colitis in severe corticosteroid-treated autoimmune hepatitis. *J Hepatol* 1992; **14**: 325-331 [PMID: 1500696 DOI: 10.1016/0168-8278(92)90178-r]

55 **Khandelwal A**, Gorsi U, Marginean EC, Papadatos D, George U. Isolated granulomatous hepatitis-A histopathological surprise mimicking cholangiocarcinoma in ulcerative colitis. *Ann Hepatol* 2013; **12**: 332-335 [PMID: 23396747]

56 **Hilzenrat N**, Lamoureux E, Sherker A, Cohen A. Cholestasis in Crohn's disease: a diagnostic challenge. *Can J Gastroenterol* 1997; **11**: 35-37 [PMID: 9113796 DOI: 10.1155/1997/203843]

57 **Patedakis Litvinov BI**, Pathak AP. Granulomatous hepatitis in a patient with Crohn's disease and cholestasis. *BMJ Case Rep* 2017; **2017** [PMID: 28882937 DOI: 10.1136/bcr-2017-220988]

58 **Kim JM**, Cheon JH. Pathogenesis and clinical perspectives of extraintestinal manifestations in inflammatory bowel diseases. *Intest Res* 2020; **18**: 249-264 [PMID: 32295331 DOI: 10.5217/ir.2019.00128]

59 **Rojas-Feria M**, Castro M, Suárez E, Ampuero J, Romero-Gómez M. Hepatobiliary manifestations in inflammatory bowel disease: the gut, the drugs and the liver. *World J Gastroenterol* 2013; **19**: 7327-7340 [PMID: 24259964 DOI: 10.3748/wjg.v19.i42.7327]

60 **Knox TA**, Kaplan MM, Gelfand JA, Wolff SM. Methotrexate treatment of idiopathic granulomatous hepatitis. *Ann Intern Med* 1995; **122**: 592-595 [PMID: 7887553 DOI: 10.7326/0003-4819-122-8-199504150-00006]

61 **Restellini S**, Chazouillères O, Frossard JL. Hepatic manifestations of inflammatory bowel diseases. *Liver Int* 2017; **37**: 475-489 [PMID: 27712010 DOI: 10.1111/liv.13265]

62 **Lorusso D**, Leo S, Mossa A, Misciagna G, Guerra V. Cholelithiasis in inflammatory bowel disease. A case-control study. *Dis Colon Rectum* 1990; **33**: 791-794 [PMID: 2202567 DOI: 10.1007/BF02052328]

63 **Fraquelli M**, Losco A, Visentin S, Cesana BM, Pometta R, Colli A, Conte D. Gallstone disease and related risk factors in patients with Crohn disease: analysis of 330 consecutive cases. *Arch Intern Med* 2001; **161**: 2201-2204 [PMID: 11575976]

64 **Bargiggia S**, Maconi G, Elli M, Molteni P, Ardizzone S, Parente F, Todaro I, Greco S, Manzionna G, Bianchi Porro G. Sonographic prevalence of liver steatosis and biliary tract stones in patients with inflammatory bowel disease: study of 511 subjects at a single center. *J Clin Gastroenterol* 2003; **36**:

417-420 [PMID: 12702985 DOI: 10.1097/00004836-200305000-00012]
65 **Kratzer W**, Haenle MM, Mason RA, von Tirpitz C, Kaechele V. Prevalence of cholelithiasis in patients with chronic inflammatory bowel disease. *World J Gastroenterol* 2005; **11**: 6170-6175 [PMID: 16273645 DOI: 10.3748/wjg.v11.i39.6170]
66 **Zhang FM**, Xu CF, Shan GD, Chen HT, Xu GQ. Is gallstone disease associated with inflammatory bowel diseases? *J Dig Dis* 2015; **16**: 634-641 [PMID: 26332254 DOI: 10.1111/1751-2980.12286]
67 **Sturdik I**, Krajcovicova A, Jalali Y, Adamcova M, Tkacik M, Sekac J, Koller T, Huorka M, Payer J, Hlavaty T. Pathophysiology and risk factors for cholelithiasis in patients with Crohn's disease. *Physiol Res* 2019; **68**: S173-S182 [PMID: 31842581 DOI: 10.33549/physiolres.934302]
68 **Fagagnini S**, Heinrich H, Rossel JB, Biedermann L, Frei P, Zeitz J, Spalinger M, Battegay E, Zimmerli L, Vavricka SR, Rogler G, Scharl M, Misselwitz B. Risk factors for gallstones and kidney stones in a cohort of patients with inflammatory bowel diseases. *PLoS One* 2017; **12**: e0185193 [PMID: 29023532 DOI: 10.1371/journal.pone.0185193]
69 **Chen CH**, Lin CL, Kao CH. Association between Inflammatory Bowel Disease and Cholelithiasis: A Nationwide Population-Based Cohort Study. *Int J Environ Res Public Health* 2018; **15** [PMID: 29538289 DOI: 10.3390/ijerph15030513]
70 **Parente F**, Pastore L, Bargiggia S, Cucino C, Greco S, Molteni M, Ardizzone S, Porro GB, Sampietro GM, Giorgi R, Moretti R, Gallus S. Incidence and risk factors for gallstones in patients with inflammatory bowel disease: a large case-control study. *Hepatology* 2007; **45**: 1267-1274 [PMID: 17464998 DOI: 10.1002/hep.21537]
71 **Lapidus A**, Einarsson C. Bile composition in patients with ileal resection due to Crohn's disease. *Inflamm Bowel Dis* 1998; **4**: 89-94 [PMID: 9589295 DOI: 10.1002/ibd.3780040204]
72 **Heaton KW**. Disturbances of bile acid metabolism in intestinal disease. *Clin Gastroenterol* 1977; **6**: 69-89 [PMID: 330053]
73 **Dietrich CG**, Rau M, Geier A. Screening for nonalcoholic fatty liver disease-when, who and how? *World J Gastroenterol* 2021; **27**: 5803-5821 [PMID: 34629804 DOI: 10.3748/wjg.v27.i35.5803]
74 **Ge X**, Zheng L, Wang M, Du Y, Jiang J. Prevalence trends in non-alcoholic fatty liver disease at the global, regional and national levels, 1990-2017: a population-based observational study. *BMJ Open* 2020; **10**: e036663 [PMID: 32747349 DOI: 10.1136/bmjopen-2019-036663]
75 **Voss J**, Schneider CV, Kleinjans M, Bruns T, Trautwein C, Strnad P. Hepatobiliary phenotype of individuals with chronic intestinal disorders. *Sci Rep* 2021; **11**: 19954 [PMID: 34620902 DOI: 10.1038/s41598-021-98843-7]
76 **Yen HH**, Su PY, Huang SP, Wu L, Hsu TC, Zeng YH, Chen YY. Evaluation of non-alcoholic fatty liver disease in patients with inflammatory bowel disease using controlled attenuation parameter technology: A Taiwanese retrospective cohort study. *PLoS One* 2021; **16**: e0252286 [PMID: 34043691 DOI: 10.1371/journal.pone.0252286]
77 **Likhitsup A**, Dundulis J, Ansari S, Patibandla S, Hutton C, Kennedy K, Helzberg JH, Chhabra R. High prevalence of non-alcoholic fatty liver disease in patients with inflammatory bowel disease receiving anti-tumor necrosis factor therapy. *Ann Gastroenterol* 2019; **32**: 463-468 [PMID: 31474792 DOI: 10.20524/aog.2019.0405]
78 **Lapumnuaypol K**, Kanjanahattakij N, Pisarcik D, Thongprayoon C, Wijarnpreecha K, Cheungpasitporn W. Effects of inflammatory bowel disease treatment on the risk of nonalcoholic fatty liver disease: a meta-analysis. *Eur J Gastroenterol Hepatol* 2018; **30**: 854-860 [PMID: 29697458 DOI: 10.1097/MEG.0000000000001144]
79 **Hoffmann P**, Jung V, Behnisch R, Gauss A. Prevalence and risk factors of nonalcoholic fatty liver disease in patients with inflammatory bowel diseases: A cross-sectional and longitudinal analysis. *World J Gastroenterol* 2020; **26**: 7367-7381 [PMID: 33362390 DOI: 10.3748/wjg.v26.i46.7367]
80 **Sartini A**, Gitto S, Bianchini M, Verga MC, Di Girolamo M, Bertani A, Del Buono M, Schepis F, Lei B, De Maria N, Villa E. Non-alcoholic fatty liver disease phenotypes in patients with inflammatory bowel disease. *Cell Death Dis* 2018; **9**: 87 [PMID: 29367619 DOI: 10.1038/s41419-017-0124-2]
81 **Lin JN**, Lin CL, Lin MC, Lai CH, Lin HH, Kao CH. Pyogenic liver abscess in patients with inflammatory bowel disease: a nationwide cohort study. *Liver Int* 2016; **36**: 136-144 [PMID: 26012730 DOI: 10.1111/liv.12875]
82 **McGreal S**, Sayers R, Wurm P, West K. Crohn's disease presenting with pyogenic liver abscess: a case report. *Case Rep Gastrointest Med* 2012; **2012**: 762480 [PMID: 22928123 DOI: 10.1155/2012/762480]
83 **Navot-Mintzer D**, Koren A, Shahbari A, Nussinson E, Sakran W. Liver abscesses as the presenting manifestation of Crohn's disease in an adolescent. *Inflamm Bowel Dis* 2006; **12**: 666-667 [PMID: 16804405 DOI: 10.1097/01.MIB.0000225336.06732.90]
84 **Margalit M**, Elinav H, Ilan Y, Shalit M. Liver abscess in inflammatory bowel disease: report of two cases and review of the literature. *J Gastroenterol Hepatol* 2004; **19**: 1338-1342 [PMID: 15610305 DOI: 10.1111/j.1440-1746.2004.03368.x]
85 **Grainge MJ**, West J, Card TR. Venous thromboembolism during active disease and remission in inflammatory bowel disease: a cohort study. *Lancet* 2010; **375**: 657-663 [PMID: 20149425 DOI: 10.1016/S0140-6736(09)61963-2]
86 **Talbot RW**, Heppell J, Dozois RR, Beart RW Jr. Vascular complications of inflammatory bowel disease. *Mayo Clin Proc* 1986; **61**: 140-145 [PMID: 3080643 DOI: 10.1016/s0025-6196(12)65200-8]

87 **Gupta S**, Hidalgo J, Singh B, Iyer A, Yang Y, Short A, Singh S, Bhatt H, Gupta S. Usage of Direct Acting Oral Anticoagulants in Cirrhotic and Non-Cirrhotic Portal Vein Thrombosis: A Systematic Review. *Cureus* 2021; **13**: e16922 [PMID: 34367844 DOI: 10.7759/cureus.16922]

88 **Remzi FH**, Fazio VW, Oncel M, Baker ME, Church JM, Ooi BS, Connor JT, Preen M, Einstein D. Portal vein thrombi after restorative proctocolectomy. *Surgery* 2002; **132**: 655-61; discussion 661 [PMID: 12407350 DOI: 10.1067/msy.2002.127689]

89 **Jackson LM**, O'Gorman PJ, O'Connell J, Cronin CC, Cotter KP, Shanahan F. Thrombosis in inflammatory bowel disease: clinical setting, procoagulant profile and factor V Leiden. *QJM* 1997; **90**: 183-188 [PMID: 9093595 DOI: 10.1093/qjmed/90.3.183]

90 **Sleutjes JAM**, van Lennep JER, van der Woude CJ, de Vries AC. Thromboembolic and atherosclerotic cardiovascular events in inflammatory bowel disease: epidemiology, pathogenesis and clinical management. *Therap Adv Gastroenterol* 2021; **14**: 17562848211032126 [PMID: 34377149 DOI: 10.1177/17562848211032126]

91 **Harbord M**, Eliakim R, Bettenworth D, Karmiris K, Katsanos K, Kopylov U, Kucharzik T, Molnár T, Raine T, Sebastian S, de Sousa HT, Dignass A, Carbonnel F; European Crohn's and Colitis Organisation [ECCO]. Third European Evidence-based Consensus on Diagnosis and Management of Ulcerative Colitis. Part 2: Current Management. *J Crohns Colitis* 2017; **11**: 769-784 [PMID: 28513805 DOI: 10.1093/ecco-jcc/jjx009]

92 **Zezos P**, Kouklakis G, Saibil F. Inflammatory bowel disease and thromboembolism. *World J Gastroenterol* 2014; **20**: 13863-13878 [PMID: 25320522 DOI: 10.3748/wjg.v20.i38.13863]

93 **Jackson CS**, Fryer J, Danese S, Vanagunas A, Polensky S, Buchman AL. Mesenteric vascular thromboembolism in inflammatory bowel disease: a single center experience. *J Gastrointest Surg* 2011; **15**: 97-100 [PMID: 20824370 DOI: 10.1007/s11605-010-1336-2]

94 **Sinagra E**, Aragona E, Romano C, Maisano S, Orlando A, Virdone R, Tesè L, Modesto I, Criscuoli V, Cottone M. The role of portal vein thrombosis in the clinical course of inflammatory bowel diseases: report on three cases and review of the literature. *Gastroenterol Res Pract* 2012; **2012**: 916428 [PMID: 23093957 DOI: 10.1155/2012/916428]

95 **Olivera PA**, Zuily S, Kotze PG, Regnault V, Al Awadhi S, Bossuyt P, Gearry RB, Ghosh S, Kobayashi T, Lacolley P, Louis E, Magro F, Ng SC, Papa A, Raine T, Teixeira FV, Rubin DT, Danese S, Peyrin-Biroulet L. International consensus on the prevention of venous and arterial thrombotic events in patients with inflammatory bowel disease. *Nat Rev Gastroenterol Hepatol* 2021 [PMID: 34453143 DOI: 10.1038/s41575-021-00492-8]

96 **Voudoukis E**, Karmiris K, Koutroubakis IE. Multipotent role of platelets in inflammatory bowel diseases: a clinical approach. *World J Gastroenterol* 2014; **20**: 3180-3190 [PMID: 24696603 DOI: 10.3748/wjg.v20.i12.3180]

97 **Koutroubakis IE**. The relationship between coagulation state and inflammatory bowel disease: current understanding and clinical implications. *Expert Rev Clin Immunol* 2015; **11**: 479-488 [PMID: 25719625 DOI: 10.1586/1744666X.2015.1019475]

98 **Chamouard P**, Grunebaum L, Wiesel ML, Frey PL, Wittersheim C, Sapin R, Baumann R, Cazenave JP. Prothrombin fragment 1 + 2 and thrombin-antithrombin III complex as markers of activation of blood coagulation in inflammatory bowel diseases. *Eur J Gastroenterol Hepatol* 1995; **7**: 1183-1188 [PMID: 8789309 DOI: 10.1097/00042737-199512000-00010]

99 **Dolapcioglu C**, Soylu A, Kendir T, Ince AT, Dolapcioglu H, Purisa S, Bolukbas C, Sokmen HM, Dalay R, Ovunc O. Coagulation parameters in inflammatory bowel disease. *Int J Clin Exp Med* 2014; **7**: 1442-1448 [PMID: 24995109]

100 **Magro F**, Soares JB, Fernandes D. Venous thrombosis and prothrombotic factors in inflammatory bowel disease. *World J Gastroenterol* 2014; **20**: 4857-4872 [PMID: 24803797 DOI: 10.3748/wjg.v20.i17.4857]

101 **European Association for the Study of the Liver**. Clinical Practice Guideline Panel: Chair:; Panel members; EASL Governing Board representative: EASL Clinical Practice Guidelines: Drug-induced liver injury. *J Hepatol* 2019; **70**: 1222-1261 [PMID: 30926241 DOI: 10.1016/j.jhep.2019.02.014]

102 **Azad Khan AK**, Nurazzaman M, Truelove SC. The effect of the acetylator phenotype on the metabolism of sulphasalazine in man. *J Med Genet* 1983; **20**: 30-36 [PMID: 6133000 DOI: 10.1136/jmg.20.1.30]

103 **Torres J**, Bonovas S, Doherty G, Kucharzik T, Gisbert JP, Raine T, Adamina M, Armuzzi A, Bachmann O, Bager P, Biancone L, Bokemeyer B, Bossuyt P, Burisch J, Collins P, El-Hussuna A, Ellul P, Frei-Lanter C, Furfaro F, Gingert C, Gionchetti P, Gomollon F, González-Lorenzo M, Gordon H, Hlavaty T, Juillerat P, Katsanos K, Kopylov U, Krustins E, Lytras T, Maaser C, Magro F, Marshall JK, Myrelid P, Pellino G, Rosa I, Sabino J, Savarino E, Spinelli A, Stassen L, Uzzan M, Vavricka S, Verstockt B, Warusavitarne J, Zmora O, Fiorino G. ECCO Guidelines on Therapeutics in Crohn's Disease: Medical Treatment. *J Crohns Colitis* 2020; **14**: 4-22 [PMID: 31711158 DOI: 10.1093/ecco-jcc/jjz180]

104 **Ransford RA**, Langman MJ. Sulphasalazine and mesalazine: serious adverse reactions re-evaluated on the basis of suspected adverse reaction reports to the Committee on Safety of Medicines. *Gut* 2002; **51**: 536-539 [PMID: 12235076 DOI: 10.1136/gut.51.4.536]

105 **Ham M**, Moss AC. Mesalamine in the treatment and maintenance of remission of ulcerative colitis. *Expert Rev Clin Pharmacol* 2012; **5**: 113-123 [PMID: 22390554 DOI: 10.1586/ecp.12.2]

106 **Namias A**, Bhalotra R, Donowitz M. Reversible sulfasalazine-induced granulomatous hepatitis. *J Clin Gastroenterol* 1981; **3**: 193-198 [PMID: 6113258 DOI: 10.1097/00004836-198106000-00017]

107 **Marinos G**, Riley J, Painter DM, McCaughan GW. Sulfasalazine-induced fulminant hepatic failure. *J Clin Gastroenterol* 1992; **14**: 132-135 [PMID: 1348259 DOI: 10.1097/00004836-199203000-00012]

108 **Hirten R**, Sultan K, Thomas A, Bernstein DE. Hepatic manifestations of non-steroidal inflammatory bowel disease therapy. *World J Hepatol* 2015; **7**: 2716-2728 [PMID: 26644815 DOI: 10.4254/wjh.v7.i27.2716]

109 **Cuffari C**, Théorêt Y, Latour S, Seidman G. 6-Mercaptopurine metabolism in Crohn's disease: correlation with efficacy and toxicity. *Gut* 1996; **39**: 401-406 [DOI: 10.1136/gut.39.3.401]

110 **Axelrad JE**, Roy A, Lawlor G, Korelitz B, Lichtiger S. Thiopurines and inflammatory bowel disease: Current evidence and a historical perspective. *World J Gastroenterol* 2016; **22**: 10103-10117 [PMID: 28028358 DOI: 10.3748/wjg.v22.i46.10103]

111 **Bastida G**, Nos P, Aguas M, Beltrán B, Rubín A, Dasí F, Ponce J. Incidence, risk factors and clinical course of thiopurine-induced liver injury in patients with inflammatory bowel disease. *Aliment Pharmacol Ther* 2005; **22**: 775-782 [PMID: 16225485 DOI: 10.1111/j.1365-2036.2005.02636.x]

112 **Tran-Minh ML**, Sousa P, Maillet M, Allez M, Gornet JM. Hepatic complications induced by immunosuppressants and biologics in inflammatory bowel disease. *World J Hepatol* 2017; **9**: 613-626 [PMID: 28539989 DOI: 10.4254/wjh.v9.i13.613]

113 **Gisbert JP**, González-Lama Y, Maté J. Thiopurine-induced liver injury in patients with inflammatory bowel disease: a systematic review. *Am J Gastroenterol* 2007; **102**: 1518-1527 [PMID: 17391318 DOI: 10.1111/j.1572-0241.2007.01187.x]

114 **Gisbert JP**, Chaparro M, Gomollón F. Common misconceptions about 5-aminosalicylates and thiopurines in inflammatory bowel disease. *World J Gastroenterol* 2011; **17**: 3467-3478 [PMID: 21941413 DOI: 10.3748/wjg.v17.i30.3467]

115 **Sousa P**, Estevinho MM, Dias CC, Ministro P, Kopylov U, Danese S, Peyrin-Biroulet L, Magro F. Thiopurines' Metabolites and Drug Toxicity: A Meta-Analysis. *J Clin Med* 2020; **9** [PMID: 32668748 DOI: 10.3390/jcm9072216]

116 **Thomsen SB**, Allin KH, Burisch J, Jensen CB, Hansen S, Gluud LL, Theede K, Kiszka-Kanowitz M, Nielsen AM, Jess T. Outcome of concomitant treatment with thiopurines and allopurinol in patients with inflammatory bowel disease: A nationwide Danish cohort study. *United European Gastroenterol J* 2020; **8**: 68-76 [PMID: 32213059 DOI: 10.1177/2050640619868387]

117 **Warner B**, Johnston E, Arenas-Hernandez M, Marinaki A, Irving P, Sanderson J. A practical guide to thiopurine prescribing and monitoring in IBD. *Frontline Gastroenterol* 2018; **9**: 10-15 [PMID: 29484155 DOI: 10.1136/flgastro-2016-100738]

118 **Shaye OA**, Yadegari M, Abreu MT, Poordad F, Simon K, Martin P, Papadakis KA, Ippoliti A, Vasiliauskas E, Tran TT. Hepatotoxicity of 6-mercaptopurine (6-MP) and Azathioprine (AZA) in adult IBD patients. *Am J Gastroenterol* 2007; **102**: 2488-2494 [PMID: 17764490 DOI: 10.1111/j.1572-0241.2007.01515.x]

119 **Ribaldone DG**, Adriani A, Caviglia GP, Nicolò A, Agnesod D, Simiele M, Riganò D, Pellicano R, Canaparo R, Perri GD, D'Avolio A, Luzza F, Saracco GM, Astegiano M. Correlation between Thiopurine S-Methyltransferase Genotype and Adverse Events in Inflammatory Bowel Disease Patients. *Medicina (Kaunas)* 2019; **55** [PMID: 31387318 DOI: 10.3390/medicina55080441]

120 **Liu YP**, Wu HY, Yang X, Xu HQ, Li YC, Shi DC, Huang JF, Huang Q, Fu WL. Association between thiopurine S-methyltransferase polymorphisms and thiopurine-induced adverse drug reactions in patients with inflammatory bowel disease: a meta-analysis. *PLoS One* 2015; **10**: e0121745 [PMID: 25799415 DOI: 10.1371/journal.pone.0121745]

121 **Moran GW**, Dubeau MF, Kaplan GG, Yang H, Eksteen B, Ghosh S, Panaccione R. Clinical predictors of thiopurine-related adverse events in Crohn's disease. *World J Gastroenterol* 2015; **21**: 7795-7804 [PMID: 26167079 DOI: 10.3748/wjg.v21.i25.7795]

122 **McLean LP**, Cross RK. Adverse events in IBD: to stop or continue immune suppressant and biologic treatment. *Expert Rev Gastroenterol Hepatol* 2014; **8**: 223-240 [PMID: 24490595 DOI: 10.1586/17474124.2014.881715]

123 **Philips CA**, Ahamed R, Rajesh S, George T, Mohanan M, Augustine P. Comprehensive review of hepatotoxicity associated with traditional Indian Ayurvedic herbs. *World J Hepatol* 2020; **12**: 574-595 [PMID: 33033566 DOI: 10.4254/wjh.v12.i9.574]

124 **Aithal GP**. Hepatotoxicity related to antirheumatic drugs. *Nat Rev Rheumatol* 2011; **7**: 139-150 [PMID: 21263458 DOI: 10.1038/nrrheum.2010.214]

125 **Renna S**, Cottone M, Orlando A. Optimization of the treatment with immunosuppressants and biologics in inflammatory bowel disease. *World J Gastroenterol* 2014; **20**: 9675-9690 [PMID: 25110407 DOI: 10.3748/wjg.v20.i29.9675]

126 **Meijer B**, Mulder CJ, van Bodegraven AA, de Boer NK. How I treat my inflammatory bowel disease-patients with thiopurines? *World J Gastrointest Pharmacol Ther* 2016; **7**: 524-530 [PMID: 27867685 DOI: 10.4292/wjgpt.v7.i4.524]

127 **Lamb CA**, Kennedy NA, Raine T, Hendy PA, Smith PJ, Limdi JK, Hayee B, Lomer MCE, Parkes GC, Selinger C, Barrett KJ, Davies RJ, Bennett C, Gittens S, Dunlop MG, Faiz O, Fraser A, Garrick V, Johnston PD, Parkes M, Sanderson J, Terry H; IBD guidelines eDelphi consensus group, Gaya DR, Iqbal TH, Taylor SA, Smith M, Brookes M, Hansen R, Hawthorne AB. British Society of Gastroenterology consensus guidelines on the management of inflammatory bowel disease in adults. *Gut* 2019; **68**: s1-s106 [DOI: 10.1136/gutjnl-2019-318484]

128 **Egan LJ**, Sandborn WJ. Methotrexate for inflammatory bowel disease: pharmacology and preliminary results. *Mayo Clin Proc* 1996; **71**: 69-80 [PMID: 8538238]

129 **Patel V**, Wang Y, MacDonald JK, McDonald JW, Chande N. Methotrexate for maintenance of remission in Crohn's disease. *Cochrane Database Syst Rev* 2014; CD006884 [PMID: 25157445 DOI: 10.1002/14651858.CD006884.pub3]

130 **Wang Y**, MacDonald JK, Vandermeer B, Griffiths AM, El-Matary W. Methotrexate for maintenance of remission in ulcerative colitis. *Cochrane Database Syst Rev* 2015; CD007560 [PMID: 26263042 DOI: 10.1002/14651858.CD007560.pub3]

131 **Coskun M**, Steenholdt C, de Boer NK, Nielsen OH. Pharmacology and Optimization of Thiopurines and Methotrexate in Inflammatory Bowel Disease. *Clin Pharmacokinet* 2016; **55**: 257-274 [PMID: 26255287 DOI: 10.1007/s40262-015-0316-9]

132 **Almeyda J**, Baker H, Levene GM, Barnardo D, Landells JW. Methotrexate, alcohol, and liver damage. *Br Med J* 1971; **2**: 167 [PMID: 5581500 DOI: 10.1136/bmj.2.5754.167-b]

133 **Prey S**, Paul C. Effect of folic or folinic acid supplementation on methotrexate-associated safety and efficacy in inflammatory disease: a systematic review. *Br J Dermatol* 2009; **160**: 622-628 [PMID: 18945303 DOI: 10.1111/j.1365-2133.2008.08876.x]

134 **Visser K**, Katchamart W, Loza E, Martinez-Lopez JA, Salliot C, Trudeau J, Bombardier C, Carmona L, van der Heijde D, Bijlsma JW, Boumpas DT, Canhao H, Edwards CJ, Hamuryudan V, Kvien TK, Leeb BF, Martín-Mola EM, Mielants H, Müller-Ladner U, Murphy G, Østergaard M, Pereira IA, Ramos-Remus C, Valentini G, Zochling J, Dougados M. Multinational evidence-based recommendations for the use of methotrexate in rheumatic disorders with a focus on rheumatoid arthritis: integrating systematic literature research and expert opinion of a broad international panel of rheumatologists in the 3E Initiative. *Ann Rheum Dis* 2009; **68**: 1086-1093 [PMID: 19033291 DOI: 10.1136/ard.2008.094474]

135 **Seow CH**, de Silva S, Kaplan GG, Devlin SM, Ghosh S, Panaccione R. Managing the risks of IBD therapy. *Curr Gastroenterol Rep* 2009; **11**: 509-517 [PMID: 19903428 DOI: 10.1007/s11894-009-0077-4]

136 **Barbero-Villares A**, Mendoza J, Trapero-Marugan M, Gonzalez-Alvaro I, Daudén E, Gisbert JP, Moreno-Otero R. Evaluation of liver fibrosis by transient elastography in methotrexate treated patients. *Med Clin (Barc)* 2011; **137**: 637-639 [PMID: 21719043 DOI: 10.1016/j.medcli.2010.12.024]

137 **Tobon GJ**, Cañas C, Jaller JJ, Restrepo JC, Anaya JM. Serious liver disease induced by infliximab. *Clin Rheumatol* 2007; **26**: 578-581 [PMID: 16547695 DOI: 10.1007/s10067-005-0169-y]

138 **Lopetuso LR**, Mocci G, Marzo M, D'Aversa F, Rapaccini GL, Guidi L, Armuzzi A, Gasbarrini A, Papa A. Harmful Effects and Potential Benefits of Anti-Tumor Necrosis Factor (TNF)-α on the Liver. *Int J Mol Sci* 2018; **19** [PMID: 30060508 DOI: 10.3390/ijms19082199]

139 **Hagel S**, Bruns T, Theis B, Herrmann A, Stallmach A. Subacute liver failure induced by adalimumab. *Int J Clin Pharmacol Ther* 2011; **49**: 38-40 [PMID: 21176723 DOI: 10.5414/cpp49038]

140 **Shelton E**, Chaudrey K, Sauk J, Khalili H, Masia R, Nguyen DD, Yajnik V, Ananthakrishnan AN. New onset idiosyncratic liver enzyme elevations with biological therapy in inflammatory bowel disease. *Aliment Pharmacol Ther* 2015; **41**: 972-979 [PMID: 25756190 DOI: 10.1111/apt.13159]

141 **Ghabril M**, Bonkovsky HL, Kum C, Davern T, Hayashi PH, Kleiner DE, Serrano J, Rochon J, Fontana RJ, Bonacini M; US Drug-Induced Liver Injury Network. Liver injury from tumor necrosis factor-α antagonists: analysis of thirty-four cases. *Clin Gastroenterol Hepatol* 2013; **11**: 558-564.e3 [PMID: 23333219 DOI: 10.1016/j.cgh.2012.12.025]

142 **FDA**. U.S. Food and Drug Administration. [cited 17 January 2021]. Available from: https://www.fda.gov/science-research/Liver-toxicity-knowledge-base-ltkb/drug-induced-liver-injury-rank-dilirank-dataset

143 **Shah P**, Sundaram V, Björnsson E. Biologic and Checkpoint Inhibitor-Induced Liver Injury: A Systematic Literature Review. *Hepatol Commun* 2020; **4**: 172-184 [PMID: 32025603 DOI: 10.1002/hep4.1465]

144 **Biswas S**, Bryant RV, Travis S. Interfering with leukocyte trafficking in Crohn's disease. *Best Pract Res Clin Gastroenterol* 2019; **38-39**: 101617 [PMID: 31327399 DOI: 10.1016/j.bpg.2019.05.004]

145 **Sandborn WJ**, Colombel JF, Enns R, Feagan BG, Hanauer SB, Lawrance IC, Panaccione R, Sanders M, Schreiber S, Targan S, van Deventer S, Goldblum R, Despain D, Hogge GS, Rutgeerts P; International Efficacy of Natalizumab as Active Crohn's Therapy (ENACT-1) Trial Group; Evaluation of Natalizumab as Continuous Therapy (ENACT-2) Trial Group. Natalizumab induction and maintenance therapy for Crohn's disease. *N Engl J Med* 2005; **353**: 1912-1925 [DOI: 10.1056/NEJMoa043335]

146 **Targan SR**, Feagan BG, Fedorak RN, Lashner BA, Panaccione R, Present DH, Spehlmann ME, Rutgeerts PJ, Tulassay Z, Volfova M, Wolf DC, Hernandez C, Bornstein J, Sandborn WJ; International Efficacy of Natalizumab in Crohn's Disease Response and Remission (ENCORE) Trial Group. Natalizumab for the treatment of active Crohn's disease: results of the ENCORE Trial. *Gastroenterology* 2007; **132**: 1672-1683 [PMID: 17484865 DOI: 10.1053/j.gastro.2007.03.024]

147 **Wyatt NJ**, Speight RA, Stewart CJ, Kirby JA, Lamb CA. Targeting Leukocyte Trafficking in Inflammatory Bowel Disease. *BioDrugs* 2021; **35**: 473-503 [PMID: 34613592 DOI: 10.1007/s40259-021-00496-5]

148 **Lenti MV**, Levison S, Eliadou E, Willert R, Kemp K, Carter A, Stansfield C, Assadsangabi A,

Singh S, Crooks B, Tattersall S, Fairhurst F, Kenneth C, Subramanian S, Probert C, Storey D, Gregg B, Smith P, Liu E, Limdi JK, Johnston A, Hamlin PJ, Selinger CP. A real-world, long-term experience on effectiveness and safety of vedolizumab in adult patients with inflammatory bowel disease: The Cross Pennine study. *Dig Liver Dis* 2018; **50**: 1299-1304 [PMID: 30077465 DOI: 10.1016/j.dld.2018.07.007]

149 **Clinton JW**, Kiparizoska S, Aggarwal S, Woo S, Davis W, Lewis JH. Drug-Induced Liver Injury: Highlights and Controversies in the Recent Literature. *Drug Saf* 2021; **44**: 1125-1149 [PMID: 34533782 DOI: 10.1007/s40264-021-01109-4]

150 LiverTox: Clinical and Research Information on Drug-Induced Liver Injury [Internet]. Bethesda (MD): National Institute of Diabetes and Digestive and Kidney Diseases; 2012–. [PMID: 31643176]

151 **Sandborn WJ**, Feagan BG, Rutgeerts P, Hanauer S, Colombel JF, Sands BE, Lukas M, Fedorak RN, Lee S, Bressler B, Fox I, Rosario M, Sankoh S, Xu J, Stephens K, Milch C, Parikh A; GEMINI 2 Study Group. Vedolizumab as induction and maintenance therapy for Crohn's disease. *N Engl J Med* 2013; **369**: 711-721 [DOI: 10.1056/NEJMoa1215739]

152 **Feagan BG**, Rutgeerts P, Sands BE, Hanauer S, Colombel JF, Sandborn WJ, Van Assche G, Axler J, Kim HJ, Danese S, Fox I, Milch C, Sankoh S, Wyant T, Xu J, Parikh A; GEMINI 1 Study Group. Vedolizumab as induction and maintenance therapy for ulcerative colitis. *N Engl J Med* 2013; **369**: 699-710 [DOI: 10.1056/NEJMoa1215734]

153 **Sandborn WJ**, Gasink C, Gao LL, Blank MA, Johanns J, Guzzo C, Sands BE, Hanauer SB, Targan S, Rutgeerts P, Ghosh S, de Villiers WJ, Panaccione R, Greenberg G, Schreiber S, Lichtiger S, Feagan BG; CERTIFI Study Group. Ustekinumab induction and maintenance therapy in refractory Crohn's disease. *N Engl J Med* 2012; **367**: 1519-1528 [PMID: 23075178 DOI: 10.1056/NEJMoa1203572]

154 **Sands BE**, Sandborn WJ, Panaccione R, O'Brien CD, Zhang H, Johanns J, Adedokun OJ, Li K, Peyrin-Biroulet L, Van Assche G, Danese S, Targan S, Abreu MT, Hisamatsu T, Szapary P, Marano C; UNIFI Study Group. Ustekinumab as Induction and Maintenance Therapy for Ulcerative Colitis. *N Engl J Med* 2019; **381**: 1201-1214 [PMID: 31553833 DOI: 10.1056/NEJMoa1900750]

155 LiverTox: Clinical and Research Information on Drug-Induced Liver Injury [Internet]. Bethesda (MD): National Institute of Diabetes and Digestive and Kidney Diseases; 2012–. Ustekinumab. [PMID: 31643346]

156 **Chiu HY**, Chen CH, Wu MS, Cheng YP, Tsai TF. The safety profile of ustekinumab in the treatment of patients with psoriasis and concurrent hepatitis B or C. *Br J Dermatol* 2013; **169**: 1295-1303 [PMID: 23746170 DOI: 10.1111/bjd.12461]

157 **FDA**. U.S. Food and Drug Administration. FDA approves new treatment for moderately to severely active ulcerative colitis. 2018 May 1. [cited 06 February 2021]. Available from: https://www.fda.gov/news-events/press-announcements/fda-approves-new-treatment-moderately-severely-active-ulcerative-colitis

158 **Harris C**, Cummings JRF. JAK1 inhibition and inflammatory bowel disease. *Rheumatology (Oxford)* 2021; **60**: ii45-ii51 [PMID: 33950226 DOI: 10.1093/rheumatology/keaa896]

159 **van Vollenhoven RF**, Fleischmann R, Cohen S, Lee EB, García Meijide JA, Wagner S, Forejtova S, Zwillich SH, Gruben D, Koncz T, Wallenstein GV, Krishnaswami S, Bradley JD, Wilkinson B; ORAL Standard Investigators. Tofacitinib or adalimumab *vs* placebo in rheumatoid arthritis. *N Engl J Med* 2012; **367**: 508-519 [DOI: 10.1056/NEJMoa1112072]

160 **Fleischmann R**, Cutolo M, Genovese MC, Lee EB, Kanik KS, Sadis S, Connell CA, Gruben D, Krishnaswami S, Wallenstein G, Wilkinson BE, Zwillich SH. Phase IIb dose-ranging study of the oral JAK inhibitor tofacitinib (CP-690,550) or adalimumab monotherapy *vs* placebo in patients with active rheumatoid arthritis with an inadequate response to disease-modifying antirheumatic drugs. *Arthritis Rheum* 2012; **64**: 617-629 [PMID: 21952978 DOI: 10.1002/art.33383]

161 **Burmester GR**, Blanco R, Charles-Schoeman C, Wollenhaupt J, Zerbini C, Benda B, Gruben D, Wallenstein G, Krishnaswami S, Zwillich SH, Koncz T, Soma K, Bradley J, Mebus C; ORAL Step investigators. Tofacitinib (CP-690,550) in combination with methotrexate in patients with active rheumatoid arthritis with an inadequate response to tumour necrosis factor inhibitors: a randomised phase 3 trial. *Lancet* 2013; **381**: 451-460 [PMID: 23294500 DOI: 10.1016/S0140-6736(12)61424-X]

162 **Sandborn WJ**, Su C, Sands BE, D'Haens GR, Vermeire S, Schreiber S, Danese S, Feagan BG, Reinisch W, Niezychowski W, Friedman G, Lawendy N, Yu D, Woodworth D, Mukherjee A, Zhang H, Healey P, Panés J; OCTAVE Induction 1, OCTAVE Induction 2, and OCTAVE Sustain Investigators. Tofacitinib as Induction and Maintenance Therapy for Ulcerative Colitis. *N Engl J Med* 2017; **376**: 1723-1736 [PMID: 28467869 DOI: 10.1056/NEJMoa1606910]

163 **Lair-Mehiri L**, Stefanescu C, Vaysse T, Laharie D, Roblin X, Rosa I, Treton X, Abitbol V, Amiot A, Bouguen G, Dib N, Fumery M, Pariente B, Carbonnel F, Peyrin-Biroulet L, Simon M, Viennot S, Bouhnik Y. Real-world evidence of tofacitinib effectiveness and safety in patients with refractory ulcerative colitis. *Dig Liver Dis* 2020; **52**: 268-273 [PMID: 31732444 DOI: 10.1016/j.dld.2019.10.003]

164 **D'Amico F**, Parigi TL, Fiorino G, Peyrin-Biroulet L, Danese S. Tofacitinib in the treatment of ulcerative colitis: efficacy and safety from clinical trials to real-world experience. *Therap Adv Gastroenterol* 2019; **12**: 1756284819848631 [PMID: 31205486 DOI: 10.1177/1756284819848631]

165 **Deepak P**, Alayo QA, Khatiwada A, Lin B, Fenster M, Dimopoulos C, Bader G, Weisshof R, Jacobs M, Gutierrez A, Ciorba MA, Christophi GP, Patel A, Hirten RP, Colombel JF, Rubin DT, Ha C, Beniwal-Patel P, Ungaro RC, Syal G, Pekow J, Cohen BL, Yarur A. Safety of Tofacitinib in a Real-

World Cohort of Patients With Ulcerative Colitis. *Clin Gastroenterol Hepatol* 2021; **19**: 1592-1601.e3 [PMID: 32629130 DOI: 10.1016/j.cgh.2020.06.050]

166 **Coban S**, Kekilli M, Köklü S. Approach and management of patients with chronic hepatitis B and C during the course of inflammatory bowel disease. *Inflamm Bowel Dis* 2014; **20**: 2142-2150 [PMID: 25072501 DOI: 10.1097/MIB.0000000000000126]

167 **Chevaux JB**, Bigard MA, Bensenane M, Oussalah A, Jarlot S, Belle A, Nani A, Bronowicki JP, Peyrin-Biroulet L. Inflammatory bowel disease and hepatitis B and C. *Gastroenterol Clin Biol* 2009; **33**: 1082-1093 [PMID: 19896313 DOI: 10.1016/j.gcb.2009.03.021]

168 **Sansone S**, Guarino M, Castiglione F, Rispo A, Auriemma F, Loperto I, Rea M, Caporaso N, Morisco F. Hepatitis B and C virus reactivation in immunosuppressed patients with inflammatory bowel disease. *World J Gastroenterol* 2014; **20**: 3516-3524 [PMID: 24707134 DOI: 10.3748/wjg.v20.i13.3516]

169 **Rahier JF**, Magro F, Abreu C, Armuzzi A, Ben-Horin S, Chowers Y, Cottone M, de Ridder L, Doherty G, Ehehalt R, Esteve M, Katsanos K, Lees CW, Macmahon E, Moreels T, Reinisch W, Tilg H, Tremblay L, Veereman-Wauters G, Viget N, Yazdanpanah Y, Eliakim R, Colombel JF; European Crohn's and Colitis Organisation (ECCO). Second European evidence-based consensus on the prevention, diagnosis and management of opportunistic infections in inflammatory bowel disease. *J Crohns Colitis* 2014; **8**: 443-468 [PMID: 24613021 DOI: 10.1016/j.crohns.2013.12.013]

170 **Borman ZA**, Côté-Daigneault J, Colombel JF. The risk for opportunistic infections in inflammatory bowel disease with biologics: an update. *Expert Rev Gastroenterol Hepatol* 2018; **12**: 1101-1108 [PMID: 30277409 DOI: 10.1080/17474124.2018.1530983]

171 **Jiang HY**, Wang SY, Deng M, Li YC, Ling ZX, Shao L, Ruan B. Immune response to hepatitis B vaccination among people with inflammatory bowel diseases: A systematic review and meta-analysis. *Vaccine* 2017; **35**: 2633-2641 [PMID: 28404358 DOI: 10.1016/j.vaccine.2017.03.080]

172 **Craviotto V**, Furfaro F, Loy L, Zilli A, Peyrin-Biroulet L, Fiorino G, Danese S, Allocca M. Viral infections in inflammatory bowel disease: Tips and tricks for correct management. *World J Gastroenterol* 2021; **27**: 4276-4297 [PMID: 34366605 DOI: 10.3748/wjg.v27.i27.4276]

173 **Morisco F**, Castiglione F, Rispo A, Stroffolini T, Sansone S, Vitale R, Guarino M, Biancone L, Caruso A, D'Inca R, Marmo R, Orlando A, Riegler G, Donnarumma L, Camera S, Zorzi F, Renna S, Bove V, Tontini G, Vecchi M, Caporaso N. Effect of immunosuppressive therapy on patients with inflammatory bowel diseases and hepatitis B or C virus infection. *J Viral Hepat* 2013; **20**: 200-208 [PMID: 23383659 DOI: 10.1111/j.1365-2893.2012.01643.x]

174 **Imperatore N**, Castiglione F, Rispo A, Sessa A, Caporaso N, Morisco F. Timing Strategies of Direct-Acting Antivirals and Biologics Administration in HCV-Infected Subjects with Inflammatory Bowel Diseases. *Front Pharmacol* 2017; **8**: 867 [PMID: 29209223 DOI: 10.3389/fphar.2017.00867]

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REVIEW

Gastrointestinal and hepatic side effects of potential treatment for COVID-19 and vaccination in patients with chronic liver diseases

Man Fai Law, Rita Ho, Kimmy Wan Tung Law, Carmen Ka Man Cheung

ORCID number: Man Fai Law 0000-0003-2462-6625.; Rita Ho 0000-0001-5966-2680; Kimmy Wan Tung Law 0000-0002-8740-0896; Carmen Ka Man Cheung 0000-0001-9386-506X.

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Man Fai Law, Carmen Ka Man Cheung, Department of Medicine and Therapeutics, Prince of Wales Hospital, The Chinese University of Hong Kong, Hong Kong, China

Rita Ho, Department of Medicine, North District Hospital, Hong Kong, China

Kimmy Wan Tung Law, West Island School, Hong Kong, China

Corresponding author: Man Fai Law, MRCP, Staff Physician, Department of Medicine and Therapeutics, Prince of Wales Hospital, The Chinese University of Hong Kong, 30-32 Ngan Shing Street, Shatin, Hong Kong, China. mflaw99@yahoo.com.hk

Abstract

The outbreak of coronavirus disease 2019 (COVID-19) is a global pandemic. Many clinical trials have been performed to investigate potential treatments or vaccines for this disease to reduce the high morbidity and mortality. The drugs of higher interest include umifenovir, bromhexine, remdesivir, lopinavir/ritonavir, steroid, tocilizumab, interferon alpha or beta, ribavirin, fivapiravir, nitazoxanide, ivermectin, molnupiravir, hydroxychloroquine/chloroquine alone or in combination with azithromycin, and baricitinib. Gastrointestinal (GI) symptoms and liver dysfunction are frequently seen in patients with COVID-19, which can make it difficult to differentiate disease manifestations from treatment adverse effects. GI symptoms of COVID-19 include anorexia, dyspepsia, nausea, vomiting, diarrhea and abdominal pain. Liver injury can be a result of systemic inflammation or cytokine storm, or due to the adverse drug effects in patients who have been receiving different treatments. Regular monitoring of liver function should be performed. COVID-19 vaccines have been rapidly developed with different technologies including mRNA, viral vectors, inactivated viruses, recombinant DNA, protein subunits and live attenuated viruses. Patients with chronic liver disease or inflammatory bowel disease and liver transplant recipients are encouraged to receive vaccination as the benefits outweigh the risks. Vaccination against COVID-19 is also recommended to family members and healthcare professionals caring for these patients to reduce exposure to the severe acute respiratory syndrome coronavirus 2 virus.

Key Words: COVID-19 treatment; Gastrointestinal side effects; Hepatic side effects; COVID-19 vaccine; Chronic liver disease; Liver transplantation

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Core Tip: Gastrointestinal symptoms such as anorexia, dyspepsia, nausea, vomiting, diarrhea and abdominal pain are common among patients with coronavirus disease 2019 (COVID-19). Liver injury can be a result of systemic inflammation or cytokine storm, or due to the adverse drug reactions of different treatments. Regular monitoring of liver function is recommended. Patients with inflammatory bowel disease, chronic liver diseases or liver transplant recipients are encouraged to receive the COVID-19 vaccine, and the benefits will outweigh the risks in the vast majority of patients.

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INTRODUCTION

The outbreak of coronavirus disease 2019 (COVID-19) is a global pandemic caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). It is a very contagious virus and has infected millions of people worldwide causing numerous deaths. There are many clinical trials investigating potential treatments or vaccines for this disease to reduce the high morbidity and mortality.

Drugs with potential utility include remdesivir, lopinavir/ritonavir (LPV/r), steroids, tocilizumab, interferon alpha or beta, ribavirin, hydroxychloroquine/chloroquine alone or in combination with azithromycin, and baricitinib. Gastrointestinal (GI) symptoms and liver dysfunction are frequently seen in COVID-19 which can make it difficult to differentiate disease manifestations from treatment side effects[1,2].

The common GI symptoms in patients with COVID-19 include anorexia, dyspepsia, nausea, vomiting, diarrhea and abdominal pain[3-11]. The pooled prevalence of GI symptoms is 17.6% according to a recent meta-analysis[12]. The hepatic manifestations of COVID-19 include elevated liver enzymes and less commonly elevated bilirubin levels. The incidence of liver injury ranges from 14.8% to 53% as indicated by abnormal alanine transaminase (ALT)/aspartate aminotransferase (AST) levels with slight elevation of bilirubin levels[2,7]. Patients with liver dysfunction also tend to have severe COVID-19, and the liver injury in these patients can be a result of systemic inflammation or cytokine storm, or due to the adverse drug reactions in severe COVID-19 patients who have been receiving different treatments. While cholangiocytes may contribute to hepatic regeneration and immune response, it has been suggested that bile duct epithelial cells play a greater role in hepatic injury due to SARS-CoV-2 infection than cholangiocytes do[13]. The aim of the current article is to review the GI and hepatic side effects associated with the potential agents for the treatment of COVID-19, focusing particularly on redemsivir, LPV/r and steroids which have shown beneficial effects in the treatment of COVID-19. COVID-19 vaccines are now available in many countries and an increasing number of people are getting vaccinated. We will discuss their side effects and the current views on whether patients with chronic liver diseases (CLD), liver transplantation or inflammatory bowel disease (IBD) should receive the vaccine.

COVID-19 TREATMENTS

The agents used for COVID-19 treatment can be classified according to the type of agents, such as antiviral, antiparasitic, antibacterial and immunomodulatory agents, or according to the site of action on the SARS-CoV-2 virus such as blocking the entry of virus, inhibition of viral replication and anti-inflammatory effect.

Viral entry can be blocked by proteins, peptides, or small molecule compounds that bind to the viral S protein, thereby preventing the virus from interacting with the host membrane. Examples are umifenovir and bromhexine[14].

Inhibitors of viral nucleic acid synthesis are the best represented class of antiviral drugs that suppress viral replication in host cells[15]. Examples include lopinavir-

ritonavir, remdesivir, ribavirin, chloroquine or hydroxychloroquine, favipiravir, nitazoxanide, ivermectin and molnupiravir.

The RNA-dependent RNA polymerase (RdRp) is found in the core of the coronavirus replication machinery, nsp12 protein, and has an important role in the viral life cycle[16]. Inhibition of RdRp is a possible target for therapeutic interventions. Examples of RdRP inhibitors include favipiravir and ribavirin.

Excessive inflammatory responses and cytokine release are found in patients with severe cases of COVID-19. This mechanism contributes to the worsening of the disease and stimulates lung and other systemic injuries. The early modulation of these responses can reduce the risk of acute respiratory distress[17]. Examples of agents that target the inflammatory response include steroids, tocilizumab [an anti-interleukin (IL)-6 monoclonal antibody] and baricitinib. The mechanisms of agents used for the treatment of COVID-19 are shown in Figure 1.

AGENTS AGAINST THE ENTRY OF VIRUS

Umifenovir

Umifenovir is used for the treatment of some enveloped and non-enveloped viral infection. It can also effectively block SARS-CoV-2 entry into cells and inhibits post-entry stages of infection[18]. The efficacy of the drug was assessed in an open-label randomized controlled trial (RCT). One hundred patients were randomly assigned to two treatment groups receiving either hydroxychloroquine followed by LPV/r or hydroxychloroquine followed by umifenovir[19]. The primary outcome was hospitalization duration and clinical improvement 7 d after admission.

Umifenovir significantly improved clinical and laboratory parameters including peripheral oxygen saturation, intensive care unit (ICU) admission rate, duration of hospitalization, white blood cell (WBC), and erythrocyte sedimentation rate when compared with LPV/r. The duration of hospitalization in the umifenovir group was significantly shorter than in the LPV/r arm (7.2 d *vs* 9.6 d; $P = 0.02$)[19].

Nausea, vomiting and liver function test (LFT) derangements are the major GI and hepatic abnormalities that can occur in patients receiving umifenovir. Clinicians should use the drug with caution in those patients with hepatic impairment.

Bromhexine

SARS-CoV-2 invades the human body through the angiotensin-converting enzyme 2 (ACE-2)/transmembrane protease serine 2 (TMPRSS2). In addition to host cell entry, TMPRSS2 is involved in the maturation and release of the virus, which ultimately increase the viral infectivity[20]. Therefore, a possible useful therapeutic approach for COVID-19 is the inhibition of TMPRSS2[21].

Bromhexine has strong inhibitory effect on TMPRSS2 and can be used to block pulmonary virus infection[22]. Therefore, it may exert a protective effect against COVID-19-induced acute lung injury. The effect and safety of bromhexine was assessed in patients with mild or moderate COVID-19 who were randomly assigned to a bromhexine group or a control group at a 2:1 ratio[22]. The primary end points were the time to clinical recovery and the rate of deterioration after initiation of medications.

There were no significant differences in the outcomes between the two treatment groups. The side effects include LFT derangement (38.9%), gingivitis (11.1%), insomnia (11.1%), headache (5.6%), and elevated WBCs in urine (5.6%). However, all side effects were mild and no patient stopped the treatment because of the adverse effects[22].

Another randomized, open-label clinical trial study involving 78 patients was performed to assess the efficacy of bromhexine. Patients were randomized to the bromhexine group or the control group. The primary outcomes were the rate of ICU admissions, intubation and then mechanical ventilation, and 28-d mortality[23]. When compared with the standard treatment group, the bromhexine-treated group showed a significant reduction in ICU admissions (5.1% *vs* 28.2%, $P = 0.006$), intubation (2.6% *vs* 23.1%, $P = 0.007$) and death (0 *vs* 5, $P = 0.027$)[23].

INHIBITORS OF VIRAL REPLICATION

LPV/r

LPV/r is a co-formulation of two structurally related protease inhibitor (PI) antiret-

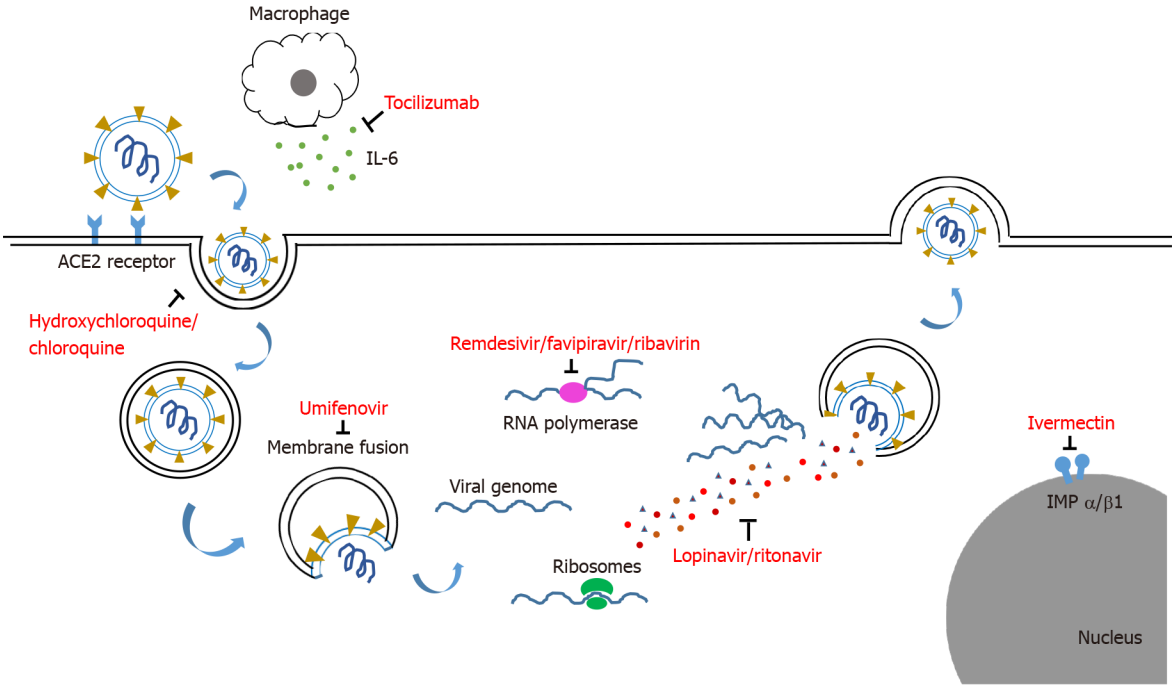

Figure 1 The mechanism of potential treatment of coronavirus disease 2019. ACE: Angiotensin-converting enzyme; IL-6: Interleukin-6.

roviral agents widely used to treat HIV infections[24]. Ritonavir substantially increases the half-life of lopinavir by inhibiting cytochrome P450 (CYP) isoenzyme 3A4[25]. PIs prevent cleavage of gag and gag-pol protein precursors in infected cells, arresting maturation and inhibiting the formation of infectious virions, thereby preventing subsequent waves of infection[26].

Lopinavir demonstrated *in vitro* inhibitory activity against SARS-CoV and Middle East respiratory syndrome coronavirus[27-29]. Addition of LPV/r to ribavirin in treating SARS patients showed a reduction of adverse outcomes [death or development of acute respiratory distress syndrome (ARDS) requiring intensive care] compared to ribavirin alone[30]. Conflicting results of published data have stirred controversy concerning the use of LPV/r in COVID-19 patients. Cao *et al*[31] conducted a RCT in Wuhan, China to assess the efficacy and safety of LPV/r in 199 severe COVID-19 patients. Patients were randomly assigned in a 1:1 ratio to receive either LPV/r (400/100 mg, orally) twice daily or supportive care alone. Treatment with LPV/r was not associated with a difference from standard care in the time to clinical improvement [hazard ratio (HR) for clinical improvement, 1.31; 95% confidence interval (CI): 0.95 to 1.80]. The 28-d mortality rate and the percentages of patients with detectable viral RNA at various time points were similar. In a modified intention-to-treat analysis, which excluded three patients with early death, antiviral treatment shortened the median time to clinical improvement by 1 day compared with standard care (15 d *vs* 16 d, HR, 1.39; 95%CI: 1.00 to 1.91)[31]. Another RCT included 86 patients with mild to moderate disease; the use of LPV/r did not shorten the time of positive-to-negative conversion of SARS-CoV-2 nucleic acid in respiratory specimen, nor symptoms or radiological improvement[32]. On the other hand, Yan *et al*[33] reported data from a retrospective study including 129 non-critically ill patients with COVID-19. They showed that the median duration of SARS-CoV-2 shedding in the LPV/r treatment group was 22 d [interquartile range (IQR) 18-29], which was significantly shorter than in group that did not receive LPV/r treatment (28.5 d, IQR 19.5-38) (log-rank P = 0.009). Subgroup analysis revealed that the administration of LPV/r treatment within 10 d of symptom onset, but not later administration, could shorten the duration of SARS-CoV-2 RNA shedding compared with no LPV/r treatment[33]. Ye *et al*[34] studied the clinical efficacy of LPV/r in 47 patients and showed that patients in the active treatment group returned to normal body temperature in a shorter time compared with the control group (4.8 ± 1.94 d *vs* 7.3 ± 1.53 d, P = 0.0364).

GI adverse events were common in patients receiving LPV/r. The most common GI adverse event in patients receiving LPV/r was diarrhea (occurring in 20% of patients); others included nausea, vomiting abdominal pain and gastroenteritis[35]. In the study by Cao *et al*[31], 14% of patients were unable to complete the full 14-d course of LPV/r

because of GI adverse events (Table 1). In the study by Li *et al*[32], one patient withdrew from the study due to severe diarrhea. Twice-daily dosing of LPV/r is associated with a reduced frequency of moderate to severe diarrhea compared with once daily [36]. The majority of patients who develop diarrhea can be managed conservatively and may not require antidiarrheal treatment[37]. Hypokalemia, secondary to diarrhea or emesis, should be treated according to standard local protocols[38]. If patients develop significant adverse effects, lower dosages of LPV/r (*e.g.*, 200/100 mg twice a day) can be considered, with the understanding that lower doses may not markedly alleviate toxicities[34].

Ritonavir use is associated with a 5-fold higher incidence of severe hepatotoxicity compared with other PIs[39]. Hepatitis including elevation of AST, ALT, and gamma-glutamyl transferase levels has been reported in 3.5% of patients taking LPV/r, according to the package insert[35]. This drug is principally metabolized by the hepatic CYP3A4 isoenzyme[40] and therefore, caution should be exercised when administering this drug to patients with hepatic impairment. Safety data on LPV/r use in patients with cirrhosis do exist[41]. Coinfection with hepatitis B virus (HBV) and/or hepatitis C virus (HCV) increases the risk hepatotoxicity and patients with such infections should be monitored closely[42]. Patients with severe liver disease such as cirrhosis or those with significant elevation of liver enzyme were excluded from RCTs [31,32]. Concomitant use of tenofovir with LPV/r is not recommended since this will lead to elevated levels of tenofovir. Physicians may consider switching from tenofovir to entecavir during treatment with LPV/r.

Remdesivir

Remdesivir was initially under clinical development for the treatment of Ebola virus disease[43]. It is a monophosphoramidate prodrug of an adenosine analog, which is then metabolized in cells to an active nucleoside triphosphate that inhibits viral RdRp early in the viral infectious cycle. It has demonstrated antiviral activity against coronavirus including SARS-CoV-2[44-47]. Other potential antiviral mechanisms involve lethal mutagenesis and chain termination[48,49].

Remdesivir was used to treat the first case of COVID-19 infection in the United States[3]. Thereafter, numerous clinical trials focusing on its efficacy and safety have been published. In a multicenter RCT led by Beigel *et al*[50] including 1059 hospitalized patients with evidence of lower respiratory tract involvement, remdesivir was administered intravenously as a 200-mg loading dose on day 1, followed by 100-mg daily on days 2 through 10 or until hospital discharge or death. Patients who received treatment had a shorter time to recovery than patients who received placebo (median 11 d *vs* 15 d; rate ratio for recovery, 1.32; 95%CI: 1.12 to 1.55; $P < 0.001$). Recovery was defined as patients not requiring supplemental oxygen or ongoing medical care except for infection-control reasons. Mortality was numerically lower in the treatment group than the placebo group, but the difference was not significant (HR for death, 0.70; 95%CI: 0.47 to 1.04)[50]. Another RCT from China enrolled 237 patients, but failed to demonstrate a significant difference in the time to clinical improvement with remdesivir in severe patients [21.0 d in remdesivir group *vs* 23.0 d in the control group, HR 1.23 (95%CI: 0.87 to 1.75)][51]. Nevertheless, the results should be interpreted with caution as the power of this study was limited by failure to complete full enrolment due to control of the outbreak in Wuhan.

Several studies have compared the efficacy and safety of 5 d *vs* 10 d of remdesivir treatment in patients with COVID-19[52,53]. Goldman *et al*[52] enrolled 397 COVID-19 patients with evidence of pneumonia and reduced oxygen levels but not requiring mechanical ventilation or extracorporeal membrane oxygenation. Similar clinical improvement was observed in the 5-d group and 10-d group based on assessment on day 14 ($P = 0.14$). The most common GI/hepatic adverse events were nausea (10% in the 5-d group *vs* 9% in the 10-d group), increased ALT (6% *vs* 8%), and constipation (7% in both groups)[52]. Spinner *et al*[53] randomized 596 patients with moderate COVID-19 to a 10-d course of remdesivir, a 5-d course of remdesivir, or standard care in a 1:1:1 ratio. At 11 d after starting treatment, those randomized to the 5-d course of remdesivir had a statistically significant difference in clinical status compared with standard care[53]. However, those receiving the 10-d course of remdesivir did not have a statistically significant difference in clinical outcome compared with standard care. Common side effects included nausea, hypokalemia, and headache. Elevated liver enzymes were observed in one-third of patients, and were of grade ≥ 3 severity in 2% of patients[53].

GI/hepatic adverse events were similar in the treatment and control arms of the two RCTs described above[50,51]. One patient receiving remdesivir developed a hemorrhage of the lower digestive tract and three patients discontinued treatment as a

Table 1 Gastrointestinal adverse events in key studies investigating treatments for coronavirus disease 2019

Ref.	Dosage	*n*	Age, yr	Gender, male (%)	Incidence of adverse events in treatment *vs* control arm, *n* (%)						
					Diarrhea	Vomiting	Abdominal pain	Constipation	Increased AST	Increased ALT	Drug termination due to AE
Lopinavir/ritonavir											
Cao *et al*[31]	400/100 mg twice a day for 14 d	Tx 99; control 100	Median 58 (IQR 49-68)	120 (60.3)	4 (4.2) *vs* 0	6 (6.3) *vs* 0	4 (4.2) *vs* 2 (2.1)	NA	2 (2.1) *vs* 5 (5.1)	1 (1.1) *vs* 4 (4.0)	14%
Li *et al*[32]	200/50 mg, twice a day for 7-14 d	Tx 34; control 17	mean ± SD, 49.4 ± 14.7	40 (46.5)	9/34 (26.5) *vs* 0	NA	NA	NA	NA	1/21 (4.8) *vs* 0	1/34 (2.94)
Remdesivir											
Beigel *et al* [50]	200 mg daily on day 1, followed by 100 mg daily on day 2-10	Tx 538; control 521	mean ± SD, 58.9 ± 15.0	684 (64.3)	NA	NA	NA	NA	15 (2.8) *vs* 20 (3.8)	8 (1.5) *vs* 9 (1.7)	49 (9.1)
Wang *et al* [51]	200 mg daily on day 1, followed by 100 mg daily on day 2-10	Tx 158; control 79	Median (IQR) 65 (56-71)	89 (56)	5 (3) *vs* 2 (3)	4 (3) *vs* 2 (3%)	NA	21 (14) *vs* 12 (15)	7 (5) *vs* 9 (12)	NA	18 (12)
Spinner *et al* [53]	200 mg daily on day 1, followed by 100 mg daily on day 2-5 or day 2-10	193; 193; 200	Median (IQR) 56 (45-66)	118 (61), 114 (60)	5% *vs* 6% *vs* 7%	NA	NA	NA	32 *vs* 32 *vs* 33	32 *vs* 34 *vs* 39	31 (7.8)
Hydroxychloroquine											
Cavalcanti *et al*[70]	400 mg daily	Tx 221; control 227	mean ± SD, 50.3 ± 14.6	388 (55.3)	NA	0 *vs* 1 (0.6)	NA	NA	17 (8.5) *vs* 6 (3.4)	NA	NA
Boulware *et al*[71]	800 mg once, followed by 600 mg	Tx 414; control 407	Median (IQR) 41 (33-51)	196 (47.3)	81 (23.2) *vs* 15 (4.3) for diarrhoea or abdominal pain or vomiting	81 (23.2) *vs* 15 (4.3) for diarrhoea or abdominal pain or vomiting	81 (23.2) *vs* 15 (4.3) for diarrhoea or abdominal pain or vomiting	NA	NA	NA	17 (4.1)
Favipiravir											
Chen *et al* [80]	1600 mg twice a day on day 1, followed by 600 mg twice daily on day 2-10	Tx 116; control 120	NA	59 (50.86)	NA	NA	NA	NA	10 (8.62)	NA	Nil
Nitazoxanide											
Rocco *et al* [82]	500 mg 3 times per day	Tx 194; control 198	18-77	101 (52)	57 (29.4) *vs* 49 (24.7)	9 (4.6) *vs* 3 (1.5)	10 (5.2) *vs* 5 (2.5)	NA	NA	NA	Nil
Tocilizumab											

Stone *et al* [120]	Tocilizumab 8 mg/kg IV inf not to exceed 800 mg	Tx 161; control 82	Median (IQR) 61.6 (46.4-69.7)	96 (60)	NA	NA	NA	NA	6 (3.7) *vs* 3 (3.7) for grade 3 or 4	8 (5.0) *vs* 4 (4.9) for grade 3 or 4	NA

AE: Adverse event; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; inf: Infusion; IQR: Interquartile range; IV: Intravenous; NA: Not available; Tx: Treatment.

result of liver enzyme elevation in the study by Wang *et al*[51]. No serious grade 3 or 4 liver dysfunction was reported in either arm[51].

GI and hepatic adverse events have also been reported in case series of patients receiving remdesivir. In a remdesivir compassionate use program (n = 53), 12 patients (23%) developed elevated hepatic enzymes, and 5 (9%) had diarrhea[54]. Two patients (3.8%) discontinued remdesivir prematurely because of elevated aminotransferases [54]. In another case series in 35 patients who received compassionate remdesivir treatment in Italy, hepatotoxicity was the most frequent adverse event, with a grade 3 to 4 increase in transaminase levels observed in 42.8% of the patients[55]. In the first 12 COVID-19 patients in United States, all 3 patients who received remdesivir experienced transient transaminitis and GI symptoms including nausea, vomiting, gastroparesis or rectal bleeding[56]. Another case series of critically ill patients receiving remdesivir in Italy reported that three of these four patients had elevated ALT and AST levels, ranging from 5 times to 8 times the upper limit of normal[57].

Hepatic adverse events are not unexpected with nucleoside analogues; these agents can cause direct hepatotoxicity by inducing mitochondrial dysfunction and/or idiosyncratic hepatotoxicity *via* an acute hypersensitivity reaction or the production of toxic intermediates[58]. Asymptomatic grade 1 or 2 ALT elevations were observed in healthy individuals who received remdesivir in phase 1 studies[59]. Pharmacokinetic studies in patients with hepatic impairment were limited, but remdesivir should be used with caution in patients with existing liver disease, and only if the potential benefit outweighs the risk[60]. Regular monitoring of liver function should be performed if possible[61].

Hydroxychloroquine/chloroquine ± azithromycin

Hydroxychloroquine/chloroquine are drugs commonly used in the management of rheumatoid arthritis, systemic lupus erythematosus and malaria. SARS-CoV-2 enters cells by binding to the ACE-2 receptor. Chloroquine may inhibit terminal glycosylation, thus preventing the virus from binding to the ACE-2 receptor[62]. Hydroxychloroquine prevents SARS-CoV-2 from binding to gangliosides which in turn prevents the virion from engaging with the ACE-2 receptor[63].

The use of hydroxychloroquine/chloroquine in the treatment of COVID-19 is controversial[64-71]. A multicenter, RCT was conducted in 504 hospitalized patients with COVID-19 who were receiving either no supplemental oxygen or a maximum of 4 L/min of supplemental oxygen. Patients were randomly assigned in a 1:1:1 ratio to receive standard care, standard care plus hydroxychloroquine 400 mg twice daily, or

standard care plus hydroxychloroquine 400 mg twice daily and azithromycin 500 mg once daily for 7 d[70]. Active treatment had no effect on patients' clinical status at 15 d compared with standard care. The proportional odds of having a higher score on the seven-point ordinal scale at 15 d was not increased by either hydroxychloroquine alone [odds ratio (OR) 1.21; 95%CI: 0.69 to 2.11; P = 1.00] or hydroxychloroquine plus azithromycin (OR, 0.99; 95%CI: 0.57 to 1.73; P = 1.00). In addition, a higher proportion of patients receiving hydroxychloroquine alone (8.5%) or with azithromycin (10.9%) developed elevated liver enzymes compared those who did not receive either agent (3.4%)[70]. Further randomized studies are needed to clarify the efficacy of hydroxychloroquine or chloroquine in the treatment of COVID-19.

These drugs also have a number of side effects. Apart from the well-known arrhythmogenic cardiotoxicity of the drugs, the most common adverse events of hydroxychloroquine and chloroquine are GI, including GI upset, nausea, vomiting, diarrhea, abdominal cramps, and a metallic taste[72-74]. In a study evaluating the use of chloroquine, nearly 24% of patients suffered from nausea or abdominal cramps and 17% reported diarrhea as side effects[75]. Up to 50% of patients receiving hydroxychloroquine in another study reported some GI side effects; the frequency was dose-dependent with GI events occurring more commonly with loading doses of 800 mg or higher[76].

Chloroquine and hydroxychloroquine should be administered with food to reduce nausea and vomiting. At the same time, chloroquine can be crushed and mixed with flavored syrups to mask the bitter taste. It is also recommended to avoid taking antacids within 4 h of chloroquine because of a potential for chelation and reduced bioavailability, but this drug interaction does not occur with hydroxychloroquine.

Azithromycin is a semisynthetic macrolide antibiotic that is commonly prescribed to treat infections with Gram-positive, Gram-negative and atypical pathogens. It has been used for the treatment of COVID-19 in combination with hydroxychloroquine or chloroquine and has produced synergistic effects in the context of combination therapy[77]. Azithromycin may cause GI side effects such as nausea and vomiting.

Ribavirin

Ribavirin is a guanine derivative used for the treatment of respiratory syncytial virus and HCV infections. It has been used in combination with other agents for the treatment of COVID-19[78]. In a prospective study of patients with mild to moderate COVID-19, the combination of interferon-beta, oral LPV/r and ribavirin produced a significantly shorter median time from start of study treatment to negative nasopharyngeal swab compared with LPV/r alone[78]. Patients in the combination group also had earlier relief of symptoms compared with the control group (4 d *vs* 8 d, P < 0.0001). This study suggests that combination therapy is more potent than single-agent antiviral therapy against COVID-19[78].

The common side effects observed in the combination therapy group included diarrhea (40%), fever (37%), nausea (35%) and elevated ALT levels (13%)[78]. Since CYP enzymes are not involved in the metabolism and elimination of ribavirin, there is minimal potential for drug-drug interactions.

Favipiravir

Favipiravir is an RdRp inhibitor[79]. Once inside cells, favipiravir is converted into an active phosphoribosylated form, which acts as a substrate for viral RNA polymerase, and then inhibits RNA polymerase activity. It is a broad-spectrum antiviral drug approved in Japan for the treatment of influenza. It has also been used for the treatment of Ebola and Lassa virus infection.

Chen *et al*[80] conducted a prospective, randomized, open-label multicenter clinical trial involving 240 adult patients with COVID-19 comparing the efficacy and safety of favipiravir *vs* umifenovir. The clinical recovery rate on day 7 was better in the favipiravir arm than in the umifenovir arm (71.43% *vs* 55.86%, P = 0.01). Favipiravir significantly shortened the latency to relief for pyrexia and cough compared with umifenovir, and dyspnea was significantly (P = 0.017) less common in the favipiravir group than in the umifenovir group. Deranged LFT is a common side effect of favipiravir and was found in 8.6% of patients.

Cai *et al*[81] conducted an open-label study in 80 patients with mild to moderate COVID-19 and assessed the effects of favipiravir in comparison with LPV/r for the treatment of COVID-19. Favipiravir was shown to have shorter viral clearance time (median 4 d *vs* 11 d). In addition, a higher proportion of patients in the favipiravir than the LPV/r groups showed improvement in chest imaging (91.43% *vs* 62.22%; P = 0.004), particularly in the group with viral clearance within 7 d of starting treatment. Multivariable Cox regression showed that favipiravir was significantly (P = 0.026)

associated with faster viral clearance[81].

The most common side effects of favipiravir were liver enzyme abnormalities, GI symptoms like diarrhea, and serum uric acid elevations. We would be cautious about prescribing favipiravir in patients with abnormal LFT results.

Nitazoxanide

Nitazoxanide is an antiparasitic prodrug with antiviral properties that is approved by the U.S. Food and Drug Administration (FDA). The effects of nitazoxanide against COVID-19 were examined in a multicenter, randomized, double-blind, placebo-controlled trial recruiting 392 patients presenting up to 3 d after onset of symptoms including fever, dry cough, and/or fatigue. The patients were randomized in a 1:1 ratio to receive either nitazoxanide 500 mg 3 times/d or matching placebo for 5 d after the diagnosis of SARS-CoV2 infection was made by reverse transcription polymerase chain reaction (RT-PCR) on a nasopharyngeal sample[82].

Although there was no difference between the nitazoxanide and placebo groups in the resolution of symptoms at the 5-d study visit, a significantly higher proportion of patients in the nitazoxanide group (29.9%) returned a negative PCR result for SARS-CoV-2 compared with the placebo group (18.2%; $P = 0.009$). There was also significantly greater reduction in viral load between the start and end of therapy in patients receiving nitazoxanide (55%) compared with placebo (45%; $P = 0.013$). GI side effects included nausea (14.4%), vomiting (4.6%), diarrhea (29.4%), and abdominal pain (5.2%) were reported in patients receiving nitazoxanide in the study[82].

Ivermectin

Ivermectin is an antiparasitic drug and was found to have a broad range of antiviral activity against many RNA and DNA viruses *in vitro*. It was also shown to be highly effective *in vitro* against SARS-CoV-2[83].

It was shown that the combined use of ivermectin, nitazoxanide and ribavirin plus zinc supplement achieved better clearance of the SARS-COV2 from the nasopharynx in a shorter time than symptomatic therapy in a non-RCT[84]. The viral clearance rates on the 7th day were 0% and 58.1%, respectively, in the groups receiving supportive treatment and combined antiviral therapy, and were 13.7% and 73.1%, respectively, on the 15th day. The corresponding cumulative viral clearance rates on the 15th day were 13.7% and 88.7%, respectively. Overall, 11.3% of patients had elevation of LFTs and 22.6% of developed GI upset during the study period.

Rajter *et al*[85] performed a retrospective study of 280 COVID-19 patients to assess the efficacy of ivermectin, in which 173 had been treated with ivermectin and 107 had not. Most patients in both groups also received hydroxychloroquine, azithromycin, or both. Mortality was significantly lower in the ivermectin group (13.3% *vs* 24.5%; $P < 0.05$). Mortality was also lower among ivermectin-treated patients with severe pulmonary involvement (38.8% *vs* 80.7%; $P = 0.001$). Eleven percent of phas a broad range of antiviral activity against many RNA and DNA viruses *in vitro* has a broad range of antiviral activity against many RNA and DNA viruses *in vitro*. Ivermectin has a broad range of antiviral activity against many RNA and DNA viruses *in vitro*.

Molnupiravir

Molnupiravir is an oral, direct-acting antiviral agent which was shown to be highly effective in reducing nasopharyngeal SARS-CoV-2 infectious virus and viral RNA. It is well absorbed after oral administration. Fischer *et al*[86] randomized 202 patients to molnupiravir (200, 400 or 800 mg) or placebo twice-daily for 5 d. Antiviral activity was assessed as time to undetectable levels of viral RNA by RT-PCR and time to elimination of infectious virus isolation from nasopharyngeal swabs.

The results showed a significant reduction in virus isolation in participants receiving 800 mg molnupiravir (1.9%) *vs* placebo (16.7%) at day 3 ($P = 0.02$). Virus was not isolated from any patient receiving 400 mg or 800 mg molnupiravir while 11.1% of patients receiving placebo had virus isolated at day 5 ($P = 0.03$).

There was decrease in the time to viral RNA clearance in patients given 800 mg molnupiravir compared with placebo (14 d *vs* 27 d, $P = 0.001$). There was also a higher rate of overall clearance in patients receiving molnupiravir. The side effects of molnupiravir include headache, insomnia, and increased ALT. We would be cautious using molnupiravir in patient with hepatic dysfunction.

Immunomodulatory agents

Cytokine storm is an important pathogenic process in COVID-19 patients[87]. SARS-CoV-2 binds to the toll-like receptor, activating the nuclear factor (NF)-κB pathway

and pro-inflammatory cytokines[88]. Cytokines are signalling molecules that recruit immune cells to the site of inflammation, induce vascular leakage and exudation, and stimulate the generation of free radicals and proteases[89]. Pro-inflammatory cytokines induce alveolar injury and reduced alveolar fluid clearance resulted in ARDS[90]. Compared with mild or moderate cases, patients with severe COVID-19 have higher levels of circulating IL-2, IL-6, IL-7, IL-10, interferon gamma, granulocyte colony stimulating factor, interferon-inducible protein 10, monocyte chemoattractant peptide , macrophage inflammatory protein-1A, and tumor necrosis factor (TNF)-α[7, 91-93]. This raises the possibility of using immunomodulatory agents to control the inflammatory response, and thereby improve the prognosis of COVID-19[94].

Corticosteroids

Corticosteroids inhibit NF-κB signalling and various pro-inflammatory cytokines such as IL-1β, IL-2, IL-6, TNF-α, and IL-17. It also reduces the proliferation, activation, differentiation, and survival of T cells and macrophages[95]. Steroids may play a protective role in the respiratory and digestive systems by activating ACE-2 and suppressing the cytokine storm, in particular reducing IL-6 levels, in patients with severe or critical COVID-19[96]. Corticosteroids were used in early reports from Wuhan, China, where they were used in an attempt to reduce inflammation-induced lung injury[90].

Dexamethasone is the first treatment that has been shown to reduce mortality in severely ill COVID-19 patients[97,98]. The randomized evaluation of COVID-19 therapy (RECOVERY) trial compared 2104 patients receiving oral or intravenous dexamethasone (at a dose of 6 mg once daily) for up to 10 d with 4321 patients receiving usual care alone. The 28-d mortality rate was lower in the group receiving dexamethasone compared with usual care group in patients who were receiving invasive mechanical ventilation (29.3% *vs* 41.4%; rate ratio, 0.64; 95%CI: 0.51 to 0.81) or receiving oxygen without invasive mechanical ventilation (23.3% *vs* 26.2%; rate ratio, 0.82; 95%CI: 0.72 to 0.94). No survival benefit was seen among those who were receiving no respiratory support at randomization. Dexamethasone also reduced mortality in patients with symptoms for more than 7 d but not in those with more recent symptom onset[97].

The positive impact of steroids was confirmed in a prospective meta-analysis of seven clinical trials involving 1703 critically ill patients with COVID-19 conducted in 12 countries[99]. The meta-analysis showed that the use of systemic corticosteroids reduced all-cause 28-d mortality compared with usual care or placebo. The number of deaths was 222 in those receiving corticosteroids compared to 425 deaths in the usual care or placebo group. Dexamethasone could significantly suppress the odds of all-cause mortality.

The preliminary report of the RECOVERY study did not describe side effects. Previously reported side effects of steroids include hyperglycemia, hypokalemia, delayed viral clearance, risk of secondary bacterial infection, psychosis and avascular osteonecrosis[100-104]. Corticosteroids may induce various GI adverse events such as gastritis, peptic ulcer formation and GI bleeding, with the risk of bleeding significantly increased by concomitant non-steroidal anti-inflammatory drug use[105,106]. Direct SARS-CoV-2 invasion of the GI tract, causing erosion and ulcers in severe patients, may increase the risk further[1]. Prophylactic proton pump inhibitors should be considered in patients who receive dexamethasone[107].

Steroids increase the risk of acute pancreatitis by an unknown mechanism[108]. Steroids activate triglyceride synthesis and accumulation, increase fatty acid uptake and inhibit fatty acid beta-oxidation in the liver, while they also increase lipolysis, lipogenesis and the secretion of non-esterified fatty acids and adipokines in adipose tissue, which results in hepatic steatosis[109]. Diabetes and obesity are associated with the development of non-alcoholic fatty liver disease[110]. These metabolic risk factors may result in deleterious effects on host immunity, and are closely related to disease severity and mortality in patients with COVID-19[111-115]. Regular monitoring of liver function and glucose level is recommended for this high-risk group of patients receiving dexamethasone.

Tocilizumab

COVID-19 can trigger aggressive an inflammatory response resulting in cytokine release syndrome (CRS), which is associated with an unfavorable prognosis[116]. A meta-analysis of 6 studies including 1302 patients demonstrated 2.9-fold higher levels of IL-6 in patients with complicated COVID-19 compared with patients with non-complicated disease[117]. IL-6 is an important cytokine responsible for an inflammatory storm that leads to impaired oxygen diffusion in the lungs[7]. Tocilizumab is a

recombinant humanized monoclonal antibody against the IL-6 receptor and reduces the effects of CRS. This led to speculation that it could be used in the treatment of COVID-19, especially in severe patients with high IL-6 levels.

A retrospective, observational cohort study was carried out to investigate mortality in 544 patients with severe COVID-19 requiring support in the ICU; 179 patients received tocilizumab and 365 patients received standard care. There was an improvement in median overall survival from time of hospital admission in patients receiving tocilizumab when compared with the standard care cohort (20% *vs* 7%; $P < 0.001$)[118].

Another multicenter retrospective cohort study investigated outcomes in 4485 adults with COVID-19 admitted to ICU in 68 hospitals. Among critically ill patients, the risk of in-hospital mortality was lower in patients treated with tocilizumab in the first 2 d of ICU admission compared with patients whose early treatment did not include tocilizumab (HR, 0.71; 95%CI: 0.56 to 0.92)[119].

However, similar favorable results were not seen in a RCT involving 243 patients with hyperinflammatory states. Tocilizumab was not shown to be effective enough to prevent intubation or death in moderately ill, hospitalized COVID-19 patients in this trial[120]. Further research in RCTs is needed.

Reports have emerged of liver injury with an increase in transaminase levels associated with tocilizumab use in COVID-19 patients[121], and increases in liver enzyme levels were seen in 5% of patients in one of the cohort studies described above and in 1% of patients in the RCT[118,120]. In the cohort study by Gupta *et al*[119], 16.6% of patients receiving tocilizumab developed an AST of more than 250 U/L and 8.5% developed an ALT level of more than 500 U/L. Tocilizumab can interfere with serum concentrations of CYP3A4 substrates. It should be used with caution and liver function regularly monitored, especially when used in combination with another hepatotoxic drug or in patients receiving multiple concomitant medications.

Baricitinib

Baricitinib is a selective inhibitor of Janus kinase (JAK) 1 and 2, and orally administered. It was originally developed for the treatment of rheumatoid arthritis. Inhibition of JAK blocks intracellular signal transmission from cytokine or growth factor receptors and leads to reduced hematopoiesis[17]. This inhibition of signal transmission prevents phosphorylation and then activation of signal transducers and activators of transcription.

Baricitinib was used in combination with remdesivir in a RCT involving 1033 patients with COVID-19. The rationale for combining these two therapies is that clinical outcomes would be improved by reducing the immune response and preventing a hyperinflammatory state[122]. The combination was found to be significantly better than remdesivir alone in reducing recovery time and accelerating clinical improvement in patients with COVID-19. This effect was more marked in patients receiving high-flow oxygen or non-invasive ventilation. The time to recovery was 10 d in patients who received combination treatment compared with 18 d in patients who received remdesivir alone. The 28-d mortality was 5.1% in the combination group and 7.8% in the control group (HR for death, 0.65; 95%CI: 0.39 to 1.09).

The combination was associated with fewer serious adverse events. Transaminases increased in 1.2% of patients receiving combination therapy and 2% of patients receiving remdesivir, and bilirubin increased in 0.4% and 1.6%, respectively. Regular monitoring of liver function is recommended, especially when used in combination with remdesivir.

A summary of the side effects of the potential treatments for COVID-19 is shown in Table 2.

COVID-19 VACCINES AND LIVER AND GI DISEASES

Vaccination is an important method to protect the population from COVID-19 and is likely to be especially important in high-risk individuals, such as those with pre-existing health conditions. A minimum vaccine efficacy of 50% is necessary to get regulatory approval from the World Health Organization (WHO). Patients with chronic diseases have a higher mortality when they get infected with COVID-19. Therefore, this group of patients will benefit more from the vaccination. However, the phase 1-3 studies of the COVID-19 vaccines mainly recruited healthy individuals, so data are limited in patients with chronic diseases. The decision to be vaccinated may

Table 2 Gastrointestinal and hepatic side effects of potential treatments for coronavirus disease 2019

Drug name	Gastrointestinal and hepatic side effects
Remdesivir	Elevation of liver enzymes
Lopinavir-ritonavir	Nausea, vomiting, abdominal pain, gastroenteritis
Hydroxychloroquine/chloroquine	Nausea, vomiting, abdominal pain, diarrhea
Steroids	Epigastric pain, peptic ulcer, risk of HBV reactivation
Interferon	Diarrhea, nausea, elevated alanine aminotransferase level
Ribavirin	Elevated liver enzyme levels
Umifenovir	Nausea, vomiting and deranged liver function
Bromhexine	Deranged liver function
Favipiravir	Diarrhoea, liver enzyme abnormalities
Nitazoxanide	Nausea, vomiting, diarrhoea and abdominal pain
Imervectin	Elevation of liver enzymes
Molnupiravir	Elevated alanine aminotransferase
Tocilizumab	Liver dysfunction
Baricitinib	Nausea, liver dysfunction
Azithromycin	Nausea, vomiting

also depend on the stability of the patient's chronic illness and the prevalence of COVID-19 in the relevant country or region.

TYPES OF VACCINES

Different technologies were applied to the development of the vaccines including mRNA, viral vectors, inactivated viruses, recombinant DNA, protein subunits and live attenuated viruses.

The BNT162b2 mRNA vaccine (manufactured by Pfizer BioNTec) and the mRNA-1273 mRNA vaccine (manufactured by Moderna-NIH) was developed based on mRNAs that encode variants of the SARS-CoV-2 spike glycoprotein and are encapsulated into lipid nanoparticles[123-125]. The ChAdOx1 nCoV-19 vaccine (manufactured by AstraZeneca) uses an adenoviral vector and is approved by the WHO is currently being used in Europe, the United States and many other countries [126]. Another WHO-approved COVID-19 vaccine is Ad26.COV2.S, developed by Janssen (Johnson & Johnson); this is a single-dose viral vector vaccine based on a human adenovirus that has been modified to contain the gene for making the spike protein of the SARS-CoV-2 virus[127]. However, the use of this vaccine was stopped by the WHO because of the risk of thrombotic complications.

The two mRNA vaccines described above got the earliest approval from the WHO and are now being used, but these vaccines must be stored in very low temperature freezers. Common acute side effects of the vaccines include myalgia, fatigue, low-grade fever, headache, nausea and redness or soreness at the injection site. There do not appear to be many GI and hepatic side effects.

BNT162b2 was chosen by Pfizer/BioNTec as the most promising of two potential mRNA vaccine candidates based on safety and immunogenicity data from phase I studies in younger and older adults[123]. A two-dose regimen of BNT162b2 confirmed a 95% protection rate against COVID-19 in persons 16 years of age or older. The side effect profile was characterized mainly by fatigue, mild to moderate pain at the injection site, and headache[124].

A phase III study of the mRNA-1273 vaccine was carried out in 30420 healthy individuals aged 18 or above randomly assigned in a 1:1 ratio to receive either vaccine or placebo. It showed an efficacy of 94.1% at preventing COVID-19 illness, including severe disease[125]. There were no major safety concerns apart from transient local and systemic reactions.

The third approved vaccine is ChAdOx1 nCoV-19 vaccine (AZD1222) which was developed at Oxford University. It consists of a replication-deficient chimpanzee adenoviral vector ChAdOx1 which contains the SARS-CoV-2 structural surface glycoprotein antigen (spike protein; nCoV-19) gene. After receiving two standard doses of vaccine, the efficacy of the vaccine was 62.1% *vs* 1.6% of 4455 participants in the control group[126].

Recently, however, safety concerns have emerged about the thrombotic risk associated with the vaccine. A pathogenic PF4-dependent syndrome, which was unrelated to the use of heparin, was identified after the administration of the vaccine [128]. Clinicians should pay particular attention to individuals with thrombotic risk factors.

The fifth vaccine is an inactivated vaccine developed by Sinovac Life Sciences and is being used in some countries. CoronaVac was well tolerated and induced humoral responses against SARS-CoV-2, and it was approved for emergency use in China and some other countries and regions. Efficacy and safety were demonstrated in two phase I/II double-blind, placebo-controlled RCTs in healthy adults aged 18-59 years and 60 years or older[129,130]. A phase III, randomized, multicenter, double-blind, placebo-controlled clinical study is being carried out to assess the efficacy and safety of the adsorbed vaccine COVID-19 (inactivated) produced by Sinovac in two age groups: 18 years to 59 years and 60 years or more[131].

Another vaccine, Sinopharm, which is an inactivated vaccine developed in China, has been approved and used in some countries and regions. It showed promising results in phase I/II trials[132]. The phase III trial data will provide more information on the safety, efficacy and immunogenicity of the vaccine. A summary of the available COVID-19 vaccines is shown in Table 3. There are ongoing studies for these and other vaccines and more choices will become available over time.

COVID-19 VACCINES AND CLD

Patients with CLD, liver cirrhosis, hepatobiliary malignancies, and candidates for liver transplantation are at higher risk of COVID-19 infections. At the same time, these groups of patients have a lower immune response to vaccines.

The benefits and risks of vaccination for patients with chronic disease or immunocompromised patients should be weighed individually, taking into account the incidence of the infection in the country or community, the vaccine formulation, the type of immunosuppressive therapy (*e.g.*, chemotherapy, transplantation) the patient is receiving, and the extent of their immunosuppression.

There is a reduction of immune memory against and immune responses to certain vaccines as patients age and their CLD progresses[133]. Moreover, patients with alcohol-associated liver disease, CLD and cirrhosis may have an impaired immune response to vaccination. At the same time, they are more susceptible to infections and infection-related complications[134].

Patients with immunosuppressive conditions or liver diseases were usually excluded from the studies of the COVID-19 vaccines. A post-marketing study in a nationwide mass vaccination setting in Israel suggests that the BNT162b2 mRNA vaccine is effective for a wide range of COVID-19-related outcomes, a finding consistent with that of the randomized trial[135]. All persons who were newly vaccinated were matched to unvaccinated controls in a 1:1 ratio according to demographic and clinical characteristics. Each study group included 596618 persons, and the vaccinated population included 9699 (1.6%) patients with liver disease and 435 (0.1%) patients with solid organ transplantation[135].

There are currently limited published data on specific patient subgroups. Investigators have performed subgroup analyses, each time restricting the matching process to persons with a specific condition of interest, in order to maximize the sample size [136]. The results on the subgroup with CLD are not yet known.

Patients with CLD infected with SARS-CoV-2 infection have higher risk of adverse outcome than the general population. There are on-going trials in patients with liver diseases worldwide and the results are pending[137].

In view of the high rate of complications and decompensation caused by COVID 19 in CLD, we recommend SARS-CoV-2 vaccination in patients with CLD, and in candidates for liver transplantation, with prioritization of patients with risk factors for severe COVID-19.

In general, professional bodies like the European Association for the Study of the Liver and the American Association for the Study of Liver Disease recommend

Table 3 Summary of the ata for the currently used coronavirus disease 2019 vaccines

Vaccine	Mechanism	Number of participants	Efficacy
mRNA-1273 (Moderna)[125]	RNA (embedded in lipid nanoparticles)encodes a variant of the SARS-CoV-2 spike protein	30420 participants (randomized 1:1 vaccine *vs* placebo)	Efficacy 94.1% (11 vaccinated *vs* 185 controls with COVID-19)
BNT162b2 (BioNTech and Pfizer)[124]	RNA (embedded in lipid nanoparticles) encodes a variant of the SARS-CoV-2 spike protein	43548 participants (randomized 1:1 vaccine *vs* placebo)	Efficacy 95% (9 vaccinated *vs* 169 controls with COVID-19)
ChAdOx1 nCoV-19 (AZD122; AstraZenenca and University of Oxford)[126]	Replication-deficient chimpanzee adenovirus vector, containing the full-length codon-optimized coding sequence of SARS-CoV-2 spike protein	23848 participants (randomized 1:1 vaccine *vs* placebo)	Efficacy 70.4% [30 (0.5%) of 5807 vaccine recipients *vs* 101 (1.7%) of 5829 controls with COVID-19]
CoronaVac (Sinovac Life Sciences, Beijing, China) [129,131]	Inactivated vaccine candidate against COVID-19	600 participants	Seroconversion was seen in 114 (97%) of 117 in the 3 μg group, 118 (100%) of 118 in the 6 μg group, and none (0%) of 59 in the placebo group
Sinopharm vaccine[132]	Inactivated vaccine candidate against COVID-19	448 participants	Neutralizing antibodies were detected in 100% of recipients

COVID-19: Coronavirus disease 2019; SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2.

COVID-19 vaccination for patients with CLD as the benefits likely outweigh the risks [138,139].

Rituximab may be used for the treatment of CLD such as autoimmune hepatitis and its efficacy is shown in a recent retrospective study[140]. There is usually a blunted vaccine response after vaccination in patients with lymphoma[141-144] or autoimmune disorders[145-148] treated with rituximab. B cells are required for the development of humoral immune responses to neoantigens. Therefore, depletion of B cells following rituximab will likely reduce the humoral immune responses to the COVID-19 vaccine. Both T cell-dependent and -independent responses are also significantly impaired for at least 6 mo after rituximab treatment[148].

Assuming that immunological response to the COVID-19 vaccine correlates with disease protection, it is recommended that vaccination be performed at least 6 mo after rituximab infusion.

EFFICACY AND SAFETY OF VACCINES IN SOLID ORGAN TRANSPLANT RECIPIENTS

Solid organ transplant (SOT) recipients are on immunosuppression to prevent graft rejection, so they are at a higher risk of infection and infective complications. Vaccination is useful to prevent infections and the associated complications in transplant recipients.

COVID-19 vaccination is recommended for all SOT recipients including liver transplant recipients, and vaccination can be given 3-6 mo after SOT. Since the current approved vaccines do not contain live or attenuated virus, they are likely to be safe in immunosuppressed patients[139,149].

The immunogenicity of vaccines in SOT recipients is lower than in immunocompetent individuals because of the immunosuppressive therapy and the underlying chronic disease. Therefore, vaccination against COVID-19 is recommended for family members and healthcare professionals caring for these patients to reduce exposure to SARS-CoV-2[138].

COVID-19 VACCINE AND IBD

IBD is an umbrella term for the immune-mediated inflammatory conditions of Crohn's disease and ulcerative colitis.

IBD patients may receive immunosuppressive drugs such as high-dose corticosteroids, immunomodulators (thiopurines, methotrexate, and calcineurin inhibitors), anticytokine therapies (including anti-TNF and anti-IL-12p40 biologics), anti-integrin

therapies (vedolizumab), and small-molecule inhibitors of signalling (tofacitinib), which could leave them susceptible to infection.

Immunosuppressive drugs may reduce the humoral response to vaccines and thus their effectiveness, which could have major implications for the safety of immunosuppressed patients in the COVID-19 era. The risks associated with current COVID-19 vaccines are low, and guidelines recommend vaccination for patients with IBD[150, 151].

COVID-19 vaccination is also advocated for IBD patients younger than 16 years. Although pediatric patients may experience milder illness if they get infected by SARS-CoV-2[152,153], they can be the source of ongoing outbreaks and transmission [154]. The cessation of the COVID-19 pandemic relies on maximal community uptake of the COVID-19 vaccine in order to achieve herd immunity. On May 10, 2021, the U.S. FDA expanded the Emergency Use Authorization for the BNT162b2 mRNA vaccine to include people aged 12 years to 15 years[155]. This is based on the results of an RCT enrolling 2260 adolescents (12-15-year-old) who were randomized 1:1 to receive the BNT162b2 or placebo[156]. In 7 d after the second dose of BNT162b2, there were zero new case of COVID-19, translating into 100% vaccine efficacy, while there were 16 confirmed cases in the placebo group. Vaccinated adolescents 12- to 15-year-old had higher geometric mean titers of SARS-CoV-2 neutralizing antibodies (1239.5 *vs* 705.1) compared with recipients aged 16 years to 25 years. A favorable safety and side effect profile, similar to other age groups, was also demonstrated in the 12- to 15-year-old recipients of BNT162b2[156].

The use of COVID-19 vaccines is not recommended in pregnant women and there are no safety data of the vaccines in these women to date.

Another point to consider is that patients with IBD are at risk of thromboembolic complications, and COVID-19 increases the risk of thromboembolic events. Studies have shown that prophylactic anticoagulation can reduce the 30-d mortality risk in patients with COVID-19[157].

RECOMMENDATIONS

COVID-19 is a pandemic infection with a high burden of morbidity and mortality. Various drugs are under investigation for the treatment of the disease, but many are associated with GI and hepatic side effects. Caution and careful monitoring should be exercised when prescribing these therapies in patients with GI symptoms like diarrhea and vomiting. As liver impairment is a common observation among patients with COVID-19, we recommend that all patients with COVID-19 and liver impairment undergo investigations for potential causes of liver disease, including viral hepatitis serology, particularly in areas where HBV is prevalent.

Furthermore, increasing rates of liver dysfunction have been correlated with the severity of COVID-19[158]. We need to maintain a high index of suspicion as hepatotoxic drug effects may be difficult to detect in this condition.

High-dose corticosteroids and tocilizumab have been used for the treatment of patients with severe COVID-19. There is a risk of HBV reactivation, hepatitis flare, and even acute liver failure in patients with chronic HBV infection receiving this regimen. Screening for HBsAg is recommended, and antiviral prophylaxis with nucleoside analogs should be given to patients with COVID-19 who are positive for HBsAg during steroid therapy.

COVID-19 vaccines have been rapidly developed. Patients with CLD or IBD and liver transplant recipients are encouraged to receive vaccination. The benefits will outweigh the risks.

Vaccination against COVID-19 is also recommended for family members and healthcare professionals caring for these patients to reduce exposure to SARS-CoV-2. The vaccination against COVID-19 is encouraged for all individuals at risk of SARS-CoV-2 infection, including those with underlying chronic diseases. Recommendations by professional bodies, governments and health authorities will be important driver of COVID-19 vaccination[159].

CONCLUSION

Extensive research has been performed to identify potential treatments for SARS-CoV-2 infection. GI symptoms and liver dysfunction in COVID-19 patients could be due to disease manifestations or treatment side effects, which physicians should take into

consideration when choosing the best therapeutic strategy. The development of effective and safe vaccines is the light at the end of the tunnel to end the pandemic and should be encouraged, including for patients with CLD, IBD, liver transplant recipients their family members, and healthcare professionals.

REFERENCES

1 **Lin L**, Jiang X, Zhang Z, Huang S, Fang Z, Gu Z, Gao L, Shi H, Mai L, Liu Y, Lin X, Lai R, Yan Z, Li X, Shan H. Gastrointestinal symptoms of 95 cases with SARS-CoV-2 infection. *Gut* 2020; **69**: 997-1001 [PMID: 32241899 DOI: 10.1136/gutjnl-2020-321013]

2 **Fan Z**, Chen L, Li J, Cheng X, Yang J, Tian C, Zhang Y, Huang S, Liu Z, Cheng J. Clinical Features of COVID-19-Related Liver Functional Abnormality. *Clin Gastroenterol Hepatol* 2020; **18**: 1561-1566 [PMID: 32283325 DOI: 10.1016/j.cgh.2020.04.002]

3 **Holshue ML**, DeBolt C, Lindquist S, Lofy KH, Wiesman J, Bruce H, Spitters C, Ericson K, Wilkerson S, Tural A, Diaz G, Cohn A, Fox L, Patel A, Gerber SI, Kim L, Tong S, Lu X, Lindstrom S, Pallansch MA, Weldon WC, Biggs HM, Uyeki TM, Pillai SK; Washington State 2019-nCoV Case Investigation Team. First Case of 2019 Novel Coronavirus in the United States. *N Engl J Med* 2020; **382**: 929-936 [PMID: 32004427 DOI: 10.1056/NEJMoa2001191]

4 **Guan WJ**, Ni ZY, Hu Y, Liang WH, Ou CQ, He JX, Liu L, Shan H, Lei CL, Hui DSC, Du B, Li LJ, Zeng G, Yuen KY, Chen RC, Tang CL, Wang T, Chen PY, Xiang J, Li SY, Wang JL, Liang ZJ, Peng YX, Wei L, Liu Y, Hu YH, Peng P, Wang JM, Liu JY, Chen Z, Li G, Zheng ZJ, Qiu SQ, Luo J, Ye CJ, Zhu SY, Zhong NS; China Medical Treatment Expert Group for Covid-19. Clinical Characteristics of Coronavirus Disease 2019 in China. *N Engl J Med* 2020; **382**: 1708-1720 [PMID: 32109013 DOI: 10.1056/NEJMoa2002032]

5 **Wong SH**, Lui RN, Sung JJ. Covid-19 and the digestive system. *J Gastroenterol Hepatol* 2020; **35**: 744-748 [PMID: 32215956 DOI: 10.1111/jgh.15047]

6 **Chan JF**, Yuan S, Kok KH, To KK, Chu H, Yang J, Xing F, Liu J, Yip CC, Poon RW, Tsoi HW, Lo SK, Chan KH, Poon VK, Chan WM, Ip JD, Cai JP, Cheng VC, Chen H, Hui CK, Yuen KY. A familial cluster of pneumonia associated with the 2019 novel coronavirus indicating person-to-person transmission: a study of a family cluster. *Lancet* 2020; **395**: 514-523 [PMID: 31986261 DOI: 10.1016/S0140-6736(20)30154-9]

7 **Huang C**, Wang Y, Li X, Ren L, Zhao J, Hu Y, Zhang L, Fan G, Xu J, Gu X, Cheng Z, Yu T, Xia J, Wei Y, Wu W, Xie X, Yin W, Li H, Liu M, Xiao Y, Gao H, Guo L, Xie J, Wang G, Jiang R, Gao Z, Jin Q, Wang J, Cao B. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet* 2020; **395**: 497-506 [PMID: 31986264 DOI: 10.1016/S0140-6736(20)30183-5]

8 **Chen N**, Zhou M, Dong X, Qu J, Gong F, Han Y, Qiu Y, Wang J, Liu Y, Wei Y, Xia J, Yu T, Zhang X, Zhang L. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. *Lancet* 2020; **395**: 507-513 [PMID: 32007143 DOI: 10.1016/S0140-6736(20)30211-7]

9 **Luo S**, Zhang X, Xu H. Don't Overlook Digestive Symptoms in Patients With 2019 Novel Coronavirus Disease (COVID-19). *Clin Gastroenterol Hepatol* 2020; **18**: 1636-1637 [PMID: 32205220 DOI: 10.1016/j.cgh.2020.03.043]

10 **Jin X**, Lian JS, Hu JH, Gao J, Zheng L, Zhang YM, Hao SR, Jia HY, Cai H, Zhang XL, Yu GD, Xu KJ, Wang XY, Gu JQ, Zhang SY, Ye CY, Jin CL, Lu YF, Yu X, Yu XP, Huang JR, Xu KL, Ni Q, Yu CB, Zhu B, Li YT, Liu J, Zhao H, Zhang X, Yu L, Guo YZ, Su JW, Tao JJ, Lang GJ, Wu XX, Wu WR, Qv TT, Xiang DR, Yi P, Shi D, Chen Y, Ren Y, Qiu YQ, Li LJ, Sheng J, Yang Y. Epidemiological, clinical and virological characteristics of 74 cases of coronavirus-infected disease 2019 (COVID-19) with gastrointestinal symptoms. *Gut* 2020; **69**: 1002-1009 [PMID: 32213556 DOI: 10.1136/gutjnl-2020-320926]

11 **Pan L**, Mu M, Yang P, Sun Y, Wang R, Yan J, Li P, Hu B, Wang J, Hu C, Jin Y, Niu X, Ping R, Du Y, Li T, Xu G, Hu Q, Tu L. Clinical Characteristics of COVID-19 Patients With Digestive Symptoms in Hubei, China: A Descriptive, Cross-Sectional, Multicenter Study. *Am J Gastroenterol* 2020; **115**: 766-773 [PMID: 32287140 DOI: 10.14309/ajg.0000000000000620]

12 **Cheung KS**, Hung IFN, Chan PPY, Lung KC, Tso E, Liu R, Ng YY, Chu MY, Chung TWH, Tam AR, Yip CCY, Leung KH, Fung AY, Zhang RR, Lin Y, Cheng HM, Zhang AJX, To KKW, Chan KH, Yuen KY, Leung WK. Gastrointestinal Manifestations of SARS-CoV-2 Infection and Virus Load in Fecal Samples From a Hong Kong Cohort: Systematic Review and Meta-analysis. *Gastroenterology* 2020; **159**: 81-95 [PMID: 32251668 DOI: 10.1053/j.gastro.2020.03.065]

13 **Banales JM**, Huebert RC, Karlsen T, Strazzabosco M, LaRusso NF, Gores GJ. Cholangiocyte pathobiology. *Nat Rev Gastroenterol Hepatol* 2019; **16**: 269-281 [PMID: 30850822 DOI: 10.1038/s41575-019-0125-y]

14 **Shyr ZA**, Gorshkov K, Chen CZ, Zheng W. Drug Discovery Strategies for SARS-CoV-2. *J Pharmacol Exp Ther* 2020; **375**: 127-138 [PMID: 32723801 DOI: 10.1124/jpet.120.000123]

15 **Hoenen T**, Groseth A, Feldmann H. Therapeutic strategies to target the Ebola virus life cycle. *Nat Rev Microbiol* 2019; **17**: 593-606 [PMID: 31341272 DOI: 10.1038/s41579-019-0233-2]

16 **Shannon A**, Selisko B, Le N, Huchting J, Touret F, Piorkowski G, Fattorini V, Ferron F, Decroly E, Meier C, Coutard B, Peersen O, Canard B. Favipiravir strikes the SARS-CoV-2 at its Achilles heel,

the RNA polymerase. *bioRxiv* 2020 [PMID: 32511380 DOI: 10.1101/2020.05.15.098731]
17 **Barlow A**, Landolf KM, Barlow B, Yeung SYA, Heavner JJ, Claassen CW, Heavner MS. Review of Emerging Pharmacotherapy for the Treatment of Coronavirus Disease 2019. *Pharmacotherapy* 2020; **40**: 416-437 [PMID: 32259313 DOI: 10.1002/phar.2398]
18 **Wang X**, Cao R, Zhang H, Liu J, Xu M, Hu H, Li Y, Zhao L, Li W, Sun X, Yang X, Shi Z, Deng F, Hu Z, Zhong W, Wang M. The anti-influenza virus drug, arbidol is an efficient inhibitor of SARS-CoV-2 in vitro. *Cell Discov* 2020; **6**: 28 [PMID: 32373347 DOI: 10.1038/s41421-020-0169-8]
19 **Nojomi M**, Yassin Z, Keyvani H, Makiani MJ, Roham M, Laali A, Dehghan N, Navaei M, Ranjbar M. Effect of Arbidol (Umifenovir) on COVID-19: a randomized controlled trial. *BMC Infect Dis* 2020; **20**: 954 [PMID: 33317461 DOI: 10.1186/s12879-020-05698-w]
20 **Limburg H**, Harbig A, Bestle D, Stein DA, Moulton HM, Jaeger J, Janga H, Hardes K, Koepke J, Schulte L, Koczulla AR, Schmeck B, Klenk HD, Böttcher-Friebertshäuser E. TMPRSS2 Is the Major Activating Protease of Influenza A Virus in Primary Human Airway Cells and Influenza B Virus in Human Type II Pneumocytes. *J Virol* 2019; **93** [PMID: 31391268 DOI: 10.1128/JVI.00649-19]
21 **Hoffmann M**, Kleine-Weber H, Schroeder S, Krüger N, Herrler T, Erichsen S, Schiergens TS, Herrler G, Wu NH, Nitsche A, Müller MA, Drosten C, Pöhlmann S. SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor. *Cell* 2020; **181**: 271-280.e8 [PMID: 32142651 DOI: 10.1016/j.cell.2020.02.052]
22 **Li T**, Sun L, Zhang W, Zheng C, Jiang C, Chen M, Chen D, Dai Z, Bao S, Shen X. Bromhexine Hydrochloride Tablets for the Treatment of Moderate COVID-19: An Open-Label Randomized Controlled Pilot Study. *Clin Transl Sci* 2020; **13**: 1096-1102 [PMID: 32881359 DOI: 10.1111/cts.12881]
23 **Ansarin K**, Tolouian R, Ardalan M, Taghizadieh A, Varshochi M, Teimouri S, Vaezi T, Valizadeh H, Saleh P, Safiri S, Chapman KR. Effect of bromhexine on clinical outcomes and mortality in COVID-19 patients: A randomized clinical trial. *Bioimpacts* 2020; **10**: 209-215 [PMID: 32983936 DOI: 10.34172/bi.2020.27]
24 **Cvetkovic RS**, Goa KL. Lopinavir/ritonavir: a review of its use in the management of HIV infection. *Drugs* 2003; **63**: 769-802 [PMID: 12662125 DOI: 10.2165/00003495-200363080-00004]
25 **Mangum EM**, Graham KK. Lopinavir-Ritonavir: a new protease inhibitor. *Pharmacotherapy* 2001; **21**: 1352-1363 [PMID: 11714208 DOI: 10.1592/phco.21.17.1352.34419]
26 **Flexner C**. HIV-protease inhibitors. *N Engl J Med* 1998; **338**: 1281-1292 [PMID: 9562584 DOI: 10.1056/NEJM199804303381808]
27 **Chen F**, Chan KH, Jiang Y, Kao RY, Lu HT, Fan KW, Cheng VC, Tsui WH, Hung IF, Lee TS, Guan Y, Peiris JS, Yuen KY. In vitro susceptibility of 10 clinical isolates of SARS coronavirus to selected antiviral compounds. *J Clin Virol* 2004; **31**: 69-75 [PMID: 15288617 DOI: 10.1016/j.jcv.2004.03.003]
28 **de Wilde AH**, Jochmans D, Posthuma CC, Zevenhoven-Dobbe JC, van Nieuwkoop S, Bestebroer TM, van den Hoogen BG, Neyts J, Snijder EJ. Screening of an FDA-approved compound library identifies four small-molecule inhibitors of Middle East respiratory syndrome coronavirus replication in cell culture. *Antimicrob Agents Chemother* 2014; **58**: 4875-4884 [PMID: 24841269 DOI: 10.1128/AAC.03011-14]
29 **Chu CM**, Cheng VC, Hung IF, Wong MM, Chan KH, Chan KS, Kao RY, Poon LL, Wong CL, Guan Y, Peiris JS, Yuen KY; HKU/UCH SARS Study Group. Role of lopinavir/ritonavir in the treatment of SARS: initial virological and clinical findings. *Thorax* 2004; **59**: 252-256 [PMID: 14985565 DOI: 10.1136/thorax.2003.012658]
30 **Chan KS**, Lai ST, Chu CM, Tsui E, Tam CY, Wong MM, Tse MW, Que TL, Peiris JS, Sung J, Wong VC, Yuen KY. Treatment of severe acute respiratory syndrome with lopinavir/ritonavir: a multicentre retrospective matched cohort study. *Hong Kong Med J* 2003; **9**: 399-406 [PMID: 14660806]
31 **Cao B**, Wang Y, Wen D, Liu W, Wang J, Fan G, Ruan L, Song B, Cai Y, Wei M, Li X, Xia J, Chen N, Xiang J, Yu T, Bai T, Xie X, Zhang L, Li C, Yuan Y, Chen H, Li H, Huang H, Tu S, Gong F, Liu Y, Wei Y, Dong C, Zhou F, Gu X, Xu J, Liu Z, Zhang Y, Shang L, Wang K, Li K, Zhou X, Dong X, Qu Z, Lu S, Hu X, Ruan S, Luo S, Wu J, Peng L, Cheng F, Pan L, Zou J, Jia C, Liu X, Wang S, Wu X, Ge Q, He J, Zhan H, Qiu F, Guo L, Huang C, Jaki T, Hayden FG, Horby PW, Zhang D, Wang C. A Trial of Lopinavir-Ritonavir in Adults Hospitalized with Severe Covid-19. *N Engl J Med* 2020; **382**: 1787-1799 [PMID: 32187464 DOI: 10.1056/NEJMoa2001282]
32 **Li Y**, Xie Z, Lin W, Cai W, Wen C, Guan Y, Mo X, Wang J, Wang Y, Peng P, Chen X, Hong W, Xiao G, Liu J, Zhang L, Hu F, Li F, Zhang F, Deng X, Li L. Efficacy and Safety of Lopinavir/Ritonavir or Arbidol in Adult Patients with Mild/Moderate COVID-19: An Exploratory Randomized Controlled Trial. *Med (N Y)* 2020; **1**: 105-113.e4 [PMID: 32838353 DOI: 10.1016/j.medj.2020.04.001]
33 **Yan D**, Liu XY, Zhu YN, Huang L, Dan BT, Zhang GJ, Gao YH. Factors associated with prolonged viral shedding and impact of lopinavir/ritonavir treatment in hospitalised non-critically ill patients with SARS-CoV-2 infection. *Eur Respir J* 2020; **56** [PMID: 32430428 DOI: 10.1183/13993003.00799-2020]
34 **Ye XT**, Luo YL, Xia SC, Sun QF, Ding JG, Zhou Y, Chen W, Wang XF, Zhang WW, Du WJ, Ruan ZW, Hong L. Clinical efficacy of lopinavir/ritonavir in the treatment of Coronavirus disease 2019. *Eur Rev Med Pharmacol Sci* 2020; **24**: 3390-3396 [PMID: 32271456 DOI:

10.26355/eurrev_202003_20706]
35 **AbbVie Inc**. KALETRA (lopinavir and ritonavir) [package insert] North Chicago, IL: AbbVie Inc., 2016
36 **Molina JM**, Podsadecki TJ, Johnson MA, Wilkin A, Domingo P, Myers R, Hairrell JM, Rode RA, King MS, Hanna GJ. A lopinavir/ritonavir-based once-daily regimen results in better compliance and is non-inferior to a twice-daily regimen through 96 wk. *AIDS Res Hum Retroviruses* 2007; **23**: 1505-1514 [PMID: 18160008 DOI: 10.1089/aid.2007.0107]
37 **Guest JL**, Ruffin C, Tschampa JM, DeSilva KE, Rimland D. Differences in rates of diarrhea in patients with human immunodeficiency virus receiving lopinavir-ritonavir or nelfinavir. *Pharmacotherapy* 2004; **24**: 727-735 [PMID: 15222662 DOI: 10.1592/phco.24.8.727.36071]
38 **Liu F**, Xu A, Zhang Y, Xuan W, Yan T, Pan K, Yu W, Zhang J. Patients of COVID-19 may benefit from sustained Lopinavir-combined regimen and the increase of Eosinophil may predict the outcome of COVID-19 progression. *Int J Infect Dis* 2020; **95**: 183-191 [PMID: 32173576 DOI: 10.1016/j.ijid.2020.03.013]
39 **Sulkowski MS**, Thomas DL, Chaisson RE, Moore RD. Hepatotoxicity associated with antiretroviral therapy in adults infected with human immunodeficiency virus and the role of hepatitis C or B virus infection. *JAMA* 2000; **283**: 74-80 [PMID: 10632283 DOI: 10.1001/jama.283.1.74]
40 **Rock BM**, Hengel SM, Rock DA, Wienkers LC, Kunze KL. Characterization of ritonavir-mediated inactivation of cytochrome P450 3A4. *Mol Pharmacol* 2014; **86**: 665-674 [PMID: 25274602 DOI: 10.1124/mol.114.094862]
41 **Casado JL**, Del Palacio M, Moya J, Rodriguez JM, Moreno A, Perez-Elías MJ, Belso A, Dronda F, Moreno S. Safety and pharmacokinetics of lopinavir in HIV/HCV coinfected patients with advanced liver disease. *HIV Clin Trials* 2011; **12**: 235-243 [PMID: 22180521 DOI: 10.1310/hct1205-235]
42 **Sulkowski MS**. Hepatotoxicity associated with antiretroviral therapy containing HIV-1 protease inhibitors. *Semin Liver Dis* 2003; **23**: 183-194 [PMID: 12800071 DOI: 10.1055/s-2003-39949]
43 **Mulangu S**, Dodd LE, Davey RT Jr, Tshiani Mbaya O, Proschan M, Mukadi D, Lusakibanza Manzo M, Nzolo D, Tshomba Oloma A, Ibanda A, Ali R, Coulibaly S, Levine AC, Grais R, Diaz J, Lane HC, Muyembe-Tamfum JJ; PALM Writing Group, Sivahera B, Camara M, Kojan R, Walker R, Dighero-Kemp B, Cao H, Mukumbayi P, Mbala-Kingebeni P, Ahuka S, Albert S, Bonnett T, Crozier I, Duvenhage M, Proffitt C, Teitelbaum M, Moench T, Aboulhab J, Barrett K, Cahill K, Cone K, Eckes R, Hensley L, Herpin B, Higgs E, Ledgerwood J, Pierson J, Smolskis M, Sow Y, Tierney J, Sivapalasingam S, Holman W, Gettinger N, Vallée D, Nordwall J; PALM Consortium Study Team. A Randomized, Controlled Trial of Ebola Virus Disease Therapeutics. *N Engl J Med* 2019; **381**: 2293-2303 [PMID: 31774950 DOI: 10.1056/NEJMoa1910993]
44 **Brown AJ**, Won JJ, Graham RL, Dinnon KH 3rd, Sims AC, Feng JY, Cihlar T, Denison MR, Baric RS, Sheahan TP. Broad spectrum antiviral remdesivir inhibits human endemic and zoonotic deltacoronaviruses with a highly divergent RNA dependent RNA polymerase. *Antiviral Res* 2019; **169**: 104541 [PMID: 31233808 DOI: 10.1016/j.antiviral.2019.104541]
45 **Sheahan TP**, Sims AC, Graham RL, Menachery VD, Gralinski LE, Case JB, Leist SR, Pyrc K, Feng JY, Trantcheva I, Bannister R, Park Y, Babusis D, Clarke MO, Mackman RL, Spahn JE, Palmiotti CA, Siegel D, Ray AS, Cihlar T, Jordan R, Denison MR, Baric RS. Broad-spectrum antiviral GS-5734 inhibits both epidemic and zoonotic coronaviruses. *Sci Transl Med* 2017; **9** [PMID: 28659436 DOI: 10.1126/scitranslmed.aal3653]
46 **Zhang L**, Zhou R. Structural Basis of the Potential Binding Mechanism of Remdesivir to SARS-CoV-2 RNA-Dependent RNA Polymerase. *J Phys Chem B* 2020; **124**: 6955-6962 [PMID: 32521159 DOI: 10.1021/acs.jpcb.0c04198]
47 **Wang M**, Cao R, Zhang L, Yang X, Liu J, Xu M, Shi Z, Hu Z, Zhong W, Xiao G. Remdesivir and chloroquine effectively inhibit the recently emerged novel coronavirus (2019-nCoV) in vitro. *Cell Res* 2020; **30**: 269-271 [PMID: 32020029 DOI: 10.1038/s41422-020-0282-0]
48 **Agostini ML**, Andres EL, Sims AC, Graham RL, Sheahan TP, Lu X, Smith EC, Case JB, Feng JY, Jordan R, Ray AS, Cihlar T, Siegel D, Mackman RL, Clarke MO, Baric RS, Denison MR. Coronavirus Susceptibility to the Antiviral Remdesivir (GS-5734) Is Mediated by the Viral Polymerase and the Proofreading Exoribonuclease. *mBio* 2018; **9** [PMID: 29511076 DOI: 10.1128/mBio.00221-18]
49 **Gordon CJ**, Tchesnokov EP, Feng JY, Porter DP, Götte M. The antiviral compound remdesivir potently inhibits RNA-dependent RNA polymerase from Middle East respiratory syndrome coronavirus. *J Biol Chem* 2020; **295**: 4773-4779 [PMID: 32094225 DOI: 10.1074/jbc.AC120.013056]
50 **Beigel JH**, Tomashek KM, Dodd LE, Mehta AK, Zingman BS, Kalil AC, Hohmann E, Chu HY, Luetkemeyer A, Kline S, Lopez de Castilla D, Finberg RW, Dierberg K, Tapson V, Hsieh L, Patterson TF, Paredes R, Sweeney DA, Short WR, Touloumi G, Lye DC, Ohmagari N, Oh MD, Ruiz-Palacios GM, Benfield T, Fätkenheuer G, Kortepeter MG, Atmar RL, Creech CB, Lundgren J, Babiker AG, Pett S, Neaton JD, Burgess TH, Bonnett T, Green M, Makowski M, Osinusi A, Nayak S, Lane HC; ACTT-1 Study Group Members. Remdesivir for the Treatment of Covid-19 - Final Report. *N Engl J Med* 2020; **383**: 1813-1826 [PMID: 32445440 DOI: 10.1056/NEJMoa2007764]
51 **Wang Y**, Zhang D, Du G, Du R, Zhao J, Jin Y, Fu S, Gao L, Cheng Z, Lu Q, Hu Y, Luo G, Wang K, Lu Y, Li H, Wang S, Ruan S, Yang C, Mei C, Wang Y, Ding D, Wu F, Tang X, Ye X, Ye Y, Liu B, Yang J, Yin W, Wang A, Fan G, Zhou F, Liu Z, Gu X, Xu J, Shang L, Zhang Y, Cao L, Guo T, Wan Y, Qin H, Jiang Y, Jaki T, Hayden FG, Horby PW, Cao B, Wang C. Remdesivir in adults with

severe COVID-19: a randomised, double-blind, placebo-controlled, multicentre trial. *Lancet* 2020; **395**: 1569-1578 [PMID: 32423584 DOI: 10.1016/S0140-6736(20)31022-9]

52 **Goldman JD**, Lye DCB, Hui DS, Marks KM, Bruno R, Montejano R, Spinner CD, Galli M, Ahn MY, Nahass RG, Chen YS, SenGupta D, Hyland RH, Osinusi AO, Cao H, Blair C, Wei X, Gaggar A, Brainard DM, Towner WJ, Muñoz J, Mullane KM, Marty FM, Tashima KT, Diaz G, Subramanian A; GS-US-540-5773 Investigators. Remdesivir for 5 or 10 Days in Patients with Severe Covid-19. *N Engl J Med* 2020; **383**: 1827-1837 [PMID: 32459919 DOI: 10.1056/NEJMoa2015301]

53 **Spinner CD**, Gottlieb RL, Criner GJ, Arribas López JR, Cattelan AM, Soriano Viladomiu A, Ogbuagu O, Malhotra P, Mullane KM, Castagna A, Chai LYA, Roestenberg M, Tsang OTY, Bernasconi E, Le Turnier P, Chang SC, SenGupta D, Hyland RH, Osinusi AO, Cao H, Blair C, Wang H, Gaggar A, Brainard DM, McPhail MJ, Bhagani S, Ahn MY, Sanyal AJ, Huhn G, Marty FM; GS-US-540-5774 Investigators. Effect of Remdesivir *vs* Standard Care on Clinical Status at 11 Days in Patients With Moderate COVID-19: A Randomized Clinical Trial. *JAMA* 2020; **324**: 1048-1057 [PMID: 32821939 DOI: 10.1001/jama.2020.16349]

54 **Grein J**, Ohmagari N, Shin D, Diaz G, Asperges E, Castagna A, Feldt T, Green G, Green ML, Lescure FX, Nicastri E, Oda R, Yo K, Quiros-Roldan E, Studemeister A, Redinski J, Ahmed S, Bernett J, Chelliah D, Chen D, Chihara S, Cohen SH, Cunningham J, D'Arminio Monforte A, Ismail S, Kato H, Lapadula G, L'Her E, Maeno T, Majumder S, Massari M, Mora-Rillo M, Mutoh Y, Nguyen D, Verweij E, Zoufaly A, Osinusi AO, DeZure A, Zhao Y, Zhong L, Chokkalingam A, Elboudwarej E, Telep L, Timbs L, Henne I, Sellers S, Cao H, Tan SK, Winterbourne L, Desai P, Mera R, Gaggar A, Myers RP, Brainard DM, Childs R, Flanigan T. Compassionate Use of Remdesivir for Patients with Severe Covid-19. *N Engl J Med* 2020; **382**: 2327-2336 [PMID: 32275812 DOI: 10.1056/NEJMoa2007016]

55 **Antinori S**, Cossu MV, Ridolfo AL, Rech R, Bonazzetti C, Pagani G, Gubertini G, Coen M, Magni C, Castelli A, Borghi B, Colombo R, Giorgi R, Angeli E, Mileto D, Milazzo L, Vimercati S, Pellicciotta M, Corbellino M, Torre A, Rusconi S, Oreni L, Gismondo MR, Giacomelli A, Meroni L, Rizzardini G, Galli M. Compassionate remdesivir treatment of severe Covid-19 pneumonia in intensive care unit (ICU) and Non-ICU patients: Clinical outcome and differences in post-treatment hospitalisation status. *Pharmacol Res* 2020; **158**: 104899 [PMID: 32407959 DOI: 10.1016/j.phrs.2020.104899]

56 **COVID-19 Investigation Team**. Clinical and virologic characteristics of the first 12 patients with coronavirus disease 2019 (COVID-19) in the United States. *Nat Med* 2020; **26**: 861-868 [PMID: 32327757 DOI: 10.1038/s41591-020-0877-5]

57 **Durante-Mangoni E**, Andini R, Bertolino L, Mele F, Florio LL, Murino P, Corcione A, Zampino R. Early experience with remdesivir in SARS-CoV-2 pneumonia. *Infection* 2020; **48**: 779-782 [PMID: 32418190 DOI: 10.1007/s15010-020-01448-x]

58 **National Institute of Diabetes and Digestive and Kidney Diseases**. Nucleoside Analogues. In: Bethesda (MD): National Institute of Diabetes and Digestive and Kidney Diseases. LiverTox: Clinical and Research Information on Drug-Induced Liver Injury. 2020

59 **Jorgensen SCJ**, Kebriaei R, Dresser LD. Remdesivir: Review of Pharmacology, Pre-clinical Data, and Emerging Clinical Experience for COVID-19. *Pharmacotherapy* 2020; **40**: 659-671 [PMID: 32446287 DOI: 10.1002/phar.2429]

60 **U. S. Food and Drug Administration**. Fact sheet for health care providers Emergency Use Authorization (EUA) of Remdesivir (GS-5734™). [cited 1 May 2020]. In: U.S. Food and Drug Administration [Internet]. Available from: https://www.fda.gov/news-events/press-announcements/coronavirus-covid-19-update-fda-issues-emergency-use-authorization-potential-covid-19-treatment

61 **Kang JE**, Rhie SJ. Practice considerations on the use of investigational anti-COVID-19 medications: Dosage, administration and monitoring. *J Clin Pharm Ther* 2020; **45**: 1199-1205 [PMID: 32524645 DOI: 10.1111/jcpt.13199]

62 **Vincent MJ**, Bergeron E, Benjannet S, Erickson BR, Rollin PE, Ksiazek TG, Seidah NG, Nichol ST. Chloroquine is a potent inhibitor of SARS coronavirus infection and spread. *Virol J* 2005; **2**: 69 [PMID: 16115318 DOI: 10.1186/1743-422X-2-69]

63 **Fantini J**, Di Scala C, Chahinian H, Yahi N. Structural and molecular modelling studies reveal a new mechanism of action of chloroquine and hydroxychloroquine against SARS-CoV-2 infection. *Int J Antimicrob Agents* 2020; **55**: 105960 [PMID: 32251731 DOI: 10.1016/j.ijantimicag.2020.105960]

64 **Gautret P**, Lagier JC, Parola P, Hoang VT, Meddeb L, Mailhe M, Doudier B, Courjon J, Giordanengo V, Vieira VE, Tissot Dupont H, Honoré S, Colson P, Chabrière E, La Scola B, Rolain JM, Brouqui P, Raoult D. Hydroxychloroquine and azithromycin as a treatment of COVID-19: results of an open-label non-randomized clinical trial. *Int J Antimicrob Agents* 2020; **56**: 105949 [PMID: 32205204 DOI: 10.1016/j.ijantimicag.2020.105949]

65 **Chen Z**, Hu J, Zhang Z, Jiang S, Han S, Yan D, Zhuang R, Hu B. Efficacy of hydroxychloroquine in patients with COVID-19: results of a randomized clinical trial. 2020 Preprint. Available from: medRxiv:2020.03.22.20040758 [DOI: 10.1101/2020.03.22.20040758]

66 **Molina JM**, Delaugerre C, Le Goff J, Mela-Lima B, Ponscarme D, Goldwirt L, de Castro N. No evidence of rapid antiviral clearance or clinical benefit with the combination of hydroxychloroquine and azithromycin in patients with severe COVID-19 infection. *Med Mal Infect* 2020; **50**: 384

[PMID: 32240719 DOI: 10.1016/j.medmal.2020.03.006]

67 **Gautret P**, Lagier JC, Parola P, Hoang VT, Meddeb L, Sevestre J, Mailhe M, Doudier B, Aubry C, Amrane S, Seng P, Hocquart M, Eldin C, Finance J, Vieira VE, Tissot-Dupont HT, Honoré S, Stein A, Million M, Colson P, La Scola B, Veit V, Jacquier A, Deharo JC, Drancourt M, Fournier PE, Rolain JM, Brouqui P, Raoult D. Clinical and microbiological effect of a combination of hydroxychloroquine and azithromycin in 80 COVID-19 patients with at least a six-day follow up: A pilot observational study. *Travel Med Infect Dis* 2020; **34**: 101663 [PMID: 32289548 DOI: 10.1016/j.tmaid.2020.101663]

68 **Gao J**, Tian Z, Yang X. Breakthrough: Chloroquine phosphate has shown apparent efficacy in treatment of COVID-19 associated pneumonia in clinical studies. *Biosci Trends* 2020; **14**: 72-73 [PMID: 32074550 DOI: 10.5582/bst.2020.01047]

69 **Geleris J**, Sun Y, Platt J, Zucker J, Baldwin M, Hripcsak G, Labella A, Manson DK, Kubin C, Barr RG, Sobieszczyk ME, Schluger NW. Observational Study of Hydroxychloroquine in Hospitalized Patients with Covid-19. *N Engl J Med* 2020; **382**: 2411-2418 [PMID: 32379955 DOI: 10.1056/NEJMoa2012410]

70 **Cavalcanti AB**, Zampieri FG, Rosa RG, Azevedo LCP, Veiga VC, Avezum A, Damiani LP, Marcadenti A, Kawano-Dourado L, Lisboa T, Junqueira DLM, de Barros E Silva PGM, Tramujas L, Abreu-Silva EO, Laranjeira LN, Soares AT, Echenique LS, Pereira AJ, Freitas FGR, Gebara OCE, Dantas VCS, Furtado RHM, Milan EP, Golin NA, Cardoso FF, Maia IS, Hoffmann Filho CR, Kormann APM, Amazonas RB, Bocchi de Oliveira MF, Serpa-Neto A, Falavigna M, Lopes RD, Machado FR, Berwanger O; Coalition Covid-19 Brazil I Investigators. Hydroxychloroquine with or without Azithromycin in Mild-to-Moderate Covid-19. *N Engl J Med* 2020; **383**: 2041-2052 [PMID: 32706953 DOI: 10.1056/NEJMoa2019014]

71 **Boulware DR**, Pullen MF, Bangdiwala AS, Pastick KA, Lofgren SM, Okafor EC, Skipper CP, Nascene AA, Nicol MR, Abassi M, Engen NW, Cheng MP, LaBar D, Lother SA, MacKenzie LJ, Drobot G, Marten N, Zarychanski R, Kelly LE, Schwartz IS, McDonald EG, Rajasingham R, Lee TC, Hullsiek KH. A Randomized Trial of Hydroxychloroquine as Postexposure Prophylaxis for Covid-19. *N Engl J Med* 2020; **383**: 517-525 [PMID: 32492293 DOI: 10.1056/NEJMoa2016638]

72 **Drent M**, Proesmans VLJ, Elfferich MDP, Jessurun NT, de Jong SMG, Ebner NM, Lewis EDO, Bast A. Ranking Self-reported Gastrointestinal Side Effects of Pharmacotherapy in Sarcoidosis. *Lung* 2020; **198**: 395-403 [PMID: 31960165 DOI: 10.1007/s00408-020-00323-8]

73 **Srinivasa A**, Tosounidou S, Gordon C. Increased Incidence of Gastrointestinal Side Effects in Patients Taking Hydroxychloroquine: A Brand-related Issue? *J Rheumatol* 2017; **44**: 398 [PMID: 28250164 DOI: 10.3899/jrheum.161063]

74 **Tétu P**, Hamelin A, Lebrun-Vignes B, Soria A, Barbaud A, Francès C, Chasset F. [Prevalence of hydroxychloroquine-induced side-effects in dermatology patients: A retrospective survey of 102 patients]. *Ann Dermatol Venereol* 2018; **145**: 395-404 [PMID: 29792286 DOI: 10.1016/j.annder.2018.03.168]

75 **Arnaout A**, Robertson SJ, Pond GR, Lee H, Jeong A, Ianni L, Kroeger L, Hilton J, Coupland S, Gottlieb C, Hurley B, McCarthy A, Clemons M. A randomized, double-blind, window of opportunity trial evaluating the effects of chloroquine in breast cancer patients. *Breast Cancer Res Treat* 2019; **178**: 327-335 [PMID: 31392517 DOI: 10.1007/s10549-019-05381-y]

76 **Furst DE**, Lindsley H, Baethge B, Botstein GR, Caldwell J, Dietz F, Ettlinger R, Golden HE, McLaughlin GE, Moreland LW, Roberts WN, Rooney TW, Rothschild B, Sack M, Sebba AI, Weisman M, Welch KE, Yocum D. Dose-loading with hydroxychloroquine improves the rate of response in early, active rheumatoid arthritis: a randomized, double-blind six-week trial with eighteen-week extension. *Arthritis Rheum* 1999; **42**: 357-365 [PMID: 10025931 DOI: 10.1002/1529-0131(199902)42:2<357::AID-ANR19>3.0.CO;2-J]

77 **Arshad S**, Kilgore P, Chaudhry ZS, Jacobsen G, Wang DD, Huitsing K, Brar I, Alangaden GJ, Ramesh MS, McKinnon JE, O'Neill W, Zervos M; Henry Ford COVID-19 Task Force. Treatment with hydroxychloroquine, azithromycin, and combination in patients hospitalized with COVID-19. *Int J Infect Dis* 2020; **97**: 396-403 [PMID: 32623082 DOI: 10.1016/j.ijid.2020.06.099]

78 **Hung IF**, Lung KC, Tso EY, Liu R, Chung TW, Chu MY, Ng YY, Lo J, Chan J, Tam AR, Shum HP, Chan V, Wu AK, Sin KM, Leung WS, Law WL, Lung DC, Sin S, Yeung P, Yip CC, Zhang RR, Fung AY, Yan EY, Leung KH, Ip JD, Chu AW, Chan WM, Ng AC, Lee R, Fung K, Yeung A, Wu TC, Chan JW, Yan WW, Chan JF, Lie AK, Tsang OT, Cheng VC, Que TL, Lau CS, Chan KH, To KK, Yuen KY. Triple combination of interferon beta-1b, lopinavir-ritonavir, and ribavirin in the treatment of patients admitted to hospital with COVID-19: an open-label, randomised, phase 2 trial. *Lancet* 2020; **395**: 1695-1704 [PMID: 32401715 DOI: 10.1016/S0140-6736(20)31042-4]

79 **Dong L**, Hu S, Gao J. Discovering drugs to treat coronavirus disease 2019 (COVID-19). *Drug Discov Ther* 2020; **14**: 58-60 [PMID: 32147628 DOI: 10.5582/ddt.2020.01012]

80 **Chen C**, Zhang Y, Huang J, Yin P, Cheng Z, Wu J, Chen S, Chen Bo, Lu M, Luo Y, Ju L, Zhang J, Wang X. Favipiravir vs arbidol for COVID-19: a randomized clinical trial. 2020 Preprint. Available from: medRxiv:2020.03.17.20037432 [DOI: 10.1101/2020.03.17.20037432]

81 **Cai Q**, Yang M, Liu D, Chen J, Shu D, Xia J, Liao X, Gu Y, Cai Q, Yang Y, Shen C, Li X, Peng L, Huang D, Zhang J, Zhang S, Wang F, Liu J, Chen L, Chen S, Wang Z, Zhang Z, Cao R, Zhong W, Liu Y, Liu L. Experimental Treatment with Favipiravir for COVID-19: An Open-Label Control Study. *Engineering (Beijing)* 2020; **6**: 1192-1198 [PMID: 32346491 DOI: 10.1016/j.eng.2020.03.007]

82 **Rocco PRM**, Silva PL, Cruz FF, Melo-Junior MAC, Tierno PFGMM, Moura MA, De Oliveira LFG, Lima CC, Dos Santos EA, Junior WF, Fernandes APSM, Franchini KG, Magri E, de Moraes NF, Gonçalves JMJ, Carbonieri MN, Dos Santos IS, Paes NF, Maciel PVM, Rocha RP, de Carvalho AF, Alves PA, Proença-Módena JL, Cordeiro AT, Trivella DBB, Marques RE, Luiz RR, Pelosi P, Lapa E Silva JR; SARITA-2 investigators. Early use of nitazoxanide in mild COVID-19 disease: randomised, placebo-controlled trial. *Eur Respir J* 2021; **58** [PMID: 33361100 DOI: 10.1183/13993003.03725-2020]

83 **Caly L**, Druce JD, Catton MG, Jans DA, Wagstaff KM. The FDA-approved drug ivermectin inhibits the replication of SARS-CoV-2 in vitro. *Antiviral Res* 2020; **178**: 104787 [PMID: 32251768 DOI: 10.1016/j.antiviral.2020.104787]

84 **Elalfy H**, Besheer T, El-Mesery A, El-Gilany AH, Soliman MA, Alhawarey A, Alegezy M, Elhadidy T, Hewidy AA, Zaghloul H, Neamatallah MAM, Raafat D, El-Emshaty WM, Abo El Kheir NY, El-Bendary M. Effect of a combination of nitazoxanide, ribavirin, and ivermectin plus zinc supplement (MANS.NRIZ study) on the clearance of mild COVID-19. *J Med Virol* 2021; **93**: 3176-3183 [PMID: 33590901 DOI: 10.1002/jmv.26880]

85 **Rajter JC**, Sherman MS, Fatteh N, Vogel F, Sacks J, Rajter JJ. Use of Ivermectin Is Associated With Lower Mortality in Hospitalized Patients With Coronavirus Disease 2019: The Ivermectin in COVID Nineteen Study. *Chest* 2021; **159**: 85-92 [PMID: 33065103 DOI: 10.1016/j.chest.2020.10.009]

86 **Fischer W**, Eron JJ, Holman W, Cohen MS, Fang L, Szewczyk LJ, Sheahan TP, Baric R, Mollan KR, Wolfe CR, Duke ER, Azizad MM, Borroto-Esoda K, Wohl DA, Loftis AJ, Alabanza P, Lipansky F, Painter WP. Molnupiravir, an Oral Antiviral Treatment for COVID-19. *medRxiv* 2021 [PMID: 34159342 DOI: 10.1101/2021.06.17.21258639]

87 **Azkur AK**, Akdis M, Azkur D, Sokolowska M, van de Veen W, Brüggen MC, O'Mahony L, Gao Y, Nadeau K, Akdis CA. Immune response to SARS-CoV-2 and mechanisms of immunopathological changes in COVID-19. *Allergy* 2020; **75**: 1564-1581 [PMID: 32396996 DOI: 10.1111/all.14364]

88 **Conti P**, Ronconi G, Caraffa A, Gallenga CE, Ross R, Frydas I, Kritas SK. Induction of pro-inflammatory cytokines (IL-1 and IL-6) and lung inflammation by Coronavirus-19 (COVI-19 or SARS-CoV-2): anti-inflammatory strategies. *J Biol Regul Homeost Agents* 2020; **34**: 327-331 [PMID: 32171193 DOI: 10.23812/CONTI-E]

89 **Wu J**. Tackle the free radicals damage in COVID-19. *Nitric Oxide* 2020; **102**: 39-41 [PMID: 32562746 DOI: 10.1016/j.niox.2020.06.002]

90 **Huppert LA**, Matthay MA, Ware LB. Pathogenesis of Acute Respiratory Distress Syndrome. *Semin Respir Crit Care Med* 2019; **40**: 31-39 [PMID: 31060086 DOI: 10.1055/s-0039-1683996]

91 **Chen G**, Wu D, Guo W, Cao Y, Huang D, Wang H, Wang T, Zhang X, Chen H, Yu H, Zhang M, Wu S, Song J, Chen T, Han M, Li S, Luo X, Zhao J, Ning Q. Clinical and immunological features of severe and moderate coronavirus disease 2019. *J Clin Invest* 2020; **130**: 2620-2629 [PMID: 32217835 DOI: 10.1172/JCI137244]

92 **Liu J**, Li S, Liu J, Liang B, Wang X, Wang H, Li W, Tong Q, Yi J, Zhao L, Xiong L, Guo C, Tian J, Luo J, Yao J, Pang R, Shen H, Peng C, Liu T, Zhang Q, Wu J, Xu L, Lu S, Wang B, Weng Z, Han C, Zhu H, Zhou R, Zhou H, Chen X, Ye P, Zhu B, Wang L, Zhou W, He S, He Y, Jie S, Wei P, Zhang J, Lu Y, Wang W, Zhang L, Li L, Zhou F, Wang J, Dittmer U, Lu M, Hu Y, Yang D, Zheng X. Longitudinal characteristics of lymphocyte responses and cytokine profiles in the peripheral blood of SARS-CoV-2 infected patients. *EBioMedicine* 2020; **55**: 102763 [PMID: 32361250 DOI: 10.1016/j.ebiom.2020.102763]

93 **Gao Y**, Li T, Han M, Li X, Wu D, Xu Y, Zhu Y, Liu Y, Wang X, Wang L. Diagnostic utility of clinical laboratory data determinations for patients with the severe COVID-19. *J Med Virol* 2020; **92**: 791-796 [PMID: 32181911 DOI: 10.1002/jmv.25770]

94 **Ye Q**, Wang B, Mao J. The pathogenesis and treatment of the `Cytokine Storm' in COVID-19. *J Infect* 2020; **80**: 607-613 [PMID: 32283152 DOI: 10.1016/j.jinf.2020.03.037]

95 **Tang Y**, Liu J, Zhang D, Xu Z, Ji J, Wen C. Cytokine Storm in COVID-19: The Current Evidence and Treatment Strategies. *Front Immunol* 2020; **11**: 1708 [PMID: 32754163 DOI: 10.3389/fimmu.2020.01708]

96 **Xiang Z**, Liu J, Shi D, Chen W, Li J, Yan R, Bi Y, Hu W, Zhu Z, Yu Y, Yang Z. Glucocorticoids improve severe or critical COVID-19 by activating ACE2 and reducing IL-6 Levels. *Int J Biol Sci* 2020; **16**: 2382-2391 [PMID: 32760206 DOI: 10.7150/ijbs.47652]

97 **RECOVERY Collaborative Group**, Horby P, Lim WS, Emberson JR, Mafham M, Bell JL, Linsell L, Staplin N, Brightling C, Ustianowski A, Elmahi E, Prudon B, Green C, Felton T, Chadwick D, Rege K, Fegan C, Chappell LC, Faust SN, Jaki T, Jeffery K, Montgomery A, Rowan K, Juszczak E, Baillie JK, Haynes R, Landray MJ. Dexamethasone in Hospitalized Patients with Covid-19. *N Engl J Med* 2021; **384**: 693-704 [PMID: 32678530 DOI: 10.1056/NEJMoa2021436]

98 **Ledford H**. Coronavirus breakthrough: dexamethasone is first drug shown to save lives. *Nature* 2020; **582**: 469 [PMID: 32546811 DOI: 10.1038/d41586-020-01824-5]

99 **WHO Rapid Evidence Appraisal for COVID-19 Therapies (REACT) Working Group**, Sterne JAC, Murthy S, Diaz JV, Slutsky AS, Villar J, Angus DC, Annane D, Azevedo LCP, Berwanger O, Cavalcanti AB, Dequin PF, Du B, Emberson J, Fisher D, Giraudeau B, Gordon AC, Granholm A, Green C, Haynes R, Heming N, Higgins JPT, Horby P, Jüni P, Landray MJ, Le Gouge A, Leclerc M, Lim WS, Machado FR, McArthur C, Meziani F, Møller MH, Perner A, Petersen MW, Savovic J, Tomazini B, Veiga VC, Webb S, Marshall JC. Association Between Administration of Systemic

Corticosteroids and Mortality Among Critically Ill Patients With COVID-19: A Meta-analysis. *JAMA* 2020; **324**: 1330-1341 [PMID: 32876694 DOI: 10.1001/jama.2020.17023]

100 **Russell CD**, Millar JE, Baillie JK. Clinical evidence does not support corticosteroid treatment for 2019-nCoV lung injury. *Lancet* 2020; **395**: 473-475 [PMID: 32043983 DOI: 10.1016/S0140-6736(20)30317-2]

101 **Lee DT**, Wing YK, Leung HC, Sung JJ, Ng YK, Yiu GC, Chen RY, Chiu HF. Factors associated with psychosis among patients with severe acute respiratory syndrome: a case-control study. *Clin Infect Dis* 2004; **39**: 1247-1249 [PMID: 15486852 DOI: 10.1086/424016]

102 **Xiao JZ**, Ma L, Gao J, Yang ZJ, Xing XY, Zhao HC, Jiao JS, Li GW. [Glucocorticoid-induced diabetes in severe acute respiratory syndrome: the impact of high dosage and duration of methylprednisolone therapy]. *Zhonghua Nei Ke Za Zhi* 2004; **43**: 179-182 [PMID: 15059370]

103 **Arabi YM**, Mandourah Y, Al-Hameed F, Sindi AA, Almekhlafi GA, Hussein MA, Jose J, Pinto R, Al-Omari A, Kharaba A, Almotairi A, Al Khatib K, Alraddadi B, Shalhoub S, Abdulmomen A, Qushmaq I, Mady A, Solaiman O, Al-Aithan AM, Al-Raddadi R, Ragab A, Balkhy HH, Al Harthy A, Deeb AM, Al Mutairi H, Al-Dawood A, Merson L, Hayden FG, Fowler RA; Saudi Critical Care Trial Group. Corticosteroid Therapy for Critically Ill Patients with Middle East Respiratory Syndrome. *Am J Respir Crit Care Med* 2018; **197**: 757-767 [PMID: 29161116 DOI: 10.1164/rccm.201706-1172OC]

104 **Yang Z**, Liu J, Zhou Y, Zhao X, Zhao Q. The effect of corticosteroid treatment on patients with coronavirus infection: a systematic review and meta-analysis. *J Infect* 2020; **81**: e13-e20 [PMID: 32283144 DOI: 10.1016/j.jinf.2020.03.062]

105 **Piper JM**, Ray WA, Daugherty JR, Griffin MR. Corticosteroid use and peptic ulcer disease: role of nonsteroidal anti-inflammatory drugs. *Ann Intern Med* 1991; **114**: 735-740 [PMID: 2012355 DOI: 10.7326/0003-4819-114-9-735]

106 **Gabriel SE**, Jaakkimainen L, Bombardier C. Risk for serious gastrointestinal complications related to use of nonsteroidal anti-inflammatory drugs. A meta-analysis. *Ann Intern Med* 1991; **115**: 787-796 [PMID: 1834002 DOI: 10.7326/0003-4819-115-10-787]

107 **Caplan A**, Fett N, Rosenbach M, Werth VP, Micheletti RG. Prevention and management of glucocorticoid-induced side effects: A comprehensive review: Gastrointestinal and endocrinologic side effects. *J Am Acad Dermatol* 2017; **76**: 11-16 [PMID: 27986133 DOI: 10.1016/j.jaad.2016.02.1239]

108 **Sadr-Azodi O**, Mattsson F, Bexlius TS, Lindblad M, Lagergren J, Ljung R. Association of oral glucocorticoid use with an increased risk of acute pancreatitis: a population-based nested case-control study. *JAMA Intern Med* 2013; **173**: 444-449 [PMID: 23440105 DOI: 10.1001/jamainternmed.2013.2737]

109 **Woods CP**, Hazlehurst JM, Tomlinson JW. Glucocorticoids and non-alcoholic fatty liver disease. *J Steroid Biochem Mol Biol* 2015; **154**: 94-103 [PMID: 26241028 DOI: 10.1016/j.jsbmb.2015.07.020]

110 **Yki-Järvinen H**. Non-alcoholic fatty liver disease as a cause and a consequence of metabolic syndrome. *Lancet Diabetes Endocrinol* 2014; **2**: 901-910 [PMID: 24731669 DOI: 10.1016/S2213-8587(14)70032-4]

111 **Hu Y**, Sun J, Dai Z, Deng H, Li X, Huang Q, Wu Y, Sun L, Xu Y. Prevalence and severity of corona virus disease 2019 (COVID-19): A systematic review and meta-analysis. *J Clin Virol* 2020; **127**: 104371 [PMID: 32315817 DOI: 10.1016/j.jcv.2020.104371]

112 **Muniyappa R**, Gubbi S. COVID-19 pandemic, coronaviruses, and diabetes mellitus. *Am J Physiol Endocrinol Metab* 2020; **318**: E736-E741 [PMID: 32228322 DOI: 10.1152/ajpendo.00124.2020]

113 **Simonnet A**, Chetboun M, Poissy J, Raverdy V, Noulette J, Duhamel A, Labreuche J, Mathieu D, Pattou F, Jourdain M; LICORN and the Lille COVID-19 and Obesity study group. High Prevalence of Obesity in Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) Requiring Invasive Mechanical Ventilation. *Obesity (Silver Spring)* 2020; **28**: 1195-1199 [PMID: 32271993 DOI: 10.1002/oby.22831]

114 **Yang J**, Hu J, Zhu C. Obesity aggravates COVID-19: A systematic review and meta-analysis. *J Med Virol* 2021; **93**: 257-261 [PMID: 32603481 DOI: 10.1002/jmv.26237]

115 **Zhou Y**, Chi J, Lv W, Wang Y. Obesity and diabetes as high-risk factors for severe coronavirus disease 2019 (Covid-19). *Diabetes Metab Res Rev* 2021; **37**: e3377 [PMID: 32588943 DOI: 10.1002/dmrr.3377]

116 **Ragab D**, Salah Eldin H, Taeimah M, Khattab R, Salem R. The COVID-19 Cytokine Storm; What We Know So Far. *Front Immunol* 2020; **11**: 1446 [PMID: 32612617 DOI: 10.3389/fimmu.2020.01446]

117 **Coomes EA**, Haghbayan H. Interleukin-6 in Covid-19: A systematic review and meta-analysis. *Rev Med Virol* 2020; **30**: 1-9 [PMID: 32845568 DOI: 10.1002/rmv.2141]

118 **Guaraldi G**, Meschiari M, Cozzi-Lepri A, Milic J, Tonelli R, Menozzi M, Franceschini E, Cuomo G, Orlando G, Borghi V, Santoro A, Di Gaetano M, Puzzolante C, Carli F, Bedini A, Corradi L, Fantini R, Castaniere I, Tabbì L, Girardis M, Tedeschi S, Giannella M, Bartoletti M, Pascale R, Dolci G, Brugioni L, Pietrangelo A, Cossarizza A, Pea F, Clini E, Salvarani C, Massari M, Viale PL, Mussini C. Tocilizumab in patients with severe COVID-19: a retrospective cohort study. *Lancet Rheumatol* 2020; **2**: e474-e484 [PMID: 32835257 DOI: 10.1016/S2665-9913(20)30173-9]

119 **Gupta S**, Wang W, Hayek SS, Chan L, Mathews KS, Melamed ML, Brenner SK, Leonberg-Yoo A, Schenck EJ, Radbel J, Reiser J, Bansal A, Srivastava A, Zhou Y, Finkel D, Green A, Mallappallil M, Faugno AJ, Zhang J, Velez JCQ, Shaefi S, Parikh CR, Charytan DM, Athavale AM, Friedman AN,

Redfern RE, Short SAP, Correa S, Pokharel KK, Admon AJ, Donnelly JP, Gershengorn HB, Douin DJ, Semler MW, Hernán MA, Leaf DE; STOP-COVID Investigators. Association Between Early Treatment With Tocilizumab and Mortality Among Critically Ill Patients With COVID-19. *JAMA Intern Med* 2021; **181**: 41-51 [PMID: 33080002 DOI: 10.1001/jamainternmed.2020.6252]

120 **Stone JH**, Frigault MJ, Serling-Boyd NJ, Fernandes AD, Harvey L, Foulkes AS, Horick NK, Healy BC, Shah R, Bensaci AM, Woolley AE, Nikiforow S, Lin N, Sagar M, Schrager H, Huckins DS, Axelrod M, Pincus MD, Fleisher J, Sacks CA, Dougan M, North CM, Halvorsen YD, Thurber TK, Dagher Z, Scherer A, Wallwork RS, Kim AY, Schoenfeld S, Sen P, Neilan TG, Perugino CA, Unizony SH, Collier DS, Matza MA, Yinh JM, Bowman KA, Meyerowitz E, Zafar A, Drobni ZD, Bolster MB, Kohler M, D'Silva KM, Dau J, Lockwood MM, Cubbison C, Weber BN, Mansour MK; BACC Bay Tocilizumab Trial Investigators. Efficacy of Tocilizumab in Patients Hospitalized with Covid-19. *N Engl J Med* 2020; **383**: 2333-2344 [PMID: 33085857 DOI: 10.1056/NEJMoa2028836]

121 **Muhović D**, Bojović J, Bulatović A, Vukčević B, Ratković M, Lazović R, Smolović B. First case of drug-induced liver injury associated with the use of tocilizumab in a patient with COVID-19. *Liver Int* 2020; **40**: 1901-1905 [PMID: 32478465 DOI: 10.1111/liv.14516]

122 **Kalil AC**, Patterson TF, Mehta AK, Tomashek KM, Wolfe CR, Ghazaryan V, Marconi VC, Ruiz-Palacios GM, Hsieh L, Kline S, Tapson V, Iovine NM, Jain MK, Sweeney DA, El Sahly HM, Branche AR, Regalado Pineda J, Lye DC, Sandkovsky U, Luetkemeyer AF, Cohen SH, Finberg RW, Jackson PEH, Taiwo B, Paules CI, Arguinchona H, Erdmann N, Ahuja N, Frank M, Oh MD, Kim ES, Tan SY, Mularski RA, Nielsen H, Ponce PO, Taylor BS, Larson L, Rouphael NG, Saklawi Y, Cantos VD, Ko ER, Engemann JJ, Amin AN, Watanabe M, Billings J, Elie MC, Davey RT, Burgess TH, Ferreira J, Green M, Makowski M, Cardoso A, de Bono S, Bonnett T, Proschan M, Deye GA, Dempsey W, Nayak SU, Dodd LE, Beigel JH; ACTT-2 Study Group Members. Baricitinib plus Remdesivir for Hospitalized Adults with Covid-19. *N Engl J Med* 2021; **384**: 795-807 [PMID: 33306283 DOI: 10.1056/NEJMoa2031994]

123 **Walsh EE**, Frenck RW Jr, Falsey AR, Kitchin N, Absalon J, Gurtman A, Lockhart S, Neuzil K, Mulligan MJ, Bailey R, Swanson KA, Li P, Koury K, Kalina W, Cooper D, Fontes-Garfias C, Shi PY, Türeci Ö, Tompkins KR, Lyke KE, Raabe V, Dormitzer PR, Jansen KU, Şahin U, Gruber WC. Safety and Immunogenicity of Two RNA-Based Covid-19 Vaccine Candidates. *N Engl J Med* 2020; **383**: 2439-2450 [PMID: 33053279 DOI: 10.1056/NEJMoa2027906]

124 **Polack FP**, Thomas SJ, Kitchin N, Absalon J, Gurtman A, Lockhart S, Perez JL, Pérez Marc G, Moreira ED, Zerbini C, Bailey R, Swanson KA, Roychoudhury S, Koury K, Li P, Kalina WV, Cooper D, Frenck RW Jr, Hammitt LL, Türeci Ö, Nell H, Schaefer A, Ünal S, Tresnan DB, Mather S, Dormitzer PR, Şahin U, Jansen KU, Gruber WC; C4591001 Clinical Trial Group. Safety and Efficacy of the BNT162b2 mRNA Covid-19 Vaccine. *N Engl J Med* 2020; **383**: 2603-2615 [PMID: 33301246 DOI: 10.1056/NEJMoa2034577]

125 **Baden LR**, El Sahly HM, Essink B, Kotloff K, Frey S, Novak R, Diemert D, Spector SA, Rouphael N, Creech CB, McGettigan J, Khetan S, Segall N, Solis J, Brosz A, Fierro C, Schwartz H, Neuzil K, Corey L, Gilbert P, Janes H, Follmann D, Marovich M, Mascola J, Polakowski L, Ledgerwood J, Graham BS, Bennett H, Pajon R, Knightly C, Leav B, Deng W, Zhou H, Han S, Ivarsson M, Miller J, Zaks T; COVE Study Group. Efficacy and Safety of the mRNA-1273 SARS-CoV-2 Vaccine. *N Engl J Med* 2021; **384**: 403-416 [PMID: 33378609 DOI: 10.1056/NEJMoa2035389]

126 **Voysey M**, Clemens SAC, Madhi SA, Weckx LY, Folegatti PM, Aley PK, Angus B, Baillie VL, Barnabas SL, Bhorat QE, Bibi S, Briner C, Cicconi P, Collins AM, Colin-Jones R, Cutland CL, Darton TC, Dheda K, Duncan CJA, Emary KRW, Ewer KJ, Fairlie L, Faust SN, Feng S, Ferreira DM, Finn A, Goodman AL, Green CM, Green CA, Heath PT, Hill C, Hill H, Hirsch I, Hodgson SHC, Izu A, Jackson S, Jenkin D, Joe CCD, Kerridge S, Koen A, Kwatra G, Lazarus R, Lawrie AM, Lelliott A, Libri V, Lillie PJ, Mallory R, Mendes AVA, Milan EP, Minassian AM, McGregor A, Morrison H, Mujadidi YF, Nana A, O'Reilly PJ, Padayachee SD, Pittella A, Plested E, Pollock KM, Ramasamy MN, Rhead S, Schwarzbold AV, Singh N, Smith A, Song R, Snape MD, Sprinz E, Sutherland RK, Tarrant R, Thomson EC, Török ME, Toshner M, Turner DPJ, Vekemans J, Villafana TL, Watson MEE, Williams CJ, Douglas AD, Hill AVS, Lambe T, Gilbert SC, Pollard AJ; Oxford COVID Vaccine Trial Group. Safety and efficacy of the ChAdOx1 nCoV-19 vaccine (AZD1222) against SARS-CoV-2: an interim analysis of four randomised controlled trials in Brazil, South Africa, and the UK. *Lancet* 2021; **397**: 99-111 [PMID: 33306989 DOI: 10.1016/S0140-6736(20)32661-1]

127 **Sadoff J**, Le Gars M, Shukarev G, Heerwegh D, Truyers C, de Groot AM, Stoop J, Tete S, Van Damme W, Leroux-Roels I, Berghmans PJ, Kimmel M, Van Damme P, de Hoon J, Smith W, Stephenson KE, De Rosa SC, Cohen KW, McElrath MJ, Cormier E, Scheper G, Barouch DH, Hendriks J, Struyf F, Douoguih M, Van Hoof J, Schuitemaker H. Interim Results of a Phase 1-2a Trial of Ad26.COV2.S Covid-19 Vaccine. *N Engl J Med* 2021; **384**: 1824-1835 [PMID: 33440088 DOI: 10.1056/NEJMoa2034201]

128 **Scully M**, Singh D, Lown R, Poles A, Solomon T, Levi M, Goldblatt D, Kotoucek P, Thomas W, Lester W. Pathologic Antibodies to Platelet Factor 4 after ChAdOx1 nCoV-19 Vaccination. *N Engl J Med* 2021; **384**: 2202-2211 [PMID: 33861525 DOI: 10.1056/NEJMoa2105385]

129 **Zhang Y**, Zeng G, Pan H, Li C, Hu Y, Chu K, Han W, Chen Z, Tang R, Yin W, Chen X, Liu X, Jiang C, Li J, Yang M, Song Y, Wang X, Gao Q, Zhu F. Safety, tolerability, and immunogenicity of an inactivated SARS-CoV-2 vaccine in healthy adults aged 18-59 years: a randomised, double-blind, placebo-controlled, phase 1/2 clinical trial. *Lancet Infect Dis* 2021; **21**: 181-192 [PMID: 33217362

DOI: 10.1016/S1473-3099(20)30843-4]

130 **Wu Z**, Hu Y, Xu M, Chen Z, Yang W, Jiang Z, Li M, Jin H, Cui G, Chen P, Wang L, Zhao G, Ding Y, Zhao Y, Yin W. Safety, tolerability, and immunogenicity of an inactivated SARS-CoV-2 vaccine (CoronaVac) in healthy adults aged 60 years and older: a randomised, double-blind, placebo-controlled, phase 1/2 clinical trial. *Lancet Infect Dis* 2021; **21**: 803-812 [PMID: 33548194 DOI: 10.1016/S1473-3099(20)30987-7]

131 **Palacios R**, Patiño EG, de Oliveira Piorelli R, Conde MTRP, Batista AP, Zeng G, Xin Q, Kallas EG, Flores J, Ockenhouse CF, Gast C. Double-Blind, Randomized, Placebo-Controlled Phase III Clinical Trial to Evaluate the Efficacy and Safety of treating Healthcare Professionals with the Adsorbed COVID-19 (Inactivated) Vaccine Manufactured by Sinovac - PROFISCOV: A structured summary of a study protocol for a randomised controlled trial. *Trials* 2020; **21**: 853 [PMID: 33059771 DOI: 10.1186/s13063-020-04775-4]

132 **Xia S**, Zhang Y, Wang Y, Wang H, Yang Y, Gao GF, Tan W, Wu G, Xu M, Lou Z, Huang W, Xu W, Huang B, Wang W, Zhang W, Li N, Xie Z, Ding L, You W, Zhao Y, Yang X, Liu Y, Wang Q, Huang L, Xu G, Luo B, Liu P, Guo W. Safety and immunogenicity of an inactivated SARS-CoV-2 vaccine, BBIBP-CorV: a randomised, double-blind, placebo-controlled, phase 1/2 trial. *Lancet Infect Dis* 2021; **21**: 39-51 [PMID: 33069281 DOI: 10.1016/S1473-3099(20)30831-8]

133 **McMahon BJ**, Wainwright K, Bulkow L, Parkinson AJ, Lindenbaum M, Wainwright R, Helminiak C. Response to hepatitis B vaccine in Alaska natives with chronic alcoholism compared with non-alcoholic control subjects. *Am J Med* 1990; **88**: 460-464 [PMID: 2140010 DOI: 10.1016/0002-9343(90)90423-b]

134 **European Association for the Study of the Liver**. EASL Clinical Practice Guidelines for the management of patients with decompensated cirrhosis. *J Hepatol* 2018; **69**: 406-460 [PMID: 29653741 DOI: 10.1016/j.jhep.2018.03.024]

135 **Dagan N**, Barda N, Kepten E, Miron O, Perchik S, Katz MA, Hernán MA, Lipsitch M, Reis B, Balicer RD. BNT162b2 mRNA Covid-19 Vaccine in a Nationwide Mass Vaccination Setting. *N Engl J Med* 2021; **384**: 1412-1423 [PMID: 33626250 DOI: 10.1056/NEJMoa2101765]

136 **Barda N**, Dagan N, Balicer RD. BNT162b2 mRNA Covid-19 Vaccine in a Nationwide Mass Vaccination Setting. Reply. *N Engl J Med* 2021; **384**: 1970 [PMID: 33882227 DOI: 10.1056/NEJMc2104281]

137 **Sharma A**, Patnaik I, Kumar A, Gupta R. COVID-19 vaccines in patients with chronic liver disease. *J Clin Exp Hepatol* 2021 [PMID: 34177192 DOI: 10.1016/j.jceh.2021.06.013]

138 **Cornberg M**, Buti M, Eberhardt CS, Grossi PA, Shouval D. EASL position paper on the use of COVID-19 vaccines in patients with chronic liver diseases, hepatobiliary cancer and liver transplant recipients. *J Hepatol* 2021; **74**: 944-951 [PMID: 33563499 DOI: 10.1016/j.jhep.2021.01.032]

139 **Fix OK**, Blumberg EA, Chang KM, Chu J, Chung RT, Goacher EK, Hameed B, Kaul DR, Kulik LM, Kwok RM, McGuire BM, Mulligan DC, Price JC, Reau NS, Reddy KR, Reynolds A, Rosen HR, Russo MW, Schilsky ML, Verna EC, Ward JW, Fontana RJ; AASLD COVID-19 Vaccine Working Group. American Association for the Study of Liver Diseases Expert Panel Consensus Statement: Vaccines to Prevent Coronavirus Disease 2019 Infection in Patients With Liver Disease. *Hepatology* 2021; **74**: 1049-1064 [PMID: 33577086 DOI: 10.1002/hep.31751]

140 **Than NN**, Hodson J, Schmidt-Martin D, Taubert R, Wawman RE, Botter M, Gautam N, Bock K, Jones R, Appanna GD, Godkin A, Montano-Loza AJ, Lammert F, Schramm C, Manns MP, Swain M, Burak KW, Adams DH, Hirschfield GM, Oo YH. Efficacy of rituximab in difficult-to-manage autoimmune hepatitis: Results from the International Autoimmune Hepatitis Group. *JHEP Rep* 2019; **1**: 437-445 [PMID: 32039395 DOI: 10.1016/j.jhepr.2019.10.005]

141 **Yri OE**, Torfoss D, Hungnes O, Tierens A, Waalen K, Nordøy T, Dudman S, Kilander A, Wader KF, Ostenstad B, Ekanger R, Meyer P, Kolstad A. Rituximab blocks protective serologic response to influenza A (H1N1) 2009 vaccination in lymphoma patients during or within 6 mo after treatment. *Blood* 2011; **118**: 6769-6771 [PMID: 22058114 DOI: 10.1182/blood-2011-08-372649]

142 **Bedognetti D**, Zoppoli G, Massucco C, Zanardi E, Zupo S, Bruzzone A, Sertoli MR, Balleari E, Racchi O, Messina M, Caltabiano G, Icardi G, Durando P, Marincola FM, Boccardo F, Ferrarini M, Ansaldi F, De Maria A. Impaired response to influenza vaccine associated with persistent memory B cell depletion in non-Hodgkin's lymphoma patients treated with rituximab-containing regimens. *J Immunol* 2011; **186**: 6044-6055 [PMID: 21498665 DOI: 10.4049/jimmunol.1004095]

143 **Takata T**, Suzumiya J, Ishikawa T, Takamatsu Y, Ikematsu H, Tamura K. Attenuated antibody reaction for the primary antigen but not for the recall antigen of influenza vaccination in patients with non-Hodgkin B-cell lymphoma after the administration of rituximab-CHOP. *J Clin Exp Hematop* 2009; **49**: 9-13 [PMID: 19474512 DOI: 10.3960/jslrt.49.9]

144 **van der Kolk LE**, Baars JW, Prins MH, van Oers MH. Rituximab treatment results in impaired secondary humoral immune responsiveness. *Blood* 2002; **100**: 2257-2259 [PMID: 12200395]

145 **Bingham CO 3rd**, Looney RJ, Deodhar A, Halsey N, Greenwald M, Codding C, Trzaskoma B, Martin F, Agarwal S, Kelman A. Immunization responses in rheumatoid arthritis patients treated with rituximab: results from a controlled clinical trial. *Arthritis Rheum* 2010; **62**: 64-74 [PMID: 20039397 DOI: 10.1002/art.25034]

146 **Kim W**, Kim SH, Huh SY, Kong SY, Choi YJ, Cheong HJ, Kim HJ. Reduced antibody formation after influenza vaccination in patients with neuromyelitis optica spectrum disorder treated with rituximab. *Eur J Neurol* 2013; **20**: 975-980 [PMID: 23521577 DOI: 10.1111/ene.12132]

147 **Eisenberg RA**, Jawad AF, Boyer J, Maurer K, McDonald K, Prak ET, Sullivan KE. Rituximab-

treated patients have a poor response to influenza vaccination. *J Clin Immunol* 2013; **33**: 388-396 [PMID: 23064976 DOI: 10.1007/s10875-012-9813-x]

148 **Nazi I**, Kelton JG, Larché M, Snider DP, Heddle NM, Crowther MA, Cook RJ, Tinmouth AT, Mangel J, Arnold DM. The effect of rituximab on vaccine responses in patients with immune thrombocytopenia. *Blood* 2013; **122**: 1946-1953 [PMID: 23851398 DOI: 10.1182/blood-2013-04-494096]

149 **Marjot T**, Webb GJ, Barritt AS, Ginès P, Lohse AW, Moon AM, Pose E, Trivedi P, Barnes E. SARS-CoV-2 vaccination in patients with liver disease: responding to the next big question. *Lancet Gastroenterol Hepatol* 2021; **6**: 156-158 [PMID: 33444545 DOI: 10.1016/S2468-1253(21)00008-X]

150 **Alexander JL**, Moran GW, Gaya DR, Raine T, Hart A, Kennedy NA, Lindsay JO, MacDonald J, Segal JP, Sebastian S, Selinger CP, Parkes M, Smith PJ, Dhar A, Subramanian S, Arasaradnam R, Lamb CA, Ahmad T, Lees CW, Dobson L, Wakeman R, Iqbal TH, Arnott I, Powell N; Inflammatory Bowel Disease section of the British Society of Gastroenterology and the the Inflammatory Bowel Disease Clinical Research Group. SARS-CoV-2 vaccination for patients with inflammatory bowel disease: a British Society of Gastroenterology Inflammatory Bowel Disease section and IBD Clinical Research Group position statement. *Lancet Gastroenterol Hepatol* 2021; **6**: 218-224 [PMID: 33508241 DOI: 10.1016/S2468-1253(21)00024-8]

151 **Siegel CA**, Melmed GY, McGovern DP, Rai V, Krammer F, Rubin DT, Abreu MT, Dubinsky MC; International Organization for the Study of Inflammatory Bowel Disease (IOIBD); International Organization for the Study of Inflammatory Bowel Diseases (IOIBD). SARS-CoV-2 vaccination for patients with inflammatory bowel diseases: recommendations from an international consensus meeting. *Gut* 2021; **70**: 635-640 [PMID: 33472895 DOI: 10.1136/gutjnl-2020-324000]

152 **Bailey LC**, Razzaghi H, Burrows EK, Bunnell HT, Camacho PEF, Christakis DA, Eckrich D, Kitzmiller M, Lin SM, Magnusen BC, Newland J, Pajor NM, Ranade D, Rao S, Sofela O, Zahner J, Bruno C, Forrest CB. Assessment of 135 794 Pediatric Patients Tested for Severe Acute Respiratory Syndrome Coronavirus 2 Across the United States. *JAMA Pediatr* 2021; **175**: 176-184 [PMID: 33226415 DOI: 10.1001/jamapediatrics.2020.5052]

153 **Swann OV**, Holden KA, Turtle L, Pollock L, Fairfield CJ, Drake TM, Seth S, Egan C, Hardwick HE, Halpin S, Girvan M, Donohue C, Pritchard M, Patel LB, Ladhani S, Sigfrid L, Sinha IP, Olliaro PL, Nguyen-Van-Tam JS, Horby PW, Merson L, Carson G, Dunning J, Openshaw PJM, Baillie JK, Harrison EM, Docherty AB, Semple MG; ISARIC4C Investigators. Clinical characteristics of children and young people admitted to hospital with covid-19 in United Kingdom: prospective multicentre observational cohort study. *BMJ* 2020; **370**: m3249 [PMID: 32960186 DOI: 10.1136/bmj.m3249]

154 **Su L**, Ma X, Yu H, Zhang Z, Bian P, Han Y, Sun J, Liu Y, Yang C, Geng J, Gai Z. The different clinical characteristics of corona virus disease cases between children and their families in China - the character of children with COVID-19. *Emerg Microbes Infect* 2020; **9**: 707-713 [PMID: 32208917 DOI: 10.1080/22221751.2020.1744483]

155 **U S. Food and Drug Administration**. Pfizer-BioNTech COVID-19 Vaccine. [cited December 22, 2020]. In: U.S. Food and Drug Administration [Internet]. Available from: https://www.fda.gov/emergency-preparedness-and-response/coronavirus-disease-2019-covid-19/pfizer-biontech-covid-19-vaccine

156 **Frenck RW Jr**, Klein NP, Kitchin N, Gurtman A, Absalon J, Lockhart S, Perez JL, Walter EB, Senders S, Bailey R, Swanson KA, Ma H, Xu X, Koury K, Kalina WV, Cooper D, Jennings T, Brandon DM, Thomas SJ, Türeci Ö, Tresnan DB, Mather S, Dormitzer PR, Şahin U, Jansen KU, Gruber WC; C4591001 Clinical Trial Group. Safety, Immunogenicity, and Efficacy of the BNT162b2 Covid-19 Vaccine in Adolescents. *N Engl J Med* 2021; **385**: 239-250 [PMID: 34043894 DOI: 10.1056/NEJMoa2107456]

157 **Rentsch CT**, Beckman JA, Tomlinson L, Gellad WF, Alcorn C, Kidwai-Khan F, Skanderson M, Brittain E, King JT Jr, Ho YL, Eden S, Kundu S, Lann MF, Greevy RA Jr, Ho PM, Heidenreich PA, Jacobson DA, Douglas IJ, Tate JP, Evans SJW, Atkins D, Justice AC, Freiberg MS. Early initiation of prophylactic anticoagulation for prevention of coronavirus disease 2019 mortality in patients admitted to hospital in the United States: cohort study. *BMJ* 2021; **372**: n311 [PMID: 33574135 DOI: 10.1136/bmj.n311]

158 **Guo T**, Fan Y, Chen M, Wu X, Zhang L, He T, Wang H, Wan J, Wang X, Lu Z. Cardiovascular Implications of Fatal Outcomes of Patients With Coronavirus Disease 2019 (COVID-19). *JAMA Cardiol* 2020; **5**: 811-818 [PMID: 32219356 DOI: 10.1001/jamacardio.2020.1017]

159 **Wong MCS**, Wong ELY, Huang J, Cheung AWL, Law K, Chong MKC, Ng RWY, Lai CKC, Boon SS, Lau JTF, Chen Z, Chan PKS. Acceptance of the COVID-19 vaccine based on the health belief model: A population-based survey in Hong Kong. *Vaccine* 2021; **39**: 1148-1156 [PMID: 33461834 DOI: 10.1016/j.vaccine.2020.12.083]

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REVIEW

Genotype E: The neglected genotype of hepatitis B virus

Luicer Anne Olubayo Ingasia, Constance Wose Kinge, Anna Kramvis

ORCID number: Luicer Anne Olubayo Ingasia 0000-0002-7370-7272; Constance Wose Kinge 0000-0002-6221-5459; Anna Kramvis 0000-0001-6006-3765.

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Luicer Anne Olubayo Ingasia, Constance Wose Kinge, Anna Kramvis, Hepatitis Virus Diversity Research Unit, Department of Internal Medicine, School of Clinical Medicine, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg 2193, Gauteng, South Africa

Constance Wose Kinge, Department of Implementation Science, Right to Care, Johannesburg 0046, Gauteng, South Africa

Corresponding author: Anna Kramvis, BSc, PhD, Director, Professor, Hepatitis Virus Diversity Research Unit, Department of Internal Medicine, School of Clinical Medicine, Faculty of Health Sciences, University of the Witwatersrand, 7 York Road, Parktown, Johannesburg 2193, Gauteng, South Africa. anna.kramvis@wits.ac.za

Abstract

Hepatitis B virus (HBV) (sub)genotypes A1, D3 and E circulate in sub-Saharan Africa, the region with one of the highest incidences of HBV-associated hepatocellular carcinoma globally. Although genotype E was identified more than 20 years ago, and is the most widespread genotype in Africa, it has not been extensively studied. The current knowledge status and gaps in its origin and evolution, natural history of infection, disease progression, response to antiviral therapy and vaccination are discussed. Genotype E is an African genotype, with unique molecular characteristics that is found mainly in Western and Central Africa and rarely outside Africa except in individuals of African descent. The low prevalence of this genotype in the African descendant populations in the New World, phylogeographic analyses, the low genetic diversity and evidence of remnants of genotype E in ancient HBV samples suggests the relatively recent re-introduction into the population. There is scarcity of information on the clinical and virological characteristics of genotype E-infected patients, disease progression and outcomes and efficacy of anti-HBV drugs. Individuals infected with genotype E have been characterised with high hepatitis B e antigen-positivity and high viral load with a lower end of treatment response to interferon-alpha. A minority of genotype E-infected participants have been included in studies in which treatment response was monitored. Of concern is that current guidelines do not consider patients infected with genotype E. Thus, there is an urgent need for further large-scale investigations into genotype E, the neglected genotype of HBV.

Key Words: Hepatitis B virus; Genotype E; Evolution; Clinical significance; Antiviral therapy; Vaccination

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Core Tip: Although genotype E was identified more than 20 years ago, and is the most widespread genotype in Africa, it has not been extensively studied. The current knowledge status and gaps in its origin and evolution, natural history of infection, disease progression, response to antiviral therapy and vaccination discussed in this review highlight the urgent need for further more in-depth and large-scale investigations into genotype E, the neglected genotype of hepatitis B virus.

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INTRODUCTION

Hepatitis B virus (HBV), a common cause of liver disease, is the prototype member of the family *Hepadnaviridae*. Despite the availability of vaccines, HBV infection remains a public health concern causing high morbidity and mortality rates, as a result of the serious clinical consequences of cirrhosis and hepatocellular carcinoma (HCC)[1]. It is estimated that a third of the world's population is or has been infected with HBV at some point in their lives[1]. As a result of its unusual mechanism of replication by reverse transcription through an RNA intermediate, and lack of proof reading ability of its viral polymerase[2], HBV displays sequence heterogeneity, which leads to the existence of at least 9 genotypes. Four genotypes, A to D, were recognized initially, with genotypes E to I being recognized subsequently[3]. A putative 10th genotype J, has been proposed[4]. All genotypes, except E and G, are further subdivided into subgenotypes. Most HBV genotypes and, in some cases subgenotypes have a distinct geographical distribution. HBV genotypes A and D have global distributions while genotypes B and C are predominantly found in East and Southeast Asia. Genotype E is found in West and Central Africa, genotypes F and H are found among various population groups, including indigenous peoples in Central and South America[5,6], while genotype G is found in the Americas and Europe[6]. Genotype I was reported in Vietnam and Laos[6], with the most recent putative genotype J identified in a Japanese patient living in Borneo island[4].

GENOTYPE E IN AFRICA AND ITS ORIGINS

Together with south-east Asia, Africa is one of the two regions in the world where HBV remains endemic. West Africa is the only major region in the world where HBV is still hyperendemic[5] — [> 8% of hepatitis B surface antigen (HBsAg) chronic carriers in the general population] and there is a correspondingly high incidence of HCC[7]. Genotype E was first described in 1992 from a HBsAg-positive Cameroonian blood donor[8]. It predominates in sub-Saharan Africa (SSA) accounting for 97% of individual infections and 17.6% of all HBV infections globally[9-11]. It is found almost exclusively throughout the vast expanses of the Western and Central Africa crescent including Angola, Liberia, Senegal[12,13], Ivory Coast[14], the Gambia, Nigeria[15], Mali, Burkina Faso, Togo, Guinea, Benin, Democratic Republic of Congo, Cameroon [16] and Namibia. The prevalence of genotype E decreases in proportions towards Eastern Africa, where, with the exception of Madagascar (genotype E), mainly genotype A has been found[5,9,11].

Genotype E has been found only in Africa, with some rare exceptions on other continents mainly in persons with a link to Africa[17,18]. Nonetheless, two cases, where no link to Africa could be established, have been documented, one in India[19] and another in Colombia[20]. Genotype A, on the other hand, circulates on every continent, including Africa, where it has the highest genetic diversity of 4% over the complete genome compared to 3% outside Africa[21]. Despite its high genetic diversity in Africa, genotype A is rarely found in West Africa. The dispersal routes of genotype A have previously been described to coincide with the slave trade leading to the dispersal of this genotype to the Americas and the Indian subcontinent[19,21-23]. Despite the forced migrations of slaves from West Africa to the New world[3,17], only

sporadic cases of genotype E have been reported in the Americas[17,24], Northern Europe[25] including Belgium[26] and the Netherlands[27]. This may suggest that genotype E was not in circulation before and during the slave trade (9th to 19th century) and has only been introduced into the West African population after the end of the slave trade in the late 1800s[23].

The conspicuously low genetic diversity of genotype E ranging between 1.2% and 1.95%[11,16,23,28,29] further supports a short natural history in Africa[16] and relatively recent introduction into the general population[16,30]. Various times from the most recent common ancestor (tMRCA) of genotype E have been calculated using Bayesian inference, with a median tMRCA of 130 years[30] whereas in Nigeria, a more recent tMRCA was estimated to be year 1948 [95% higher posterior density (HPD): 1924-1966] (73 years), with an increase in the genotype E-infected population over the last approximately 40 years to 50 years[31]. A recent study focusing on ancient HBV estimated a median MRCA to be year 1016 (95% HPD: 712-1358)[32]. These times differ from the estimated tMRCA of 6000 years[33]. Differences in the calculation of the nucleotide substitution rate of HBV are responsible for the variance of the estimated age of genotype E. Our recent study describing the phylogeography of full genomes of genotype E showed localized transmission, and limited movements within West and Central Africa. The study showed West Africa to be the most probable origin of the genotype E epidemic, with strains dispersing to the European region from there, whereas the strains dispersed to the Americas originated in Central Africa[29].

Studies on HBV-infected mummies from the 16th century revealed a very close relationship between the ancient and modern HBV genomes dating 400-500 years[34,35]. Furthermore, studies conducted by Krause-Kyora *et al*[36] reported ancient HBV sequences in the Neolithic age, while studies by Mühlemann *et al*[32] reported archeological ancient HBV and predicted recombination breakpoints in the polymerase gene leading to the formation of genotype A with similar recombination events involved in the creation of genotypes E and G[32,36-38] in the Bronze age[32]. Concurring with Mühlemann *et al*[32]'s study, Krause showed recombination events over time and similarity between the earliest ancient HBV sequences of the Neolithic era and modern HBV genotypes E and G[36]. By comparing the sequences from the above two studies, Datta *et al*[39] was able to confirm the previous findings of the presence of remnants of genotype E in ancient sequences from the Neolithic and Bronze age[32,36,39].

At first glance, the widespread prevalence and extensive geographic distribution of genotype E[17,28,29] may be difficult to reconcile with the long natural history of genotype A in Africa. However, isolation of genotype E in indigenous isolated tribes of Africa; Pygmies[37] and Khoi San (Kramvis unpublished data), believed to be direct descendants of earliest human lineages[6,37,40], and the recent discovery of the ancient HBV sequences in the Neolithic and Bronze era from skeletal remains of humans with remnants of genotype E[32,36,39], may support the theory that genotype E pre-existed but has been re-introduced into the population thus replacing genotype A. Similarly, the presence of recombinant sequences similar to extant genotypes D (subgenotype D6) and E, which are presently endemic in certain regions of Africa[6], together with the co-existence of genotypes E/A/D in SSA, including Sudan and Cameroon, also support the aforesaid possibility[37,41,42]. Possible mechanisms of introduction and routes of transmission include mass vaccination programmes carried out in Western Africa and a high frequency of hepatitis B e antigen (HBeAg)-positivity in mothers infected with genotype E [mother to child transmission (MTCT)][43,44] leading to chronicity due to HBe/HBcAg-specific T helper cell tolerance *in utero*[44]. In constrast to genotype E, the two subgenotypes of A, A1 and A3, circulating in Africa, are characterized by early loss of HBeAg seroconversion and a high frequency of HBeAg-negativity[10].

Genotype E, closely related to human strains, has also been isolated from captive and wild born chimpanzees originating from West and Central Africa[12,41,45]. The direction of transmission was not established[17] although, it was suggested that the practice of injecting human serum into chimpanzees after their capture in Africa was the most probable explanation[41,42,46]. Thus, chimpanzees may be a possible source of separate primate to human transmission events of HBV in West Africa[41,42,46]. Moreover, a closer relationship between the Neolithic and the African non-human primate strains compared to other human strains suggests African origin of extinct HBV genotypes and reciprocal cross-species transmission in the past[38,47] supporting preceding suppositions[48].

MOLECULAR STRUCTURE OF GENOTYPE E

Genotype E is the most prevalent genotype of HBV in Africa estimated to have infected close to 20% of chronic HBV carriers globally. However, due to limited studies and the lack of surveillance data in Africa, this estimate may be higher[17]. Genotype E is the second shortest genotype after D with a complete genome length of 3212 bp (Figure 1). It has a unique three-nucleotide deletion in the preS1 that can differentiate it from other genotypes (Figure 1) and a signature pattern of amino acids in the preS1. In addition, genotype E has a putative additional start codon in the preS1, which may lead to an elongated middle hepatitis B surface protein (317 amino acids in length instead of 281 amino acids)[11]. This elongated middle HBsAg has not been detected to date. The amino acids of the preS1, preS2 and S genes are well conserved, with signature motifs $Leu^{3}SerTrpThrValProLeuGluTrp^{11}$ in the preS1 specific to genotype E [11]. Additional signature amino acids are also found at Thr^{18}, Arg^{38}, His^{44}, Thr^{52}, Met^{83}, Lys^{85} and Thr^{108} in the preS1. All genotype E strains have a His at amino acid position 15 of the preS1 but no known unique signature motifs in the pre-S2 region. Arg^{122}, Lys^{160} and Leu^{127} residues are a characteristic of the S gene in this genotype and encodes for a unique serological subtype *ayw*4[11,12]. Although the reactivity to different diagnostic assays has been determined for genotypes A to D[49], it has not been tested for genotype E. The L209V substitution in the HBsAg was described as a unique feature among all genotype E sequences deposited in GenBank to date[50]. The spacer region of the polymerase (POL) has eight amino acids unique to genotype E: Met^{64}, Glu^{16}, His^{21}, Arg^{52}, Asp^{55}, Lys^{88}, Asn^{110} and His^{111}. Within the reverse transcriptase, Met^{164} is the only unique amino acid substitution in this genotype[11]. This introduces a start codon that theoretically could be translated into a protein of 344 amino acids. Although genotype E has the T1858 mutation in the precore (preC) region it does not frequently develop the G1896A mutation[44,51], which has been shown to stabilize the encapsidation signal (ε) converting the wobble to a stable Watson-Crick T-A pair[52]. This introduces a stop codon in the HBeAg precursor leading to no expression of the mature HBeAg[10,44,51]. As a result of its unique molecular structure, genotype E has a restriction map that differentiates it from other genotypes of HBV (Figure 1).

VARIANTS AND MUTANTS OF GENOTYPE E

Variants can play a critical role in HBV epidemics. From the limited studies on genotype E, a number of variants and mutants that can hypothetically affect detection, vaccination response and pathogenicity of HBV, have been described. Within the 'a' determinant of HBsAg, the vaccine and immune escape mutations R48T, P120T and G145R have been reported in genotype E HBV isolated from infected individuals[3, 53]. The preS2 F22L mutation, associated with cirrhosis, and a risk factor for the development of HCC, was found in genotype E isolates from Sudanese HCC patients [54].

Variants can also be generated through recombination[38] within an individual co-infected, with more than one genotype, resulting in drug resistant or diverse HBV strains. Recombinants can only occur when the various genotypes co-circulate in a population. Genotype E presents high chances of recombination, with A/E and D/E recombinants found in Ghana, A/E recombinant has been reported in Cameroon[37], Guinea, Burkina Faso and Nigeria[31] while D/E recombinant has been found in Gabon, Sudan, South Africa, Niger and Guinea[55,56].

Table 1, summarizes the different recombination events of genotype E with either D or A, mostly reported within Africa with different breakpoints within the HBV genome[37,54-61].

The F22L mutation and various deletions in the preS2 and the 1753V and 1762T/1764A mutations in the basic core promoter (BCP), are mostly found in HBV strains isolated from HCC patients[62] than in those from non-HCC controls[54,63]. Deletions in the core region have been reported in HBsAg-positive genotype E asymptomatic blood donors in Guinea. Another study conducted by Yousif *et al*[54] found preS2 deletion mutations in HBV from patients infected with either genotypes D or E in Sudan. The preS deletions in genotype E were found in the HBV isolated from HCC patients, while genotype D deletion mutants were detected in non-HCC patients [54]. The significance of this difference remains to be determined. On the other hand subgenotype A1, which is mostly found in SSA[5], has been shown to have a higher carcinogenic potential compared to other (sub)genotypes[64]. A meta-analysis study associated the preS deletion mutants with a 3.77-fold increased risk of HCC[65].

Table 1 Recombination events of genotype E with breakpoints across the genome

Parental genotype	Region	Genome position (from the *EcoR*I site)	Country
D/E	*preS1*		Niger, Ghana, Gabon, and Sudan[53-58]
D/E	*preC/C*		Ireland[59] and South Africa[60]
D/E	*Pol*	978, 1230	Sudan[56]
	X	1643	
	C/Pol overlapping region	2384	
	Pol	2756	
	preS1/Pol overlapping region	3000	
D/E	*X/preC* overlapping region	1649, 1932	Niger[58]
	C/Pol overlapping region	2392, 2385	
	Pol	2831, 2836	
	Pol/preS1 overlapping region	3075, 3083	
D/E	*X*	1651	Ghana[57]
	C/Pol overlapping region	2406	
	Pol	2823	
	Pol/preS1 overlapping region	3081	
E/D	*preS*	85-505	Niger[58]
	S-Pol overlapping region	796-1306	
A/E	*C*		Ghana[57]
A/E	*Pol*	874-1062	Cameroon[37]
	X		
E/A	*Pol*	908-1026	
	X-C		
A/E	*preC/C*		Guinea[57] and France[61]
E/A	*X*		

The *precore/core* (preC/C) encodes the e antigen (HBeAg) and core protein (HBcAg); *Pol* for polymerase (reverse transcriptase), *preS1* encodes the large surface protein and *X* is a transcriptional transactivator protein.

Furthermore, a prospective study revealed the predictive value of a combination of the preS and BCP mutants in the development of HCC and pro-oncogenic role of mutated envelope proteins through their intracellular accumulation[66]. These mutations may be used as biomarkers for screening high-risk individuals in resource limited regions such as SSA, who may potentially develop HCC[67].

TRANSMISSION OF GENOTYPE E

The prevalence of chronic HBV infection varies widely according to geographic area and is closely linked with the predominant routes of HBV transmission. In regions of Africa, where genotype E prevails, transmission can occur horizontally or vertically *in utero*, intrapartum or *via* breast-feeding[68] from mother to child[69]. However, about 50% of the infection in children cannot be accounted for by MTCT and in many endemic regions, prior to the introduction of neonatal vaccination, the prevalence peaked among children aged between 7 years to 14 years[70]. In the pre-vaccine era, most chronic carriers were infected horizontally in SSA and only 10% were infected through MTCT compared to 40% in Asia[71,72]. Horizontal transmission can occur early in life mainly from HBeAg-positive family members/household contacts, playmates or by unsafe medical interventions. Very few studies have been carried out

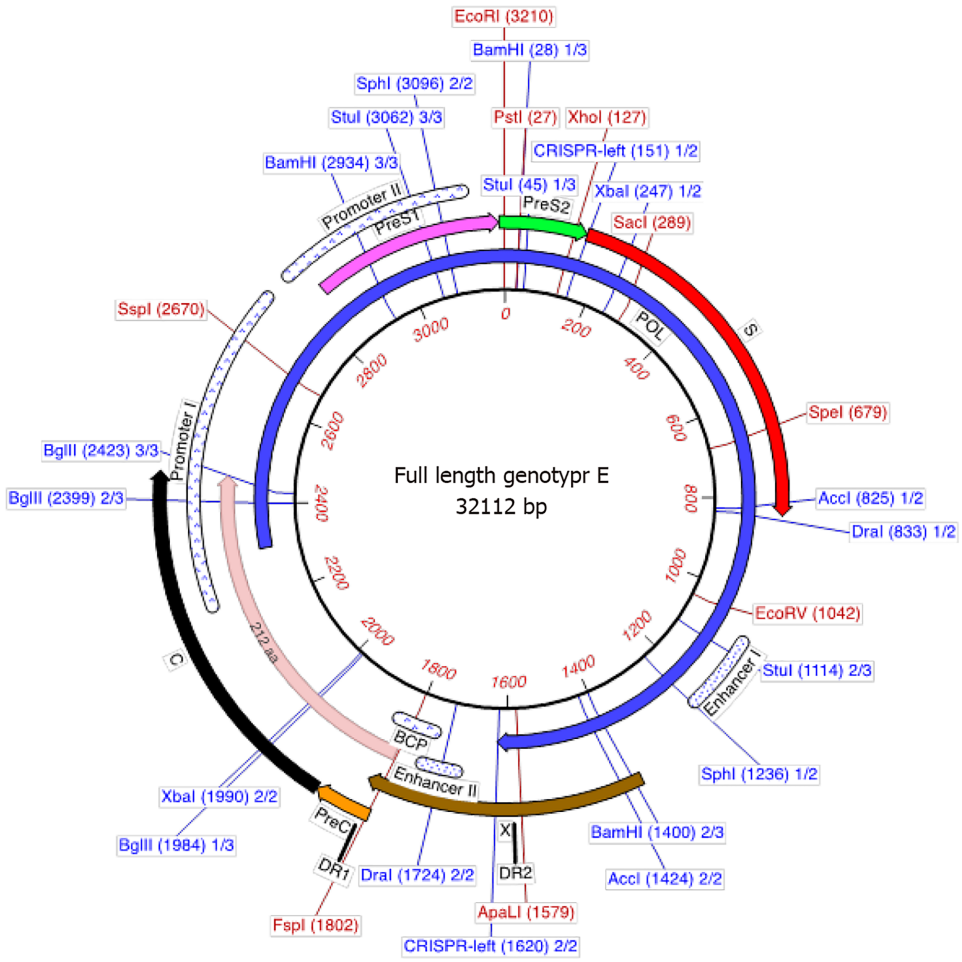

Figure 1 Organizational structure of hepatitis B virus genotype E genome. The hepatitis B virus genome consists of a partially double stranded DNA with the complete minus strand and the incomplete strand. The four open reading frames are shown: *precore*/*core* (*preC*/*C*) that encodes the e antigen (HBeAg) and core protein (HBcAg); *POL* for polymerase (reverse transcriptase), *preS1*/*preS2*/*S* for surface proteins (three forms of HBsAg, small, middle and large) and *X* for a transcriptional trans-activator protein. The promoters, enhancers and the unique restriction enzymes are shown.

in terms of identifying routes of transmission for genotype E. In the Gambia, MTCT is responsible for 16% of chronic infections and increases the risk of persistent viral replication and severe liver disease[73]. Strong evidence from a phylogenetic analysis showed intrafamilial transmission of HBV[73]. A study conducted in Ghana also concluded that the HBV is predominantly transmitted through horizontal transmission in childhood with intrafamilial, rather than interfamilial environment being the primary place of transmission[74]. However, a study conducted in Nigeria in two semi-isolated rural communities suggested that HBV transmission between siblings was not the major route of transmission with a complex pattern of transmission among the residents of the two communities[31]. So it appears that other factors may be at play in the transmission of genotype E in various communities. As has been shown in Burkina Faso, co-infection with human immunodeficiency virus (HIV), which leads to an increase in HBV viral load and frequency of HBeAg-positivity, can increase the risk of HBV transmission by as much as 2.5-fold[75,76]. Traditional cultural practices such as scarification and tattooing have been shown to be responsible for the transmission of HBV[77].

NATURAL HISTORY OF HBV GENOTYPE E INFECTION

Genotypes and subgenotypes can influence the natural history of infection. Comparing different (sub)genotypes is often difficult because the (sub)genotypes do not circulate in the same populations. The majority of the studies have compared genotypes B and C as well as A and D and have shown different clinical manifestations and the serious outcomes of disease [cirrhosis (LC) and HCC][78-81]. The natural history of infection

in individuals infected with genotype E has not been extensively studied, and has mostly been derived from anecdotal evidence. Genotype E has clinically been characterized, with high viral loads and the patients infected with this genotype are more likely to be HBeAg-positive than the patients infected with genotype D[5,10,53,54,56]. A higher HBeAg-positivity of this genotype has been shown to confer tolerance, with a milder clinical manifestation[10]. This could be the reason for the higher prevalence of genotype E in Sudanese blood donors, whereas genotype D is more prevalent in those patients with liver disease[28,54,56]. In addition, infection with genotype E has previously been linked to higher chronicity rates than other genotypes[10,54,56].

Table 2, which was compiled from limited data comparing genotype E to D in Sudan (Yousif *et al*[53,54]) and studies in the Gambia (Shimakawa *et al*[72]), summarizes the clinical manifestation of genotype E relative to other genotypes[53,54,72,82]. As is evident from this table most aspects of clinical characteristics of genotype E have not been formally studied.

In their study, Yousif *et al*[54] observed that genotype E infected liver disease patients and blood donors[56] had a higher frequency of HBeAg-positivity and higher viral loads compared to patients infected with genotype D (Table 2)[53,54]. Both genotype D and E have the 1858T, and thus can develop the G1896A mutation, however, what is puzzling is that G1896A is positively associated with genotype D and negatively associated with genotype E[51].

This lack of association may be the reason for the high frequency of HBeAg-positivity in individuals infected with genotype E compared to genotype D. A study focusing on chronic hepatitis B (CHB) and HCC in Burkina Faso showed patients infected with genotype E had lower viral loads, lower frequency of HBeAg-positivity and higher prevalence of cirrhosis than those infected with genotype C or C/E recombinants. With the majority of HCC, infected with genotype E (78%), HCC-associated risk factors were old age, male with high HBV viral load when comparing CHB in HCC patients to non-HCC patients[83]. Another longitudinal study conducted in Gambia showed that a majority of the genotyped CHB carriers were infected with genotype E[72]. Although the mean viral load and alanine aminotransferase levels were higher in carriers with HBsAg-positive mothers, a majority (47%) had undetectable viral loads with 22% of all chronic HBV infections having viral loads ranging between 50 and 200 IU/mL. HBV viral load has been used to predict progression from cirrhosis to HCC[84]. From this study, the rate at which the HBV DNA cleared was faster when compared to age progression making it difficult to predict HCC[72]. What should be noted from this study is that, the samples that were assayed for viral loads were from a different time frame (2012-2013), while the genotyped samples were from 2003. Successful genotyping would require viral loads high enough to allow amplification of the DNA and thus higher viral loads may be a factor that biases genotyping making it hard to draw any conclusion on the infecting genotype for the chronic carriers who had undetectable or low HBV DNA.

African regions in which genotype E is endemic are characterized by a higher incidence of HCC[85] and epidemiological studies have suggested the carcinogenic potential of genotype E[86]. Although the mechanisms underlying this oncogenic potential have not yet been clarified for genotype E, they could be related to immune escape phenomena[87], as well as to other possible cofounders that may be involved, such as HIV co-infection, dietary iron overload or aflatoxin consumption[85,88,89].

HBV-HIV CO-INFECTION AND OCCULT INFECTION

Globally, an estimated 10% of the 37 million HIV infected individuals are co-infected with HBV[90]. HBV/HIV co-infection in SSA accounts for 36% (2-4 million) with the highest rates reported in West- and Southern Africa[90]. Epidemiological and virological characteristics of HIV-infected individuals in West Africa showed an average of 13% prevalence of HBsAg-positivity, ranging between 1.1% in blood donors and 35.7% in pregnant women attending antenatal care[76,91-93], while 4.75% of HBV-HIV infected individuals were HBeAg-positive with the prevalence ranging between 3.2% and 7.2% in adults and anti-retroviral (ART) naïve adults, respectively[94,95]. An average HBV exposure rate of 74% (64%-81.7%) in ART naïve and adults initiating ART[90,94,96,97] has been documented. A high rate of morbidity has been reported in HBV/HIV co-infected individuals, while the progression of CHB to HCC is more rapid in genotype E HIV-positive individuals than in those with HBV alone[98]. In a study of Senegalese children, 47% who were HBV genotype E-HIV co-infected had elevated levels of drug resistance mutations (L180M, M204V/I, and S202N) to both

Table 2 Comparison of the virological and clinical characteristics of genotype E with other genotypes

	Genotypes							
	E	A	B	C	D	F	G	H
HBV DNA level	Increased	Decreased	Decreased	Increased	Not studied	Not studied	Not studied	Not studied
Frequency of precore G1896A mutation	Increased[1]	Decreased	Increased	Decreased	Increased	Not studied	Not studied	Not studied
Frequency of basic core promoter T1762A/A1764G mutation	Not studied	Increased	Decreased	Increased	Decreased	Not studied	Not studied	Not studied
Frequency of preS deletion mutation	Not studied	Increased	Decreased	Increased	Not studied	Not studied	Not studied	Not studied
Tendency of chronicity								
High		+		+				
Low			+		+			
Not studied	+					+	+	+
HBeAg positivity								
High	+			+				
Low		+	+		+			
Not studied						+	+	+
HBeAg seroconversion								
Early		+	+					
Late				+	+			
Not studied	+					+	+	+
HBsAg seroconversion								
More		+	+					
Less				+	+			
Not studied	+					+	+	+

[1]Relative to D3.

+: Classification of category; preS: Surface protein; HBeAg: Hepatitis B e antigen; HBsAg: Hepatitis B surface antigen. Adapted from Yousif *et al*[53,54], Shimakawa *et al*[72] and Schaefer *et al*[82].

HIV and HBV, significant levels of HBsAg escape mutations, HBV DNA persistence and HIV virologic failure[99]. This suggests that the use of the Tenofovir Disoproxil Fumarate regimen in the management of HBV, HIV and HBV-HIV co-infection is ideal in the SSA setting.

Occult HBV infection (OBI) is defined as the presence of replication-competent HBV DNA (*i.e.*, episomal HBV covalently closed circular DNA) in the liver and/or HBV DNA in the blood of people who test negative for HBsAg by currently available assays)[100]. OBI is frequent in HIV-infected individuals and has been described in individuals infected with genotype E, with a prevalence 10% and 15% in HIV-positive patients from the Ivory Coast and Sudan, respectively[97,101].

Biomarkers are very important in assessing risk factors for the development of serious clinical manifestations. As is evident from the above observations the same risk biomarkers may not be applicable to all (sub)genotypes and cannot be extrapolated from studies on other genotypes. Therefore, it is important that biomarkers are studied exclusively in genotype E.

TREATMENT AND RESPONSE TO ANTIVIRAL THERAPY

Current antiviral therapies, which include nucleos(t)ide analogues (NA) and interferon-alpha (IFN-α) reduce but do not eliminate the risk of liver cancer. As

curative therapies are developed, it will be important to monitor patients for progression to liver cancer, even if they have been cured of CHB infection. HBV genotype may influence the efficacy of the antiviral therapy but most studies that analyzed the role of HBV genotype in the treatment with NA mostly focused on genotypes A, B, C and D. Lamivudine (LAM) is the earliest used NA in the world and the association between HBV genotype and LAM has been demonstrated both in terms of response and the development of resistance mutations. Various response rates have been observed for various studies with genotype A being more likely to develop resistance mutations[102,103]. Studies have shown that HBeAg-positive patients infected with genotype B have a higher response rate to IFN-α than those infected with genotype C, while patients infected with genotype A have a higher response rate to IFN-α than those infected with genotype D[104].

There is a scarcity of information on the clinical and virological characteristics of genotype E-infected patients as well as on the efficacy of anti-HBV drugs[86]. However, a few studies have described genotype E's response to treatment[86,105-108] in a variety of scenarios: Treatment-naïve CHB patients initiating treatment with NA [entecavir (ETV) or tenofovir][86], HBV-HIV co-infected patients[109], rescued after LAM failure[110], adefovir phase III clinical trials[111]; a follow-up study of HBsAg decline in ETV-responding patients[107] and response to IFN[106,112]. As is evident from the above list, only one study looked at tenofovir the drug recommended by the World Health Organization (WHO), American Association for the Study of Liver Diseases, and the European Association for the Study of the Liver for antiviral therapy.

The phase III clinical trial of adefovir dipivoxil conducted by Westland *et al*[111] included a total of 6 genotype E patients and reported antiviral efficacy in patients on a 48-wk therapy regardless of the HBV genotype. Studies by Boglione *et al*[107] and Cuenca-Gómez *et al*[86] focused on genotype E treatment-naïve, CHB patients of SSA origin, on ETV or tenofovir antiviral therapy. A higher rate of HBsAg loss in patients infected with genotype E compared to genotypes A or D was observed. In addition, a high response rate to NA was reported with undetectable viral load and loss of HBeAg in a median time of 31.8 mo with no cases of HCC[86].

Two different treatment regimens were compared in CHB patients infected with genotype E, who had migrated to Italy. In the one arm, CHB patients with low viral loads, where given pegIFN for 24 wk, whereas in the second arm, CHB patients with high viral loads were treated sequentially with ETV for 12 wk and thereafter pegIFN for 24 wk. Those treated with monotherapy did not respond as well as those on dual therapy[106]. In a follow-up study, genotype E CHB patients were treated with pegIFN for varying lengths of time 48-, 72- and 96-wk. Prolonged treatment was beneficial and recommended for individuals infected with genotype E[106,108]. Thus, from these limited studies it is evident that genotype E infected individuals are unresponsive to conventional pegIFN treatment. However, in concurring with the Boglione *et al*[107] and Cuenca-Gómez *et al*[86] studies, a retrospective study conducted in Europe by Erhardt *et al*[105], focusing on HBV genotypes E-H the response to IFN-α or NAs (LAM, adefovir, ETV) therapy concluded that genotype E infected patients treated with IFN-α had lower end of treatment response but overall sustained virological response, while the patients on NAs had viral suppression within 48 wk[105]. It should be noted that the conclusion was reached with only 5 treatment-naïve genotype E mono-infected patients[103].

Taken together, the current international treatment guidelines do not consider patients with genotype E CHB. Thus, better management strategies for HBV infected patients are recommended taking into account the genotype in question. In order to deliver proper medical care, improve knowledge on the response to treatment, and the development of resistance of relatively under-studied genotypes like E, it is critical to issue proper and specific recommendations that could differ from those issued for other genotypes. Moreover, all gathered information on response to treatment of genotype E in Africa is useful, especially considering that the development of immune escape mutations[87] can have an epidemiological impact in other parts of the world with the dispersal of these strains *via* increased migration from Africa. As new finite cure strategies are developed it is important that the clinical trials include CHB patients infected with genotype E.

RESPONSE TO VACCINATION

The risk of developing chronic infection is about 90% following perinatal infection up to 6 mo but decreases to about 20%-60% between the ages of 6 mo to 5 years[68,73].

Thus, prevention of HBV infection by vaccination is very important and is most successful when it targets infants, and when prevention begins with administration of the first dose of HBV vaccine soon after birth. The HBV vaccine is about 80%-100% effective in managing HBV infection or clinical hepatitis following completion of the dose. However, inoculation will not help those chronically infected[1]. The two commonly used efficacious vaccines are either plasma-derived vaccines prepared from purified HBsAg obtained from chronic HBV patients or recombinant vaccines from synthesized HBsAg[113]. As of 2020, more than 190 WHO member states immunized infants against HBV as part of their routine vaccination schedule, and 84% of children received HBV vaccines[1]. Even with the vaccine roll out, the burden of HBV infections in SSA remains of concern attributed to the delay in the implementation, lack of birth doses and low coverage of the vaccine programme[114-117]. The high HBeAg positivity in mothers infected with genotype E is a risk factor for MTCT[118] (one in ten infants vaccinated at birth) suggesting that vertical/perinatal infection is still present in African countries[119-122]. Antenatal HBV screening is hardly performed in SSA (0%-20%)[123], with only 33% of countries having official guidelines[124]. HBV was first classified on the basis of the amino acid substitution on the HBsAg at positions 122, 127, 134 and 160. The serological subtypes contain the common 'a' determinant and one of each of the mutually exclusive determinants *d*/*y* and *w*/*r* [125]. Additional serological specificities, originally designated as subdeterminants of 'a' and subsequently as subdeterminants of *w*, have allowed the identification of ten serological subtypes *ayw*1, *ayw*2, *ayw*3, *ayw*4, *ayr*, *adw*2, *adw*3 *adw*4, *adrq*- and *adrq*+[6,8,126]. The humoral immune response following vaccination with HBV vaccines is largely directed against the common 'a' determinant, with a lesser response directed against the *d*/*y* and *r*/*w* subdeterminant epitopes[113,127].

All currently available genetically engineered HBV vaccines are produced with the subgenotype A2, serotype *adw*, which differs from the genotype E subtype *ayw*4. Available data show that current HBV-A2 vaccines are highly effective at preventing infections and clinical disease caused by all known HBV genotypes[128]. However, a study conducted on blood donors in the United States[129] questioned the ability of subgenotype A2-derived HBV vaccines to protect against non-A2 HBV (sub)genotypes. It was concluded that while breakthrough infections with non-A2 genotypes were recorded following vaccination, which only prevented clinical disease[128]. In addition, their findings suggested that the vaccine may be less effective for non-A2 infections. In view of the global variability in genotype distribution, any gap in the efficacy of A2 vaccines has potentially important implications for the ongoing protection of populations against HBV infection and its consequences[128]. Therefore, more studies need to focus on the response of genotype E to vaccination, especially considering that this is the genotype prevailing in the region of the world where the virus continues to be hyperendemic and all preventive measures should be optimized.

The emergence of HBV escape mutants may occur under medically induced immune pressure (in association with vaccine or hepatitis B immune globulin) or naturally induced immune pressure (as a result of CHB)[130]. These HBV mutants may carry multiple amino acid substitutions around- and within the HBsAg 'a' determinant, which can affect the binding of neutralizing antibodies (anti-HBsAg), with some of the former remaining undetectable by certain diagnostic tests, thus implying a potential risk in transfusion events[130]. The emergence of S escape mutants, raised concerns about the efficacy of the current vaccine on the African continent. To this day, very few studies have focused on the genotype E response to vaccination, although vaccination began over four decades ago.

CONCLUSION

In conclusion, genotype E has unique molecular and epidemiological characteristics. The natural history of genotype E has not been studied and very little is known about the virological breakthrough as a result of vaccination. Only a few studies that focused on the treatment of a limited number of genotype E infected patients exist, making it difficult to reach any firm conclusions. In addition, most of these studies have been conducted outside of Africa on a small number of individuals that had migrated from Africa, with only a minority of studies carried out on the African continent. Consequently, it is important that African CHB patients infected with genotype E are included in clinical trials focusing on new antiviral therapy, biomarkers and other possible preventive methods. There are multiple reasons for this. Western Africa, where genotype E prevails, is the only region in the world where HBV continues to be

hyperendemic. Although West Africa has a relatively long time span of vaccination against HBV, which began in the Gambia in the early 1980s, the infection is still being maintained in the community. There is a correspondingly high incidence of HBV-associated HCC, ranked fourth worldwide and in SSA, the second leading cancer for men and the third for women, with average age-standardised incidence rates of 18.9 and 8.0 per 100000 persons/year, respectively[85]. In this region, HCC presents in younger age groups and has a median survival rate of approximately 3-4 mo. Genotype E is being dispersed from high to low endemicity regions of the world as a result of migration and this may lead to changes in the natural history of HBV infection in countries of destination, where different genotypes predominate.

Toward achieving the WHO target for the worldwide elimination of viral hepatitis as a public health burden by 2030 there is an urgent need for more in-depth and large-scale investigations into genotype E, which has been under-represented in studies, resulting in the paucity of data on this neglected genotype.

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REFERENCES

1 **World Health Organization**. Hepatitis B Fact Sheet 2021. [cited 7 April 2021]. In: World Health Organization [Internet]. Available from: https://www.who.int/news-room/fact-sheets/detail/hepatitis-b

2 **Steinhauer DA**, Holland JJ. Direct method for quantitation of extreme polymerase error frequencies at selected single base sites in viral RNA. *J Virol* 1986; **57**: 219-228 [PMID: 3001347 DOI: 10.1128/JVI.57.1.219-228.1986]

3 **Norder H**, Couroucé AM, Coursaget P, Echevarria JM, Lee SD, Mushahwar IK, Robertson BH, Locarnini S, Magnius LO. Genetic diversity of hepatitis B virus strains derived worldwide: genotypes, subgenotypes, and HBsAg subtypes. *Intervirology* 2004; **47**: 289-309 [PMID: 15564741 DOI: 10.1159/000080872]

4 **Tatematsu K**, Tanaka Y, Kurbanov F, Sugauchi F, Mano S, Maeshiro T, Nakayoshi T, Wakuta M, Miyakawa Y, Mizokami M. A genetic variant of hepatitis B virus divergent from known human and ape genotypes isolated from a Japanese patient and provisionally assigned to new genotype J. *J Virol* 2009; **83**: 10538-10547 [PMID: 19640977 DOI: 10.1128/JVI.00462-09]

5 **Kramvis A**, Kew MC. Epidemiology of hepatitis B virus in Africa, its genotypes and clinical associations of genotypes. *Hepatol Res* 2007; **37**: S9-S19 [PMID: 17627641 DOI: 10.1111/j.1872-034X.2007.00098.x]

6 **Kramvis A**. Genotypes and genetic variability of hepatitis B virus. *Intervirology* 2014; **57**: 141-150 [PMID: 25034481 DOI: 10.1159/000360947]

7 **Ferlay J**, Colombet M, Soerjomataram I, Mathers C, Parkin DM, Piñeros M, Znaor A, Bray F. Estimating the global cancer incidence and mortality in 2018: GLOBOCAN sources and methods. *Int J Cancer* 2019; **144**: 1941-1953 [PMID: 30350310 DOI: 10.1002/ijc.31937]

8 **Norder H**, Hammas B, Löfdahl S, Couroucé AM, Magnius LO. Comparison of the amino acid sequences of nine different serotypes of hepatitis B surface antigen and genomic classification of the corresponding hepatitis B virus strains. *J Gen Virol* 1992; **73** (Pt 5): 1201-1208 [PMID: 1588323 DOI: 10.1099/0022-1317-73-5-1201]

9 **Velkov S**, Ott JJ, Protzer U, Michler T. The Global Hepatitis B Virus Genotype Distribution Approximated from Available Genotyping Data. *Genes (Basel)* 2018; **9** [PMID: 30326600 DOI: 10.3390/genes9100495]

10 **Kramvis A**. Molecular characteristics and clinical relevance of African genotypes and subgenotypes of hepatitis B virus. *S Afr Med J* 2018; **108**: 17-21 [PMID: 30182908]

11 **Kramvis A**, Restorp K, Norder H, Botha JF, Magnius LO, Kew MC. Full genome analysis of hepatitis B virus genotype E strains from South-Western Africa and Madagascar reveals low genetic variability. *J Med Virol* 2005; **77**: 47-52 [PMID: 16032729 DOI: 10.1002/jmv.20412]

12 **Norder H**, Couroucé AM, Magnius LO. Complete genomes, phylogenetic relatedness, and structural proteins of six strains of the hepatitis B virus, four of which represent two new genotypes. *Virology* 1994; **198**: 489-503 [PMID: 8291231 DOI: 10.1006/viro.1994.1060]

13 **Vray M**, Debonne JM, Sire JM, Tran N, Chevalier B, Plantier JC, Fall F, Vernet G, Simon F, Mb PS. Molecular epidemiology of hepatitis B virus in Dakar, Sénégal. *J Med Virol* 2006; **78**: 329-334

[PMID: 16419106 DOI: 10.1002/jmv.20544]
14 **Suzuki S**, Sugauchi F, Orito E, Kato H, Usuda S, Siransy L, Arita I, Sakamoto Y, Yoshihara N, El-Gohary A, Ueda R, Mizokami M. Distribution of hepatitis B virus (HBV) genotypes among HBV carriers in the Cote d'Ivoire: complete genome sequence and phylogenetic relatedness of HBV genotype E. *J Med Virol* 2003; **69**: 459-465 [PMID: 12601751 DOI: 10.1002/jmv.10331]
15 **Odemuyiwa SO**, Mulders MN, Oyedele OI, Ola SO, Odaibo GN, Olaleye DO, Muller CP. Phylogenetic analysis of new hepatitis B virus isolates from Nigeria supports endemicity of genotype E in West Africa. *J Med Virol* 2001; **65**: 463-469 [PMID: 11596079 DOI: 10.1002/jmv.2058]
16 **Mulders MN**, Venard V, Njayou M, Edorh AP, Bola Oyefolu AO, Kehinde MO, Muyembe Tamfum JJ, Nebie YK, Maiga I, Ammerlaan W, Fack F, Omilabu SA, Le Faou A, Muller CP. Low genetic diversity despite hyperendemicity of hepatitis B virus genotype E throughout West Africa. *J Infect Dis* 2004; **190**: 400-408 [PMID: 15216479 DOI: 10.1086/421502]
17 **Andernach IE**, Hübschen JM, Muller CP. Hepatitis B virus: the genotype E puzzle. *Rev Med Virol* 2009; **19**: 231-240 [PMID: 19475565 DOI: 10.1002/rmv.618]
18 **Bannister EG**, Yuen L, Littlejohn M, Edwards R, Sozzi V, Colledge D, Li X, Locarnini S, Hardikar W, Revill PA. Molecular characterization of hepatitis B virus (HBV) in African children living in Australia identifies genotypes and variants associated with poor clinical outcome. *J Gen Virol* 2018; **99**: 1103-1114 [PMID: 29932395 DOI: 10.1099/jgv.0.001086]
19 **Singh J**, Dickens C, Pahal V, Kumar R, Chaudhary R, Kramvis A, Kew MC. First report of genotype e of hepatitis B virus in an Indian population. *Intervirology* 2009; **52**: 235-238 [PMID: 19590227 DOI: 10.1159/000227279]
20 **Alvarado Mora MV**, Romano CM, Gomes-Gouvêa MS, Gutierrez MF, Carrilho FJ, Pinho JR. Molecular epidemiology and genetic diversity of hepatitis B virus genotype E in an isolated Afro-Colombian community. *J Gen Virol* 2010; **91**: 501-508 [PMID: 19846674 DOI: 10.1099/vir.0.015958-0]
21 **Kramvis A**, Paraskevis D. Subgenotype A1 of HBV--tracing human migrations in and out of Africa. *Antivir Ther* 2013; **18**: 513-521 [PMID: 23792935 DOI: 10.3851/IMP2657]
22 **Hassan MA**, Kim WR, Li R, Smith CI, Fried MW, Sterling RK, Ghany MG, Wahed AS, Ganova-Raeva LM, Roberts LR, Lok ASF; Hepatitis B Research Network. Characteristics of US-Born Versus Foreign-Born Americans of African Descent With Chronic Hepatitis B. *Am J Epidemiol* 2017; **186**: 356-366 [PMID: 28525625 DOI: 10.1093/aje/kwx064]
23 **Andernach IE**, Nolte C, Pape JW, Muller CP. Slave trade and hepatitis B virus genotypes and subgenotypes in Haiti and Africa. *Emerg Infect Dis* 2009; **15**: 1222-1228 [PMID: 19751583 DOI: 10.3201/eid1508.081642]
24 **Chu CJ**, Keeffe EB, Han SH, Perrillo RP, Min AD, Soldevila-Pico C, Carey W, Brown RS Jr, Luketic VA, Terrault N, Lok AS. Hepatitis B virus genotypes in the United States: results of a nationwide study. *Gastroenterology* 2003; **125**: 444-451 [PMID: 12891547 DOI: 10.1016/S0016-5085(03)00895-3]
25 **Kidd-Ljunggren K**, Couroucé AM, Oberg M, Kidd AH. Genetic conservation within subtypes in the hepatitis B virus pre-S2 region. *J Gen Virol* 1994; **75** (Pt 6): 1485-1490 [PMID: 7726888 DOI: 10.1099/0022-1317-75-6-1485]
26 **Liu HF**, Sokal E, Goubau P. Wide variety of genotypes and geographic origins of hepatitis B virus in Belgian children. *J Pediatr Gastroenterol Nutr* 2001; **32**: 274-277 [PMID: 11345175 DOI: 10.1097/00005176-200103000-00008]
27 **van Steenbergen JE**, Niesters HG, Op de Coul EL, van Doornum GJ, Osterhaus AD, Leentvaar-Kuijpers A, Coutinho RA, van den Hoek JA. Molecular epidemiology of hepatitis B virus in Amsterdam 1992-1997. *J Med Virol* 2002; **66**: 159-165 [PMID: 11782923 DOI: 10.1002/jmv.2125]
28 **Hübschen JM**, Andernach IE, Muller CP. Hepatitis B virus genotype E variability in Africa. *J Clin Virol* 2008; **43**: 376-380 [PMID: 18922739 DOI: 10.1016/j.jcv.2008.08.018]
29 **Ingasia LAO**, Kostaki EG, Paraskevis D, Kramvis A. Global and regional dispersal patterns of hepatitis B virus genotype E from and in Africa: A full-genome molecular analysis. *PLoS One* 2020; **15**: e0240375 [PMID: 33031453 DOI: 10.1371/journal.pone.0240375]
30 **Andernach IE**, Hunewald OE, Muller CP. Bayesian inference of the evolution of HBV/E. *PLoS One* 2013; **8**: e81690 [PMID: 24312336 DOI: 10.1371/journal.pone.0081690]
31 **Forbi JC**, Vaughan G, Purdy MA, Campo DS, Xia GL, Ganova-Raeva LM, Ramachandran S, Thai H, Khudyakov YE. Epidemic history and evolutionary dynamics of hepatitis B virus infection in two remote communities in rural Nigeria. *PLoS One* 2010; **5**: e11615 [PMID: 20657838 DOI: 10.1371/journal.pone.0011615]
32 **Mühlemann B**, Jones TC, Damgaard PB, Allentoft ME, Shevnina I, Logvin A, Usmanova E, Panyushkina IP, Boldgiv B, Bazartseren T, Tashbaeva K, Merz V, Lau N, Smrčka V, Voyakin D, Kitov E, Epimakhov A, Pokutta D, Vicze M, Price TD, Moiseyev V, Hansen AJ, Orlando L, Rasmussen S, Sikora M, Vinner L, Osterhaus ADME, Smith DJ, Glebe D, Fouchier RAM, Drosten C, Sjögren KG, Kristiansen K, Willerslev E. Ancient hepatitis B viruses from the Bronze Age to the Medieval period. *Nature* 2018; **557**: 418-423 [PMID: 29743673 DOI: 10.1038/s41586-018-0097-z]
33 **Paraskevis D**, Magiorkinis G, Magiorkinis E, Ho SY, Belshaw R, Allain JP, Hatzakis A. Dating the origin and dispersal of hepatitis B virus infection in humans and primates. *Hepatology* 2013; **57**: 908-916 [PMID: 22987324 DOI: 10.1002/hep.26079]
34 **Kahila Bar-Gal G**, Kim MJ, Klein A, Shin DH, Oh CS, Kim JW, Kim TH, Kim SB, Grant PR, Pappo O, Spigelman M, Shouval D. Tracing hepatitis B virus to the 16th century in a Korean

mummy. *Hepatology* 2012; **56**: 1671-1680 [PMID: 22610996 DOI: 10.1002/hep.25852]
35 **Patterson Ross Z**, Klunk J, Fornaciari G, Giuffra V, Duchêne S, Duggan AT, Poinar D, Douglas MW, Eden JS, Holmes EC, Poinar HN. Correction: The paradox of HBV evolution as revealed from a 16th century mummy. *PLoS Pathog* 2018; **14**: e1006887 [PMID: 29425240 DOI: 10.1371/journal.ppat.1006887]
36 **Krause-Kyora B**, Susat J, Key FM, Kühnert D, Bosse E, Immel A, Rinne C, Kornell SC, Yepes D, Franzenburg S, Heyne HO, Meier T, Lösch S, Meller H, Friederich S, Nicklisch N, Alt KW, Schreiber S, Tholey A, Herbig A, Nebel A, Krause J. Neolithic and medieval virus genomes reveal complex evolution of hepatitis B. *Elife* 2018; **7** [PMID: 29745896 DOI: 10.7554/eLife.36666]
37 **Kurbanov F**, Tanaka Y, Fujiwara K, Sugauchi F, Mbanya D, Zekeng L, Ndembi N, Ngansop C, Kaptue L, Miura T, Ido E, Hayami M, Ichimura H, Mizokami M. A new subtype (subgenotype) Ac (A3) of hepatitis B virus and recombination between genotypes A and E in Cameroon. *J Gen Virol* 2005; **86**: 2047-2056 [PMID: 15958684 DOI: 10.1099/vir.0.80922-0]
38 **Simmonds P**, Midgley S. Recombination in the genesis and evolution of hepatitis B virus genotypes. *J Virol* 2005; **79**: 15467-15476 [PMID: 16306618 DOI: 10.1128/JVI.79.24.15467-15476.2005]
39 **Datta S**. Excavating new facts from ancient Hepatitis B virus sequences. *Virology* 2020; **549**: 89-99 [PMID: 32858309 DOI: 10.1016/j.virol.2020.08.002]
40 **Foupouapouognigni Y**, Mba SA, Betsem à Betsem E, Rousset D, Froment A, Gessain A, Njouom R. Hepatitis B and C virus infections in the three Pygmy groups in Cameroon. *J Clin Microbiol* 2011; **49**: 737-740 [PMID: 21106785 DOI: 10.1128/JCM.01475-10]
41 **Locarnini S**, Littlejohn M, Aziz MN, Yuen L. Possible origins and evolution of the hepatitis B virus (HBV). *Semin Cancer Biol* 2013; **23**: 561-575 [PMID: 24013024 DOI: 10.1016/j.semcancer.2013.08.006]
42 **Locarnini SA**, Littlejohn M, Yuen LKW. Origins and Evolution of the Primate Hepatitis B Virus. *Front Microbiol* 2021; **12**: 653684 [PMID: 34108947 DOI: 10.3389/fmicb.2021.653684]
43 **Milich D**, Liang TJ. Exploring the biological basis of hepatitis B e antigen in hepatitis B virus infection. *Hepatology* 2003; **38**: 1075-1086 [PMID: 14578844 DOI: 10.1053/jhep.2003.50453]
44 **Kramvis A**, Kostaki EG, Hatzakis A, Paraskevis D. Immunomodulatory Function of HBeAg Related to Short-Sighted Evolution, Transmissibility, and Clinical Manifestation of Hepatitis B Virus. *Front Microbiol* 2018; **9**: 2521 [PMID: 30405578 DOI: 10.3389/fmicb.2018.02521]
45 **Takahashi K**, Brotman B, Usuda S, Mishiro S, Prince AM. Full-genome sequence analyses of hepatitis B virus (HBV) strains recovered from chimpanzees infected in the wild: implications for an origin of HBV. *Virology* 2000; **267**: 58-64 [PMID: 10648183 DOI: 10.1006/viro.1999.0102]
46 **Hu X**, Margolis HS, Purcell RH, Ebert J, Robertson BH. Identification of hepatitis B virus indigenous to chimpanzees. *Proc Natl Acad Sci U S A* 2000; **97**: 1661-1664 [PMID: 10677515 DOI: 10.1073/pnas.97.4.1661]
47 **Souza BF**, Drexler JF, Lima RS, Rosário Mde O, Netto EM. Theories about evolutionary origins of human hepatitis B virus in primates and humans. *Braz J Infect Dis* 2014; **18**: 535-543 [PMID: 24726560 DOI: 10.1016/j.bjid.2013.12.006]
48 **Littlejohn M**, Locarnini S, Yuen L. Origins and Evolution of Hepatitis B Virus and Hepatitis D Virus. *Cold Spring Harb Perspect Med* 2016; **6**: a021360 [PMID: 26729756 DOI: 10.1101/cshperspect.a021360]
49 **Hassemer M**, Finkernagel M, Peiffer KH, Glebe D, Akhras S, Reuter A, Scheiblauer H, Sommer L, Chudy M, Nübling CM, Hildt E. Comparative characterization of hepatitis B virus surface antigen derived from different hepatitis B virus genotypes. *Virology* 2017; **502**: 1-12 [PMID: 27951436 DOI: 10.1016/j.virol.2016.12.003]
50 **Mathet VL**, Cuestas ML, Ruiz V, Minassian ML, Rivero C, Trinks J, Daleoso G, León LM, Sala A, Libellara B, Corach D, Oubiña JR. Detection of hepatitis B virus (HBV) genotype E carried--even in the presence of high titers of anti-HBs antibodies--by an Argentinean patient of African descent who had received vaccination against HBV. *J Clin Microbiol* 2006; **44**: 3435-3439 [PMID: 16954295 DOI: 10.1128/JCM.00866-06]
51 **Kramvis A**, Arakawa K, Yu MC, Nogueira R, Stram DO, Kew MC. Relationship of serological subtype, basic core promoter and precore mutations to genotypes/subgenotypes of hepatitis B virus. *J Med Virol* 2008; **80**: 27-46 [PMID: 18041043 DOI: 10.1002/jmv.21049]
52 **Lok AS**, Akarca U, Greene S. Mutations in the pre-core region of hepatitis B virus serve to enhance the stability of the secondary structure of the pre-genome encapsidation signal. *Proc Natl Acad Sci U S A* 1994; **91**: 4077-4081 [PMID: 8171038 DOI: 10.1073/pnas.91.9.4077]
53 **Yousif M**, Mudawi H, Hussein W, Mukhtar M, Nemeri O, Glebe D, Kramvis A. Genotyping and virological characteristics of hepatitis B virus in HIV-infected individuals in Sudan. *Int J Infect Dis* 2014; **29**: 125-132 [PMID: 25449246 DOI: 10.1016/j.ijid.2014.07.002]
54 **Yousif M**, Mudawi H, Bakhiet S, Glebe D, Kramvis A. Molecular characterization of hepatitis B virus in liver disease patients and asymptomatic carriers of the virus in Sudan. *BMC Infect Dis* 2013; **13**: 328 [PMID: 23865777 DOI: 10.1186/1471-2334-13-328]
55 **Araujo NM**. Hepatitis B virus intergenotypic recombinants worldwide: An overview. *Infect Genet Evol* 2015; **36**: 500-510 [PMID: 26299884 DOI: 10.1016/j.meegid.2015.08.024]
56 **Mahgoub S**, Candotti D, El Ekiaby M, Allain JP. Hepatitis B virus (HBV) infection and recombination between HBV genotypes D and E in asymptomatic blood donors from Khartoum, Sudan. *J Clin Microbiol* 2011; **49**: 298-306 [PMID: 21048009 DOI: 10.1128/JCM.00867-10]
57 **Garmiri P**, Loua A, Haba N, Candotti D, Allain JP. Deletions and recombinations in the core region

of hepatitis B virus genotype E strains from asymptomatic blood donors in Guinea, west Africa. *J Gen Virol* 2009; **90**: 2442-2451 [PMID: 19535503 DOI: 10.1099/vir.0.012013-0]

58 **Abdou Chekaraou M**, Brichler S, Mansour W, Le Gal F, Garba A, Dény P, Gordien E. A novel hepatitis B virus (HBV) subgenotype D (D8) strain, resulting from recombination between genotypes D and E, is circulating in Niger along with HBV/E strains. *J Gen Virol* 2010; **91**: 1609-1620 [PMID: 20147517 DOI: 10.1099/vir.0.018127-0]

59 **Laoi BN**, Crowley B. Molecular characterization of hepatitis B virus (HBV) isolates, including identification of a novel recombinant, in patients with acute HBV infection attending an Irish hospital. *J Med Virol* 2008; **80**: 1554-1564 [PMID: 18649329 DOI: 10.1002/jmv.21273]

60 **Matlou MK**, Gaelejwe LR, Musyoki AM, Rakgole JN, Selabe SG, Amponsah-Dacosta E. A novel hepatitis B virus recombinant genotype D4/E identified in a South African population. *Heliyon* 2019; **5**: e01477 [PMID: 31008405 DOI: 10.1016/j.heliyon.2019.e01477]

61 **Martel N**, Gomes SA, Chemin I, Trépo C, Kay A. Improved rolling circle amplification (RCA) of hepatitis B virus (HBV) relaxed-circular serum DNA (RC-DNA). *J Virol Methods* 2013; **193**: 653-659 [PMID: 23928222 DOI: 10.1016/j.jviromet.2013.07.045]

62 **Mak D**, Kramvis A. Molecular characterization of hepatitis B virus isolated from Black South African cancer patients, with and without hepatocellular carcinoma. *Arch Virol* 2020; **165**: 1815-1825 [PMID: 32504396 DOI: 10.1007/s00705-020-04686-4]

63 **Cohen D**, Ghosh S, Shimakawa Y, Ramou N, Garcia PS, Dubois A, Guillot C, Kakwata-Nkor Deluce N, Tilloy V, Durand G, Voegele C, Ndow G, d'Alessandro U, Brochier-Armanet C, Alain S, Le Calvez-Kelm F, Hall J, Zoulim F, Mendy M, Thursz M, Lemoine M, Chemin I. Hepatitis B virus preS2Δ38-55 variants: A newly identified risk factor for hepatocellular carcinoma. *JHEP Rep* 2020; **2**: 100144 [PMID: 32904132 DOI: 10.1016/j.jhepr.2020.100144]

64 **Kew MC**, Kramvis A, Yu MC, Arakawa K, Hodkinson J. Increased hepatocarcinogenic potential of hepatitis B virus genotype A in Bantu-speaking sub-saharan Africans. *J Med Virol* 2005; **75**: 513-521 [PMID: 15714494 DOI: 10.1002/jmv.20311]

65 **Lin CL**, Kao JH. Clinical implications of hepatitis B virus variants. *J Formos Med Assoc* 2010; **109**: 321-325 [PMID: 20514738 DOI: 10.1016/s0929-6646(10)60059-9]

66 **Chen CH**, Hung CH, Lee CM, Hu TH, Wang JH, Wang JC, Lu SN, Changchien CS. Pre-S deletion and complex mutations of hepatitis B virus related to advanced liver disease in HBeAg-negative patients. *Gastroenterology* 2007; **133**: 1466-1474 [PMID: 17915220 DOI: 10.1053/j.gastro.2007.09.002]

67 **Fang ZL**, Sabin CA, Dong BQ, Ge LY, Wei SC, Chen QY, Fang KX, Yang JY, Wang XY, Harrison TJ. HBV A1762T, G1764A mutations are a valuable biomarker for identifying a subset of male HBsAg carriers at extremely high risk of hepatocellular carcinoma: a prospective study. *Am J Gastroenterol* 2008; **103**: 2254-2262 [PMID: 18844615 DOI: 10.1111/j.1572-0241.2008.01974.x]

68 **Beasley RP**, Hwang LY, Lee GC, Lan CC, Roan CH, Huang FY, Chen CL. Prevention of perinatally transmitted hepatitis B virus infections with hepatitis B immune globulin and hepatitis B vaccine. *Lancet* 1983; **2**: 1099-1102 [PMID: 6138642 DOI: 10.1016/s0140-6736(83)90624-4]

69 **Botha JF**, Ritchie MJ, Dusheiko GM, Mouton HW, Kew MC. Hepatitis B virus carrier state in black children in Ovamboland: role of perinatal and horizontal infection. *Lancet* 1984; **1**: 1210-1212 [PMID: 6144925 DOI: 10.1016/s0140-6736(84)91694-5]

70 **Szmuness W**. Recent advances in the study of the epidemiology of hepatitis B. *Am J Pathol* 1975; **81**: 629-650 [PMID: 1108668]

71 **Keane E**, Funk AL, Shimakawa Y. Systematic review with meta-analysis: the risk of mother-to-child transmission of hepatitis B virus infection in sub-Saharan Africa. *Aliment Pharmacol Ther* 2016; **44**: 1005-1017 [PMID: 27630001 DOI: 10.1111/apt.13795]

72 **Shimakawa Y**, Lemoine M, Njai HF, Bottomley C, Ndow G, Goldin RD, Jatta A, Jeng-Barry A, Wegmuller R, Moore SE, Baldeh I, Taal M, D'Alessandro U, Whittle H, Njie R, Thursz M, Mendy M. Natural history of chronic HBV infection in West Africa: a longitudinal population-based study from The Gambia. *Gut* 2016; **65**: 2007-2016 [PMID: 26185161 DOI: 10.1136/gutjnl-2015-309892]

73 **Dumpis U**, Holmes EC, Mendy M, Hill A, Thursz M, Hall A, Whittle H, Karayiannis P. Transmission of hepatitis B virus infection in Gambian families revealed by phylogenetic analysis. *J Hepatol* 2001; **35**: 99-104 [PMID: 11495049 DOI: 10.1016/s0168-8278(01)00064-2]

74 **Martinson FE**, Weigle KA, Royce RA, Weber DJ, Suchindran CM, Lemon SM. Risk factors for horizontal transmission of hepatitis B virus in a rural district in Ghana. *Am J Epidemiol* 1998; **147**: 478-487 [PMID: 9525535 DOI: 10.1093/oxfordjournals.aje.a009474]

75 **Sangaré L**, Sombié R, Combasséré AW, Kouanda A, Kania D, Zerbo O, Lankoandé J. [Antenatal transmission of hepatitis B virus in an area of HIV moderate prevalence, Burkina Faso]. *Bull Soc Pathol Exot* 2009; **102**: 226-229 [PMID: 19950539]

76 **Ilboudo D**, Simpore J, Ouermi D, Bisseye C, Sagna T, Odolini S, Buelli F, Pietra V, Pignatelli S, Gnoula C, Nikiema JB, Musumeci S. Towards the complete eradication of mother-to-child HIV/HBV coinfection at Saint Camille Medical Centre in Burkina Faso, Africa. *Braz J Infect Dis* 2010; **14**: 219-224 [PMID: 20835503 DOI: 10.1016/s1413-8670(10)70047-7]

77 **Eke AC**, Eke UA, Okafor CI, Ezebialu IU, Ogbuagu C. Prevalence, correlates and pattern of hepatitis B surface antigen in a low resource setting. *Virol J* 2011; **8**: 12 [PMID: 21226907 DOI: 10.1186/1743-422X-8-12]

78 **Ogawa M**, Hasegawa K, Naritomi T, Torii N, Hayashi N. Clinical features and viral sequences of various genotypes of hepatitis B virus compared among patients with acute hepatitis B. *Hepatol Res*

2002; **23**: 167-177 [PMID: 12076712 DOI: 10.1016/s1386-6346(01)00176-0]

79 **Kobayashi M**, Suzuki F, Arase Y, Akuta N, Suzuki Y, Hosaka T, Saitoh S, Kobayashi M, Tsubota A, Someya T, Ikeda K, Matsuda M, Sato J, Kumada H. Infection with hepatitis B virus genotype A in Tokyo, Japan during 1976 through 2001. *J Gastroenterol* 2004; **39**: 844-850 [PMID: 15565403 DOI: 10.1007/s00535-004-1400-3]

80 **Wai CT**, Fontana RJ, Polson J, Hussain M, Shakil AO, Han SH, Davern TJ, Lee WM, Lok AS; US Acute Liver Failure Study Group. Clinical outcome and virological characteristics of hepatitis B-related acute liver failure in the United States. *J Viral Hepat* 2005; **12**: 192-198 [PMID: 15720535 DOI: 10.1111/j.1365-2893.2005.00581.x]

81 **Zhang HW**, Yin JH, Li YT, Li CZ, Ren H, Gu CY, Wu HY, Liang XS, Zhang P, Zhao JF, Tan XJ, Lu W, Schaefer S, Cao GW. Risk factors for acute hepatitis B and its progression to chronic hepatitis in Shanghai, China. *Gut* 2008; **57**: 1713-1720 [PMID: 18755887 DOI: 10.1136/gut.2008.157149]

82 **Schaefer S**. Hepatitis B virus: significance of genotypes. *J Viral Hepat* 2005; **12**: 111-124 [PMID: 15720525 DOI: 10.1111/j.1365-2893.2005.00584.x]

83 **Wongjarupong N**, Yonli AT, Nagalo BM, Djigma FW, Somda SK, Hassan MA, Mohamed EA, Sorgho AP, Compaore TR, Soubeiga ST, Kiendrebeogo I, Sanou M, Diarra B, Yang HI, Chen CJ, Ouattara AK, Zohoncon TM, Martinson JJ, Buetow K, Chamcheu JC, Antwi SO, Borad MJ, Simpore J, Roberts LR. Characteristics of Patients With Chronic Hepatitis B Virus Infection With Genotype E Predominance in Burkina Faso. *Hepatol Commun* 2020; **4**: 1781-1792 [PMID: 33305149 DOI: 10.1002/hep4.1595]

84 **Ishikawa T**, Ichida T, Yamagiwa S, Sugahara S, Uehara K, Okoshi S, Asakura H. High viral loads, serum alanine aminotransferase and gender are predictive factors for the development of hepatocellular carcinoma from viral compensated liver cirrhosis. *J Gastroenterol Hepatol* 2001; **16**: 1274-1281 [PMID: 11903747 DOI: 10.1046/j.1440-1746.2001.02616.x]

85 **Mak D**, Kramvis A. Epidemiology and aetiology of hepatocellular carcinoma in Sub-Saharan Africa. *Hepatoma Res* 2021; **7**: 39 [DOI: 10.20517/2394-5079.2021.15]

86 **Cuenca-Gómez JÁ**, Lozano-Serrano AB, Cabezas-Fernández MT, Soriano-Pérez MJ, Vázquez-Villegas J, Estévez-Escobar M, Cabeza-Barrera I, Salas-Coronas J. Chronic hepatitis B genotype E in African migrants: response to nucleos(t)ide treatment in real clinical practice. *BMC Infect Dis* 2018; **18**: 568 [PMID: 30428845 DOI: 10.1186/s12879-018-3469-y]

87 **Malagnino V**, Salpini R, Maffongelli G, Battisti A, Fabeni L, Piermatteo L, Colagrossi L, Fini V, Ricciardi A, Sarrecchia C, Perno CF, Andreoni M, Svicher V, Sarmati L. High rates of chronic HBV genotype E infection in a group of migrants in Italy from West Africa: Virological characteristics associated with poor immune clearance. *PLoS One* 2018; **13**: e0195045 [PMID: 29596494 DOI: 10.1371/journal.pone.0195045]

88 **Okeke E**, Davwar PM, Roberts L, Sartorius K, Spearman W, Malu A, Duguru M. Epidemiology of Liver Cancer in Africa: Current and Future Trends. *Semin Liver Dis* 2020; **40**: 111-123 [PMID: 31726474 DOI: 10.1055/s-0039-3399566]

89 **Lemoine M**, Thursz MR. Battlefield against hepatitis B infection and HCC in Africa. *J Hepatol* 2017; **66**: 645-654 [PMID: 27771453 DOI: 10.1016/j.jhep.2016.10.013]

90 **Matthews PC**, Geretti AM, Goulder PJ, Klenerman P. Epidemiology and impact of HIV coinfection with hepatitis B and hepatitis C viruses in Sub-Saharan Africa. *J Clin Virol* 2014; **61**: 20-33 [PMID: 24973812 DOI: 10.1016/j.jcv.2014.05.018]

91 **Adesina O**, Oladokun A, Akinyemi O, Adedokun B, Awolude O, Odaibo G, Olaleye D, Adewole I. Human immuno-deficiency virus and hepatitis B virus coinfection in pregnancy at the University College Hospital, Ibadan. *Afr J Med Med Sci* 2010; **39**: 305-310 [PMID: 21735996]

92 **Anigilaje EA**, Olutola A. Prevalence and Clinical and Immunoviralogical Profile of Human Immunodeficiency Virus-Hepatitis B Coinfection among Children in an Antiretroviral Therapy Programme in Benue State, Nigeria. *ISRN Pediatr* 2013; **2013**: 932697 [PMID: 23691352 DOI: 10.1155/2013/932697]

93 **Tounkara A**, Sarro YS, Kristensen S, Dao S, Diallo H, Diarra B, Noumsi TG, Guindo O. Seroprevalence of HIV/HBV coinfection in Malian blood donors. *J Int Assoc Physicians AIDS Care (Chic)* 2009; **8**: 47-51 [PMID: 19182212 DOI: 10.1177/1545109708330118]

94 **Jobarteh M**, Malfroy M, Peterson I, Jeng A, Sarge-Njie R, Alabi A, Peterson K, Cotten M, Hall A, Rowland-Jones S, Whittle H, Tedder R, Jaye A, Mendy M. Seroprevalence of hepatitis B and C virus in HIV-1 and HIV-2 infected Gambians. *Virol J* 2010; **7**: 230 [PMID: 20843322 DOI: 10.1186/1743-422X-7-230]

95 **Geretti AM**, Patel M, Sarfo FS, Chadwick D, Verheyen J, Fraune M, Garcia A, Phillips RO. Detection of highly prevalent hepatitis B virus coinfection among HIV-seropositive persons in Ghana. *J Clin Microbiol* 2010; **48**: 3223-3230 [PMID: 20631103 DOI: 10.1128/JCM.02231-09]

96 **Laurent C**, Bourgeois A, Mpoudi-Ngolé E, Kouanfack C, Ciaffi L, Nkoué N, Mougnutou R, Calmy A, Koulla-Shiro S, Ducos J, Delaporte E. High rates of active hepatitis B and C co-infections in HIV-1 infected Cameroonian adults initiating antiretroviral therapy. *HIV Med* 2010; **11**: 85-89 [PMID: 19659944 DOI: 10.1111/j.1468-1293.2009.00742.x]

97 **N'Dri-Yoman T**, Anglaret X, Messou E, Attia A, Polneau S, Toni T, Chenal H, Seyler C, Gabillard D, Wakasugi N, Eholié S, Danel C. Occult HBV infection in untreated HIV-infected adults in Côte d'Ivoire. *Antivir Ther* 2010; **15**: 1029-1034 [PMID: 21041918 DOI: 10.3851/IMP1641]

98 **Thio CL**, Seaberg EC, Skolasky R Jr, Phair J, Visscher B, Muñoz A, Thomas DL; Multicenter AIDS Cohort Study. HIV-1, hepatitis B virus, and risk of liver-related mortality in the Multicenter Cohort

Study (MACS). *Lancet* 2002; **360**: 1921-1926 [PMID: 12493258 DOI: 10.1016/S0140-6736(02)11913-1]

99 **Toyé RM**, Lô G, Diop-Ndiaye H, Cissé AM, Ndiaye AJS, Kébé-Fall K, Dramé A, Cohen D, Pujol FH, Mboup S, Boye CS, Chemin I, Laborde-Balen G, Taverne B, Touré-Kane C. Prevalence and molecular characterization of hepatitis B virus infection in HIV-infected children in Senegal. *Clin Res Hepatol Gastroenterol* 2021; **45**: 101502 [PMID: 32828748 DOI: 10.1016/j.clinre.2020.07.007]

100 **Raimondo G**, Allain JP, Brunetto MR, Buendia MA, Chen DS, Colombo M, Craxì A, Donato F, Ferrari C, Gaeta GB, Gerlich WH, Levrero M, Locarnini S, Michalak T, Mondelli MU, Pawlotsky JM, Pollicino T, Prati D, Puoti M, Samuel D, Shouval D, Smedile A, Squadrito G, Trépo C, Villa E, Will H, Zanetti AR, Zoulim F. Statements from the Taormina expert meeting on occult hepatitis B virus infection. *J Hepatol* 2008; **49**: 652-657 [PMID: 18715666 DOI: 10.1016/j.jhep.2008.07.014]

101 **Mudawi H**, Hussein W, Mukhtar M, Yousif M, Nemeri O, Glebe D, Kramvis A. Overt and occult hepatitis B virus infection in adult Sudanese HIV patients. *Int J Infect Dis* 2014; **29**: 65-70 [PMID: 25449238 DOI: 10.1016/j.ijid.2014.07.004]

102 **Chen XL**, Li M, Zhang XL. HBV genotype B/C and response to lamivudine therapy: a systematic review. *Biomed Res Int* 2013; **2013**: 672614 [PMID: 24364035 DOI: 10.1155/2013/672614]

103 **Zalewska M**, Domagała M, Simon K, Gładysz A. [Hepatitis B virus genotypes and the response to lamivudine therapy]. *Pol Arch Med Wewn* 2005; **114**: 1190-1199 [PMID: 16789488]

104 **Lin CL**, Kao JH. The clinical implications of hepatitis B virus genotype: Recent advances. *J Gastroenterol Hepatol* 2011; **26** Suppl 1: 123-130 [PMID: 21199523 DOI: 10.1111/j.1440-1746.2010.06541.x]

105 **Erhardt A**, Göbel T, Ludwig A, Lau GK, Marcellin P, van Bömmel F, Heinzel-Pleines U, Adams O, Häussinger D. Response to antiviral treatment in patients infected with hepatitis B virus genotypes E-H. *J Med Virol* 2009; **81**: 1716-1720 [PMID: 19697400 DOI: 10.1002/jmv.21588]

106 **Boglione L**, Cariti G, Ghisetti V, Burdino E, Di Perri G. Extended duration of treatment with peginterferon alfa-2a in patients with chronic hepatitis B, HBeAg-negative and E genotype: A retrospective analysis. *J Med Virol* 2018; **90**: 1047-1052 [PMID: 29384201 DOI: 10.1002/jmv.25038]

107 **Boglione L**, Cardellino CS, De Nicolò A, Cariti G, Di Perri G, D'Avolio A. Different HBsAg decline after 3 years of therapy with entecavir in patients affected by chronic hepatitis B HBeAg-negative and genotype A, D and E. *J Med Virol* 2014; **86**: 1845-1850 [PMID: 25131947 DOI: 10.1002/jmv.24038]

108 **Boglione L**, Cusato J, Cariti G, Di Perri G, D'Avolio A. The E genotype of hepatitis B: clinical and virological characteristics, and response to interferon. *J Infect* 2014; **69**: 81-87 [PMID: 24631900 DOI: 10.1016/j.jinf.2014.02.018]

109 **Boyd A**, Moh R, Gabillard D, le Carrou J, Danel C, Anglaret X, Eholié SP, Maylin S, Delaugerre C, Zoulim F, Girard PM, Lacombe K; ANRS 12240 VarBVA study. Low risk of lamivudine-resistant HBV and hepatic flares in treated HIV-HBV-coinfected patients from Côte d'Ivoire. *Antivir Ther* 2015; **20**: 643-654 [PMID: 25852125 DOI: 10.3851/IMP2959]

110 **De Francesco MA**, Gargiulo F, Spinetti A, Zaltron S, Giagulli C, Caccuri F, Castelli F, Caruso A. Clinical course of chronic hepatitis B patients receiving nucleos(t)ide analogues after virological breakthrough during monotherapy with lamivudine. *New Microbiol* 2015; **38**: 29-37 [PMID: 25742145]

111 **Westland C**, Delaney W 4th, Yang H, Chen SS, Marcellin P, Hadziyannis S, Gish R, Fry J, Brosgart C, Gibbs C, Miller M, Xiong S. Hepatitis B virus genotypes and virologic response in 694 patients in phase III studies of adefovir dipivoxil1. *Gastroenterology* 2003; **125**: 107-116 [PMID: 12851876 DOI: 10.1016/s0016-5085(03)00700-5]

112 **Boglione L**, Cusato J, Cariti G, Di Perri G, D'Avolio A. Role of HBsAg decline in patients with chronic hepatitis B HBeAg-negative and E genotype treated with pegylated-interferon. *Antiviral Res* 2016; **136**: 32-36 [PMID: 27793564 DOI: 10.1016/j.antiviral.2016.10.011]

113 **Shokrgozar MA**, Shokri F. Subtype specificity of anti-HBs antibodies produced by human B-cell lines isolated from normal individuals vaccinated with recombinant hepatitis B vaccine. *Vaccine* 2002; **20**: 2215-2220 [PMID: 12009275 DOI: 10.1016/S0264-410X(02)00116-0]

114 **Spearman CW**, Sonderup MW. Preventing hepatitis B and hepatocellular carcinoma in South Africa: The case for a birth-dose vaccine. *S Afr Med J* 2014; **104**: 610-612 [PMID: 25212400 DOI: 10.7196/samj.8607]

115 **Kao JH**. Hepatitis B vaccination and prevention of hepatocellular carcinoma. *Best Pract Res Clin Gastroenterol* 2015; **29**: 907-917 [PMID: 26651252 DOI: 10.1016/j.bpg.2015.09.011]

116 **Gerlich WH**. Prophylactic vaccination against hepatitis B: achievements, challenges and perspectives. *Med Microbiol Immunol* 2015; **204**: 39-55 [PMID: 25523195 DOI: 10.1007/s00430-014-0373-y]

117 **Poorolajal J**, Mahmoodi M, Majdzadeh R, Nasseri-Moghaddam S, Haghdoost A, Fotouhi A. Long-term protection provided by hepatitis B vaccine and need for booster dose: a meta-analysis. *Vaccine* 2010; **28**: 623-631 [PMID: 19887132 DOI: 10.1016/j.vaccine.2009.10.068]

118 **Ismail AM**, Raghavendran A, Sivakumar J, Radhakrishnan M, Rose W, Abraham P. Mother to child transmission of hepatitis B virus: a cause for concern. *Indian J Med Microbiol* 2015; **33** Suppl: 140-143 [PMID: 25657134 DOI: 10.4103/0255-0857.150931]

119 **Graber-Stiehl I**. The silent epidemic killing more people than HIV, malaria or TB. *Nature* 2018; **564**: 24-26 [PMID: 30518904 DOI: 10.1038/d41586-018-07592-7]

120 **Andersson MI**, Rajbhandari R, Kew MC, Vento S, Preiser W, Hoepelman AI, Theron G, Cotton M, Cohn J, Glebe D, Lesi O, Thursz M, Peters M, Chung R, Wiysonge C. Mother-to-child transmission of hepatitis B virus in sub-Saharan Africa: time to act. *Lancet Glob Health* 2015; **3**: e358-e359 [PMID: 26087980 DOI: 10.1016/S2214-109X(15)00056-X]

121 **Keeble S**, Quested J, Barker D, Varadarajan A, Shankar AG. Immunization of babies born to HBsAg positive mothers: An audit on the delivery and completeness of follow up in Norfolk and Suffolk, United Kingdom. *Hum Vaccin Immunother* 2015; **11**: 1153-1156 [PMID: 25876072 DOI: 10.1080/21645515.2015.1019977]

122 **Ma L**, Alla NR, Li X, Mynbaev OA, Shi Z. Mother-to-child transmission of HBV: review of current clinical management and prevention strategies. *Rev Med Virol* 2014; **24**: 396-406 [PMID: 24956038 DOI: 10.1002/rmv.1801]

123 **Dionne-Odom J**, Njei B, Tita ATN. Elimination of Vertical Transmission of Hepatitis B in Africa: A Review of Available Tools and New Opportunities. *Clin Ther* 2018; **40**: 1255-1267 [PMID: 29983265 DOI: 10.1016/j.clinthera.2018.05.016]

124 **Smith S**, Harmanci H, Hutin Y, Hess S, Bulterys M, Peck R, Rewari B, Mozalevskis A, Shibeshi M, Mumba M, Le LV, Ishikawa N, Nolna D, Sereno L, Gore C, Goldberg DJ, Hutchinson S. Global progress on the elimination of viral hepatitis as a major public health threat: An analysis of WHO Member State responses 2017. *JHEP Rep* 2019; **1**: 81-89 [PMID: 32039355 DOI: 10.1016/j.jhepr.2019.04.002]

125 **Bancroft WH**, Mundon FK, Russell PK. Detection of additional antigenic determinants of hepatitis B antigen. *J Immunol* 1972; **109**: 842-848 [PMID: 4116360]

126 **Okamoto H**, Tsuda F, Sakugawa H, Sastrosoewignjo RI, Imai M, Miyakawa Y, Mayumi M. Typing hepatitis B virus by homology in nucleotide sequence: comparison of surface antigen subtypes. *J Gen Virol* 1988; **69** (Pt 10): 2575-2583 [PMID: 3171552 DOI: 10.1099/0022-1317-69-10-2575]

127 **Hauser P**, Voet P, Simoen E, Thomas HC, Pêtre J, De Wilde M, Stephenne J. Immunological properties of recombinant HBsAg produced in yeast. *Postgrad Med J* 1987; **63** Suppl 2: 83-91 [PMID: 2446304]

128 **Cassidy A**, Mossman S, Olivieri A, De Ridder M, Leroux-Roels G. Hepatitis B vaccine effectiveness in the face of global HBV genotype diversity. *Expert Rev Vaccines* 2011; **10**: 1709-1715 [PMID: 22085174 DOI: 10.1586/erv.11.151]

129 **Stramer SL**, Wend U, Candotti D, Foster GA, Hollinger FB, Dodd RY, Allain JP, Gerlich W. Nucleic acid testing to detect HBV infection in blood donors. *N Engl J Med* 2011; **364**: 236-247 [PMID: 21247314 DOI: 10.1056/NEJMoa1007644]

130 **Weber B**. Genetic variability of the S gene of hepatitis B virus: clinical and diagnostic impact. *J Clin Virol* 2005; **32**: 102-112 [PMID: 15653412 DOI: 10.1016/j.jcv.2004.10.008]

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MINIREVIEWS

One stop shop approach for the diagnosis of liver hemangioma

Larisa Daniela Sandulescu, Cristiana Marinela Urhut, Sarmis Marian Sandulescu, Ana-Maria Ciurea, Sergiu Marian Cazacu, Sevastita Iordache

ORCID number: Larisa Daniela Sandulescu 0000-0001-7696-3733; Cristiana Marinela Urhut 0000-0002-5353-4327; Sarmis Marian Sandulescu 0000-0002-9444-0618; Ana-Maria Ciurea 0000-0002-0910-475X; Sergiu Marian Cazacu 0000-0001-9623-7683; Sevastita Iordache 0000-0001-9772-7238.

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Larisa Daniela Sandulescu, Sergiu Marian Cazacu, Sevastita Iordache, Department of Gastroenterology, Research Center of Gastroenterology and Hepatology, University of Medicine and Pharmacy of Craiova, Craiova 200349, Romania

Cristiana Marinela Urhut, Department of Gastroenterology, Emergency County Hospital of Craiova, Craiova 200642, Romania

Sarmis Marian Sandulescu, Department of Surgery, Emergency County Hospital of Craiova, University of Medicine and Pharmacy of Craiova, Craiova 200349, Romania

Ana-Maria Ciurea, Department of Oncology, Emergency County Hospital of Craiova, University of Medicine and Pharmacy of Craiova, Craiova 200349, Romania

Corresponding author: Larisa Daniela Sandulescu, MD, Academic Research, Associate Professor, Department of Gastroenterology, Research Center of Gastroenterology and Hepatology, University of Medicine and Pharmacy of Craiova, Petru Rares Street 2, Craiova 200349, Romania. larisasandulescu@yahoo.com

Abstract

Hepatic hemangioma is usually detected on a routine ultrasound examination because of silent clinical behaviour. The typical ultrasound appearance of hemangioma is easily recognizable and quickly guides the diagnosis without the need for further investigation. But there is also an entire spectrum of atypical and uncommon ultrasound features and our review comes to detail these particular aspects. An atypical aspect in standard ultrasound leads to the continuation of explorations with an imaging investigation with contrast substance [ultrasound/computed tomography/or magnetic resonance imaging (MRI)]. For a clinician who practices ultrasound and has an ultrasound system in the room, the easiest, fastest, non-invasive and cost-effective method is contrast enhanced ultrasound (CEUS). Approximately 85% of patients are correctly diagnosed with this method and the patient has the correct diagnosis in about 30 min without fear of malignancy and without waiting for a computer tomography (CT)/MRI appointment. In less than 15% of patients CEUS does not provide a conclusive appearance; thus, CT scan or MRI becomes mandatory and liver biopsy is rarely required. The aim of this updated review is to synthesize the typical and atypical ultrasound aspects of hepatic hemangioma in the adult patient and to propose a fast, non-invasive and cost-effective clinical-ultrasound algorithm for the diagnosis of hepatic hemangioma.

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Key Words: Hemangioma; Liver; Ultrasound; Contrast media; Diagnosis; Algorithm

Core Tip: Liver hemangiomas are benign tumors usually found on a routine ultrasound in an asymptomatic adult patient. A high-performance ultrasonographic system equipped with contrast-enhanced ultrasound software, allows the experienced examinator to orient the diagnosis quickly, cost-effectively and non-invasively in most cases. This article reviews the typical and atypical ultrasound features of hepatic hemangioma and proposes a diagnostic algorithm for liver hemangiomas in patients referred to the hepatologist.

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INTRODUCTION

After focal fatty sparing, hepatic hemangioma (HH) is the second most common benign solid lesion of the liver[1]. The rate of detection of HHs has increased as imaging methods have become more effectiveness and accessible. The prevalence depends on the method used for detection: 2%-4% for ultrasonography, up to 5% for computed tomography and up to 7% of cases in autopsy cases[2-5]. HH are more common in women than in men[4]. It can appear at any age but are detected more frequently between 30-50 years[6]. HHs are usually single, small in size, less than 3 cm, but can also be multiple and large in size up to 20 cm.

PATHOLOGY

HHs belong to the group of non-epithelial lesions, consisting of a blood-filled space, fed by hepatic arterial circulation. HH arises from a vascular malformation and increases in size mainly by dilating the vessels inside the tumour.

The pathogenesis of hemangioma is not entirely understood, the theory of congenital disorder[7] with possible hormonal dependence has been taken into account[4]. Macroscopically, HHs are well delineated, described as flat red-blue lesions. Hemangiomas are classified into three types: Cavernous, capillary and sclerosing hemangioma. Capillary hemangiomas are usually small, less than 3 cm, while cavernous hemangiomas reach sizes over 5 cm. Sclerotic hemangioma is small, completely fibrous, therefore it can occasionally be misdiagnosed as a malignant fibrous tumor[9,10]. Microscopically, hemangiomas consist of cavernous vascular spaces padded with a flattened endothelium divided by fibrous septa of varying thicknesses that are often incomplete. Currently, according to newer classification system of the International Society for the Study of Vascular Anomalies ISSVA, last updated in 2018, HH is a vascular tumor, considered as a slow flow venous malformation[11].

NATURAL COURSE

Small hemangiomas are usually asymptomatic, detected by chance on imaging evaluation. Multiple or bulky tumors can cause symptoms, as pain in abdominal right upper quadrant secondary to infarction, haemorrhage, torsion or distention of the Glisson's capsule. Other symptoms like fullness, nausea, vomiting and early satiety may result from compression of adjacent organs[12].

Liver function tests are usually normal. The natural history of hemangiomas is variable: Most of them remain stable, some may grow or involute. In the vast majority of cases does not require treatment or monitoring.

ULTRASOUND EXAMINATION IN HH

B-mode ultrasound

In recent years, ultrasound examination is the main method of detecting HH due to the fact that it is widely available, inexpensive, rapidly performed without exposing the patient to radiation. Because ultrasonography systems are becoming more and more efficient, smaller and smaller masses are detected, from 2-3 mm, especially if a linear probe with a frequency higher than 8 MHz is used (Figure 1).

The classic sonographic appearance of hemangioma is that of a homogeneous hyperechoic mass, measuring less than 3 cm in diameter with acoustic enhancement and sharp margins[13] (Figure 2). Sometimes it outlines a central hypoechoic area (Figure 3). HHs does not have a peritumoral halo and pushes the hepatic vessels without their invasion or thrombosis (Figure 4). The acoustic enhancement is due to the blood content. When located subdiaphragmatically it produces the artifact "in the mirror" (Figure 5). The hyperechoic appearance is related to the interfaces between vascular space and the fibrous stroma[13]. HH is usualy homogenous mass, but at dimension > 5 cm may show inhomogeneous echogenicity probably because of intratumorally changes, such as thrombosis or fibrosis[14] (Figure 6). No intra-tumoral vessels are seen at color Doppler exam due very slow intralesional flows, but power Doppler technique is more sensitive in detecting blood flow[13] (Figure 7). This aspect is found in most cases of HHs and corresponds histologically to the cavernous hemangioma[14]. Most typical-looking hemangiomas measure less than 3 cm[13].

Contrast enhanced ultrasound

Contrast enhanced ultrasound (CEUS) can be performed immediately after standard ultrasound exam while focal liver lesion (FLL) is found, in the same session, using a dedicated contrast software. Currently, four contrast agents are used in the imaging assessment of FLLs[15,16].

Traditionally CEUS reveals tissue perfusion in real time, in all arterial, portal and late phases but a new contrast agent (Sonazoid) allows the assessment of an additional postvascular phase (Kupffer)[17].

The aspect of the capture in the arterial phase orients on the tumor type while the presence or absence of the wash-out in the late phase differentiates the benign tumors from the malignant masses[15,16]. For the diagnosis of HH the arterial phase is the most important. The typical CEUS feature of a hemangioma, regardless of the injected contrast agent, is peripheral nodular enhancement in the arterial phase with progressive centripetal partial or complete fill-in[16] in portal venous phase and complete enhancement in late phase (Figures 8 and 9). In the postvascular phase (specific for Levovist) hemangioma is isoenhancement or slight hypoenhancement relative to surrounding liver parenchyma[18]. The described appearance is highly suggestive of hemangioma. When the two hallmarks of haemangioma, peripheral pools and centripetal progression, are present the diagnosis of HH is most likely, the specificity of the method approaching 100% in most studies[19,20].

Not all hemangiomas have typical enhancement, thus, the overall sensitivity of CEUS for diagnosis of hemangioma is lower than specificity, approximately 86% (95% confidence interval: 81%-92%) according to a meta-analysis including 612 cases from 20 studies[20]. As the years passed, the equipment evolved, and the examiners gained more experience. Recent multicenter European studies, each with over 1000 examined FLL, reveal that CEUS correctly diagnosed 85%-90% of hemangiomas[21-24] and if a computerized image analysis is added the diagnostic accuracy reaches 93.3%[25]. Moreover, there are studies that demonstrate CEUS to be approximately equal to the computed tomography (CT)-scan or magnetic resonance imaging (MRI) regarding to assessment of tumor differentiation and specification of newly discovered liver tumors in clinical practice, including for HH[26,27].

Because it is a proven method, WFUMB (World Federation for Ultrasound in Medicine and Biology) Guidelines for CEUS in the liver – update 2020 recommends CEUS as the first line imaging technique for the characterization of incidentally, indeterminate FLLs at ultrasound in patients with non-cirrhotic liver and no history or clinical suspicion of malignancy[15]. Similarly, the EASL (European Association for the Study of the Liver) Clinical Practice Guidelines on the management of benign liver

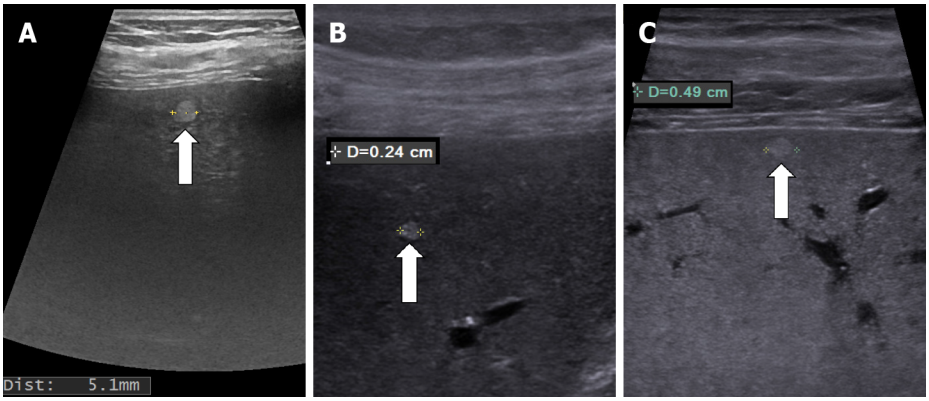

Figure 1 Very small (less than 5 mm), hyperechoic, well delimited hemangiomas showed by linear probe exam (arrows). A: Subcapsular hepatic hemangioma; B and C: Intraparenchymal hepatic hemangioma.

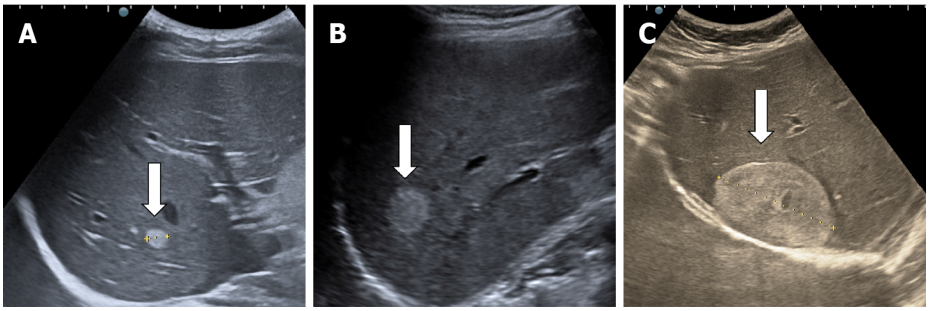

Figure 2 Typical hepatic hemangioma. Ultrasonography shows the hemangioma as a hyperechoic mass with sharp margins. A and B: Small hepatic hemangioma; C: Large hepatic hemangioma.

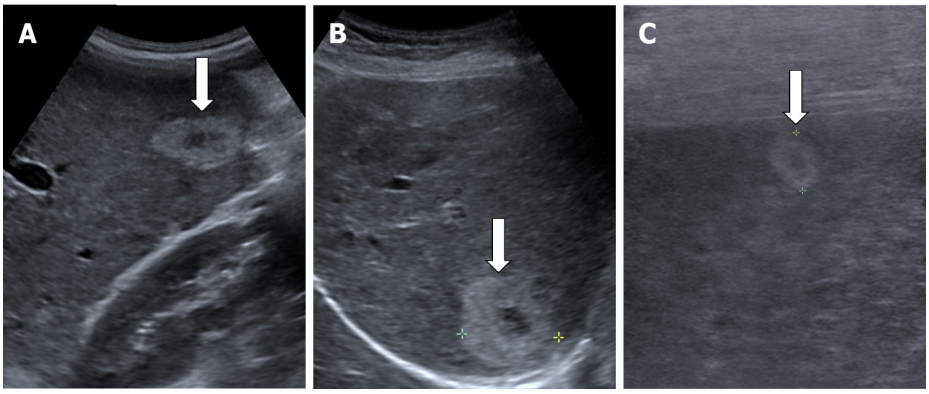

Figure 3 Examples of hyperechoic hepatic hemangioma with hypoechoic central area. A and B: Convex probe; C: Linear probe.

tumors recommends CEUS or another contrast imaging method (CT, MR) when in B-mode ultrasound the appearance is atypical, or when the lesion occurs in cancer patients or those with underlying liver disease[1].

The advantages of CEUS are related to the immediate availability in the ultrasound room where the lesion was detected, the real-time visualization of the tumor perfusion, non-ionizing technique and low financial costs[28,29]. Moreover, sonographic contrast agents have only a few contraindications and precautions, can be used regardless of renal and thyroid impairment and have excellent safety profiles[30].

There are few disadvantages of CEUS as compared to other imaging techniques: the dependence on the experience of the sonographer and providing only limited information in patients with high body mass index or bowel gas overlay. As a specific disadvantage for the diagnosis of hemangioma, CEUS with SonoVue cannot appreciate the very late phase of HH because the contrast substance is eliminated by breathing in about 5-6 min after injection.

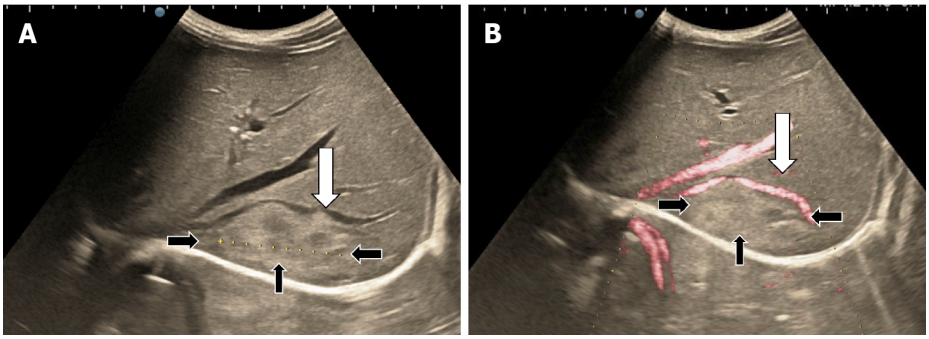

Figure 4 Subdiaphragmatic hepatic hemangioma (white arrows) that pushes the right hepatic vein (black arrows) without its invasion or thrombosis. A: B-mode ultrasound; B: Doppler ultrasound mode.

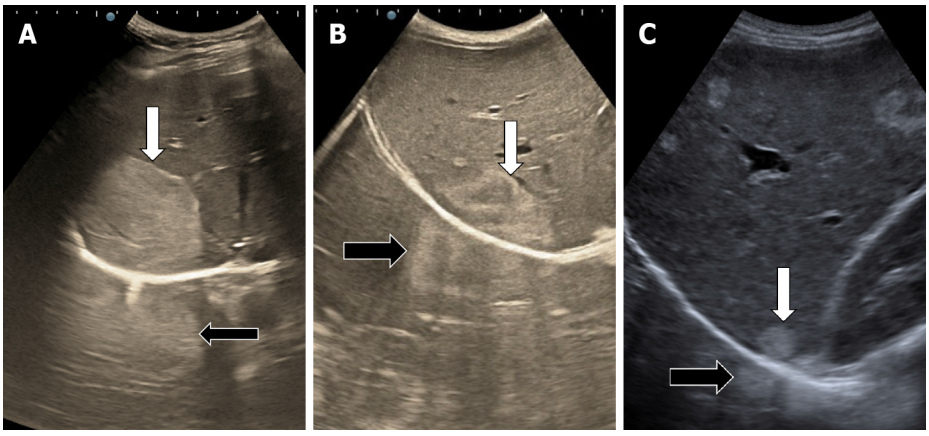

Figure 5 Examples of hepatic hemangioma located subdiaphragmatically (white arrows) with the artefact "in the mirror" (black arrows). A: Large, hyperechoic hepatic hemangioma; B: Inhomogeneous lesion; C: Small hepatic hemangioma.

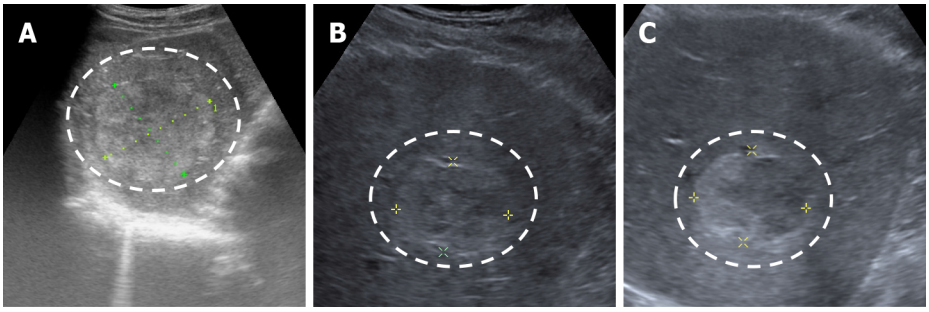

Figure 6 Illustration of hepatic hemangioma with inhomogeneous echogenicity. A-C: Hepatic hemangioma with intratumorally changes, such as fibrosis (A) or thrombosis (B and C).

In some cases, the phenomenon of pseudo-washout in the late phase observed due to hyperinsonation may induce differential diagnosis issues with malignant lesions but the typical appearance of the arterial phase is enough in clinical practice for a correct diagnosis of hemangioma (Figure 10).

HH VARIANTS

Flashfilling hemangioma

The diagnosis of HH is relatively easy if typical peripheral nodular enhancement with subsequent central fill-in is present. In about 16% of all hemangiomas, however, there is a rapid, uniform and intense homogeneous enhancement in the arterial phase, more

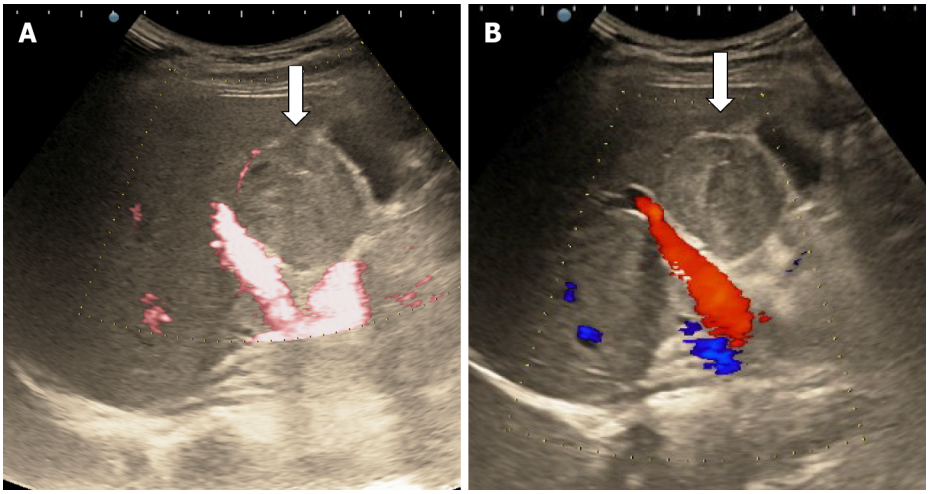

Figure 7 Doppler mode ultrasound for hepatic hemangioma. A and B: No intralesional vessels are seen at power (A) or color Doppler (B) exam due very slow intralesional flows.

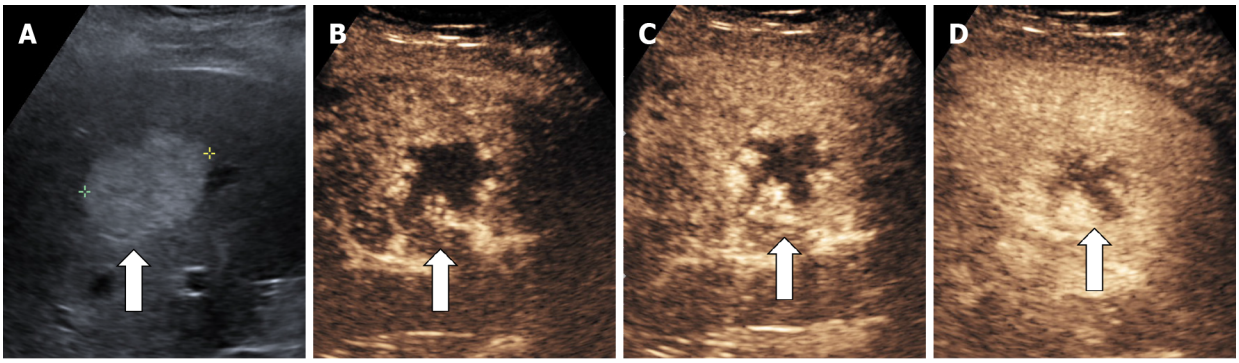

Figure 8 Typical hepatic hemangioma in B-mode ultrasound. A: Hyperechoic mass with sharp margins; B-D: After contrast agent administration the mass shows peripheral nodular enhancement in arterial phase (B and C) with partial centripetal filling in the late phase (D).

often in small hemangiomas (42% are under 1 cm in size)[13,31]. The homogeneous enhancement persists into the portal and late phases (Figure 11).

The mechanism of the enhancement is not clearly understood. The large proportion of small-sized hemangiomas with this type of loading suggests that this pattern may be due to a difference between blood spaces: the smaller the lesion, the more rapid is the spread of contrast agent within[31-33].

Rapidly filling hemangiomas could be difficult to be differentiated from hepatocellular carcinoma (HCC) and hypervascular liver metastases because they exhibit hypervascularity during the hepatic arterial phase. In the late phase, HH remains isoenhanced while metastases and most HCC show a typical washout of contrast agent during the portal and delayed phases. Differentiation remains difficult between small and well-differentiated HH and HCC, which do not show wash-out in the late phase [34].

Hemangioma with echoic border

In some cases (up to 15% of cases) HH has an echoic border, which is seen as a thick echoic rind or a thin echoic rim (Figure 12)[35]. The central part of the lesion has low echogenicity due to previous hemorrhagic necrosis, scarring, or myxomatous changes. On CEUS this type of HH often shows the typical pattern of enhancement so that the diagnosis can be made easily (Figure 13)[32,36].

Sclerosed/sclerosing hemangioma

When the HH is predominantly fibrosed with near complete loss of the vascular spaces it is called 'sclerosed/hyalinized' while partially affected lesions are called 'sclerosing/hyalinizing' hemangiomas[13,32].

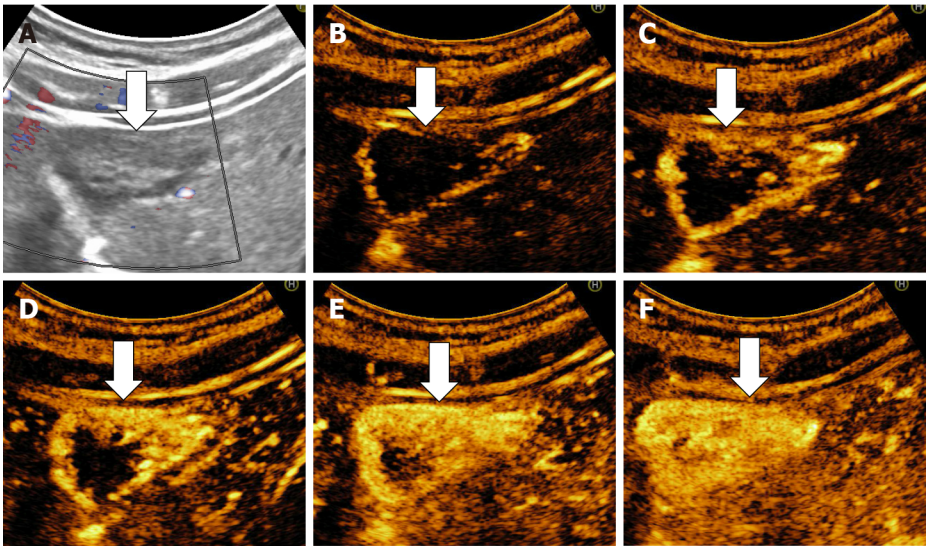

Figure 9 Example of hepatic hemangioma with inhomogeneous echogenicity. A: Gray scale ultrasound; B-E: On contrast enhanced ultrasound the hemangioma shows the typical peripheral nodular contrast enhancement (B and C) and centripetal fill-in (D and E); F: The mass shows strong homogenous enhancement in the late phase.

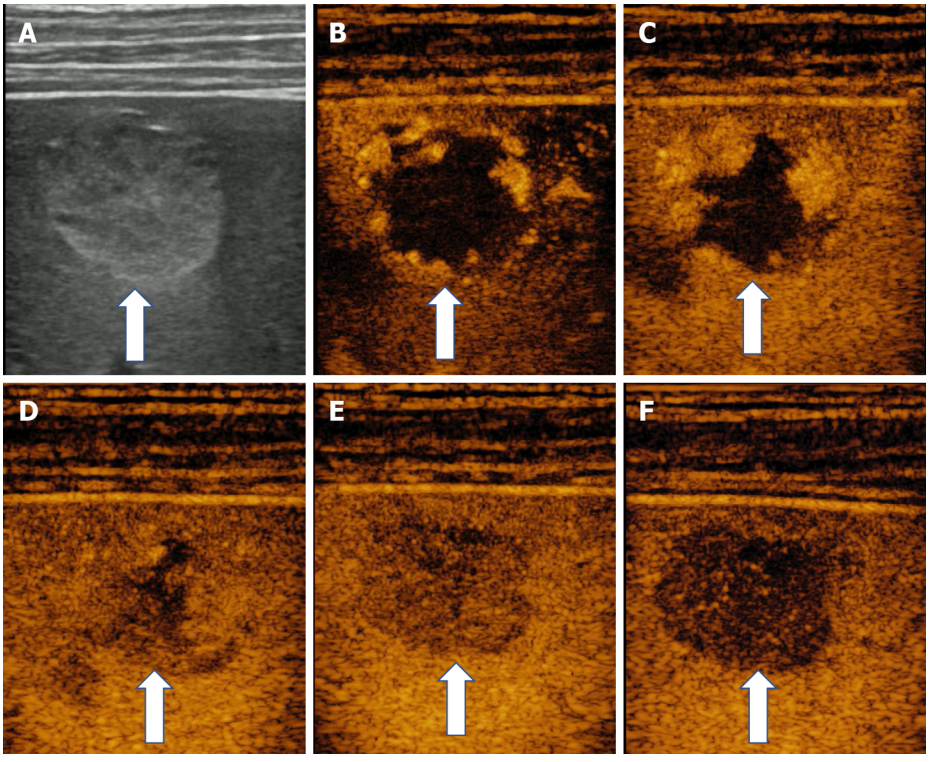

Figure 10 Ultrasound images using linear probe in a case of small, hyperechoic, subcapsular hepatic hemangioma. A: Gray scale ultrasound; B-E: A typical enhancement is showed in contrast enhanced ultrasound. Peripheral pools in arterial phase (B and C) and centripetal progression (D) followed by complete fill-in (E); F: In the late phase phenomenon of pseudo-washout is observed due to hyperinsonation determined by the proximity of the linear probe.

At ultrasound exam, sclerosed hemangioma are heterogeneous in echotexture with predominantly hypoechoic areas from sclerosis and geographic pattern [37]. When placed subcapsular HH causes capsular retraction. If the patient has been known for several years with HH and the images are evaluated dynamically, a reduction in size of the lesion over time can be observed[37].

In CEUS three patterns may be observed: no enhancement, persistent irregular ring enhancement and lack of early enhancement with slight peripheral enhancement in the late phase[33,38,39] (Figure 14). These enhancement patterns create differential diagnosis issues with the intrahepatic cholangiocarcinoma and liver metastasis[40]. In

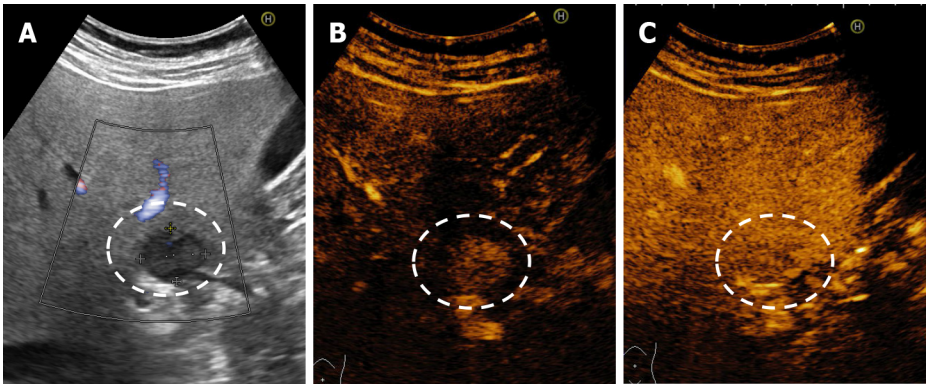

Figure 11 Example of a flashfilling hemangioma. A: On B-mode ultrasound a hypoechoic hemangioma is observed anterior of hepatic hilum; B and C: After injection of contrast agent, a rapid, uniform and intense homogeneous enhancement in the arterial phase (B) that persists into the late phases (C) is observed.

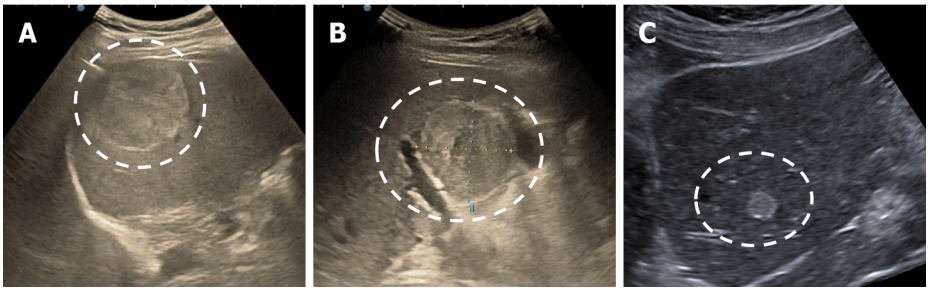

Figure 12 Illustration of hepatic hemangioma with echoic border. A-C: Hepatic hemangioma localized in the right (A and B) and left (C) liver lobe respectively.

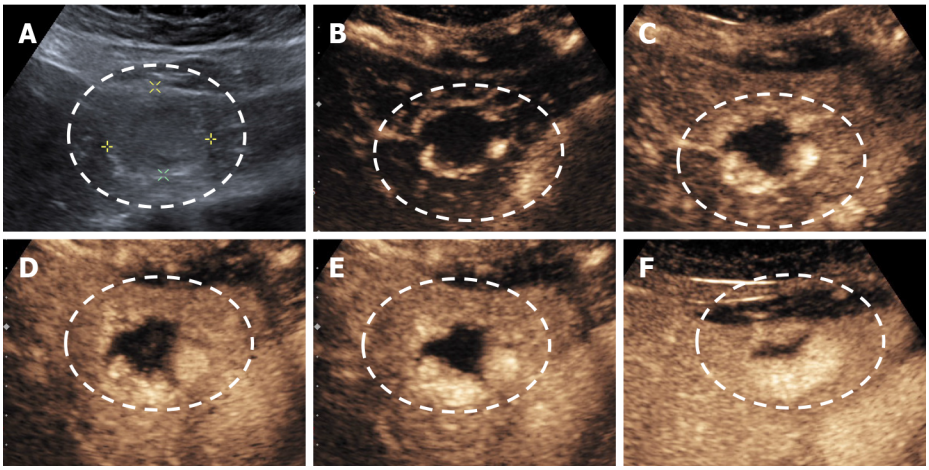

Figure 13 Example of hemangioma with echoic border. A: B-mode ultrasound; B-F: Typical pattern of enhancement: Peripheral nodular enhancement in arterial phase (B and C) centripetal filling (D and E) and incomplete enhancement in late phase (F).

a case report, reinjection of Sonasoid helped in the discriminate between the two entities[41].

Hemangioma with calcifications

In very rare cases, although the tissue is soft, HHs may have calcifications. It can appear in the marginal or central part of the lesion. There may be several spotted calcifications, which correspond to phleboliths or large coarse calcifications[13]. On post-contrast administration, calcified hemangiomas may appear poorly or no enhanced as the calcifications do not show enhancement[37] (Figure 15).

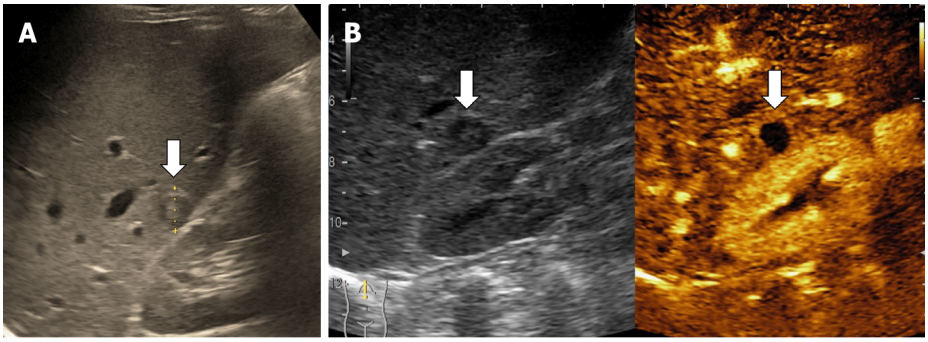

Figure 14 Sclerosed hemangioma in a 45-yr-old man detected on a routine ultrasound examination. A: B-mode ultrasound revealed a small hypoechoic lesion; B: In contrast enhanced ultrasound no enhancement is observed.

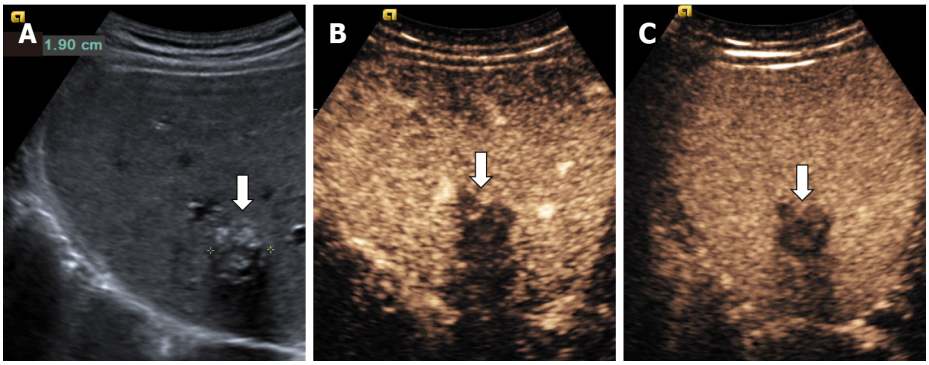

Figure 15 Hemangioma with calcifications in a 64-yr-old man detected on a routine ultrasound examination. A: On B-mode ultrasound several spotted calcifications are showed in the marginal and central part of the lesion and posterior acoustic shadow also; B and C: On post-contrast administration no enhanced is noticed in the portal (B) or the late phase (C).

Giant hemangioma

The majority of the authors define giant hemangiomas as lesions greater 12 cm in diameter[32,33,37]. On B-mode ultrasound, large hemangiomas often appear intense heterogeneous. After intravenous administration of contrast agent, the typical early, peripheral, globular enhancement is observed. However, during the venous and delayed phases, the progressive centripetal enhancement of the lesion is present but does not lead to complete filling[13,42] (Figure 16).

Cystic or multilocular hemangioma

Represents a very rare aspect of HH, cited in only few case reports[43-45]. On B-mode ultrasound appears as inhomogeneous lesion with a large central cavity that contains fluid and possible septa[13,46]. This type of hemangioma could originate from cystic degeneration caused by central thrombosis and hemorrhage[32]. The fluid cystic cavities appear anechoic on US or with hyperechoic material suggesting previous internal hemorrhage. In our experience, the typical early, peripheral, globular enhancement is observed, without centripetal progression of enhancement and the septa could have contrast enhancement as well (Figure 17). Although the appearance of B-mode ultrasound creates differential diagnosis issues with mucinous cystic neoplasm (biliary cystadenoma or cystadenocarcinoma)[47], epithelioid hemangioendothelioma[48] or angiosarcoma[49], CEUS directs the diagnosis to hemangioma.

Multiple hemangiomas and hemangiomatosis

HHs may be multiple in 10%-50% of cases[13]. In standard ultrasound multiple HH has hyperechoic, variable in size, well delimited (Figure 18). The presence of multiple FLLs in B-mode ultrasound has to be differentiated from liver metastases or other multiple malignancies.

Hemangiomatosis, also called diffuse hepatic hemangiomatosis (DHH), is a rare condition characterized by innumerable HHs distributed in the liver parenchyma[13]. In B-mode ultrasound the lesions appear frequently hyperechoic or hypoechoic and the boundary of the lesions is usually ill-defined as compared to multiple HH where

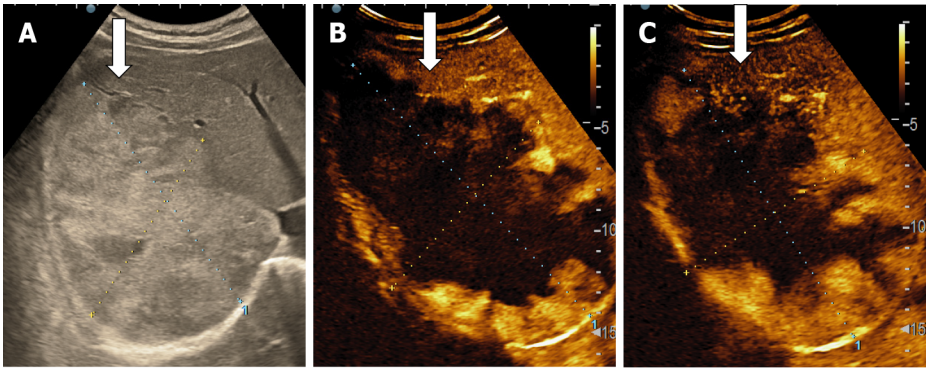

Figure 16 Oblique subcostal baseline image of the right liver lobe in a 45-yr-old woman. A: An intense heterogeneous, large hemangiomas (about 17 cm); B and C: After intravenous administration of contrast agent, the typical early, peripheral, globular enhancement (B) is observed followed by progressive centripetal incomplete enhancement of the lesion (C).

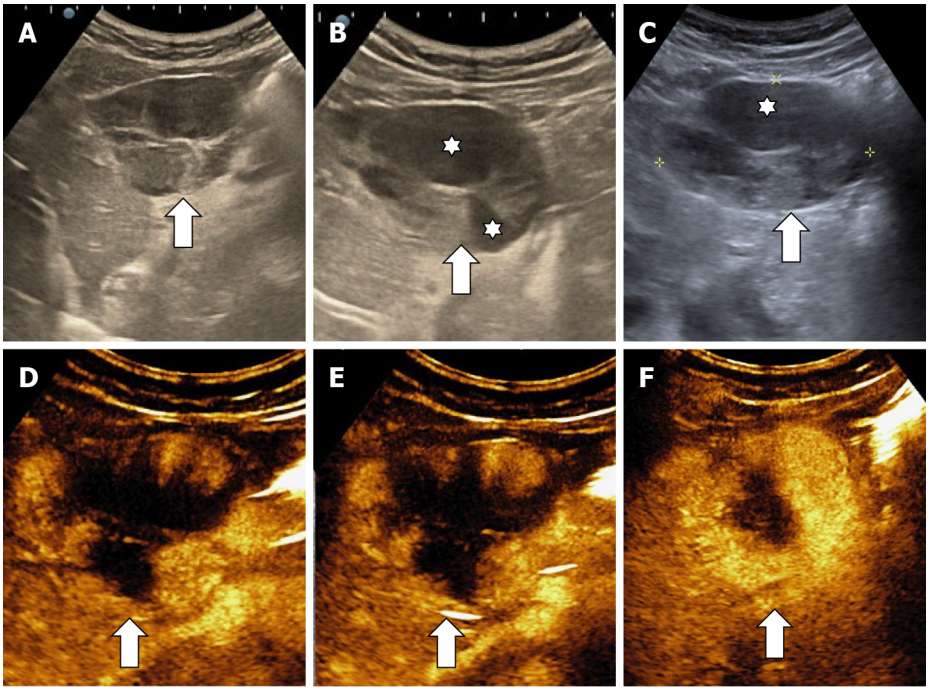

Figure 17 Multicystic hemangioma. A-C: B mode ultrasound shows an inhomogeneous lesion (A) with central cavity (stars) (B) that contains fluid and septa (C); D-F: In contrast enhanced ultrasound the mass shows a progressive (D and E) but partial filling (F) because of the presence of fluid-like cystic cavities that do not enhance.

the lesions are well delineated. DHH is more frequently seen in newborns where the entire liver is usually involved but uncommon cases of isolated DHH without extrahepatic involvement may be seen in the adult population (about 17 cases in the literature)[14,50].

Hepatic hemangiomatosis may present as two forms, a multinodular pattern consisting of multiple small discrete and coalescent nodules, and a diffuse pattern consisting of innumerable poorly defined lesions, with a tendency to confluence, replacing almost all of the liver[14]. To our knowledge, the appearance of DHH in contrast ultrasound has not yet been reported. In our experience, in DHH with multiple, small LFHs, the loading is of the "flashfilling" type (Figure 19).

HEMANGIOMA DEVELOPING IN ABNORMAL LIVER

Hemangioma in fatty liver

The incidence of liver steatosis has increased in recent years and HHs no longer have the typical ultrasound appearance in a hyperechoic liver. Most often they are

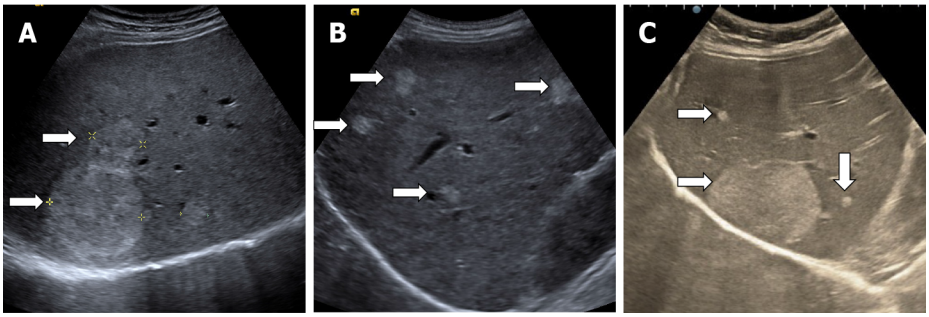

Figure 18 Illustration of multiple hepatic hemangioma in B mode ultrasound. A: Two hyperechoic lesions; B: Four small well delimited lesions; C: One large hepatic hemangioma besides two small hyperechoic lesions.

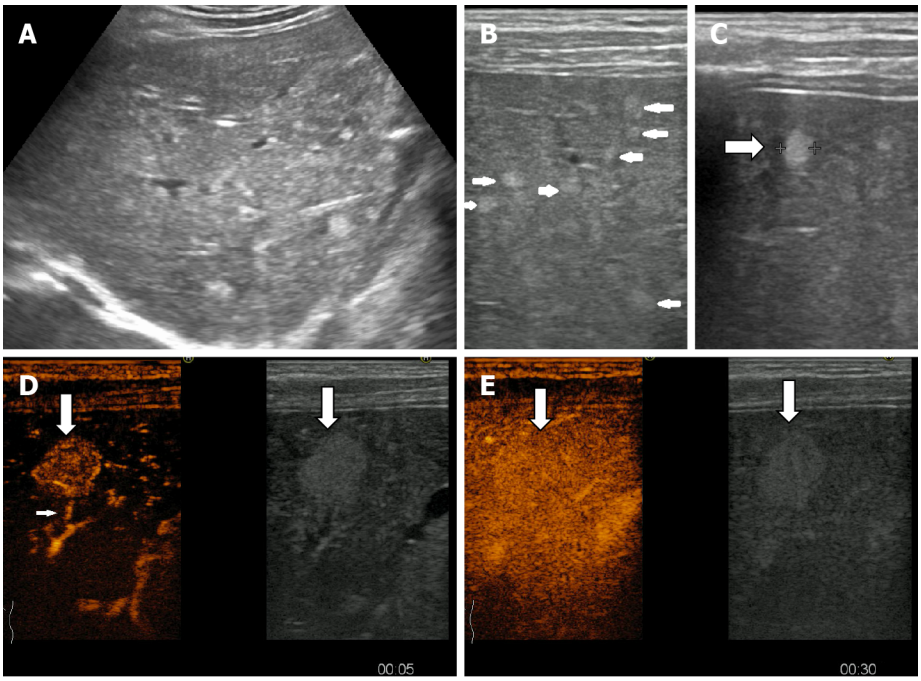

Figure 19 A multinodular pattern of hepatic hemangiomatosis on ultrasound. A: Small hyperechoic lesions are scattered throughout the right liver lobe; B and C: Multiple subcapsular infracentimetric hemangiomas on ultrasound exam using linear probe; D and E: On contrast enhanced ultrasound examination fast-filling hemangioma displaying early homogenous enhancement and visible afferent artery in the artherial phase (D), homogenous enhancement with surrounding parenchyma on early portal phase (E).

isoechoic, or hypoechoic relative to a hyperechoic, fatty liver[13]. In some cases, the area surrounding the hemangioma appears hypoechoic and resembles a halo, an appearance termed a "pseudohalo"[51] (Figure 20). Fortunately, in CEUS HH in fatty liver show a typical enhancement pattern of cavernous or flash-filling hemangioma[52-54] (Figure 21).

Hemangioma in cirrhosis

HHs in cirrhotic liver are uncommon compared to their incidence in non-cirrhotic liver [55]. It appears that the process of cirrhosis (necrosis and fibrosis) obliterates existing hemangiomas. In B-mode ultrasound, HH in cirrhotic liver had an atypical appearance, are often solitary and small in size[13,37,55] difficult to be differentiated from dysplastic nodules and HCC. In CEUS, the enhancement pattern of a cavernous hemangiomas (Figure 22) is enough for diagnosis but flash-filling enhancement of a HH is similar to the enhancement of an HCC in the arterial phase (Figure 23)[56]. Therefore, in the case of an FLL with a hyperenhancement appearance in the arterial phase, it is necessary to complete the imaging assessment.

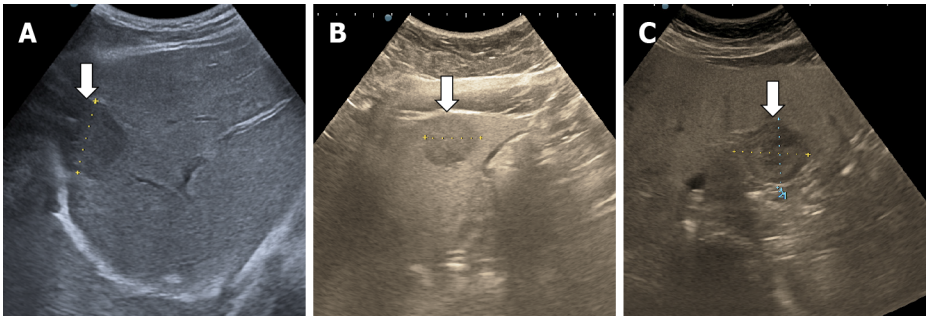

Figure 20 Examples of hypoechoic hemangioma relative to a hyperechoic, fatty liver. A and B: B mode ultrasound show a hypoechoic lesion with a subdiaphragmatic (A) and subcapsular position (B); C: Case of hepatic hemangioma in fatty liver with an area surrounding the lesion appears hypoechoic and resembles a halo, an appearance termed a " pseudohalo".

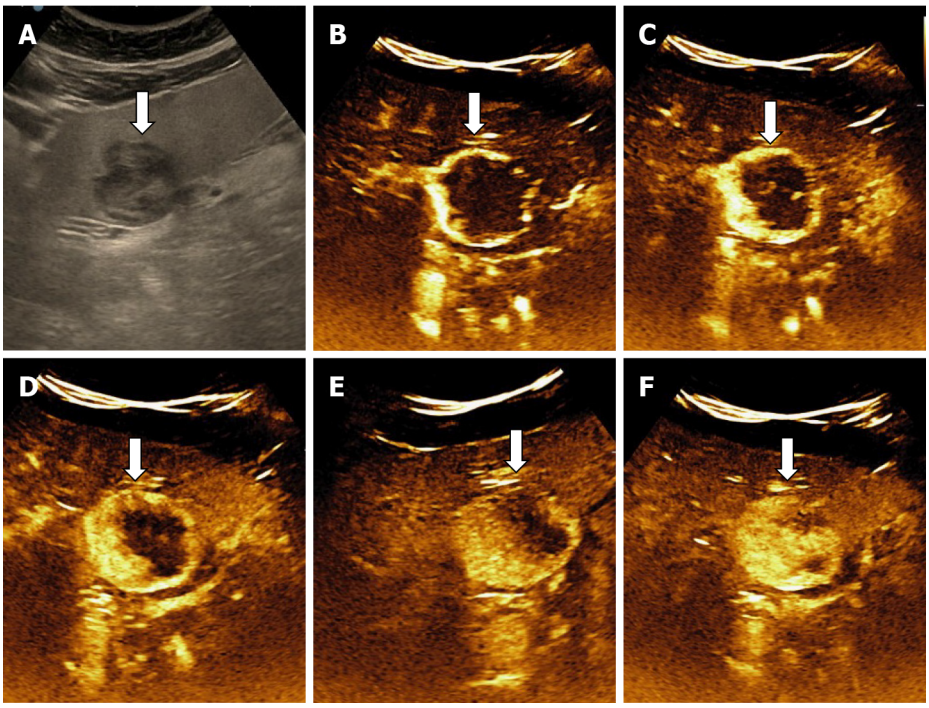

Figure 21 Hypoechoic hemangioma in 57-yr-old woman with liver steatosis. A: B mode ultrasound image; B-F: After intravenous administration of contrast agent, the typical early, peripheral, globular enhancement (B and C) is observed followed by progressive, centripetal (D and E) incomplete (F) enhancement of the lesion.

ONE STOP SHOP APPROACH

Ultrasound has been introduced into clinical practice for over 50 years. Contrast ultrasound after more than 15 years of use has been shown to provide more information than standard ultrasound in the diagnosis of liver tumors. In several countries, the hepatologist also practices ultrasonography. Thus, it has the possibility to complete on the spot the information obtained through anamnesis and clinical examination with imaging data. In an asymptomatic adult patient, without liver or oncological disease, the detection on standard ultrasound of a FLL below 3 cm with homogeneous hyperechoic appearance, sharp margin, posterior enhancement, absence of halo sign, without intra-tumoral vessels at colour Doppler directs the diagnosis to HH and does not require further investigation[1,16]. However, if ultrasound shows a lesion with features other than those described, measures over 3 cm or has been detected in oncology patients or those with underlying liver disease, contrast enhanced imaging (CEUS, CT or MRI) is required[1]. EFSUMB Guidelines for CEUS in the liver – update 2020 recommends CEUS as the first step[16]. CEUS can be performed immediately after standard ultrasound in the consulting room, without the need to assess renal function as needed in the administration of contrast agents for CT/MRI. Studies to date have shown that CEUS has similar performance to computed

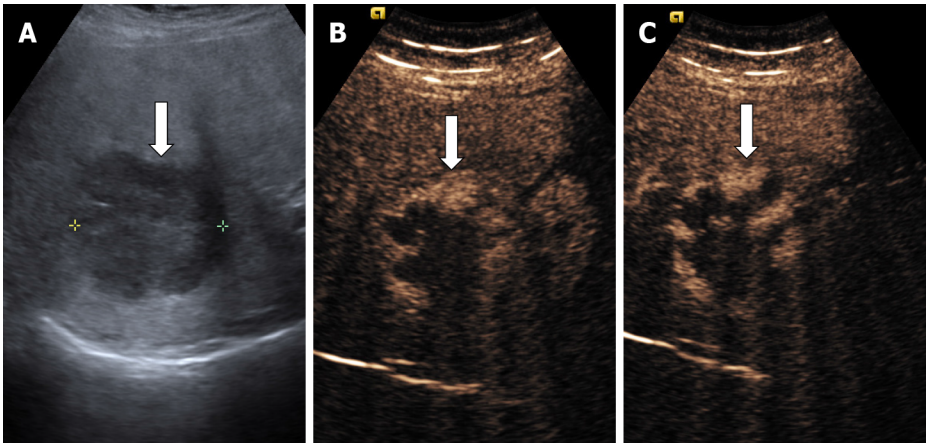

Figure 22 A case of cavernous hemangioma detected in a 64-yr-old man with liver cirrhosis. A: On B mode ultrasound is observed a hyperechoic inhomogeneous liver and a hypoechoic large lesion in the right liver lobe; B: On contrast enhanced ultrasound, the liver lesion shows a typical early, peripheral, globular enhancement; C: In the late phase incomplete enhancement is noticed.

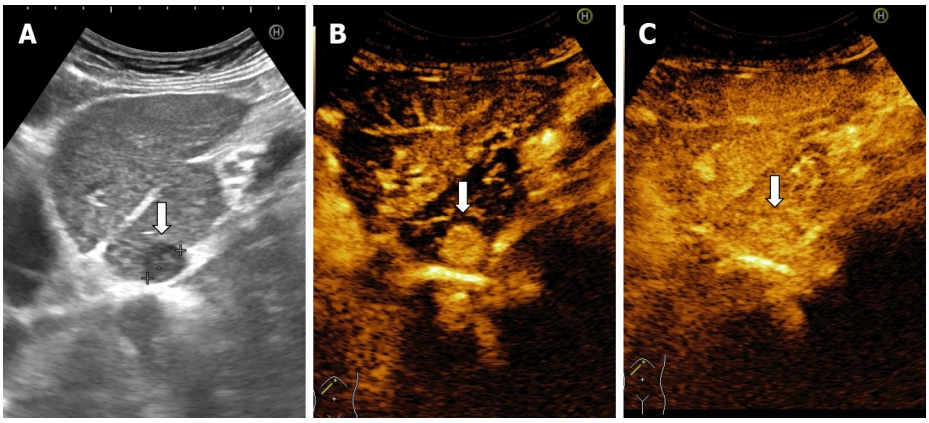

Figure 23 A difficult diagnosis in a case of a flash-filling hemangioma in a woman with hepatitis C liver cirrhosis. A: On B mode ultrasound is observed inhomogeneous liver structure and enlargement of caudate lobe. A small hypoechoic lesion is detected in the caudate lobe; B: Flash-filling enhancement in arterial phase is noticed that is similar to the enhancement of an hepatocellular carcinoma; C: Even in the late phase the liver lesion had the same enhancement comparative with liver, the segmental resection was performed. On histopathological exam the conclusion was: liver hemangioma.

tomography or MRI in the diagnosis of HH. The cost is lower[28,29,57,58], no irradiation and the contrast agent administered has lower toxic and allergic effects. A typical aspect of hemangioma in contrast ultrasound (peripheral and globular enhancement on arterial phase followed by a central enhancement on delayed phases) guides the diagnosis in a maximum of 30 min, stops further investigations and provides mental comfort to the patient. According to studies, this strategy includes approximately 85%-90% of patients[21-24]. If the appearance in the CEUS is not typical, the patient must be scheduled for further investigations. This diagnostic algorithm is applicable to the adult patient in countries where the hepatologist has an ultrasonography system equipped with CEUS software in the consulting room. CEUS saves time, is cost effective and non-invasive.

To our knowledge it is the first article to illustrate the typical and atypical aspects of HH in the adult patient by B-mode ultrasound along with CEUS. It is also for the first time when an algorithm for diagnosing HH is proposed in the consulting room, adapted according to the latest guidelines of EASL and WFUMB (Figure 24).

CONCLUSION

In conclusion, standard and contrast-enhanced ultrasound examination in a clinical context guides the diagnosis of HH in most patients.

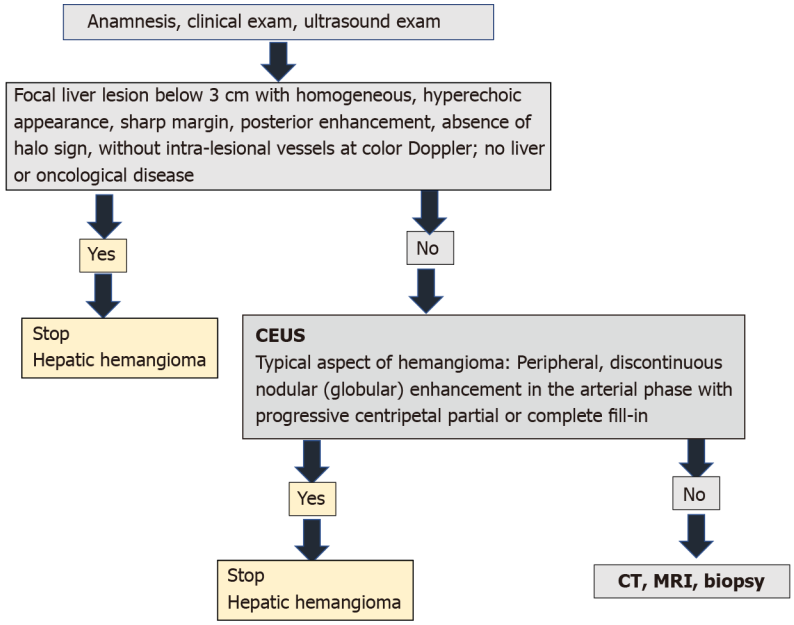

Figure 24 One stop shop approach for the diagnosis of liver hemangioma. Algorithm for diagnosing hepatic hemangioma in the consulting room, adapted according to the latest guidelines of European Association for the Study of the Liver and World Federation for Ultrasound in Medicine and Biology. CEUS: Contrast enhanced ultrasound; CT: Computed tomography; MRI: Magnetic resonance imaging.

REFERENCES

1 **European Association for the Study of the Liver (EASL)**. EASL Clinical Practice Guidelines on the management of benign liver tumours. *J Hepatol* 2016; **65**: 386-398 [PMID: 27085809 DOI: 10.1016/j.jhep.2016.04.001]

2 **Rungsinaporn K**, Phaisakamas T. Frequency of abnormalities detected by upper abdominal ultrasound. *J Med Assoc Thai* 2008; **91**: 1072-1075 [PMID: 18839847]

3 **Kaltenbach TE**, Engler P, Kratzer W, Oeztuerk S, Seufferlein T, Haenle MM, Graeter T. Prevalence of benign focal liver lesions: ultrasound investigation of 45,319 hospital patients. *Abdom Radiol (NY)* 2016; **41**: 25-32 [PMID: 26830608 DOI: 10.1007/s00261-015-0605-7]

4 **Kamyab AA**, Rezaei-Kalantari K. Hepatic Hemangioma in a Cluster of Iranian Population. *J Med Ultrasound* 2019; **27**: 97-100 [PMID: 31316220 DOI: 10.4103/JMU.JMU_98_18]

5 **Horta G**, López M, Dotte A, Cordero J, Chesta C, Castro A, Palavecino P, Poniachik J. [Benign focal liver lesions detected by computed tomography: Review of 1,184 examinations]. *Rev Med Chil* 2015; **143**: 197-202 [PMID: 25860362 DOI: 10.4067/S0034-98872015000200007]

6 **Reddy KR**, Kligerman S, Levi J, Livingstone A, Molina E, Franceschi D, Badalamenti S, Jeffers L, Tzakis A, Schiff ER. Benign and solid tumors of the liver: relationship to sex, age, size of tumors, and outcome. *Am Surg* 2001; **67**: 173-178 [PMID: 11243545]

7 **Moser C**, Hany A, Spiegel R. [Familial giant hemangiomas of the liver. Study of a family and review of the literature]. *Praxis (Bern 1994)* 1998; **87**: 461-468 [PMID: 9587222]

8 **Glinkova V**, Shevah O, Boaz M, Levine A, Shirin H. Hepatic haemangiomas: possible association with female sex hormones. *Gut* 2004; **53**: 1352-1355 [PMID: 15306599 DOI: 10.1136/gut.2003.038646]

9 **Mori H**, Ikegami T, Imura S, Shimada M, Morine Y, Kanemura H, Arakawa Y, Kanamoto M, Hanaoka J, Sugimoto K, Tokunaga T. Sclerosed hemangioma of the liver: Report of a case and review of the literature. *Hepatol Res* 2008; **38**: 529-533 [PMID: 18067557 DOI: 10.1111/j.1872-034X.2007.00306.x]

10 **Wakasugi M**, Ueshima S, Tei M, Tori M, Yoshida K, Tsujimoto M, Akamatsu H. Multiple hepatic sclerosing hemangioma mimicking metastatic liver tumor successfully treated by laparoscopic surgery: Report of a case. *Int J Surg Case Rep* 2015; **8C**: 137-140 [PMID: 25679307 DOI: 10.1016/j.ijscr.2015.01.032]

11 **Sadick M**, Müller-Wille R, Wildgruber M, Wohlgemuth WA. Vascular Anomalies (Part I): Classification and Diagnostics of Vascular Anomalies. *Rofo* 2018; **190**: 825-835 [PMID: 29874693 DOI: 10.1055/a-0620-8925]

12 **Gandolfi L**, Leo P, Solmi L, Vitelli E, Verros G, Colecchia A. Natural history of hepatic haemangiomas: clinical and ultrasound study. *Gut* 1991; **32**: 677-680 [PMID: 2060877 DOI: 10.1136/gut.32.6.677]

13 **Vilgrain V**, Brancatelli G. Liver hemangioma. In: Cioni LL, Bartolozzi C. Focal Liver Lesions. Detection, Characterization, Ablation. Springer: Verlag Berlin Heidelberg, 2005: 101-117

14 **Mamone G**, Di Piazza A, Carollo V, Cannataci C, Cortis K, Bartolotta TV, Miraglia R. Imaging of

hepatic hemangioma: from A to Z. *Abdom Radiol (NY)* 2020; **45**: 672-691 [PMID: 31686179 DOI: 10.1007/s00261-019-02294-8]

15 **Dietrich CF**, Nolsøe CP, Barr RG, Berzigotti A, Burns PN, Cantisani V, Chammas MC, Chaubal N, Choi BI, Clevert DA, Cui X, Dong Y, D'Onofrio M, Fowlkes JB, Gilja OH, Huang P, Ignee A, Jenssen C, Kono Y, Kudo M, Lassau N, Lee WJ, Lee JY, Liang P, Lim A, Lyshchik A, Meloni MF, Correas JM, Minami Y, Moriyasu F, Nicolau C, Piscaglia F, Saftoiu A, Sidhu PS, Sporea I, Torzilli G, Xie X, Zheng R. Guidelines and Good Clinical Practice Recommendations for Contrast-Enhanced Ultrasound (CEUS) in the Liver-Update 2020 WFUMB in Cooperation with EFSUMB, AFSUMB, AIUM, and FLAUS. *Ultrasound Med Biol* 2020; **46**: 2579-2604 [PMID: 32713788 DOI: 10.1016/j.ultrasmedbio.2020.04.030]

16 **Lee JY**, Minami Y, Choi BI, Lee WJ, Chou YH, Jeong WK, Park MS, Kudo N, Lee MW, Kamata K, Iijima H, Kim SY, Numata K, Sugimoto K, Maruyama H, Sumino Y, Ogawa C, Kitano M, Joo I, Arita J, Liang JD, Lin HM, Nolsoe C, Gilja OH, Kudo M. The AFSUMB Consensus Statements and Recommendations for the Clinical Practice of Contrast-Enhanced Ultrasound using Sonazoid. *Ultrasonography* 2020; **39**: 191-220 [PMID: 32447876 DOI: 10.14366/usg.20057]

17 **Barr RG**, Huang P, Luo Y, Xie X, Zheng R, Yan K, Jing X, Xu H, Fei X, Lee JM. Contrast-enhanced ultrasound imaging of the liver: a review of the clinical evidence for SonoVue and Sonazoid. *Abdom Radiol (NY)* 2020; **45**: 3779-3788 [PMID: 32424608 DOI: 10.1007/s00261-020-02573-9]

18 **Sugimoto K**, Moriyasu F, Saito K, Yoshiara H, Imai Y. Kupffer-phase findings of hepatic hemangiomas in contrast-enhanced ultrasound with sonazoid. *Ultrasound Med Biol* 2014; **40**: 1089-1095 [PMID: 24556559 DOI: 10.1016/j.ultrasmedbio.2013.12.019]

19 **Soussan M**, Aubé C, Bahrami S, Boursier J, Valla DC, Vilgrain V. Incidental focal solid liver lesions: diagnostic performance of contrast-enhanced ultrasound and MR imaging. *Eur Radiol* 2010; **20**: 1715-1725 [PMID: 20069427 DOI: 10.1007/s00330-009-1700-3]

20 **Friedrich-Rust M**, Klopffleisch T, Nierhoff J, Herrmann E, Vermehren J, Schneider MD, Zeuzem S, Bojunga J. Contrast-Enhanced Ultrasound for the differentiation of benign and malignant focal liver lesions: a meta-analysis. *Liver Int* 2013; **33**: 739-755 [PMID: 23432804 DOI: 10.1111/liv.12115]

21 **Sporea I**, Săndulescu DL, Şirli R, Popescu A, Danilă M, Spârchez Z, Mihai C, Ioaniescu S, Moga T, Timar B, Brisc C, Nedelcu D, Săftoiu A, Enăchescu V, Badea R. Contrast-Enhanced Ultrasound for the Characterization of Malignant *vs* Benign Focal Liver Lesions in a Prospective Multicenter Experience - The SRUMB Study. *J Gastrointestin Liver Dis* 2019; **28**: 191-196 [PMID: 31204417 DOI: 10.15403/jgld-180]

22 **Tranquart F**, Le Gouge A, Correas JM, Ladam Marcus V, Manzoni P, Vilgrain V, Aube C, Bellin MF, Chami L, Claudon M, Cuilleron M, Drouillard J, Gallix B, Lucidarme O, Marion D, Rode A, Tasu JP, Trillaud H, Fayault A, Rusch E, Giraudeau B. Role of contrast-enhanced ultrasound in the blinded assessment of focal lesions in comparison with MDCT and CEMRI: Results from a multicentre clinical trial. *EJC Suppl* 2008; **6**: 9-15

23 **Strobel D**, Seitz K, Blank W, Schuler A, Dietrich C, von Herbay A, Friedrich-Rust M, Kunze G, Becker D, Will U, Kratzer W, Albert FW, Pachmann C, Dirks K, Strunk H, Greis C, Bernatik T. Contrast-enhanced ultrasound for the characterization of focal liver lesions--diagnostic accuracy in clinical practice (DEGUM multicenter trial). *Ultraschall Med* 2008; **29**: 499-505 [PMID: 19241506 DOI: 10.1055/s-2008-1027806]

24 **Strobel D**, Seitz K, Blank W, Schuler A, Dietrich CF, von Herbay A, Friedrich-Rust M, Bernatik T. Tumor-specific vascularization pattern of liver metastasis, hepatocellular carcinoma, hemangioma and focal nodular hyperplasia in the differential diagnosis of 1,349 Liver lesions in contrast-enhanced ultrasound (CEUS). *Ultraschall Med* 2009; **30**: 376-382 [PMID: 19688669 DOI: 10.1055/s-0028-1109672]

25 **Sugimoto K**, Shiraishi J, Moriyasu F, Doi K. Computer-aided diagnosis of focal liver lesions by use of physicians' subjective classification of echogenic patterns in baseline and contrast-enhanced ultrasonography. *Acad Radiol* 2009; **16**: 401-411 [PMID: 19268851 DOI: 10.1016/j.acra.2008.09.018]

26 **Schwarze V**, Lindner F, Marschner C, Negrão de Figueiredo G, Rübenthaler J, Clevert DA. Single-center study: The diagnostic performance of contrast-enhanced ultrasound (CEUS) for assessing focal splenic lesions compared to CT and MRI. *Clin Hemorheol Microcirc* 2019; **73**: 65-71 [PMID: 31561333 DOI: 10.3233/CH-199204]

27 **Seitz K**, Bernatik T, Strobel D, Blank W, Friedrich-Rust M, Strunk H, Greis C, Kratzer W, Schuler A. Contrast-enhanced ultrasound (CEUS) for the characterization of focal liver lesions in clinical practice (DEGUM Multicenter Trial): CEUS vs. MRI--a prospective comparison in 269 patients. *Ultraschall Med* 2010; **31**: 492-499 [PMID: 20652854 DOI: 10.1055/s-0029-1245591]

28 **Westwood M**, Joore M, Grutters J, Redekop K, Armstrong N, Lee K, Gloy V, Raatz H, Misso K, Severens J, Kleijnen J. Contrast-enhanced ultrasound using SonoVue® (sulphur hexafluoride microbubbles) compared with contrast-enhanced computed tomography and contrast-enhanced magnetic resonance imaging for the characterisation of focal liver lesions and detection of liver metastases: a systematic review and cost-effectiveness analysis. *Health Technol Assess* 2013; **17**: 1-243 [PMID: 23611316 DOI: 10.3310/hta17160]

29 **Sirli R**, Sporea I, Martie A, Popescu A, Dănilă M. Contrast enhanced ultrasound in focal liver lesions--a cost efficiency study. *Med Ultrason* 2010; **12**: 280-285 [PMID: 21210012]

30 **Piscaglia F**, Bolondi L; Italian Society for Ultrasound in Medicine and Biology (SIUMB) Study Group on Ultrasound Contrast Agents. The safety of Sonovue in abdominal applications: retrospective

analysis of 23188 investigations. *Ultrasound Med Biol* 2006; **32**: 1369-1375 [PMID: 16965977 DOI: 10.1016/j.ultrasmedbio.2006.05.031]

31 **Hanafusa K**, Ohashi I, Himeno Y, Suzuki S, Shibuya H. Hepatic hemangioma: findings with two-phase CT. *Radiology* 1995; **196**: 465-469 [PMID: 7617862 DOI: 10.1148/radiology.196.2.7617862]

32 **Vilgrain V**, Boulos L, Vullierme MP, Denys A, Terris B, Menu Y. Imaging of atypical hemangiomas of the liver with pathologic correlation. *Radiographics* 2000; **20**: 379-397 [PMID: 10715338 DOI: 10.1148/radiographics.20.2.g00mc01379]

33 **Klotz T**, Montoriol PF, Da Ines D, Petitcolin V, Joubert-Zakeyh J, Garcier JM. Hepatic haemangioma: common and uncommon imaging features. *Diagn Interv Imaging* 2013; **94**: 849-859 [PMID: 23796395 DOI: 10.1016/j.diii.2013.04.008]

34 **Kim TK**, Lee E, Jang HJ. Imaging findings of mimickers of hepatocellular carcinoma. *Clin Mol Hepatol* 2015; **21**: 326-343 [PMID: 26770920 DOI: 10.3350/cmh.2015.21.4.326]

35 **Moody AR**, Wilson SR. Atypical hepatic hemangioma: a suggestive sonographic morphology. *Radiology* 1993; **188**: 413-417 [PMID: 8327687 DOI: 10.1148/radiology.188.2.8327687]

36 **Bartolotta TV**, Midiri M, Quaia E, Bertolotto M, Galia M, Cademartiri F, Lagalla R. Liver haemangiomas undetermined at grey-scale ultrasound: contrast-enhancement patterns with SonoVue and pulse-inversion US. *Eur Radiol* 2005; **15**: 685-693 [PMID: 15611873 DOI: 10.1007/s00330-004-2569-9]

37 **Mathew RP**, Sam M, Raubenheimer M, Patel V, Low G. Hepatic hemangiomas: the various imaging avatars and its mimickers. *Radiol Med* 2020; **125**: 801-815 [PMID: 32249391 DOI: 10.1007/s11547-020-01185-z]

38 **Doyle DJ**, Khalili K, Guindi M, Atri M. Imaging features of sclerosed hemangioma. *AJR Am J Roentgenol* 2007; **189**: 67-72 [PMID: 17579154 DOI: 10.2214/AJR.06.1076]

39 **Akahoshi S**, Yamamura K, Sato N, Oda E, Kinoshita K, Yuki H, Motohara T, Deguchi A, Komohara Y, Beppu T. A hepatic sclerosed hemangioma with drastic changes in contrast-enhanced ultrasonography. *Clin J Gastroenterol* 2020; **13**: 1252-1257 [PMID: 32705537 DOI: 10.1007/s12328-020-01194-5]

40 **Li T**, Klar MM, Alawad M, Abdul R, Zahiruddin A, Salifu MO, McFarlane IM. Hepatic Sclerosing Hemangioma Mimicking Malignancy: A Case and Literature Review. *Am J Med Case Rep* 2021; **9**: 144-146 [PMID: 33447656 DOI: 10.12691/ajmcr-9-3-2]

41 **Ando Y**, Ishigami M, Ishizu Y, Kuzuya T, Honda T, Hirooka Y. Utility of contrast-enhanced ultrasonography with perflubutane in evaluating indications for diagnostic percutaneous tumor biopsy in a case of hepatic sclerosed hemangioma. *Clin J Gastroenterol* 2018; **11**: 514-520 [PMID: 29931440 DOI: 10.1007/s12328-018-0880-2]

42 **Maruyama M**, Isokawa O, Hoshiyama K, Hoshiyama A, Hoshiyama M, Hoshiyama Y. Diagnosis and management of giant hepatic hemangioma: the usefulness of contrast-enhanced ultrasonography. *Int J Hepatol* 2013; **2013**: 802180 [PMID: 23762570 DOI: 10.1155/2013/802180]

43 **Park JS**, Kim GA, Shim JJ, Kim BS, Park SJ, Kim YW. Hepatic hemangioma presenting as a large cystic tumor. *Korean J Intern Med* 2021; **36**: 473-474 [PMID: 32539297 DOI: 10.3904/kjim.2020.115]

44 **Hussain MZ**, Ohtomo K, Hihara T, Uchiyama G, Ainoda T, Yamamoto M, Suda K. Multilocular cystic hemangioma: CT and MR appearance. *Radiat Med* 1992; **10**: 206-209 [PMID: 1438928]

45 **Cha EY**, Kim KW, Choi YJ, Song JS, Cho KJ, Lee MG. Multicystic cavernous haemangioma of the liver: ultrasonography, CT, MR appearances and pathological correlation. *Br J Radiol* 2008; **81**: e37-e39 [PMID: 18238911 DOI: 10.1259/bjr/36041107]

46 **Hanazaki K**, Koide N, Kajikawa S, Ushiyama T, Watanabe T, Adachi W, Amano J. Cavernous hemangioma of the liver with giant cyst formation: degeneration by apoptosis? *J Gastroenterol Hepatol* 2001; **16**: 352-355 [PMID: 11339432 DOI: 10.1046/j.1440-1746.2001.02344.x]

47 **Uchiyama T**, Akahane T, Watanabe M, Kitayama T, Ise H. [Case of giant liver cyst with angiogenesis mimicking hemangioma that was difficult to differentiate from cystadenocarcinoma of the liver]. *Nihon Shokakibyo Gakkai Zasshi* 2008; **105**: 1634-1639 [PMID: 18987449]

48 **Arai J**, Shimozuma Y, Otoyama Y, Sugiura I, Nakajima Y, Hayashi E, Kajiwara A, Omori R, Uozumi S, Miyashita M, Uchikoshi M, Doi H, Sakaki M, Wang T, Eguchi J, Ito T, Kurihara T, Munechika J, Gokan T, Saito K, Miura S, Tate G, Takimoto M, Yoshida H. Three cases of histologically proven hepatic epithelioid hemangioendothelioma evaluated using a second-generation microbubble contrast medium in ultrasonography: case reports. *BMC Gastroenterol* 2019; **19**: 187 [PMID: 31727083 DOI: 10.1186/s12876-019-1113-y]

49 **Raunkilde L**, Brodersen LR, Rafaelsen SA. [Imaging of hepatic angiosarcoma]. *Ugeskr Laeger* 2014; **176**: V01140046 [PMID: 25293570]

50 **Batista A**, Matos AP, Neta JO, Ramalho M. Diffuse Hepatic Hemangiomatosis in the Adult without Extra-hepatic Involvement: An Extremely Rare Occurrence. *J Clin Imaging Sci* 2014; **4**: 43 [PMID: 25250192 DOI: 10.4103/2156-7514.139733]

51 **Ito H**, Tsujimoto F, Nakajima Y, Igarashi G, Okamura T, Sakurai M, Nobuoka S, Otsubo T. Sonographic characterization of 271 hepatic hemangiomas with typical appearance on CT imaging. *J Med Ultrason (2001)* 2012; **39**: 61-68 [PMID: 27278845 DOI: 10.1007/s10396-011-0339-2]

52 **Liu GJ**, Wang W, Xie XY, Xu HX, Xu ZF, Zheng YL, Liang JY, Moriyasu F, Lu MD. Real-time contrast-enhanced ultrasound imaging of focal liver lesions in fatty liver. *Clin Imaging* 2010; **34**: 211-221 [PMID: 20416486 DOI: 10.1016/j.clinimag.2009.07.003]

53 **Liu LP**, Dong BW, Yu XL, Liang P, Zhang DK, An LC. Focal hypoechoic tumors of Fatty liver:

characterization of conventional and contrast-enhanced ultrasonography. *J Ultrasound Med* 2009; **28**: 1133-1142 [PMID: 19710210 DOI: 10.7863/jum.2009.28.9.1133]

54 **Martie A**, Bota S, Sporea I, Sirli R, Popescu A, Danila M. The contribution of contrast enhanced ultrasound for the characterization of benign liver lesions in clinical practice - a monocentric experience. *Med Ultrason* 2012; **14**: 283-287 [PMID: 23243641]

55 **Dodd GD 3rd**, Baron RL, Oliver JH 3rd, Federle MP. Spectrum of imaging findings of the liver in end-stage cirrhosis: Part II, focal abnormalities. *AJR Am J Roentgenol* 1999; **173**: 1185-1192 [PMID: 10541086 DOI: 10.2214/ajr.173.5.10541086]

56 **Renzulli M**, Brocchi S, Ierardi AM, Milandri M, Pettinari I, Lucidi V, Balacchi C, Muratori P, Marasco G, Vara G, Tovoli F, Granito A, Carrafiello G, Piscaglia F, Golfieri R. Imaging-based diagnosis of benign lesions and pseudolesions in the cirrhotic liver. *Magn Reson Imaging* 2021; **75**: 9-20 [PMID: 32926993 DOI: 10.1016/j.mri.2020.09.008]

57 **Romanini L**, Passamonti M, Aiani L, Cabassa P, Raieli G, Montermini I, Martegani A, Grazioli L, Calliada F. Economic assessment of contrast-enhanced ultrasonography for evaluation of focal liver lesions: a multicentre Italian experience. *Eur Radiol* 2007; **17** Suppl 6: F99-106 [PMID: 18376463 DOI: 10.1007/s10406-007-0234-5]

58 **Faccioli N**, D'Onofrio M, Comai A, Cugini C. Contrast-enhanced ultrasonography in the characterization of benign focal liver lesions: activity-based cost analysis. *Radiol Med* 2007; **112**: 810-820 [PMID: 17891342 DOI: 10.1007/s11547-007-0185-x]

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MINIREVIEWS

Liver function in COVID-19 infection

Dagmara Przekop, Ewa Gruszewska, Lech Chrostek

ORCID number: Dagmara Przekop 0000-0002-8001-8133; Ewa Gruszewska 0000-0002-7702-5148; Lech Chrostek 0000-0001-6701-1861.

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Dagmara Przekop, Diagnostics-Experimental Center of Sexually Transmissible Diseases, Bialystok 15-879, Poland

Ewa Gruszewska, Lech Chrostek, Department of Biochemical Diagnostics, Medical University of Bialystok, Bialystok 15-269, Poland

Corresponding author: Lech Chrostek, MD, PhD, Full Professor, Department of Biochemical Diagnostics, Medical University of Bialystok, Waszyngtona 15A, Bialystok 15-269, Poland. chrostek@umb.edu.pl

Abstract

Coronavirus disease 2019 (COVID-19) disease affects multiple organs, including anomalies in liver function. In this review we summarize the knowledge about liver injury found during severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection with special attention paid to possible mechanisms of liver damage and abnormalities in liver function tests allowing for the evaluation of the severity of liver disease. Abnormalities in liver function observed in COVID-19 disease are associated with the age and sex of patients, severity of liver injury, presence of comorbidity and pre-treatment. The method of antiviral treatment can also impact on liver function, which manifests as increasing values in liver function tests. Therefore, analysis of variations in liver function tests is necessary in evaluating the progression of liver injury to severe disease.

Key Words: COVID-19; Pathogenesis of liver injury; Angiotensin-converting enzyme 2 receptor; Liver function tests; Severe COVID-19; Treatment effect

Core Tip: The frequency of abnormalities in liver function tests (LFTs) in coronavirus disease 2019 (COVID-19) infected patients increases with age and is observed in males more than females. A pre-existing history of liver disease and comorbidity increases LFT abnormality and the likelihood of severe liver damage in COVID-19 infection. Antiviral treatment and treatment of comorbid diseases intensifies the hepatotoxic effect on the liver, which often manifests itself in higher levels in LFTs.

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INTRODUCTION

Pulmonary disease is the primary clinical manifestation in patients with coronavirus disease 2019 (COVID-19) disease. There is increasing evidence of the involvement of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection in multiple organs including the heart, kidneys, central nervous system and liver. In this paper we summarize data concerning liver injury in COVID-19 patients with special attention paid to the possible mechanisms of liver damage and laboratory tests to monitor liver injury during SARS-CoV-2 infection.

GENERAL CHARACTERISTICS

COVID-19 is an acute respiratory infectious disease caused by SARS-CoV-2[1,2]. The SARS-CoV-2 belongs to the *Coronaviridae* family of enveloped, single-stranded RNA viruses[3]. There is evidence that SARS-CoV-2 shares nearly 80% of its genomic sequence with SARS-CoV and about 50% with Middle East respiratory syndrome coronavirus[2,4]. COVID-19 is a viral infectious disease affecting all age groups (from infants to the elderly) resulting in a wide range of clinical manifestations[5-7]. The incubation period of COVID-19 tends to vary from 1 d to 14 d[8].

Multiple organ involvement

Furthermore, COVID-19 infection can present itself with differing degrees of severity, varying from asymptomatic and mild disease to viral pneumonia, in addition to various other extra-pulmonary manifestations, including for example heart, kidney, central nervous system or liver affection, with a risk of fatality[5-7]. Thus, the virus is capable of affecting any organ in the body, and in critically ill patients multiple organs are often affected. Mild cases of COVID-19 infection exhibit symptoms such as fever, dry cough, fatigue, vomiting, diarrhea, muscle weakness, and chest pain[5,7,8]. Patients may also suffer from headaches, as well as loss of smell and taste. While, in severe cases, respiratory distress and/or hypoxemia occur one week after the onset of the disease leading to deterioration into acute respiratory distress syndrome (ARDS), metabolic acidosis, septic shock, and in some cases, even death[5,7,8]. SARS-CoV-2 presents primarily as a lower tract respiratory infection transmitted *via* air droplets, but evidence of the multisystemic nature of COVID-19 is still significantly increasing [5,7,8]. The complications of COVID-19 are associated with several risk factors, namely, advancing age (> 65 years old), cardiovascular disease, hypertension, chronic respiratory disease, diabetes, and obesity[5,8]. The most common reported complication is ARDS, but other severe or even fatal complications are pneumonia, sepsis, metabolic acidosis, heart failure, and acute kidney injury[5,9-11].

Main pulmonary manifestations

Pulmonary affection is the most common serious COVID-19 manifestation[7]. There is evidence that the severity of pulmonary affection caused by SARS-CoV-2 ranges from lack of symptoms or mild pneumonia in 81% of cases, to severe cases associated with hypoxia - in 14% of cases; critical disease associated with shock, respiratory failure and multiple-organ failure - in 5% of cases; or death - in 2.3% of cases[7,12]. SARS-CoV-2 infection induces alveolar damage and interstitial inflammation. During the course of inflammation, the dendritic cells and alveolar macrophages phagocytose epithelial cells infected by SARS-CoV-2, whilst at the same time, the immune mechanisms with T cell responses are activated[7,13].

So, in patients with COVID-19 infection levels of proinflammatory cytokines and chemokines *e.g.*, interleukin 6 (IL-6), IL-1β, tumor necrosis factor, interferon γ, granulocyte stimulating factor are increased[7,8,14]. There is a suggestion that cytokine storms play a crucial role in the immunopathology of the COVID-19 infection.

Cardiac manifestations

Cardiac injury is a common characteristic of patients with COVID-19 infection. Furthermore, despite the fact that cardiovascular diseases might significantly worsen

the clinical outcome of COVID-19 patients, SARS-CoV-2 infection might also induce new cardiac complications[5,15]. Additionally, this cardiac damage might even occur without of any signs or symptoms of pneumonia and with an absence of other complications[5-7]. The major effects of SARS-CoV-2 infection on cardiomyocytes, include for example, acute myocardial injury, heart failure, impaired renal function, arrhythmias, cardiac arrest, myocarditis, sepsis, and septic shock[5,8,16]. The most frequently presented cardiac complication associated with COVID-19 infection is an acute myocardial injury with an estimated prevalence of 8%-12%[5,6,17]. Additionally, the most prevalent complications, with an estimated incidence of 16.7%, are brady- or tachyarrhythmias, also blood pressure abnormalities and dysfunction of the left ventricular[5,6,18]. Importantly, cardiac complications may occur long after viral clearance and recovery, because the inflammation can persist and evolve silently[6,7]. Confirmation of this thesis is exemplified by pulmonary fibrosis, avascular necrosis or dyslipidemia which have evolved over the long term in many survivors of SARS infection, which is closely related to COVID-19 caused by SARS-CoV-2[6]. There is evidence that about one-half of fatal cases show acute cardiac injury and heart failure [6]. These conditions are more probable in elderly patients, while in younger patients myocarditis is the more likely cause.

Although pulmonary disease is the primary clinical manifestation in patients with COVID-19, with cardiac and kidney injury also being common, as we mentioned above there is increasing evidence of its involvement in multiple organs. In this paper we summarize data concerning liver injury in COVID-19 patients.

POSSIBLE PATHOMECHANISMS OF LIVER INJURY

The alteration of hepatocyte damage biomarkers, such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), albumin, and bilirubin is a common laboratory finding in patients with COVID-19 infection. However, the pathomechanism of liver injury during infection is convoluted and not yet fully understood[8,19]. Is not clear if the liver damage is caused by the direct viral effect or if it perhaps reflects a more severe inflammatory response with hepatic injury[20,21]. The possible major pathomechanisms of liver damage are presented in Figure 1. It has been reported that the angiotensin-converting enzyme 2 (ACE2) was identified as the SARS-CoV binding site[19,20,22]. This data facilitated confirmation that SARS-CoV-2 may also directly enter the host cells through binding of its S protein to ACE2 on the surface of the host cell, although with a 10-20-fold higher affinity[2]. The ACE2 receptor expression is higher in many organs, such as lungs, heart, kidney, and it is widely expressed across a variety of cell types[8,22]. Hepatocytes and bile duct epithelial cells also express the ACE2 receptor[7,8,19]. Nevertheless, no significant altered histopathological features have been detected in such cells from COVID-19 patients[8,23]. Only single studies have claimed that the derangement of liver function is usually mild and there is not enough evidence that late-onset symptoms are related to increasing liver damage in patients with COVID-19 infection[2,19]. Additionally, recent data has suggested that SARS-CoV-2 may directly bind to ACE2 expressed in cholangiocytes, because there is evidence that ACE2 expression is displayed in 2.6% of hepatocytes and 59.7% of cholangiocytes[2,19]. Moreover, the alteration of cholangiocyte injury biomarkers, such as alkaline phosphatase (ALP) and γ-glutamyl transferase (GGT) has been observed in some cases, and consistent with biliary epithelial cell damage, and about 10% of patients with COVID-19 infection have an elevated total level of bilirubin[2,24]. There is evidence that specific expression of ACE2 in bile duct epithelial cells was about 20 times higher than in hepatocyte. Furthermore, the bile duct epithelial cells play a substantial role in immune response and liver regeneration. So, this data suggests that liver damage in COVID-19 infection results from bile duct cell injury rather than a direct viral effect in liver cells[19].

On the other hand, the liver is a vital organ for the metabolism of drugs. It is well known that patients suffering from certain viral infections caused for example, by the human immunodeficiency virus or hepatitis C virus are more prone to develop drug-induced liver injury, particularly when it is associated with highly active antiretroviral therapy[25-27]. Therefore, nowadays it is postulated that the same mechanism of liver injury could be present in COVID-19 as a result of the SARS-CoV-2 virus. Thus, hepatotoxicity during the course of the COVID-19 infection, may be initiated by the different types of antiviral drugs, antibiotics and steroids which are currently used to treat COVID-19 patients[25,28]. However, there is a lack of evidence for liver damage in chronic COVID-19 patients being completely drug-induced. A

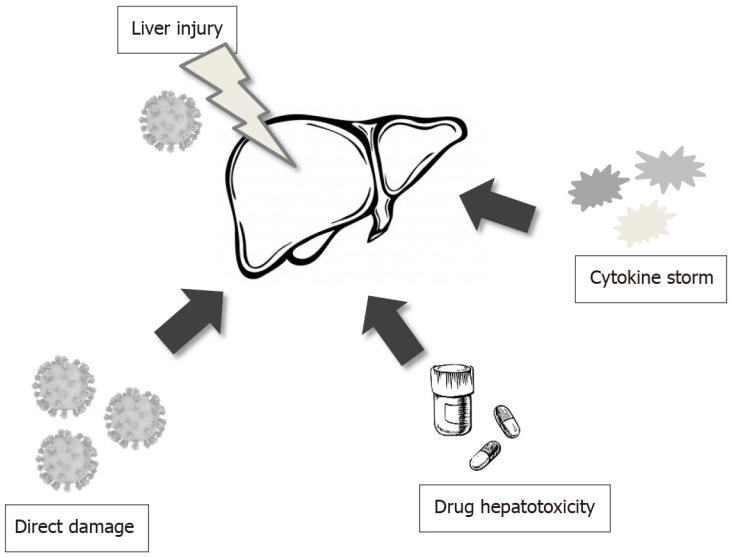

Figure 1 Possible pathomechanism of liver injury in patients with severe acute respiratory syndrome coronavirus 2 infection.

potential example of the relationship between the use of certain drugs and resulting liver damage is found in the study of Fan *et al*[29]. They reported that a high percentage of patients with abnormal values in liver function tests (LFTs) were treated with lopinavir and ritonavir during hospitalization. Similar results appeared in the study of Cai *et al*[30]. Moreover, they reported an almost four-fold increase in liver injury after lopinavir/ritonavir were used in the treatment of severe COVID-19 infection. This finding is consistent with some liver biopsy findings[31]. Certain studies have reported mild lobular and portal activity and moderate microvascular steatosis in patients who died from COVID-19[23]. Further evidence also showed minimal lymphocytic infiltration and mild sinusoidal dilatation in COVID-19 patients [24]. However, these alterations are nonspecific and may be caused by drug-induced liver injury, not excluding the possibility of hypoxemia or having come directly from the SARS-CoV-2 virus[19,23]. Considering these facts, it is very important that these patients be treated with drugs that can inhibit inflammatory response while at the same time protecting hepatic functions.

Another possible reason for liver damage in patients with COVID-19 infection may be dysregulation of the innate immune response[2,19,22]. There is evidence that inflammatory cytokine storms were found in chronically ill patients. The increased values of inflammatory indices, such as C-reactive protein (CRP), IL-6, neutrophils and lymphocytes can be observed in patients with COVID-19 infection, which suggests a relationship between liver damage and inflammatory response induced by severe COVID-19 infection.

ABNORMALITIES IN LABORATORY TESTS

There are many studies showing abnormal laboratory test results in patients with severe COVID-19 disease[32-35]. The first cases of COVID-19 patients from China with liver abnormality were documented by Chen *et al*[32]. Elevations in ALT, AST and lactate dehydrogenase (LDH) were present in 43 out of 99 patients, while most of these cases showed some mild abnormality, whilst one patient exhibited a large increase in test results (ALT of 7590 U/L and AST of 1445 U/L). Most of the participants were male, half of them with chronic diseases. LFTs not only showed abnormalities such as aminotransferases, but also noted were decreased haemoglobin, platelets, an increase of creatine kinase, LDH, ferritin, CRP and a decrease/increase in leucocytes[32].

Cai *et al*[30] conducted laboratory tests on a population of 417 patients with COVID-19 in Shenzhen hospital, China. Three hundred and eighteen patients were confirmed with abnormal liver test results, whilst another 90 had liver injury during hospitalization. The patients were qualified to the appropriate types. Abnormalities such as: hepatocellular type [elevated ALT and/or AST more than 3 × the upper limit unit of normal (ULN)], cholangiocyte type (raised ALP or GGT 2 × ULN) or mixed type (elevated ALT and/or AST more than 3 × the upper limit ULN and raised ALP or GGT

2 × ULN). The highest increase (3 × ULN) in liver enzymes such as ALT (23.4 % of patients), AST (14.8%), total bilirubin (TBIL) (11.5%) and GGT (24.4%) was noticed during the second week of hospitalization. Out of 318 cases, the mixed type dominated and there was a noted increase in all the above tests, except for ALP. In relation to the population of 90 patients, an increase was seen in ALT and GGT, while AST and TBIL were hardly visible. Mixed type patients or those with abnormal test results are at a greater risk of advanced to severe disease. Patients treated with lopinavir/ritonavir had much higher levels of TBIL and GGT, with an associated four-fold increase in the risk of liver damage[30].

A Study carried out on 292 patients in Italy led researchers to different conclusions than Cai *et al*[30]. In their opinion, LFTs are not associated with the patient's condition deteriorating to a severe form of pneumonia. Elevations in AST (18.5%), ALT (26.7%), GGT (36.2%), TBIL (10.6%) and ALP (9.2%) were inconsiderable[36]. Only ALP was not ruled out as a predictive factor, however, it may be associated with bad patient condition, systemic inflammatory response or SARS-CoV-2 tropism for the liver and ACE2 converting enzyme expression in cholangiocytes and hepatocytes. Although 250 patients were treated with lopinavir/ritonavir and 56 patients died, 82 deteriorated and 56 were admitted to intensive care, this was not in any way related to LFTs. Researchers recommended drawing conclusions carefully in the context of a complex multi-organ disease[36].

Wang *et al*[37] conducted an experiment on 156 people diagnosed with the SARS-CoV-2 virus from 2 chosen centers in China, in which they tested the correlation between the prognosis of patients and liver enzyme abnormalities, or lack of such abnormalities. Sixty-four of them had elevated AST and ALT which correlated with disease severity, higher alveolar-arterial oxygen partial pressure difference, growth of GGT, lower albumin and CD4+ T cells and B lymphocytes. The histological trial revealed severe liver apoptosis. Cytopathy in hepatocytes showed ultrastructural features such as endoplasmic reticulum dilatation, mitochondrial swelling and an impaired cell membrane. The above evidence shows that the virus has an influence on the increase in the value of liver enzymes. The most important observation was an association between a very high level of alveolar-arterial oxygen tension difference (A-aDO2) and elevated transaminases. According to this study, SARS-CoV-2 virus infection is a direct factor in liver disease[37].

Conclusions from a study carried out on 5771 adult patients from 10 hospitals in Wuhan indicated a need for monitoring hepatic parameters during hospitalization [38]. On admission to the hospital, chronically ill patients had AST levels significantly higher than ALT. Abnormalities in LFTs have been additionally associated with males, treatment, chronic liver disease, lymphocyte, neutrophil and platelet count. Abnormalities in LFTs, such as AST, ALT, TBIL, GGT, were related to mortality, however AST had the highest correlation. A significantly higher level of AST compared to ALT was also confirmed in the study of Guan *et al*[39] and Chu *et al*[40].

The medical records of 838 patients hospitalized in China indicated an increased level of AST and GGT[40]. Anomalies in LFTs (AST, GGT) were associated with organ injuries, hypoxia, inflammation and the use of antiviral drugs. The level of AST, ALT, GGT and total bilirubin displayed no significant difference between patients who were treated or not treated with umifenovir. By way of contrast, patients who underwent lopinavir/ritonavir treatment had higher levels of AST and GGT. Among the total number of COVID-19 patients, 48.8% showed normal liver function and 51.2% liver injury. Fan *et al*[29] observed abnormal liver function defined as increased LFTs in 57.8% of SARS-CoV-2 patients treated with lopinavir/ritonavir. Moreover, research in Italy suggested that remdesivir may be significant in the origin of hepatocellular injury [41]. Four out of five patients who switched from lopinavir/ritonavir to remdesivir had a reduced level of bilirubin, and significantly increased levels of AST and ALT.

In a study of 2115 people conducted in China, a more notable level of liver injury was uncovered in the group treated with lopinavir/ritonavir than in the untreated group[42]. Patients with COVID-19 and with pre-existing liver injury had more severe disease and a higher prevalence of mortality. However, the observed changes did not mimic the so-called 'cytokine storm' because the absolute lymphocyte count was lower and ESR was higher in the liver injury group than that of the non-liver injury group.

Hundt *et al*[43] observed abnormal liver tests at admission (AST 66.9%, ALT 41.6%, ALP 13.5%, and TBIL 4.3%) and peak of hospitalization (AST 83.4%, ALT 61.6%, ALP 22.7%, and TBIL 16.1%). Moreover, the type of treatment used (hydroxychloroquine, lopinavir/ritonavir, remdesivir, tocilizumab) was associated with abnormal liver transaminase elevations during hospitalization. The results of liver tests were associated with intensive care unit (ICU) admission, mechanical ventilation and death, as well as age, sex and comorbidities. Patients with severe COVID-19 showed an

increase in the total of bilirubin and regardless of severity, a significant rise in transaminases and decrease in albumin was observed[43]. Studies conducted on the Indian population also confirmed the link between laboratory test abnormalities and the severity of the disease[44]. Kumar *et al*[44] included 91 patients in their study, excluding those with pre-existing liver disease (hepatitis B and C, alcoholics, those on known hepatotoxic treatment). The analysis of patients divided into groups (I. asymptomatic, II. mild, III. moderate, IV. severe) showed that the level of transaminases was highest in group IV, ALP was highest in group III but for total bilirubin growth there was no difference between the groups. This study showed that AST and ALP are better tests for indicating the severity of liver damage in COVID-19 than ALT and TBIL.

LFT abnormality was confirmed in 17.6% of Chinese patients with the COVID-19 infection (a population of 159 patients)[45]. The authors concluded that frequency of LFT abnormality was greater in patients with chronic disease than those with mild/moderate illness, especially in older patients. In the another study (148 cases) abnormal liver function was noted in 37.2% of patients on admission and nearly half of those were over 50 years old, half of the 37.2% being men[44]. The patients with abnormal liver function had higher inflammatory indexes (CRP and procalcitonin). On admission, patients who received lopinavir/ritonavir treatment displayed a higher frequency of abnormal LFTs than those with normal liver function. The effect of antiviral treatment on liver function was observed in the study of Zampino *et al*[41]. Treatment of COVID-19 patients with remdesivir can cause hepatocellular injury with aminotransferase elevation, in contrast to the trend of bilirubin elevation with lopinavir/ritonavir treatment.

Abnormally raised liver enzymes were seen in about half of patients with COVID-19 disease[46]. AST and/or ALT > 3 × ULN, and/or ALP and/or GGT > 2 × ULN was seen in 53.5% of patients with hepatocellular injury. In addition, an association between LFTs and markers of inflammation (CRP and ferritin) was observed. Total protein and albumin, were significantly reduced in patients with abnormal liver enzymes and in patients with liver injury, in contrast to the total bilirubin level, which was significantly increased in these patients. Hepatocellular and cholestatic liver injury was more frequent in patients below the age of 50, whereas in patients over 50 years old, more common was the mixed type of liver injury.

Among a French cohort of 281 patients, 102 of them had increased liver enzymes (36.3%)[47]. The most common was an increase in GGT, followed by AST and ALT. Cases with elevated LFTs and CRP value were associated with higher rates of admission to ICU and mortality. Age, sex, diabetes and hypertension were not associated with disease severity. High levels of ALT or AST are associated with disease severity. The authors suggested that liver abnormalities are due to sepsis and tissue hypoxemia, which is documented by apoptotic injuries visualized in the histological examination (vesicular steatosis and watery degeneration). In summary, liver test abnormalities are associated with a poorer prognosis in patients with the coronavirus disease 2019[47].

A study conducted in Istanbul confirmed that liver test abnormalities, especially the AST/ALT ratio, was a good marker of mortality risk and the need for ICU admission [48]. A poorer prognosis rate was associated with higher levels of AST and ALT in the mixed pattern group followed by the hepatocellular injury group and the cholestatic injury group. Mortality in patients with abnormal AST and ALT was higher than that of patients with normal results. The patients with increased AST and ALT showed elevated levels of CRP, procalcitonin, ferritin, D-dimer, lactate and TBIL, which ultimately extended the hospitalization period[48]. The percentage of people in the ICU with elevated aminotransferases was higher than those with normal test results. Patients with ratio AST/ALT > 1 had a higher level of CRP, fibrinogen, LDH, APTT, d-dimer and lower levels of lymphocyte, albumin and GGT. This study showed that low albumin may be marker of severity in SARS-CoV-2 during the hospital admission. Abnormalities in LFTs are more common in men compared to women.

Comorbidities in people with liver diseases are a huge problem, which may have an impact on the severity of COVID-19. A prime example is obesity, in which a person is more prone to develop non-alcoholic fatty liver diseases (NAFLD)[49]. In adipose tissue, there may be a greater expression of ACE2, which increases the risk of severe COVID-19. Chronic liver disease also affects the severity of the disease. This may be related to low levels of blood platelets and lymphocytes[50]. A higher index of cytokines has also been reported, which may influence the progression of NAFLD[51]. In the course of liver cirrhosis, attention should be paid to the activation of cytokines, which leads to hepatocyte necrosis. A study population, from 9 hospitals in Lombardy showed higher mortality (17 out of 50 respondents died)[52]. There was a decrease in

albumin in patients and a significant increase in bilirubin, creatinine and prothrombin. Zou *et al*[53] detected elevated LFTs in 105 Wuhan patients with chronic HBV infection and coexisting SARS-CoV-2 (ALT 20.95%, AST 27.62%, TBIL and GGT 6.67%). These values changed during hospitalization, where 28.57% of the subjects developed acute or chronic liver failure[53]. Research carried out on 9 pregnant women showed lymphopenia (< 10×10^9 cells per L) in 5 of them, elevated CRP (> 10 mg/L) in 6 and 3 had raised AST and ALT[54]. One patient demonstrated a very high level of AST (1263 U/L) and ALT (2093 U/L).

Liver injury in severe COVID-19

The liver test abnormalities mentioned above are more frequently found in severe COVID-19 infection than in mild courses of the same infection. A few studies have demonstrated a relationship between liver test abnormalities, disease severity and mortality of patients with COVID-19[30,55]. A higher rate of LFT abnormalities was observed in severe COVID-19 infection. The higher liver test markers such as ALT, AST, GGT and total bilirubin were reported more in severe patients than in non-severe ones[56,57]. A large cohort study totalling 1099 patients, reported a much higher level of ALT and AST in severe patients (28% and 39%, respectively) than in non-severe patients (20% and 18%, respectively)[39]. So-called weighted mean difference for AST, ALT, total bilirubin and for albumin were associated with a significant increase in the severity of COVID-19 infection[58]. Among the 3381 patients included in the retrospective cohort study, 67.2% of them who were positive for SARS-CoV-2 had higher initial and peak of ALT than those who were negative[59]. Additionally, severe acute liver injury was significantly associated with elevated inflammatory markers including ferritin and IL-6. Besides ferritin and IL-6, other tests such as WBC count, lymphocyte count and platelet count were strong discriminators for severe disease[60].

There is a discrepancy between the frequency of liver test abnormalities and the liver injury in COVID-19 patients. For example, elevated liver damage markers were present in 76.3% of hospitalised patients but only 21.5% of them had liver injury[30]. This variance can be explained by pre-existing liver diseases, which contributed to the severity of liver injury during COVID-19 infection[61,62]. Finally, patients with severe liver injury are more likely to have a poorer prognosis[21]. On the other hand, pre-existing liver disease can increase the risk of COVID-19 infection[63].

CONCLUSION

Not all COVID-19 patients have liver injury and abnormalities in LFTs. However, after measuring the wide variations in these tests, the clinicians can come to some conclusions about the severity of the liver disease and improve the prognosis for patients with liver damage.

REFERENCES

1 **Bogoch II**, Watts A, Thomas-Bachli A, Huber C, Kraemer MUG, Khan K. Potential for global spread of a novel coronavirus from China. *J Travel Med* 2020; **27** [PMID: 31985790 DOI: 10.1093/jtm/taaa011]

2 **Tian D**, Ye Q. Hepatic complications of COVID-19 and its treatment. *J Med Virol* 2020; **92**: 1818-1824 [PMID: 32437004 DOI: 10.1002/jmv.26036]

3 **Cui J**, Li F, Shi ZL. Origin and evolution of pathogenic coronaviruses. *Nat Rev Microbiol* 2019; **17**: 181-192 [PMID: 30531947 DOI: 10.1038/s41579-018-0118-9]

4 **Zhu N**, Zhang D, Wang W, Li X, Yang B, Song J, Zhao X, Huang B, Shi W, Lu R, Niu P, Zhan F, Ma X, Wang D, Xu W, Wu G, Gao GF, Tan W; China Novel Coronavirus Investigating and Research Team. A Novel Coronavirus from Patients with Pneumonia in China, 2019. *N Engl J Med* 2020; **382**: 727-733 [PMID: 31978945 DOI: 10.1056/NEJMoa2001017]

5 **Baj J**, Karakuła-Juchnowicz H, Teresiński G, Buszewicz G, Ciesielka M, Sitarz E, Forma A, Karakuła K, Flieger W, Portincasa P, Maciejewski R. COVID-19: Specific and Non-Specific Clinical Manifestations and Symptoms: The Current State of Knowledge. *J Clin Med* 2020; **9** [PMID: 32516940 DOI: 10.3390/jcm9061753]

6 **Jain U**. Effect of COVID-19 on the Organs. *Cureus* 2020; **12**: e9540 [PMID: 32905500 DOI: 10.7759/cureus.9540]

7 **Gavriatopoulou M**, Korompoki E, Fotiou D, Ntanasis-Stathopoulos I, Psaltopoulou T, Kastritis E, Terpos E, Dimopoulos MA. Organ-specific manifestations of COVID-19 infection. *Clin Exp Med* 2020; **20**: 493-506 [PMID: 32720223 DOI: 10.1007/s10238-020-00648-x]

8 **Ciaccio M**, Agnello L. Biochemical biomarkers alterations in Coronavirus Disease 2019 (COVID-19). *Diagnosis (Berl)* 2020; **7**: 365-372 [PMID: 32589600 DOI: 10.1515/dx-2020-0057]

9 **Chellasamy G**, Arumugasamy SK, Govindaraju S, Yun K. Analytical insights of COVID-19 pandemic. *Trends Analyt Chem* 2020; **133**: 116072 [PMID: 33100439 DOI: 10.1016/j.trac.2020.116072]

10 **Fu L**, Wang B, Yuan T, Chen X, Ao Y, Fitzpatrick T, Li P, Zhou Y, Lin YF, Duan Q, Luo G, Fan S, Lu Y, Feng A, Zhan Y, Liang B, Cai W, Zhang L, Du X, Li L, Shu Y, Zou H. Clinical characteristics of coronavirus disease 2019 (COVID-19) in China: A systematic review and meta-analysis. *J Infect* 2020; **80**: 656-665 [PMID: 32283155 DOI: 10.1016/j.jinf.2020.03.041]

11 **Chen T**, Wu D, Chen H, Yan W, Yang D, Chen G, Ma K, Xu D, Yu H, Wang H, Wang T, Guo W, Chen J, Ding C, Zhang X, Huang J, Han M, Li S, Luo X, Zhao J, Ning Q. Clinical characteristics of 113 deceased patients with coronavirus disease 2019: retrospective study. *BMJ* 2020; **368**: m1091 [PMID: 32217556 DOI: 10.1136/bmj.m1091]

12 **Wu Z**, McGoogan JM. Characteristics of and Important Lessons From the Coronavirus Disease 2019 (COVID-19) Outbreak in China: Summary of a Report of 72 314 Cases From the Chinese Center for Disease Control and Prevention. *JAMA* 2020; **323**: 1239-1242 [PMID: 32091533 DOI: 10.1001/jama.2020.2648]

13 **Channappanavar R**, Zhao J, Perlman S. T cell-mediated immune response to respiratory coronaviruses. *Immunol Res* 2014; **59**: 118-128 [PMID: 24845462 DOI: 10.1007/s12026-014-8534-z]

14 **Huang SH**. What We Know So Far (As of March 26, 2020) About COVID-19-An MRT Point of View. *J Med Imaging Radiat Sci* 2020; **51**: 200-203 [PMID: 32279977 DOI: 10.1016/j.jmir.2020.03.004]

15 **Bansal M**. Cardiovascular disease and COVID-19. *Diabetes Metab Syndr* 2020; **14**: 247-250 [PMID: 32247212 DOI: 10.1016/j.dsx.2020.03.013]

16 **Lippi G**, Lavie CJ, Sanchis-Gomar F. Cardiac troponin I in patients with coronavirus disease 2019 (COVID-19): Evidence from a meta-analysis. *Prog Cardiovasc Dis* 2020; **63**: 390-391 [PMID: 32169400 DOI: 10.1016/j.pcad.2020.03.001]

17 **Kang Y**, Chen T, Mui D, Ferrari V, Jagasia D, Scherrer-Crosbie M, Chen Y, Han Y. Cardiovascular manifestations and treatment considerations in COVID-19. *Heart* 2020; **106**: 1132-1141 [PMID: 32354800 DOI: 10.1136/heartjnl-2020-317056]

18 **Madjid M**, Safavi-Naeini P, Solomon SD, Vardeny O. Potential Effects of Coronaviruses on the Cardiovascular System: A Review. *JAMA Cardiol* 2020; **5**: 831-840 [PMID: 32219363 DOI: 10.1001/jamacardio.2020.1286]

19 **Ali N**, Hossain K. Liver injury in severe COVID-19 infection: current insights and challenges. *Expert Rev Gastroenterol Hepatol* 2020; **14**: 879-884 [PMID: 32649840 DOI: 10.1080/17474124.2020.1794812]

20 **Garrido I**, Liberal R, Macedo G. Review article: COVID-19 and liver disease-what we know on 1st May 2020. *Aliment Pharmacol Ther* 2020; **52**: 267-275 [PMID: 32402090 DOI: 10.1111/apt.15813]

21 **Feng G**, Zheng KI, Yan QQ, Rios RS, Targher G, Byrne CD, Poucke SV, Liu WY, Zheng MH. COVID-19 and Liver Dysfunction: Current Insights and Emergent Therapeutic Strategies. *J Clin Transl Hepatol* 2020; **8**: 18-24 [PMID: 32274342 DOI: 10.14218/JCTH.2020.00018]

22 **Alqahtani SA**, Schattenberg JM. Liver injury in COVID-19: The current evidence. *United European Gastroenterol J* 2020; **8**: 509-519 [PMID: 32450787 DOI: 10.1177/2050640620924157]

23 **Xu Z**, Shi L, Wang Y, Zhang J, Huang L, Zhang C, Liu S, Zhao P, Liu H, Zhu L, Tai Y, Bai C, Gao T, Song J, Xia P, Dong J, Zhao J, Wang FS. Pathological findings of COVID-19 associated with acute respiratory distress syndrome. *Lancet Respir Med* 2020; **8**: 420-422 [PMID: 32085846 DOI: 10.1016/S2213-2600(20)30076-X]

24 **Zhang Y**, Zheng L, Liu L, Zhao M, Xiao J, Zhao Q. Liver impairment in COVID-19 patients: A retrospective analysis of 115 cases from a single centre in Wuhan city, China. *Liver Int* 2020; **40**: 2095-2103 [PMID: 32239796 DOI: 10.1111/liv.14455]

25 **Boeckmans J**, Rodrigues RM, Demuyser T, Piérard D, Vanhaecke T, Rogiers V. COVID-19 and drug-induced liver injury: a problem of plenty or a petty point? *Arch Toxicol* 2020; **94**: 1367-1369 [PMID: 32266419 DOI: 10.1007/s00204-020-02734-1]

26 **Naidoo K**, Hassan-Moosa R, Mlotshwa P, Yende-Zuma N, Govender D, Padayatchi N, Abdool-Karim SSS. High Rates of Drug-induced Liver Injury in People Living With HIV Coinfected With Tuberculosis (TB) Irrespective of Antiretroviral Therapy Timing During Antituberculosis Treatment: Results From the Starting Antiretroviral Therapy at Three Points in TB Trial. *Clin Infect Dis* 2020; **70**: 2675-2682 [PMID: 31622456 DOI: 10.1093/cid/ciz732]

27 **Bonacini M**. Liver injury during highly active antiretroviral therapy: the effect of hepatitis C coinfection. *Clin Infect Dis* 2004; **38** Suppl 2: S104-S108 [PMID: 14986282 DOI: 10.1086/381453]

28 **Zhang C**, Shi L, Wang FS. Liver injury in COVID-19: management and challenges. *Lancet Gastroenterol Hepatol* 2020; **5**: 428-430 [PMID: 32145190 DOI: 10.1016/S2468-1253(20)30057-1]

29 **Fan Z**, Chen L, Li J, Cheng X, Yang J, Tian C, Zhang Y, Huang S, Liu Z, Cheng J. Clinical Features of COVID-19-Related Liver Functional Abnormality. *Clin Gastroenterol Hepatol* 2020; **18**: 1561-1566 [PMID: 32283325 DOI: 10.1016/j.cgh.2020.04.002]

30 **Cai Q**, Huang D, Yu H, Zhu Z, Xia Z, Su Y, Li Z, Zhou G, Gou J, Qu J, Sun Y, Liu Y, He Q, Chen J, Liu L, Xu L. COVID-19: Abnormal liver function tests. *J Hepatol* 2020; **73**: 566-574 [PMID: 32298767 DOI: 10.1016/j.jhep.2020.04.006]

31 **Luglio M**, Tannuri U, de Carvalho WB, Bastos KLM, Rodriguez IS, Johnston C, Delgado AF.

COVID-19 and Liver Damage: Narrative Review and Proposed Clinical Protocol for Critically ill Pediatric Patients. *Clinics (Sao Paulo)* 2020; **75**: e2250 [PMID: 33206767 DOI: 10.6061/clinics/2020/e2250]

32 **Chen N**, Zhou M, Dong X, Qu J, Gong F, Han Y, Qiu Y, Wang J, Liu Y, Wei Y, Xia J, Yu T, Zhang X, Zhang L. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. *Lancet* 2020; **395**: 507-513 [PMID: 32007143 DOI: 10.1016/S0140-6736(20)30211-7]

33 **Yang W**, Cao Q, Qin L, Wang X, Cheng Z, Pan A, Dai J, Sun Q, Zhao F, Qu J, Yan F. Clinical characteristics and imaging manifestations of the 2019 novel coronavirus disease (COVID-19):A multi-center study in Wenzhou city, Zhejiang, China. *J Infect* 2020; **80**: 388-393 [PMID: 32112884 DOI: 10.1016/j.jinf.2020.02.016]

34 **Zhang JJ**, Dong X, Cao YY, Yuan YD, Yang YB, Yan YQ, Akdis CA, Gao YD. Clinical characteristics of 140 patients infected with SARS-CoV-2 in Wuhan, China. *Allergy* 2020; **75**: 1730-1741 [PMID: 32077115 DOI: 10.1111/all.14238]

35 **Wang D**, Hu B, Hu C, Zhu F, Liu X, Zhang J, Wang B, Xiang H, Cheng Z, Xiong Y, Zhao Y, Li Y, Wang X, Peng Z. Clinical Characteristics of 138 Hospitalized Patients With 2019 Novel Coronavirus-Infected Pneumonia in Wuhan, China. *JAMA* 2020; **323**: 1061-1069 [PMID: 32031570 DOI: 10.1001/jama.2020.1585]

36 **Vespa E**, Pugliese N, Piovani D, Capogreco A, Danese S, Aghemo A; Humanitas Covid-19 Task Force. Liver tests abnormalities in COVID-19: trick or treat? *J Hepatol* 2020; **73**: 1275-1276 [PMID: 32473194 DOI: 10.1016/j.jhep.2020.05.033]

37 **Wang Y**, Liu S, Liu H, Li W, Lin F, Jiang L, Li X, Xu P, Zhang L, Zhao L, Cao Y, Kang J, Yang J, Li L, Liu X, Li Y, Nie R, Mu J, Lu F, Zhao S, Lu J, Zhao J. SARS-CoV-2 infection of the liver directly contributes to hepatic impairment in patients with COVID-19. *J Hepatol* 2020; **73**: 807-816 [PMID: 32437830 DOI: 10.1016/j.jhep.2020.05.002]

38 **Lei F**, Liu YM, Zhou F, Qin JJ, Zhang P, Zhu L, Zhang XJ, Cai J, Lin L, Ouyang S, Wang X, Yang C, Cheng X, Liu W, Li H, Xie J, Wu B, Luo H, Xiao F, Chen J, Tao L, Cheng G, She ZG, Zhou J, Wang H, Lin J, Luo P, Fu S, Ye P, Xiao B, Mao W, Liu L, Yan Y, Chen G, Huang X, Zhang BH, Yuan Y. Longitudinal Association Between Markers of Liver Injury and Mortality in COVID-19 in China. *Hepatology* 2020; **72**: 389-398 [PMID: 32359177 DOI: 10.1002/hep.31301]

39 **Guan WJ**, Ni ZY, Hu Y, Liang WH, Ou CQ, He JX, Liu L, Shan H, Lei CL, Hui DSC, Du B, Li LJ, Zeng G, Yuen KY, Chen RC, Tang CL, Wang T, Chen PY, Xiang J, Li SY, Wang JL, Liang ZJ, Peng YX, Wei L, Liu Y, Hu YH, Peng P, Wang JM, Liu JY, Chen Z, Li G, Zheng ZJ, Qiu SQ, Luo J, Ye CJ, Zhu SY, Zhong NS; China Medical Treatment Expert Group for Covid-19. Clinical Characteristics of Coronavirus Disease 2019 in China. *N Engl J Med* 2020; **382**: 1708-1720 [PMID: 32109013 DOI: 10.1056/NEJMoa2002032]

40 **Chu H**, Bai T, Chen L, Hu L, Xiao L, Yao L, Zhu R, Niu X, Li Z, Zhang L, Han C, Song S, He Q, Zhao Y, Zhu Q, Chen H, Schnabl B, Yang L, Hou X. Multicenter Analysis of Liver Injury Patterns and Mortality in COVID-19. *Front Med (Lausanne)* 2020; **7**: 584342 [PMID: 33195339 DOI: 10.3389/fmed.2020.584342]

41 **Zampino R**, Mele F, Florio LL, Bertolino L, Andini R, Galdo M, De Rosa R, Corcione A, Durante-Mangoni E. Liver injury in remdesivir-treated COVID-19 patients. *Hepatol Int* 2020; **14**: 881-883 [PMID: 32725454 DOI: 10.1007/s12072-020-10077-3]

42 **Yadav DK**, Singh A, Zhang Q, Bai X, Zhang W, Yadav RK, Zhiwei L, Adhikari VP, Liang T. Involvement of liver in COVID-19: systematic review and meta-analysis. *Gut* 2021; **70**: 807-809 [PMID: 32669289 DOI: 10.1136/gutjnl-2020-322072]

43 **Hundt MA**, Deng Y, Ciarleglio MM, Nathanson MH, Lim JK. Abnormal Liver Tests in COVID-19: A Retrospective Observational Cohort Study of 1,827 Patients in a Major U.S. Hospital Network. *Hepatology* 2020; **72**: 1169-1176 [PMID: 32725890 DOI: 10.1002/hep.31487]

44 **Kumar A**, Kumar P, Dungdung A, Kumar Gupta A, Anurag A, Kumar A. Pattern of liver function and clinical profile in COVID-19: A cross-sectional study of 91 patients. *Diabetes Metab Syndr* 2020; **14**: 1951-1954 [PMID: 33039937 DOI: 10.1016/j.dsx.2020.10.001]

45 **Li S**, Li J, Zhang Z, Tan L, Shao T, Li M, Li X, Holmes JA, Lin W, Han M. COVID-19 induced liver function abnormality associates with age. *Aging (Albany NY)* 2020; **12**: 13895-13904 [PMID: 32721928 DOI: 10.18632/aging.103720]

46 **Saini RK**, Saini N, Ram S, Soni SL, Suri V, Malhotra P, Kaur J, Verma I, Sharma S, Zohmangaihi D. COVID-19 associated variations in liver function parameters: a retrospective study. *Postgrad Med J* 2020 epub ahead of print [PMID: 33184141 DOI: 10.1136/postgradmedj-2020-138930]

47 **Chaibi S**, Boussier J, Hajj WE, Abitbol Y, Taieb S, Horaist C, Jouannaud V, Wang P, Piquet J, Maurer C, Lahmek P, Nahon S. Liver function test abnormalities are associated with a poorer prognosis in Covid-19 patients: Results of a French cohort. *Clin Res Hepatol Gastroenterol* 2021; **45**: 101556 [PMID: 33139241 DOI: 10.1016/j.clinre.2020.10.002]

48 **Medetalibeyoglu A**, Catma Y, Senkal N, Ormeci A, Cavus B, Kose M, Bayramlar OF, Yildiz G, Akyuz F, Kaymakoglu S, Tukek T. The effect of liver test abnormalities on the prognosis of COVID-19. *Ann Hepatol* 2020; **19**: 614-621 [PMID: 32920162 DOI: 10.1016/j.aohep.2020.08.068]

49 **Zheng KI**, Gao F, Wang XB, Sun QF, Pan KH, Wang TY, Ma HL, Chen YP, Liu WY, George J, Zheng MH. Letter to the Editor: Obesity as a risk factor for greater severity of COVID-19 in patients with metabolic associated fatty liver disease. *Metabolism* 2020; **108**: 154244 [PMID: 32320741 DOI: 10.1016/j.metabol.2020.154244]

50 **Qi X**, Liu Y, Wang J, Fallowfield JA, Li X, Shi J, Pan H, Zou S, Zhang H, Chen Z, Li F, Luo Y, Mei M, Liu H, Wang Z, Li J, Yang H, Xiang H, Liu T, Zheng MH, Liu C, Huang Y, Xu D, Kang N, He Q, Gu Y, Zhang G, Shao C, Liu D, Zhang L, Kawada N, Jiang Z, Wang F, Xiong B, Takehara T, Rockey DC; COVID-Cirrhosis-CHESS Group. Clinical course and risk factors for mortality of COVID-19 patients with pre-existing cirrhosis: a multicentre cohort study. *Gut* 2021; **70**: 433-436 [PMID: 32434831 DOI: 10.1136/gutjnl-2020-321666]

51 **Prins GH**, Olinga P. Potential implications of COVID-19 in non-alcoholic fatty liver disease. *Liver Int* 2020; **40**: 2568 [PMID: 32306495 DOI: 10.1111/Liv.14484]

52 **Iavarone M**, D'Ambrosio R, Soria A, Triolo M, Pugliese N, Del Poggio P, Perricone G, Massironi S, Spinetti A, Buscarini E, Viganò M, Carriero C, Fagiuoli S, Aghemo A, Belli LS, Lucà M, Pedaci M, Rimondi A, Rumi MG, Invernizzi P, Bonfanti P, Lampertico P. High rates of 30-day mortality in patients with cirrhosis and COVID-19. *J Hepatol* 2020; **73**: 1063-1071 [PMID: 32526252 DOI: 10.1016/j.jhep.2020.06.001]

53 **Zou X**, Fang M, Li S, Wu L, Gao B, Gao H, Ran X, Bian Y, Li R, ShanshanYu, Ling J, Li D, Tian D, Huang J. Characteristics of Liver Function in Patients With SARS-CoV-2 and Chronic HBV Coinfection. *Clin Gastroenterol Hepatol* 2021; **19**: 597-603 [PMID: 32553907 DOI: 10.1016/j.cgh.2020.06.017]

54 **Chen H**, Guo J, Wang C, Luo F, Yu X, Zhang W, Li J, Zhao D, Xu D, Gong Q, Liao J, Yang H, Hou W, Zhang Y. Clinical characteristics and intrauterine vertical transmission potential of COVID-19 infection in nine pregnant women: a retrospective review of medical records. *Lancet* 2020; **395**: 809-815 [PMID: 32151335 DOI: 10.1016/S0140-6736(20)30360-3]

55 **Bloom PP**, Meyerowitz EA, Reinus Z, Daidone M, Gustafson J, Kim AY, Schaefer E, Chung RT. Liver Biochemistries in Hospitalized Patients With COVID-19. *Hepatology* 2021; **73**: 890-900 [PMID: 32415860 DOI: 10.1002/hep.31326]

56 **Huang C**, Wang Y, Li X, Ren L, Zhao J, Hu Y, Zhang L, Fan G, Xu J, Gu X, Cheng Z, Yu T, Xia J, Wei Y, Wu W, Xie X, Yin W, Li H, Liu M, Xiao Y, Gao H, Guo L, Xie J, Wang G, Jiang R, Gao Z, Jin Q, Wang J, Cao B. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet* 2020; **395**: 497-506 [PMID: 31986264 DOI: 10.1016/S0140-6736(20)30183-5]

57 **Richardson S**, Hirsch JS, Narasimhan M, Crawford JM, McGinn T, Davidson KW; the Northwell COVID-19 Research Consortium, Barnaby DP, Becker LB, Chelico JD, Cohen SL, Cookingham J, Coppa K, Diefenbach MA, Dominello AJ, Duer-Hefele J, Falzon L, Gitlin J, Hajizadeh N, Harvin TG, Hirschwerk DA, Kim EJ, Kozel ZM, Marrast LM, Mogavero JN, Osorio GA, Qiu M, Zanos TP. Presenting Characteristics, Comorbidities, and Outcomes Among 5700 Patients Hospitalized With COVID-19 in the New York City Area. *JAMA* 2020; **323**: 2052-2059 [PMID: 32320003 DOI: 10.1001/jama.2020.6775]

58 **Parohan M**, Yaghoubi S, Seraji A. Liver injury is associated with severe coronavirus disease 2019 (COVID-19) infection: A systematic review and meta-analysis of retrospective studies. *Hepatol Res* 2020; **50**: 924-935 [PMID: 32386449 DOI: 10.1111/hepr.13510]

59 **Phipps MM**, Barraza LH, LaSota ED, Sobieszczyk ME, Pereira MR, Zheng EX, Fox AN, Zucker J, Verna EC. Acute Liver Injury in COVID-19: Prevalence and Association with Clinical Outcomes in a Large U.S. Cohort. *Hepatology* 2020; **72**: 807-817 [PMID: 32473607 DOI: 10.1002/hep.31404]

60 **Henry BM**, de Oliveira MHS, Benoit S, Plebani M, Lippi G. Hematologic, biochemical and immune biomarker abnormalities associated with severe illness and mortality in coronavirus disease 2019 (COVID-19): a meta-analysis. *Clin Chem Lab Med* 2020; **58**: 1021-1028 [PMID: 32286245 DOI: 10.1515/cclm-2020-0369]

61 **Singh S**, Khan A. Clinical Characteristics and Outcomes of Coronavirus Disease 2019 Among Patients With Preexisting Liver Disease in the United States: A Multicenter Research Network Study. *Gastroenterology* 2020; **159**: 768-771.e3 [PMID: 32376408 DOI: 10.1053/j.gastro.2020.04.064]

62 **Moon AM**, Webb GJ, Aloman C, Armstrong MJ, Cargill T, Dhanasekaran R, Genescà J, Gill US, James TW, Jones PD, Marshall A, Mells G, Perumalswami PV, Qi X, Su F, Ufere NN, Barnes E, Barritt AS, Marjot T. High mortality rates for SARS-CoV-2 infection in patients with pre-existing chronic liver disease and cirrhosis: Preliminary results from an international registry. *J Hepatol* 2020; **73**: 705-708 [PMID: 32446714 DOI: 10.1016/j.jhep.2020.05.013]

63 **Liang W**, Guan W, Chen R, Wang W, Li J, Xu K, Li C, Ai Q, Lu W, Liang H, Li S, He J. Cancer patients in SARS-CoV-2 infection: a nationwide analysis in China. *Lancet Oncol* 2020; **21**: 335-337 [PMID: 32066541 DOI: 10.1016/S1470-2045(20)30096-6]

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MINIREVIEWS

Potential role of noninvasive biomarkers during liver fibrosis

Navneet Kaur, Gitanjali Goyal, Ravinder Garg, Chaitanya Tapasvi, Sonia Chawla, Rajneet Kaur

ORCID number: Navneet Kaur 0000-0002-1766-1636; Gitanjali Goyal 0000-0002-2105-1312; Ravinder Garg 0000-0003-0916-2412; Chaitanya Tapasvi 0000-0002-0244-1200; Sonia Chawla 0000-0002-4746-0261; Rajneet Kaur 0000-0003-3216-8089.

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Navneet Kaur, Gitanjali Goyal, Sonia Chawla, Rajneet Kaur, Department of Biochemistry, Guru Gobind Singh Medical College and Hospital, Baba Farid University of Health Sciences, Faridkot 151203, Punjab, India

Ravinder Garg, Department of Medicine, Guru Gobind Singh Medical College and Hospital, Baba Farid University of Health Sciences, Faridkot 151203, Punjab, India

Chaitanya Tapasvi, Department of Radiodiagnosis, Guru Gobind Singh Medical College and Hospital, Baba Farid University of Health Sciences, Faridkot 151203, Punjab, India

Corresponding author: Gitanjali Goyal, MD, Chief Doctor, Department of Biochemistry, Guru Gobind Singh Medical College and Hospital, Baba Farid University of Health Sciences, Sadiq Road, Faridkot 151203, Punjab, India. gitanjaligoyal@ggsmch.org

Abstract

Various types of liver disease exist, such as hepatitis and alcoholic liver disease. These liver diseases can result in scarring of liver tissue, cirrhosis, and finally liver failure. During liver fibrosis, there is an excess and disorganized accumulation of extracellular matrix (ECM) components which cause the loss of normal liver cell functions. For patients with chronic liver disease, fibrosis prediction is an essential part of the assessment and management. To diagnose liver fibrosis, several invasive and noninvasive markers have been proposed. However, the adoption of invasive markers remains limited due to their inherent characteristics and poor patient acceptance rate. In contrast, noninvasive markers can expedite the clinical decision through informed judgment about disease stage and prognosis. These noninvasive markers are classified into two types: Imaging techniques and serum biomarkers. However, the diagnostic values of biomarkers associated with liver fibrosis have also been analyzed. For example, the serum levels of ECM proteins can react to either matrix accumulation or degradation. During virus-host interactions, several regulatory steps take place to control gene expression, such as the change in cellular microRNA expression profiles. MicroRNAs are a class of non-coding RNAs (18-20 long nucleotides) that function by post-transcriptional regulation of gene expression. Although various noninvasive markers have been suggested in recent years, certain limitations have restricted their clinical applications. Understanding the potential of non-invasive biomarkers as a therapeutic option to treat liver fibrosis is still in progress.

Key Words: Liver fibrosis; Non-invasive biomarkers; Viral hepatitis; MicroRNA; Cirrhosis; Fibroscan

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Core Tip: Liver disease is quite common these days. Hepatitis, alcoholic liver disease, and non-alcoholic fatty liver disease can lead to liver cirrhosis. Liver fibrosis assessment is a crucial step for diagnosis and treatment purposes. Various markers have been proposed, including both invasive and non-invasive markers. Liver biopsy is the gold standard method but due to its invasiveness, it is not preferred these days. Non-invasive methods include serum biomarkers and imaging techniques. Combinational panels along with microRNAs are also used for the identification of liver fibrosis. Besides their cost-effectiveness, these panels are more dependable when compared with an individual biomarker.

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INTRODUCTION

The liver is the main organ of our body. The functions of the liver include synthetic functions, metabolic functions, and most importantly the detoxification and excretion of toxic substances. The synthetic functions include the synthesis of cholesterol, triglycerides, plasma proteins, and lipoproteins. The metabolic functions include the metabolism of carbohydrates, lipids, and proteins. Ammonia is converted to urea in the liver. Any injury to liver cells will lead to the alteration in these functions. Various types of liver disease exist, such as acute and chronic hepatitis, alcoholic liver disease, and non-alcoholic fatty liver disease (NAFLD). Hepatitis is essentially the inflammation of the liver, a condition that can be self-limiting, although it can progress to other adverse situations, including fibrosis, cirrhosis, or even liver cancer. There are various causes of this condition, and the most implicated ones include infections, certain drugs, toxic substances, and autoimmune diseases. Mainly, there are five different types of hepatitis, namely, A, B, C, D, and E. Alcoholic liver disease occurs due to excessive consumption of alcohol. All these diseases lead to injury of the liver parenchyma which is studied based on their stages. The stage and degree of liver disease are fundamental in the diagnosis, prognosis, treatment, as well as follow-up of all hepatic diseases.

STAGES OF LIVER DISEASE

The progression of liver disease passes through various stages, as depicted in Figure 1. The figure also shows the factors promoting liver cell injury and thereafter the progression of the disease. The stages of liver disease are discussed below.

Inflammation stage

There are many types of liver failure, but despite the type, the progression towards full-blown disease is the same. The first stage is associated with inflammation and typically denotes the immune system's reaction to the offending agents like toxins. In this case, the hepatitis C virus (HCV) would be responsible[1]. In the process of inflammation, the liver becomes tender and greatly enlarged. Before inflammation, massive viral infection leads to an increase in the production of inflammatory cytokines, and chemokine levels are also shown to increase (they are the inflammatory biomarkers).

Fibrosis

The second stage is associated with fibrosis, which is stimulated by chronic inflammation. Fibrosis usually occurs as a result of the liver's healing process, and it happens continuously with the regeneration of the liver's damaged areas. Fibrosis is a way that wound healing takes place with a balance between fibrogenesis and fibrinolysis[2]. The process of inflammation causes quiescent hepatic stellate cells (HSCs) to be

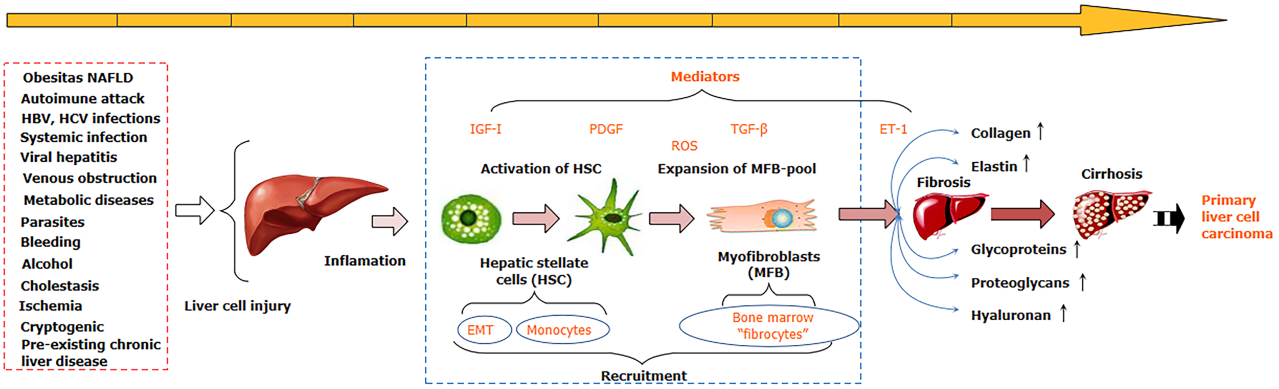

Figure 1 Factors promoting liver cell injury leading to fibrosis, cirrhosis, and carcinoma. NAFLD: Non-alcoholic fatty liver disease; HBV: Hepatitis B virus; HCV: Hepatitis C virus; PDGF: Platelet growth factor; IGF: Insulin-like growth factor; TGF: Tissue growth factor; ROS: Reactive oxygen species; ET-1: Endothelin-1; EMT: Epithelial-mesenchymal transition.

activated, which then differentiate and form myofibroblasts[3].

Myofibroblasts are important in fibrogenesis and are responsible for producing several components of the extracellular matrix (ECM), which then replace the damaged tissues. When the ECM is deposited excessively, it leads to scar formation, which can be altered by fibrolysis[4]. The process of fibrosis is dynamic, and it is bound to be reversed upon the resolution of the HCV infection[5]. The chronic damage that stimulates fibrogenesis and insufficient fibrolysis is linked to a reduction of the reversibility potential.

Cirrhosis stage

Cirrhosis is the point where the liver is completely scarred and is beyond the self-healing ability. The development of cirrhosis is long due and could even take decades, meaning that interventions can be started in the initial stages before getting to this point. After several injurious exposure or inflammatory responses by the different mediators, HSCs undergo a transition from the quiescent to the activated state. The damaged hepatocytes lead to the release of reactive oxygen species, and apoptosis could occur[6].

Cirrhosis occurs in two stages: Compensated cirrhosis and decompensated cirrhosis (end-stage liver disease). During the compensated cirrhosis, there is liver damage, but it is not severe enough to hinder some of the cells' functioning. At this stage, one can be asymptomatic, although portal hypertension may be present[7]. The chronicity of the infection could induce G1 arrest and then impair the functioning of hepatic cells, limiting regeneration.

Recent studies have determined that shortening of the liver's telomeres and their senescence results in fibrotic tissue formation in the cirrhosis stage of liver disease. During the cirrhosis stage, some clinical features become apparent: Increased propensity to bleeding, possible development of insulin resistance, sensitivity to some medications, skin itch, and water build-up leading to edema. It is also possible for the build-up of toxins in the brain, affecting memory and other mental functions.

End-stage liver disease (decompensated cirrhosis)

This is the stage where the liver has completely failed, and neither can the cells heal; it can be both acute and chronic[8]. In HCV infection, it is a chronic occurrence. This is also called decompensated cirrhosis, and it follows inflammation of the hepatocytes, which leads to fibrosis and then disruption of the liver structure and function. During this stage, there is the development of complications like jaundice, variceal bleeding, ascites, and hepatic encephalopathy.

Clinical evidence has revealed that the median survival age for decompensated cirrhosis is about 2 years, and it is a common predictor of death in patients with cirrhosis. It has also been shown that decompensation can improve once the offending agent has been eliminated[8]. Failure to remove the offending agent, therefore, means that liver transplant is the only remaining solution.

ASSESSMENT OF LIVER FIBROSIS

For assessment of liver fibrosis, various methods have been proposed, including both invasive and non-invasive methods (Figure 2). However, in clinical practice, finding the most effective and the best method for evaluating liver impairment in patients remains a major challenge. This is mainly because the prognosis and effective treatment are dependent on the assessment of liver damage as well as the extent of liver fibrosis in patients. Historically, all these parameters were provided through liver biopsy. Liver biopsy is among the oldest, effective, and most accurate assessment methods of evaluating liver histology and the progression of liver damage. The comparison of the main features of both invasive and non-invasive methods is shown in Table 1.

INVASIVE METHOD (LIVER BIOPSY)

As discussed by Shrivastava *et al*[9], liver biopsy is a process that is considered by many experts in determining the best therapeutic approaches for patients. This is also the best approach in dealing with hepatitis C especially when it comes to chronic hepatitis. It is an invasive procedure for liver assessment[10]. Consequently, liver biopsy as an assessment method of liver damage in hepatitis C patients brings forth several risks as well as sampling errors. Sampling errors in liver biopsy occur due to suboptimal biopsy size. Due to the increased risks of liver biopsy and sampling errors among other pitfalls of this assessment method, different markers have been developed. Research shows that during the pathological progression of liver fibrosis, especially in patients with hepatitis C, there is an excessive buildup of the matrix. The serum levels of different biomarkers tend to change[9]. According to the authors, there are physical and biological non-invasive approaches that are based on serum biomarkers that have been proposed.

Scoring system for liver fibrosis

The scoring system of liver fibrosis assessment based on three methods, *i.e.*, International Association of Study of Liver (IASL), Batts-Ludwig, and METAVIR scores are depicted in Table 2[11].

Limitations of liver biopsy

There are several limitations of liver biopsy that have led to the development and replacement of the assessment method with non-invasive biomarkers as an assessment method of liver damage and liver fibrosis in patients with hepatitis. One of the limitations of liver biopsy is that this method does not efficiently reflect the different fibrotic changes that may be occurring in the entire liver. This is mainly because any optimally sized liver biopsy contains a small number of complete portal tracks that reflect a small volume of the liver[12]. Besides, the process of hepatic fibrosis is not liners. As a result, to cover hepatic fibrosis in the entire liver, biopsies have to be conducted on different areas of the liver. Besides, research shows that liver biopsies may miss cirrhosis in patients with hepatitis C. This is mainly because liver biopsy cannot differentiate between early and progressed cirrhosis. Consequently, liver biopsy cannot be relied upon as an ideal and accurate prognostic predictor[12].

Research shows that there are several risks of complications that tend to arise from liver biopsy[13]. Most of these complications, however, carry symptoms such as injury to the biliary system, mild abdominal pain, and severe hemorrhage. The occurrence of such complications as a result of liver biopsy may increase hospitalization. There is variability in the interpretation of pathologists which is yet another limitation of liver biopsy. Research shows that biopsy cannot be conducted in hepatitis patients with diabetes, ascites, metabolic syndrome, and coagulopathy. Although liver biopsy has been considered as a keystone for the diagnosis of liver damage in patients with liver diseases such as hepatitis C, the invasive procedure has significant limitations mainly due to surgical complications and sampling heterogeneity.

NON-INVASIVE TECHNIQUES FOR LIVER DAMAGE ASSESSMENT

There are various methods in which non-invasive biomarkers are used to assess the damages in the liver. A conclusion reveals that through these assessments, experts can

Table 1 Comparison of characteristics of invasive and non-invasive methods

No.	Feature	Invasive	Non-invasive
1	Invasiveness	Yes	No
2	Sampling error	Yes	No
3	Cost-effective	No	Yes
4	Patient-friendly	No	Yes
5	Hospitalization required	Yes	No

Table 2 Scoring systems for liver fibrosis

Stage	IASL	Batts-Ludwig	METAVIR
No fibrosis	No fibrosis	Stage 0	F0
Fibrosis portal expansion	Mild fibrosis	Stage 1	F1
Few bridges or septa	Moderate fibrosis	Stage 2	F2
Numerous bridges or septa	Severe fibrosis	Stage 3	F3
Cirrhosis	Cirrhosis	Stage 4	F4

IASL: International Association for the Study of the Liver.

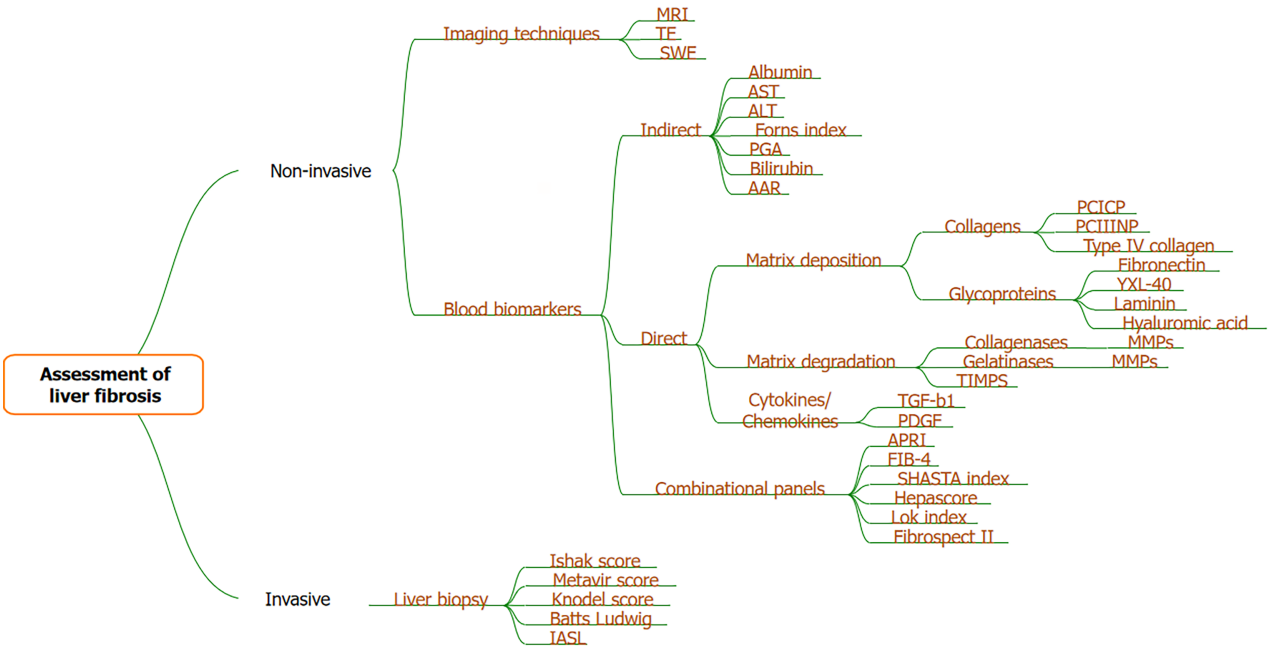

Figure 2 Various methods for assessment of liver fibrosis. MRI: Magnetic resonance imaging; TE: Transient elastography; SWE: Shear wave elastography; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; AAR: Aspartate aminotransferase aspartate aminotransferase/alanine aminotransferase ratio; TGF-β: Transforming growth factor β; PDGF: Platelet growth factor; APRI: Aspartate aminotransferase to platelet count ratio; FIB-4: Fibrosis-4; PCICP: Procollagen type 1; PCIIINP: Procollagen type 3; MMP: Matrix metalloproteinase.

understand more about liver disease and analyze the various approaches which can be relied upon in managing the condition of the patient[13]. These methods are distinctively classified into two, the natural or physical approach and the biological approach. The physical approach is majorly used with various imaging techniques while the biological method is based on the popular serum biomarkers[14]. The two methods are quite distinct in the way that the conditions are valued and assessed but they are both based on conceptions and rationales that are quite different.

PHYSICAL APPROACH

There are many types of physical approaches that experts rely on in assessing liver conditions. These physical approaches include Doppler analysis, computed tomography, acoustic radiation force impulse imaging, transient elastography (TE), ultrasonography, magnetic resonance imaging, and real-time elastography. Menessy *et al*[13] also discuss that most of these methods are based on scanning and imaging techniques by which the experts analyze the liver and the condition of the systematic process. There are some of these methods that are widely considered more than others. There are the ones that are quite fast enough for experts while there are the slow ones. Some provide a distinct value of images or scans that can be relied on comfortably.

Transient elastography

TE is the most appropriate approach due to its speed. Fallatah[15] discusses that on top of that, the approach is quite reproducible and at the same time does not depend on operators. The approach is also quite common among many hepatitis experts since it provides and measures the stiffness of the liver and compares the same stiffness and its elasticity. With such considerations, it is quite easy to analyze the conditions of hepatitis and also conduct the corresponding analysis of its physical properties, which is highly genuine. The technique is also considered for its ability to predict the issues around severe fibrosis and also its accuracy in identifying cases of liver cirrhosis that are underlying the hepatitis condition. There are, however, issues of the method's examination of fibrosis which are mostly associated with this disease. In some cases, the approach is unable to provide information that is quite sufficient for experts to diagnose cases of significant fibrosis especially with the main consideration being the hepatitis C condition. This means that the technique does not provide distinct stages and processes for the analysis of the condition, and that there should be experts to analyze and interpret the information provided through the technique despite the results from the basic approach being straightforward. This means that an expert, who has been aware of and dealt with the clinical background of the patient, especially with his or her case of hepatitis C, should be at the center of measurements and results [15].

When compared with the METAVIR score of liver biopsy, the sensitivity and specificity of the cut-off value of TE are shown in Table 3[16].

Shear wave elastography

This has been a recently developed method for measuring liver elasticity. It has been considered that it is a reliable non-invasive tool for monitoring liver stiffness in HCV patients with an accuracy of 97.6%. It is a novel, rapid, and noninvasive method for measuring liver stiffness. It determines liver stiffness by estimating the velocity of shear waves emitted in the liver tissue. Moreover, the velocity of this shear wave (*i.e.*, lateral wave) is calculated. The benefit of this mode of assessment is that the real-time images are seen with the help of a normal B-mode ultrasound probe[17].

The area under the receiver operating characteristic curve (AUROC) for F > 2 and F4 were found to be 0.87 and 0.93, respectively[18]. Shear wave elastography was 85% specific and 79% sensitive when compared with the METAVIR score by taking a cut-off value of 1.34 for the F2 stage of fibrosis[19,20].

BIOLOGICAL APPROACH

Many developments have been realized across all industries. Among these industries are the medicine and clinical areas. A new era of biotechnology and biomedicine has taken a central part in developing our clinical and medical worlds. Stasi and Milani[21] make consideration that over the years, the world of medicine has seen major developments with tremendous strides having been realized in both the biotechnology and biomedical world[13]. This has brought up a new generation of medical approaches that are characterized by rapid, novel, and non-invasive approaches. These approaches have brought up some challenging ideas of the previous settings of medicine with major changes being recognized in the invasive diagnostic and therapeutic approaches. Some characteristics need to be fulfilled by the non-invasive methods, with most of these being the factors of accessibility, simplicity, high accuracy, and being liver-specific, satisfactorily validated, and easily interpretable[14].

Class I biomarkers (direct) to assess liver fibrosis are the remnants of liver matrix components. These are formed by HSCs during ECM remodeling. These markers

Table 3 Correlation of transient elastography cutoffs with METAVIRscore

METAVIR score	Cutoff TE score (kPa)	Sensitivity	Specificity	NPV	PPV
F ≥ 2 (F0-F1 *vs* F2-4)	7.1	0.67	0.89	0.48	0.95
F ≥ 3 (F0-F1-F2 *vs* F3, 4)	9.5	0.73	0.91	0.81	0.87
F ≥ 4 (F0-F1-F2-F3 *vs* F4)	12.5	0.87	0.91	0.95	0.77

TE: Transient elastography; NPV: Negative predictive value; PPV: Positive predictive value.

directly reflect either deposition or removal of ECM[22].

Whereas indirect (class II) markers include routine investigations such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), serum bilirubin, gamma-glutamyltransferase (GGT), haptoglobin, and α_2-macroglobulin. These markers are not specific for assessing intermediate stages of fibrosis[23].

Combinational panels by computing indirect markers have also been studied. These include fibrosis-4 (FIB-4), APRI (AST to platelet count ratio), SHASTA index, Fibroscore, Hepascore, and Lok index.

Class I biomarkers (direct)

Over the years, there have been major demands to understand the pathophysiology of the liver better. This has prompted and enabled many scientists and experts in this field to establish major research while investigating the major developments in the area. Class 1 biomarkers are therefore types of non-invasive biomarkers that mimic the liver metabolism and its ECM. It has been considered that though majorly associated with the fibrosis stages, these biomarkers are also associated with the fibrogenic cells and the changes that are majorly seen in the same[10]. It has been discussed that besides measuring and assessing the conditions of the liver concerning the hepatitis C condition, these biomarkers have another clinical usefulness in which they assess the rate at which other underlying issues progress besides staging the liver fibrosis[24]. As revealed by Stasi and Milani[21] with such assessments done by the biomarkers, the same data and measurements from the assessment are turned or else translated into prognostic information that is quite effective. This is then made as a tool in which responses are evaluated. In the long run, they also help in monitoring the efficiency of the associated ant fibrotic drugs. This is where the data that is provided in these circumstances gets to be used as variables for the performance and availability measurements. The direct markers are classified as below.

Direct markers linked with matrix deposition: Collagens and glycoproteins

Collagens: These direct markers are found in the connective tissues and have three types. Pro-collagen is the precursor of the collagen which is cleaved by two different enzymes at amino (type 3) and carboxyl (type1) terminal ends to form collagens[25]. The collagens formed are procollagen type 1 (PCICP) and procollagen type 3 (PCIIINP). PCICP is the main component of connective tissue[25]. The upper limit of normal values is 202 and 170 μg in males and females, respectively[26-28]. It is increased in cirrhosis progression. PCIINP is increased with fibrotic stage and correlates well with bilirubin levels in cirrhosis cases[29-31]. The only drawback of this marker is that it increases in other medical conditions also. Also, the efficacy is decreased as compared to hyaluronic acid (HA)[27,31]. Type IV collagen is the third collagen serving as a direct marker. It acts as a surrogate marker to assess liver fibrosis [32]. Its levels are manifold increased in liver diseases and correlate well with fibrosis [33,34]. An area under the curve (AUC) of 0.82 with a negative predictive value (NPV) of 83.6% was found with a cut-off value of greater than 5.0 ng/mL in NAFLD[34].

Glycoproteins: HA is an example of a direct serum marker used in the diagnosis of liver damage in patients[35]. It is integrated and dispersed all over the extracellular space. This process is done by the HSCs. The damaged liver tends to provide HA in high quantities. As a result, this marker is used to predict the level of liver damage based on elevated serum levels. This is because the levels of HA correlate with liver fibrosis[36]. Research shows that the HA serum direct marker is more accurate than most non-invasive indices. However, this method of diagnosis works best when combined with other liver markers. NPV was 98%-100% in cirrhosis[35-38]. Also, HA levels start decreasing with the treatment of liver disease[39-41]. Laminin is a

glycoprotein that is non-collagenous and is formed by the HSCs[10]. In a patient with liver fibrosis, elevated levels of laminin correlate well with the degree of the fibrosis. However, its diagnostic value is not of much significance when compared with HA. The cut-off value of 1.45 was proposed by Sebastiani[32] for detecting fibrosis and cirrhosis. It is 77% accurate for detecting fibrosis in HCV cases. YKL-40 is another diagnostic tool used to assess liver damage in patients with hepatitis C. It is a mammalian homologue of bacterial chitinases which are involved in the remodeling or degradation of ECM[21]. The levels of YKL-40 correlate with the severity of fibrosis. Fibronectin (FN) is a high molecular weight glycoprotein of the ECM which binds to integrins (receptor proteins). It is synthesized by various cells but mainly by hepatocytes. In blood, FN exists in two major forms, *i.e.*, cellular FN (cFN) and plasma FN (pFN)[42].

Direct markers that are associated with matrix degradation: Collagenases, gelatinases, and tissue inhibitors of matrix metallo proteinases

Collagenases: Metalloproteinase-1 (MMP-1) is found to be inversely correlated with necrosis as well as fibrosis[43].

Gelatinases: Two matrix metalloproteinases MMP-2 and MMP-9 have been found. They are also known as gelatinases, *i.e.*, gelatinase A and B, respectively. Previously, MMP-2 was found to have no significant association with liver fibrosis stage[44,45]. But later Boeker *et al*[44] found an accuracy of 92% for detecting cirrhosis in HCV patients. It is increased by 2.4 folds in HCV patients as compared to controls. MMP-9 is inversely correlated with histological severity in hepatitis. Its levels start decreasing as cirrhosis progresses[46,47].

Tissue inhibitors of matrix metallo proteinases: They interact with MMP functioning and further lead to ECM degeneration inhibition. It shows a positive correlation with fibrosis stage[45-48].

Cytokines/chemokines in liver fibrosis

These include transforming growth factor (TGF)-β1, TGF-α, and platelet growth factor (PDGF). TGF-β1 correlates well with fibrosis in HCV-infected patients. The value of < 75 ng/mL is considered to be normal[49,50]. TGF-α is found to be more correlated with fibrotic stage in hepatocellular carcinoma (HCC)[51]. PDGF levels are associated with liver fibrosis and a cut-off value of 40.50 ng/L is an indicator for inflammation and fibrosis[52].

Class II biomarkers (indirect)

Back in the day, the first approach that majorly assessed the conditions of the liver and issues like hepatitis C and liver fibrosis included hematological tests and routine biochemical tests which are classified as non-invasive biomarkers. Class II biomarkers are also referred to as indirect biomarkers. They are mostly based on common functional alterations in the liver and the evaluations that are attached to the same[13]. These alterations, however, do not reflect the turnover and changes associated with the fibrogenic cells. For the class II biomarkers, the basis of the measurements and evaluation is algorithmic and single elaboration. These are mainly based on the alterations that have been observed in the liver and its functions.

AST/ALT ratio: The AST/ALT ratio (AAR) index is an example of an indirect serum marker used in the diagnosis of liver damage in patients with hepatitis C. However, it is important to note that when the stages of fibrosis are not advanced, the performance of the AAR index is low[13]. Haukeland *et al*[53] validated this test in different liver diseases. The ratio of more than 1 predicts liver cirrhosis[54,55].

APRI: It provides a quick estimate for predicting severe fibrosis or cirrhosis[56]. This is among the most validated noninvasive biomarkers[13]. APRI was calculated as [AST level/AST (upper limit of normal)]/[platelet count (10^9/L)] × 100. It was originally developed by Wai *et al*[57] in 2003. The AUC was 0.8 and 0.89 for fibrosis and cirrhosis, respectively. Loaeza-del-Castillo *et al*[56] found that it is not a diagnostic marker in autoimmune hepatitis.

BARD score: This is the combination of AAR and body mass index (BMI) and other measures of diabetic patients. NPVs of 96% and 81.3% were found[58].

ALT: Due to its high sensitivity as well as specificity, it is used as a better indicator of liver disease[59].

Forns index: It involves parameters like age, platelet count, cholesterol, and GGT[60]. Forns index was calculated as [7.811 - 3.131 × ln(platelet count)] + [0.781 × ln(GGT in IU/L)] + [3.467 × ln(age) - 0.014 × cholesterol in mg%]. It differentiates mild fibrosis from severe fibrosis.

PGA and PGAA index: PGA is used to assess fibrosis in alcoholics[61]. A combination of prothrombin index, GGT, and apolipoprotein A is used in calculating PGA. It is considered 65% accurate in detecting liver fibrosis. Furthermore, a2 macroglobulin was added and PGAA was invented. It has a 70% accuracy in detecting fibrosis[62].

FIB-4: It is a simple, fast, and cheap test that gives immediate results[23]. It is a validated test used for detecting hepatitis B and C. The AUC of 0.85 and 0.81 for detecting severe fibrosis was found in HCV and HBV, respectively[63,64]. IB-4 was calculated as [Age (years) × AST (U/L)]/[Platelet count × √ALT (U/L)]

Fibroindex: It is a simple scoring system[65]. It showed an AUC of 0.83 for fibrosis detection. Also, a cutoff value of 2.25 was strongly associated with F2-F3 fibrosis stage with an NPV of 90%[65]. Fibroindex was calculated as [1.738 - 0.064 × platelet count ($104/mm^3$)] + [0.005 × AST (U/L)] + [0.463 × gamma globulin (g/dL)].

Fibrotest: It includes certain parameters like age, gender, haptoglobin, a2 macroglobulins, apolipoprotein A1, GGT, and serum bilirubin[66,67]. This is considered as a most validated marker for detecting liver fibrosis[68,69].

Acti test: A simple addition of ALT in Fibrotest was made which reflects liver fibrosis as well as necro-inflammatory activity[70,71]. Acti test is a parameter that was initially validated for patients with chronic hepatitis B and C. It was used in collaboration with the Fibrotest as an alternative to liver biopsy. The Acti test combines five components of the Fibrotest and ALT. The assessment is crucial for treatment prescription especially in patients with moderate or severe necro-inflammatory activity as well as cirrhotic patients.

Tests for NAFLD: Initially, the simplest test was developed by using age, BMI, platelet count, ALT: AST ratio, serum albumin, and glycemic status[72]. AUC was calculated as 0.88 with an NPV of 93%. Steato test was later proposed by combining fibrotest and Acti test[73]. A cut-off value was fixed at 0.7 with a 90% specificity.

MICRORNAS AND THEIR BIOSYNTHESIS

MicroRNAs (miRNAs) are also nowadays considered potential biomarkers in assessing liver fibrosis. They are small non-coding strands of RNA, responsible for the regulation of the expression of genes after the transcription process. They usually target and regulate the biological processes and then influence the complex programs of the expression of genes in several cellular processes[74]. Notably, miRNAs are deemed principal regulators that control main cell functions in several physiological and pathophysiological processes.

The biogenesis of miRNAs is made up of two cleavage pathways; after forming the mature miRNA, there is one nuclear and one cytoplasmic. The miRNA precursors are sorted into different pathways. However, the process is unclear but appears to be determined by the site where the miRNA originates, the sequence, and even the thermodynamic stability[75]. Regulatory functions of miRNAs occur through the silencing complex induced by RNA, specific for a particular miRNA.

MiRNAs are usually transcribed from the introns and exons of the genes responsible for protein-coding or the intergenic areas. The transcription of the miRNA genes is the basis of primary transcripts, which contain the hairpin structure that consists of a terminal loop and a double-stranded stem. Later, there is then cleavage of the stem-loop structure with the help of the RNase III-like enzymes that are known as Drosha and the binding partner DGCR8[76]. The result is the formation of the precursor miRNA (pre-miRNA).

There is then the transfer of pre-miRNA from the nucleus into the cytoplasm, and this is helped by exportin-5 and the accompanying co-factor Ran-GTP. The GTP is bound to the Ras-related nuclear protein. The cofactor is then processed into a structure that is duplex by the RNA polymerase II dicer. When an miRNA binds to its

target, it leads to the degradation of the target mRNA or the suppression of the mRNA translation[76]. Figure 3 depicts the entire process of miRNA biogenesis.

More than 1500 miRNAs have been determined in the human genome, which are involved in the cell processes, including the development, differentiation, and proliferation of cells, the process of death, the pathology, and defense against viruses.

MiRNAs are essential in the process of the pathogenesis of HCV infection through the control of the signaling pathway. In this regard, they play a role in the response of both the innate and adaptive immune systems. MiR-122 has been determined to be the most abundant miRNA in the normal liver parenchyma, and it accounts for more than 70% of the miRNAs found in the hepatocytes[77]. The miR-21 gene is located on chromosome 17, and it is highly conserved. Inside the cell, miRNA-21 is found in the cytosol and the extracellular exosome. At the organ level, miRNA is located in the bone marrow, lungs, kidney, peripheral blood, colon, intestines, and thyroid.

When miR-122 binds to a 5′-untranslated region (5′-UTR) of the genomic constituent of HCV RNA, which is critical for the replication of the virus, it then stimulates translation of the viral protein and then protects HCV RNA that is uncapped from the process of degradation. Over time, the upregulation of the miR-21 leads to the feedback of inhibition of type I interferon, which is mediated by the antiviral response. This then promotes viral replication[78]. Moreover, miR-21 is detected in the oncogenic miRNA and controls the process of cell cycle and tumorigenesis.

As indicated above, miR-21 is a contributor to the development of fibrogenesis in the muscles and various organs, including the liver. Clinical data has demonstrated that miR-21 is always upregulated in the liver of patients who have biliary atresia-induced liver fibrosis. MiR-21 can induce fibrosis through activation of HSCs and then collagen synthesis. The overexpression of miR-21 leads to the promotion of oxidation, and this then increases the production of collagen, which in return, activates angiotensin. MiR-21 can affect the expression of several proteins by binding to the 3′-UTR of specific mRNAs. This results in a complex interaction network as a result of downstream effects of the signaling pathways[76]. Various signaling pathways have been identified to be the basis of the pathophysiological fibrosis process, including the phosphoinositide 3-kinase, TGF-β/Smads, and the extracellular signal-regulated kinase (ERK)/mitogen-activated protein kinase pathways[79].

Activation of angiotensin occurs through several pathways: Spry1/ERK/NF-κB, PTEN/Akt, programmed cell death 4/AP-1, and Smad7/Smad2/3/NADPH oxidase 4. In recent findings, research has been able to elucidate that a moiety that is deficient in the methionine choline diet of NASH is linked to liver damage[79]. MiR-21 then results in a decrease of steatosis, lipo-apoptosis, and inflammation with impairment of fibrosis. Recent findings have shown that antisense inhibition or the deletion of genes of miR-21 does not alter the HSC activation or fibrosis. MiR-21 is frequently upregulated in human beings with solid malignancies like breast, colon, pancreas, lung, and liver tumors[79]. MiR-21 has also been shown to be a survival factor in the course of liver injury and the development of HCC.

MiR-449a is found to be dysregulated in hepatitis C infection only. Its significance is not found in alcoholics and NAFLD. It regulates YKL-40 by targeting the NOTCH signaling pathway in HCV infection[80]. Also, the expression of miR-155 was significantly increased, which further led to tumorigenesis by modulating the Wnt signaling pathway[81].

NOVEL FINDINGS SUPPORTING IMPORTANCE OF NONINVASIVE MARKERS

According to Menessy *et al*[13], noninvasive markers are crucial. This is mainly because these procedures are effective in the evaluation of the stage of liver fibrosis in patients with hepatitis C whereby there are no clear indications for liver biopsy. Liver biopsy is not ideal for frequent development. Given the rapid development of new medications for the treatment of hepatitis C, there is an increased need for frequent evaluations of liver damage and liver fibrosis. Consequently, the use of non-invasive assessment methods for liver fibrosis in patients with hepatitis C is crucial.

For HCV infection, there are high chances of developing liver cirrhosis and liver fibrosis in some patients. This means that physicians examining a patient should be keen to verify the infections that are underlying in cases of the main condition which is hepatitis C. The presence of non-invasive biomarkers makes all these possible by establishing a process in which the necrotic processes and the inflammatory activities are considerably detected and analyzed. These biomarkers help in establishing a clear

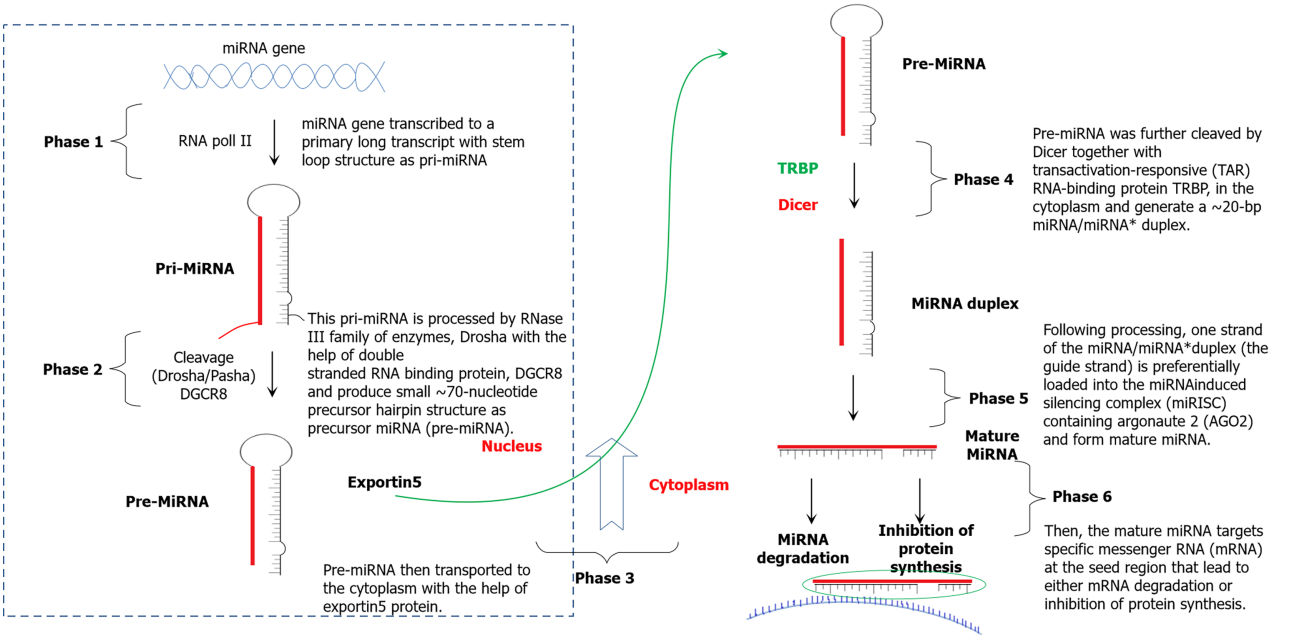

Figure 3 Process of microRNA biogenesis. miRNA: MicroRNA.

process of detecting the major changes in the liver as the patient deals with hepatitis C. The non-invasive biomarkers generally help in forecasting the main course that the HCV takes[13].

Similarly, Stasi and Milani[21] assert that non-invasive assessment methods for liver fibrosis tend to be readily available, simple, reliable, safe, inexpensive, and well-validated. As a result, they are effective in evaluating the progression of liver disease. Non-invasive biomarkers offer numerous advantages over liver biopsies. Some of these advantages include the absence of adverse effects and reduced risks of sampling errors. These bring about objectiveness when it comes to the interpretation of the results. Noninvasive biomarkers lack any reported ceiling effect hence effective as compared to liver biopsy Noninvasive assessment methods are appropriate as they allow for repeated assessment.

Various researchers argue that by definition, noninvasive biomarkers, however, cannot outperform liver biopsy even though they tend to be more accurate in the assessment of liver fibrosis. This is because of the method as well as its limitations. Some of its limitations are unreliability and feasibility especially in obese patients or under limited operator experience. The procedure is also contradicted during ascites, pregnancy, and implanted cardiac pacemaker patients. Besides, the knowledge of noninvasive biomarkers is still incomplete. This poses a challenge to clinical practice since it greatly hinders the development of accurate treatment and noninvasive diagnostic means with adequate sensitivity for liver fibrosis[24].

Similarly, Oksuz *et al*[82] affirm that for the assessment of necroinflammatory histological activity, few biomarkers have been proposed. Fallatah[15] argues that improving the accuracy of noninvasive biomarkers is essential for a correct diagnosis of liver damage in patients. This can be done using serum-based algorithms as sequential and simultaneous procedures. In a study, the comparison of TE to liver fibrosis was done[83]. The authors found that TE performed better in predicting all stages of fibrosis as well as severe fibrosis. Fibroscan values showed a good correlation with the levels of fibrosis markers. Also, the Fibroscan value of 15KpA was a significant separation limit for differentiating advanced fibrosis stages (F3 and F4). They suggested that these Fibroscan values are clinically useful to predict fibrosis stages in chronic hepatitis patients[84]. Other researchers correlated Fibroscan with fibrosis degree in liver biopsy and stated that it can be used as a noninvasive tool to diagnose moderate fibrosis[85]. Recently, there has been increased interest in detecting liver fibrosis through the application of non-invasive techniques. The APRI is the most useful score to predict fibrosis[56]. Attallah *et al*[86] found that FN discriminant scores based on FN, APRI, and albumin can be used to predict liver fibrosis (Table 4).

Table 4 Sensitivity and specificity of non-invasive biomarkers in liver fibrosis

Marker	Parameters involved	Disease	AUROC for liver fibrosis	Sensitivity	Specificity	Ref.
AST/ALT ratio	AST and ALT	NAFLD; HCV	0.83; -	74; 47	78; 96	McPherson *et al*[87]; Park *et al*[88]
BARD score	BMI, AST, ALT, DM	NAFLD	0.76	74	66	Sun *et al*[89]
APRI	AST, platelet count	NAFLD	0.67	27	89	McPherson *et al*[87]
ALT	ALT	HCV	0.716-0.815	-	-	Pradat *et al*[59]
Forns index	Age, platelet count, GGT, cholesterol	HCV	0.81-0.86	94	51	Forns *et al*[60]
PGA and PGAA	Prothrombin time, GGT, apolipoprotein A1, α2 macroglobulin	Acute liver disease	0.84-0.86	-	-	Nguyen-Khac *et al*[90]
FIB-4	Platelet count, AST, ALT, age	HCV; NAFLD	0.74-0.77; 0.85	67; 84	71; 69	Sebastiani[23]; Sun *et al*[89]
Fibro test	Haptoglobin, apolipoprotein A1, α2 macroglobulin, GGT, bilirubin, age, and gender	HBV; HCV; ALD	0.84; 0.87; 0.83	61; 75; -	80; 85; -	Salkic *et al*[91]; Imbert-Bismut *et al*[66]; Naveau *et al*[62]
Hepascore	GGT, bilirubin, HA, α2 macroglobulin, age, and gender	HCV	0.82	-	-	Naveau *et al*[62], Adams *et al*[92]
SHASTA index	HA, AST, and albumin	HCV	0.87	50	94	Kelleher *et al*[93]
Fibrospect II	α2 macroglobulin, HA, and TIMP-1	HCV	0.82-0.83	77-83	66-73	Patel *et al*[94]

AUROC: Area under receiver operating curve; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; NAFLD: Non-alcoholic fatty liver disease; HBV: Hepatitis B virus; HCV: Hepatitis C virus; BMI: Body mass index; DM: Diabetes mellitus; APRI: Aspartate aminotransferase to platelet count ratio; FIB-4: Fibrosis-4; GGT: Gamma-glutamyltransferase; HA: Hyaluronic acid; TIMP-1: Tissue inhibitors of metalloproteinases-1.

PROS AND CONS OF NON-INVASIVE BIOMARKERS

Various authors had made the remarks that non-invasive biomarkers can be used instead of liver biopsy because its acceptance has faced some key resistance from different sectors[14]. Some of the factors that bring the cases of resistance are attached to the paucity of well-designed studies and literature that discuss the non-invasive methods extensively giving a view of both sides. There are also issues with the validation of some of the non-invasive biomarkers and proposals for some of them in terms of the lack of validated data. With the ones that their proposals have been provided, some changes in terms of assessing the severity and the growth rate have not been discussed and analyzed extensively[12]. As per Menessy *et al*[13] for others, there has not been enough time to validate them in terms of testing and analysis in their use when it comes to the cases of hepatitis C[9]. What is needed in most of these cases is the specific etiology validation, especially for most of these non-invasive biomarkers. In these cases, each etiology should be considered to deal with the issues of the specific pathogenesis, associated comorbidities, and natural history.

In the clinical practice related to the hepatitis condition, there should be a careful evaluation of all risk factors that are attached to failure and errors that can be associated with the specific non-invasive tools or biomarkers. A careful evaluation is needed to interpret the result and measurements adequately[21]. For the liver biopsy, a key concern for most experts is to note the role that these non-invasive biomarkers play in achieving the right clinical practice. With these biomarkers, most of these experts can create a cost-effective and attractive approach that is quite better and advantageous than the liver biopsy.

It has been revealed that the biomarkers are substantially less invasive, which provides a different experience for the clinical experts[9]. Besides the same advantage, other significant factors make them better than the biopsy. First, they practically have no or fewer sampling errors which enable a sufficient and efficient approach in the analysis and assessments. On the other hand, they also have very few complications that are related to health and clinical advancements. Shrivastava *et al*[9] make a point that the observer-related variability is also very small, which explains the high considerations from different experts. Lastly, the measurements and assessments may be

performed and considered repeatedly even from different labs, and the instruments and the equipment for this process do not need to be complicated. This means that they can allow for the dynamic monitoring of the health condition and other issues related to liver damage. This underlines the huge role that biomarkers play in assessing and proposing the conditions of the liver which is the main body part affected by the disease.

CONCLUSION

We agree with the above discussions that the use of two or more noninvasive biomarker methods will increase the accuracy of an individual to be assessed for fibrosis. In such case, the choice of the algorithm to be used in the combination in clinical practice should be based on some specific considerations. Considerations that must be made include what is locally available, what is not related to the patient's co-morbidities, what is recently validated, and the method that the physician feels comfortable to use. We have found that a combinational panel of noninvasive biomarkers is cheap and simple as compared to the use of individual biomarkers and liver biopsy. Finally, we would suggest that one or more direct biomarkers along with one imaging technique can be used for the assessment of liver fibrosis.

REFERENCES

1 **Li H**, Huang MH, Jiang JD, Peng ZG. Hepatitis C: From inflammatory pathogenesis to anti-inflammatory/hepatoprotective therapy. *World J Gastroenterol* 2018; **24**: 5297-5311 [PMID: 30598575 DOI: 10.3748/wjg.v24.i47.5297]

2 **Nallagangula KS**, Nagaraj SK, Venkataswamy L, Chandrappa M. Liver fibrosis: a compilation on the biomarkers status and their significance during disease progression. *Future Sci OA* 2018; **4**: FSO250 [PMID: 29255622 DOI: 10.4155/fsoa-2017-0083]

3 **Brenner DA**. Molecular pathogenesis of liver fibrosis. *Trans Am Clin Climatol Assoc* 2009; **120**: 361-368 [PMID: 19768189]

4 **Bataller R**, Brenner DA. Liver fibrosis. *J Clin Invest* 2005; **115**: 209-218 [PMID: 15690074 DOI: 10.1172/JCI24282]

5 **Khatun M**, Ray RB. Mechanisms Underlying Hepatitis C Virus-Associated Hepatic Fibrosis. *Cells* 2019; **8** [PMID: 31615075 DOI: 10.3390/cells8101249]

6 **Roberts RA**, Ganey PE, Ju C, Kamendulis LM, Rusyn I, Klaunig JE. Role of the Kupffer cell in mediating hepatic toxicity and carcinogenesis. *Toxicol Sci* 2007; **96**: 2-15 [PMID: 17122412 DOI: 10.1093/toxsci/kfl173]

7 **Mansour D**, McPherson S. Management of decompensated cirrhosis. *Clin Med (Lond)* 2018; **18**: s60-s65 [PMID: 29700095 DOI: 10.7861/clinmedicine.18-2-s60]

8 **Tasnif YO**, Hebert MF. Complications of end-stage liver disease. Koda-Kimble and Young's Applied Therapeutics: The Clinical Use of Drugs, 2013: 720-742

9 **Shrivastava S**, Mukherjee A, Ray RB. Hepatitis C virus infection, microRNA and liver disease progression. *World J Hepatol* 2013; **5**: 479-486 [PMID: 24073299 DOI: 10.4254/wjh.v5.i9.479]

10 **Sebastiani G**, Gkouvatsos K, Pantopoulos K. Chronic hepatitis C and liver fibrosis. *World J Gastroenterol* 2014; **20**: 11033-11053 [PMID: 25170193 DOI: 10.3748/wjg.v20.i32.11033]

11 **Ghany MG**, Strader DB, Thomas DL, Seeff LB; American Association for the Study of Liver Diseases. Diagnosis, management, and treatment of hepatitis C: an update. *Hepatology* 2009; **49**: 1335-1374 [PMID: 19330875 DOI: 10.1002/hep.22759]

12 **Sumida Y**, Nakajima A, Itoh Y. Limitations of liver biopsy and non-invasive diagnostic tests for the diagnosis of nonalcoholic fatty liver disease/nonalcoholic steatohepatitis. *World J Gastroenterol* 2014; **20**: 475-485 [PMID: 24574716 DOI: 10.3748/wjg.v20.i2.475]

13 **Menessy AN**, Ahmed NA, Abdallah NI, Arif SS. Noninvasive predictors of hepatic fibrosis in patients with chronic hepatitis C virus in comparison with liver biopsy. *Benha Med J* 2018; **35**: 282-286 [DOI: 10.4103/bmfj.bmfj_132_17]

14 **Valva P**, Ríos DA, De Matteo E, Preciado MV. Chronic hepatitis C virus infection: Serum biomarkers in predicting liver damage. *World J Gastroenterol* 2016; **22**: 1367-1381 [PMID: 26819506 DOI: 10.3748/wjg.v22.i4.1367]

15 **Fallatah HI**. Noninvasive biomarkers of liver fibrosis: an overview. *Adv Hepatol* 2014; 357287 [DOI: 10.1155/2014/357287]

16 **Castéra L**, Vergniol J, Foucher J, Le Bail B, Chanteloup E, Haaser M, Darriet M, Couzigou P, De Lédinghen V. Prospective comparison of transient elastography, Fibrotest, APRI, and liver biopsy for the assessment of fibrosis in chronic hepatitis C. *Gastroenterology* 2005; **128**: 343-350 [PMID: 15685546 DOI: 10.1053/j.gastro.2004.11.018]

17 **Serra C**, Grasso V, Conti F, Felicani C, Mazzotta E, Lenzi M, Verucchi G, D'errico A, Andreone P.

A New Two-Dimensional Shear Wave Elastography for Noninvasive Assessment of Liver Fibrosis in Healthy Subjects and in Patients with Chronic Liver Disease. *Ultraschall Med* 2018; **39**: 432-439 [PMID: 29458217 DOI: 10.1055/s-0043-119356]

18 **Friedrich-Rust M**, Nierhoff J, Lupsor M, Sporea I, Fierbinteanu-Braticevici C, Strobel D, Takahashi H, Yoneda M, Suda T, Zeuzem S, Herrmann E. Performance of Acoustic Radiation Force Impulse imaging for the staging of liver fibrosis: a pooled meta-analysis. *J Viral Hepat* 2012; **19**: e212-e219 [PMID: 22239521 DOI: 10.1111/j.1365-2893.2011.01537.x]

19 **Bota S**, Sporea I, Peck-Radosavljevic M, Sirli R, Tanaka H, Iijima H, Saito H, Ebinuma H, Lupsor M, Badea R, Fierbinteanu-Braticevici C, Petrisor A, Friedrich-Rust M, Sarrazin C, Takahashi H, Ono N, Piscaglia F, Marinelli S, D'Onofrio M, Gallotti A, Salzl P, Popescu A, Danila M. The influence of aminotransferase levels on liver stiffness assessed by Acoustic Radiation Force Impulse Elastography: a retrospective multicentre study. *Dig Liver Dis* 2013; **45**: 762-768 [PMID: 23510533 DOI: 10.1016/j.dld.2013.02.008]

20 **Guo Y**, Parthasarathy S, Goyal P, McCarthy RJ, Larson AC, Miller FH. Magnetic resonance elastography and acoustic radiation force impulse for staging hepatic fibrosis: a meta-analysis. *Abdom Imaging* 2015; **40**: 818-834 [PMID: 24711064 DOI: 10.1007/s00261-014-0137-6]

21 **Stasi C**, Milani S. Non-invasive assessment of liver fibrosis: Between prediction/prevention of outcomes and cost-effectiveness. *World J Gastroenterol* 2016; **22**: 1711-1720 [PMID: 26819535 DOI: 10.3748/wjg.v22.i4.1711]

22 **Baranova A**, Lal P, Birerdinc A, Younossi ZM. Non-invasive markers for hepatic fibrosis. *BMC Gastroenterol* 2011; **11**: 91 [PMID: 21849046 DOI: 10.1186/1471-230X-11-91]

23 **Sterling RK**, Lissen E, Clumeck N, Sola R, Correa MC, Montaner J, S Sulkowski M, Torriani FJ, Dieterich DT, Thomas DL, Messinger D, Nelson M; APRICOT Clinical Investigators. Development of a simple noninvasive index to predict significant fibrosis in patients with HIV/HCV coinfection. *Hepatology* 2006; **43**: 1317-1325 [PMID: 16729309 DOI: 10.1002/hep.21178]

24 **Pinzani M**, Rombouts K, Colagrande S. Fibrosis in chronic liver diseases: diagnosis and management. *J Hepatol* 2005; **42** Suppl: S22-S36 [PMID: 15777570 DOI: 10.1016/j.jhep.2004.12.008]

25 **Gallorini A**, Plebani M, Pontisso P, Chemello L, Masiero M, Mantovani G, Alberti A. Serum markers of hepatic fibrogenesis in chronic hepatitis type C treated with alfa-2A interferon. *Liver* 1994; **14**: 257-264 [PMID: 7997085 DOI: 10.1111/j.1600-0676.1994.tb00085.x]

26 **Trinchet JC**, Hartmann DJ, Pateron D, Laarif M, Callard P, Ville G, Beaugrand M. Serum type I collagen and N-terminal peptide of type III procollagen in chronic hepatitis. Relationship to liver histology and conventional liver tests. *J Hepatol* 1991; **12**: 139-144 [PMID: 1711064 DOI: 10.1016/0168-8278(91)90929-6]

27 **Niemelä O**, Blake JE, Orrego H. Serum type I collagen propeptide and severity of alcoholic liver disease. *Alcohol Clin Exp Res* 1992; **16**: 1064-1067 [PMID: 1471760 DOI: 10.1111/j.1530-0277.1992.tb00700.x]

28 **Lieber CS**, Weiss DG, Paronetto F; Veterans Affairs Cooperative Study 391 Group. Value of fibrosis markers for staging liver fibrosis in patients with precirrhotic alcoholic liver disease. *Alcohol Clin Exp Res* 2008; **32**: 1031-1039 [PMID: 18422837 DOI: 10.1111/j.1530-0277.2008.00664.x]

29 **Tamura H**, Matsuda A, Kidoguchi N, Matsumura O, Mitarai T, Isoda K. A family with two sisters with collagenofibrotic glomerulonephropathy. *Am J Kidney Dis* 1996; **27**: 588-595 [PMID: 8678072 DOI: 10.1016/s0272-6386(96)90172-9]

30 **Annoni G**, Colombo M, Cantaluppi MC, Khlat B, Lampertico P, Rojkind M. Serum type III procollagen peptide and laminin (Lam-P1) detect alcoholic hepatitis in chronic alcohol abusers. *Hepatology* 1989; **9**: 693-697 [PMID: 2707736 DOI: 10.1002/hep.1840090506]

31 **Sebastiani G**, Alberti A. Non invasive fibrosis biomarkers reduce but not substitute the need for liver biopsy. *World J Gastroenterol* 2006; **12**: 3682-3694 [PMID: 16773685 DOI: 10.3748/wjg.v12.i23.3682]

32 **Sebastiani G**. Non-invasive assessment of liver fibrosis in chronic liver diseases: implementation in clinical practice and decisional algorithms. *World J Gastroenterol* 2009; **15**: 2190-2203 [PMID: 19437558 DOI: 10.3748/wjg.15.2190]

33 **Guéchot J**, Laudat A, Loria A, Serfaty L, Poupon R, Giboudeau J. Diagnostic accuracy of hyaluronan and type III procollagen amino-terminal peptide serum assays as markers of liver fibrosis in chronic viral hepatitis C evaluated by ROC curve analysis. *Clin Chem* 1996; **42**: 558-563 [PMID: 8605673]

34 **Sakugawa H**, Nakayoshi T, Kobashigawa K, Yamashiro T, Maeshiro T, Miyagi S, Shiroma J, Toyama A, Kinjo F, Saito A. Clinical usefulness of biochemical markers of liver fibrosis in patients with nonalcoholic fatty liver disease. *World J Gastroenterol* 2005; **11**: 255-259 [PMID: 15633226 DOI: 10.3748/wjg.v11.i2.255]

35 **Murawaki Y**, Ikuta Y, Okamoto K, Koda M, Kawasaki H. Diagnostic value of serum markers of connective tissue turnover for predicting histological staging and grading in patients with chronic hepatitis C. *J Gastroenterol* 2001; **36**: 399-406 [PMID: 11428586 DOI: 10.1007/s005350170084]

36 **McHutchison JG**, Blatt LM, de Medina M, Craig JR, Conrad A, Schiff ER, Tong MJ. Measurement of serum hyaluronic acid in patients with chronic hepatitis C and its relationship to liver histology. Consensus Interferon Study Group. *J Gastroenterol Hepatol* 2000; **15**: 945-951 [PMID: 11022838 DOI: 10.1046/j.1440-1746.2000.02233.x]

37 **Bourlière M**, Pénaranda G, Adhoute X, Oules V, Castellani P. Combining non-invasive methods for assessment of liver fibrosis. *Gastroenterol Clin Biol* 2008; **32**: 73-79 [PMID: 18973849 DOI:

10.1016/S0399-8320(08)73996-4]
38 **Halfon P**, Bourlière M, Pénaranda G, Deydier R, Renou C, Botta-Fridlund D, Tran A, Portal I, Allemand I, Rosenthal-Allieri A, Ouzan D. Accuracy of hyaluronic acid level for predicting liver fibrosis stages in patients with hepatitis C virus. *Comp Hepatol* 2005; **4**: 6 [PMID: 16008833 DOI: 10.1186/1476-5926-4-6]
39 **Guéchot J**, Loria A, Serfaty L, Giral P, Giboudeau J, Poupon R. Serum hyaluronan as a marker of liver fibrosis in chronic viral hepatitis C: effect of alpha-interferon therapy. *J Hepatol* 1995; **22**: 22-26 [PMID: 7751583 DOI: 10.1016/0168-8278(95)80255-x]
40 **Montazeri G**, Estakhri A, Mohamadnejad M, Nouri N, Montazeri F, Mohammadkani A, Derakhshan MH, Zamani F, Samiee S, Malekzadeh R. Serum hyaluronate as a non-invasive marker of hepatic fibrosis and inflammation in HBeAg-negative chronic hepatitis B. *BMC Gastroenterol* 2005; **5**: 32 [PMID: 16221307 DOI: 10.1186/1471-230x-5-32]
41 **Walsh KM**, Fletcher A, MacSween RN, Morris AJ. Basement membrane peptides as markers of liver disease in chronic hepatitis C. *J Hepatol* 2000; **32**: 325-330 [PMID: 10707874 DOI: 10.1016/s0168-8278(00)80079-3]
42 **Lucena S**, Arocha Piñango CL, Guerrero B. [Fibronectin. Structure and functions associated to hemostasis. Review]. *Invest Clin* 2007; **48**: 249-262 [PMID: 17598647]
43 **Murawaki Y**, Ikuta Y, Idobe Y, Kawasaki H. Serum matrix metalloproteinase-1 in patients with chronic viral hepatitis. *J Gastroenterol Hepatol* 1999; **14**: 138-145 [PMID: 10029294 DOI: 10.1046/j.1440-1746.1999.01821.x]
44 **Boeker KH**, Haberkorn CI, Michels D, Flemming P, Manns MP, Lichtinghagen R. Diagnostic potential of circulating TIMP-1 and MMP-2 as markers of liver fibrosis in patients with chronic hepatitis C. *Clin Chim Acta* 2002; **316**: 71-81 [PMID: 11750276 DOI: 10.1016/s0009-8981(01)00730-6]
45 **Walsh KM**, Timms P, Campbell S, MacSween RN, Morris AJ. Plasma levels of matrix metalloproteinase-2 (MMP-2) and tissue inhibitors of metalloproteinases -1 and -2 (TIMP-1 and TIMP-2) as noninvasive markers of liver disease in chronic hepatitis C: comparison using ROC analysis. *Dig Dis Sci* 1999; **44**: 624-630 [PMID: 10080160 DOI: 10.1023/a:1026630129025]
46 **Hayasaka A**, Suzuki N, Fujimoto N, Iwama S, Fukuyama E, Kanda Y, Saisho H. Elevated plasma levels of matrix metalloproteinase-9 (92-kd type IV collagenase/gelatinase B) in hepatocellular carcinoma. *Hepatology* 1996; **24**: 1058-1062 [PMID: 8903375 DOI: 10.1053/jhep.1996.v24.pm0008903375]
47 **Badra G**, Lotfy M, El-Refaie A, Obada M, Abdelmonem E, Kandeel S, Fathy A. Significance of serum matrix metalloproteinase-9 and tissue inhibitor of metalloproteinase-1 in chronic hepatitis C patients. *Acta Microbiol Immunol Hung* 2010; **57**: 29-42 [PMID: 20350877 DOI: 10.1556/AMicr.57.2010.1.3]
48 **Iredale JP**, Goddard S, Murphy G, Benyon RC, Arthur MJ. Tissue inhibitor of metalloproteinase-I and interstitial collagenase expression in autoimmune chronic active hepatitis and activated human hepatic lipocytes. *Clin Sci (Lond)* 1995; **89**: 75-81 [PMID: 7671571 DOI: 10.1042/cs0890075]
49 **Kanzler S**, Baumann M, Schirmacher P, Dries V, Bayer E, Gerken G, Dienes HP, Lohse AW. Prediction of progressive liver fibrosis in hepatitis C infection by serum and tissue levels of transforming growth factor-beta. *J Viral Hepat* 2001; **8**: 430-437 [PMID: 11703574 DOI: 10.1046/j.1365-2893.2001.00314.x]
50 **Nelson DR**, Gonzalez-Peralta RP, Qian K, Xu Y, Marousis CG, Davis GL, Lau JY. Transforming growth factor-beta 1 in chronic hepatitis C. *J Viral Hepat* 1997; **4**: 29-35 [PMID: 9031062 DOI: 10.1046/j.1365-2893.1997.00124.x]
51 **Harada K**, Shiota G, Kawasaki H. Transforming growth factor-alpha and epidermal growth factor receptor in chronic liver disease and hepatocellular carcinoma. *Liver* 1999; **19**: 318-325 [PMID: 10459631 DOI: 10.1111/j.1478-3231.1999.tb00056.x]
52 **Zhang BB**, Cai WM, Weng HL, Hu ZR, Lu J, Zheng M, Liu RH. Diagnostic value of platelet derived growth factor-BB, transforming growth factor-beta1, matrix metalloproteinase-1, and tissue inhibitor of matrix metalloproteinase-1 in serum and peripheral blood mononuclear cells for hepatic fibrosis. *World J Gastroenterol* 2003; **9**: 2490-2496 [PMID: 14606082 DOI: 10.3748/wjg.v9.i11.2490]
53 **Haukeland JW**, Schreiner LT, Lorgen I, Frigstad SO, Bang C, Raknerud N, Konopski Z. ASAT/ALAT ratio provides prognostic information independently of Child-Pugh class, gender and age in non-alcoholic cirrhosis. *Scand J Gastroenterol* 2008; **43**: 1241-1248 [PMID: 18609128 DOI: 10.1080/00365520802158614]
54 **Giannini E**, Risso D, Botta F, Chiarbonello B, Fasoli A, Malfatti F, Romagnoli P, Testa E, Ceppa P, Testa R. Validity and clinical utility of the aspartate aminotransferase-alanine aminotransferase ratio in assessing disease severity and prognosis in patients with hepatitis C virus-related chronic liver disease. *Arch Intern Med* 2003; **163**: 218-224 [PMID: 12546613 DOI: 10.1001/archinte.163.2.218]
55 **Sebastiani G**, Vario A, Guido M, Alberti A. Sequential algorithms combining non-invasive markers and biopsy for the assessment of liver fibrosis in chronic hepatitis B. *World J Gastroenterol* 2007; **13**: 525-531 [PMID: 17278217 DOI: 10.3748/wjg.v13.i4.525]
56 **Loaeza-del-Castillo A**, Paz-Pineda F, Oviedo-Cárdenas E, Sánchez-Avila F, Vargas-Vorácková F. AST to platelet ratio index (APRI) for the noninvasive evaluation of liver fibrosis. *Ann Hepatol* 2008; **7**: 350-357 [PMID: 19034235]
57 **Wai CT**, Greenson JK, Fontana RJ, Kalbfleisch JD, Marrero JA, Conjeevaram HS, Lok AS. A simple noninvasive index can predict both significant fibrosis and cirrhosis in patients with chronic hepatitis

C. *Hepatology* 2003; **38**: 518-526 [PMID: 12883497 DOI: 10.1053/jhep.2003.50346]

58 **Harrison SA**, Oliver D, Arnold HL, Gogia S, Neuschwander-Tetri BA. Development and validation of a simple NAFLD clinical scoring system for identifying patients without advanced disease. *Gut* 2008; **57**: 1441-1447 [PMID: 18390575 DOI: 10.1136/gut.2007.146019]

59 **Pradat P**, Alberti A, Poynard T, Esteban JI, Weiland O, Marcellin P, Badalamenti S, Trépo C. Predictive value of ALT levels for histologic findings in chronic hepatitis C: a European collaborative study. *Hepatology* 2002; **36**: 973-977 [PMID: 12297846 DOI: 10.1053/jhep.2002.35530]

60 **Forns X**, Ampurdanes S, Llovet JM, Aponte J, Quintó L, Martínez-Bauer E, Bruguera M, Sánchez-Tapias JM, Rodés J. 2002, Identification of chronic hepatitis patients without hepatic fibrosis by a simple predictive model. *Hepatology* 2002; **36**: 986-992 [DOI: 10.1053/jhep.2002.36128]

61 **Poynard T**, Aubert A, Bedossa P, Abella A, Naveau S, Paraf F, Chaput JC. A simple biological index for detection of alcoholic liver disease in drinkers. *Gastroenterology* 1991; **100**: 1397-1402 [PMID: 1672859]

62 **Naveau S**, Gaudé G, Asnacios A, Agostini H, Abella A, Barri-Ova N, Dauvois B, Prévot S, Ngo Y, Munteanu M, Balian A, Njiké-Nakseu M, Perlemuter G, Poynard T. Diagnostic and prognostic values of noninvasive biomarkers of fibrosis in patients with alcoholic liver disease. *Hepatology* 2009; **49**: 97-105 [PMID: 19053048 DOI: 10.1002/hep.22576]

63 **Vallet-Pichard A**, Mallet V, Nalpas B, Verkarre V, Nalpas A, Dhalluin-Venier V, Fontaine H, Pol S. FIB-4: an inexpensive and accurate marker of fibrosis in HCV infection. comparison with liver biopsy and fibrotest. *Hepatology* 2007; **46**: 32-36 [PMID: 17567829 DOI: 10.1002/hep.21669]

64 **Mallet V**, Dhalluin-Venier V, Roussin C, Bourliere M, Pettinelli ME, Giry C, Vallet-Pichard A, Fontaine H, Pol S. The accuracy of the FIB-4 index for the diagnosis of mild fibrosis in chronic hepatitis B. *Aliment Pharmacol Ther* 2009; **29**: 409-415 [PMID: 19035983 DOI: 10.1111/j.1365-2036.2008.03895.x]

65 **Koda M**, Matunaga Y, Kawakami M, Kishimoto Y, Suou T, Murawaki Y. FibroIndex, a practical index for predicting significant fibrosis in patients with chronic hepatitis C. *Hepatology* 2007; **45**: 297-306 [PMID: 17256741 DOI: 10.1002/hep.21520]

66 **Imbert-Bismut F**, Ratziu V, Pieroni L, Charlotte F, Benhamou Y, Poynard T; MULTIVIRC Group. Biochemical markers of liver fibrosis in patients with hepatitis C virus infection: a prospective study. *Lancet* 2001; **357**: 1069-1075 [PMID: 11297957 DOI: 10.1016/S0140-6736(00)04258-6]

67 **Imbert-Bismut F**, Messous D, Thibault V, Myers RB, Piton A, Thabut D, Devers L, Hainque B, Mercadier A, Poynard T. Intra-laboratory analytical variability of biochemical markers of fibrosis (Fibrotest) and activity (Actitest) and reference ranges in healthy blood donors. *Clin Chem Lab Med* 2004; **42**: 323-333 [PMID: 15080567 DOI: 10.1515/CCLM.2004.058]

68 **Poynard T**, Morra R, Ingiliz P, Imbert-Bismut F, Thabut D, Messous D, Munteanu M, Massard J, Benhamou Y, Ratziu V. Biomarkers of liver fibrosis. *Adv Clin Chem* 2008; **46**: 131-160 [PMID: 19004189 DOI: 10.1016/s0065-2423(08)00404-6]

69 **Ratziu V**, Massard J, Charlotte F, Messous D, Imbert-Bismut F, Bonyhay L, Tahiri M, Munteanu M, Thabut D, Cadranel JF, Le Bail B, de Ledinghen V, Poynard T; LIDO Study Group; CYTOL study group. Diagnostic value of biochemical markers (FibroTest-FibroSURE) for the prediction of liver fibrosis in patients with non-alcoholic fatty liver disease. *BMC Gastroenterol* 2006; **6**: 6 [PMID: 16503961 DOI: 10.1186/1471-230x-6-6]

70 **Poynard T**, Lassailly G, Diaz E, Clement K, Caïazzo R, Tordjman J, Munteanu M, Perazzo H, Demol B, Callafe R, Pattou F, Charlotte F, Bedossa P, Mathurin P, Ratziu V; FLIP consortium. Performance of biomarkers FibroTest, ActiTest, SteatoTest, and NashTest in patients with severe obesity: meta analysis of individual patient data. *PLoS One* 2012; **7**: e30325 [PMID: 22431959 DOI: 10.1371/journal.pone.0030325]

71 **Halfon P**, Imbert-Bismut F, Messous D, Antoniotti G, Benchetrit D, Cart-Lamy P, Delaporte G, Doutheau D, Klump T, Sala M, Thibaud D, Trepo E, Thabut D, Myers RP, Poynard T. A prospective assessment of the inter-laboratory variability of biochemical markers of fibrosis (FibroTest) and activity (ActiTest) in patients with chronic liver disease. *Comp Hepatol* 2002; **1**: 3 [PMID: 12537583 DOI: 10.1186/1476-5926-1-3]

72 **Angulo P**, Hui JM, Marchesini G, Bugianesi E, George J, Farrell GC, Enders F, Saksena S, Burt AD, Bida JP, Lindor K, Sanderson SO, Lenzi M, Adams LA, Kench J, Therneau TM, Day CP. The NAFLD fibrosis score: a noninvasive system that identifies liver fibrosis in patients with NAFLD. *Hepatology* 2007; **45**: 846-854 [PMID: 17393509 DOI: 10.1002/hep.21496]

73 **Poynard T**, Ratziu V, Naveau S, Thabut D, Charlotte F, Messous D, Capron D, Abella A, Massard J, Ngo Y, Munteanu M, Mercadier A, Manns M, Albrecht J. The diagnostic value of biomarkers (SteatoTest) for the prediction of liver steatosis. *Comp Hepatol* 2005; **4**: 10 [PMID: 16375767 DOI: 10.1186/1476-5926-4-10]

74 **Brochado-Kith Ó**, Gómez Sanz A, Real LM, Crespo García J, Ryan Murúa P, Macías J, Cabezas González J, Troya J, Pineda JA, Arias Loste MT, Díez Viñas V, Jiménez-Sousa MÁ, Medrano de Dios LM, Cuesta De la Plaza I, Monzón Fernández S, Resino García S, Fernández-Rodríguez A. MicroRNA Profile of HCV Spontaneous Clarified Individuals, Denotes Previous HCV Infection. *J Clin Med* 2019; **8** [PMID: 31207946 DOI: 10.3390/jcm8060849]

75 **Liu J**, Luo Q, Zhang X, Zhang Q, Cheng Y. Identification of vital candidate microRNA/mRNA pairs regulating ovule development using high-throughput sequencing in hazel. *BMC Dev Biol* 2020; **20**: 13 [PMID: 32605594 DOI: 10.1186/s12861-020-00219-z]

76 **Liu RH**, Ning B, Ma XE, Gong WM, Jia TH. Regulatory roles of microRNA-21 in fibrosis through

interaction with diverse pathways (Review). *Mol Med Rep* 2016; **13**: 2359-2366 [PMID: 26846276 DOI: 10.3892/mmr.2016.4834]

77 **Demerdash HM**, Hussien HM, Hassouna E, Arida EA. Detection of MicroRNA in Hepatic Cirrhosis and Hepatocellular Carcinoma in Hepatitis C Genotype-4 in Egyptian Patients. *Biomed Res Int* 2017; **2017**: 1806069 [PMID: 28642869 DOI: 10.1155/2017/1806069]

78 **Zhang T**, Yang Z, Kusumanchi P, Han S, Liangpunsakul S. Critical Role of microRNA-21 in the Pathogenesis of Liver Diseases. *Front Med (Lausanne)* 2020; **7**: 7 [PMID: 32083086 DOI: 10.3389/fmed.2020.00007]

79 **Ekambaram P**, Lee JL, Hubel NE, Hu D, Yerneni S, Campbell PG, Pollock N, Klei LR, Concel VJ, Delekta PC, Chinnaiyan AM, Tomlins SA, Rhodes DR, Priedigkeit N, Lee AV, Oesterreich S, McAllister-Lucas LM, Lucas PC. The CARMA3-Bcl10-MALT1 Signalosome Drives NFκB Activation and Promotes Aggressiveness in Angiotensin II Receptor-Positive Breast Cancer. *Cancer Res* 2018; **78**: 1225-1240 [PMID: 29259013 DOI: 10.1158/0008-5472.CAN-17-1089]

80 **Sarma NJ**, Tiriveedhi V, Subramanian V, Shenoy S, Crippin JS, Chapman WC, Mohanakumar T. Hepatitis C virus mediated changes in miRNA-449a modulates inflammatory biomarker YKL40 through components of the NOTCH signaling pathway. *PLoS One* 2012; **7**: e50826 [PMID: 23226395 DOI: 10.1371/journal.pone.0050826]

81 **Zhang Y**, Wei W, Cheng N, Wang K, Li B, Jiang X, Sun S. Hepatitis C virus-induced up-regulation of microRNA-155 promotes hepatocarcinogenesis by activating Wnt signaling. *Hepatology* 2012; **56**: 1631-1640 [PMID: 22610915 DOI: 10.1002/hep.25849]

82 **Oksuz Z**, Serin MS, Kaplan E, Dogen A, Tezcan S, Aslan G, Emekdas G, Sezgin O, Altintas E, Tiftik EN. Serum microRNAs; miR-30c-5p, miR-223-3p, miR-302c-3p and miR-17-5p could be used as novel non-invasive biomarkers for HCV-positive cirrhosis and hepatocellular carcinoma. *Mol Biol Rep* 2015; **42**: 713-720 [PMID: 25391771 DOI: 10.1007/s11033-014-3819-9]

83 **Sporea I**, Bota S, Peck-Radosavljevic M, Sirli R, Tanaka H, Iijima H, Badea R, Lupsor M, Fierbinteanu-Braticevici C, Petrisor A, Saito H, Ebinuma H, Friedrich-Rust M, Sarrazin C, Takahashi H, Ono N, Piscaglia F, Borghi A, D'Onofrio M, Gallotti A, Ferlitsch A, Popescu A, Danila M. Acoustic Radiation Force Impulse elastography for fibrosis evaluation in patients with chronic hepatitis C: an international multicenter study. *Eur J Radiol* 2012; **81**: 4112-4118 [PMID: 23000186 DOI: 10.1016/j.ejrad.2012.08.018]

84 **Takemoto R**, Nakamuta M, Aoyagi Y, Fujino T, Yasutake K, Koga K, Yoshimoto T, Miyahara T, Fukuizumi K, Wada Y, Takami Y, Saitsu H, Harada N, Nakashima M, Enjoji M. Validity of FibroScan values for predicting hepatic fibrosis stage in patients with chronic HCV infection. *J Dig Dis* 2009; **10**: 145-148 [PMID: 19426398 DOI: 10.1111/j.1751-2980.2009.00377.x]

85 **Saadany ES**, Soliman H, Ziada HD, Hamisa M, Hefeda M, Selim A, Goraba H. Fibroscan *vs* liver biopsy in the evaluation of response among the Egyptian HCV infected patients to treatment. *Egypt J Radiol Nucl Med* 2016; **47**: 1-7 [DOI: 10.1016/j.ejrnm.2015.12.007]

86 **Attallah AM**, Abdallah SO, Attallah AA, Omran MM, Farid K, Nasif WA, Shiha GE, Abdel-Aziz AA, Rasafy N, Shaker YM. Diagnostic value of fibronectin discriminant score for predicting liver fibrosis stages in chronic hepatitis C virus patients. *Ann Hepatol* 2013; **12**: 44-53 [PMID: 23293193]

87 **McPherson S**, Stewart SF, Henderson E, Burt AD, Day CP. Simple non-invasive fibrosis scoring systems can reliably exclude advanced fibrosis in patients with non-alcoholic fatty liver disease. *Gut* 2010; **59**: 1265-1269 [PMID: 20801772 DOI: 10.1136/gut.2010.216077]

88 **Park GJ**, Lin BP, Ngu MC, Jones DB, Katelaris PH. Aspartate aminotransferase: alanine aminotransferase ratio in chronic hepatitis C infection: is it a useful predictor of cirrhosis? *J Gastroenterol Hepatol* 2000; **15**: 386-390 [PMID: 10824882 DOI: 10.1046/j.1440-1746.2000.02172.x]

89 **Sun W**, Cui H, Li N, Wei Y, Lai S, Yang Y, Yin X, Chen DF. Comparison of FIB-4 index, NAFLD fibrosis score and BARD score for prediction of advanced fibrosis in adult patients with non-alcoholic fatty liver disease: A meta-analysis study. *Hepatol Res* 2016; **46**: 862-870 [PMID: 26763834 DOI: 10.1111/hepr.12647]

90 **Nguyen-Khac E**, Chatelain D, Tramier B, Decrombecque C, Robert B, Joly JP, Brevet M, Grignon P, Lion S, Le Page L, Dupas JL. Assessment of asymptomatic liver fibrosis in alcoholic patients using fibroscan: prospective comparison with seven non-invasive laboratory tests. *Aliment Pharmacol Ther* 2008; **28**: 1188-1198 [PMID: 18705692 DOI: 10.1111/j.1365-2036.2008.03831.x]

91 **Salkic NN**, Jovanovic P, Hauser G, Brcic M. FibroTest/Fibrosure for significant liver fibrosis and cirrhosis in chronic hepatitis B: a meta-analysis. *Am J Gastroenterol* 2014; **109**: 796-809 [PMID: 24535095 DOI: 10.1038/ajg.2014.21]

92 **Adams LA**, Bulsara M, Rossi E, DeBoer B, Speers D, George J, Kench J, Farrell G, McCaughan GW, Jeffrey GP. Hepascore: an accurate validated predictor of liver fibrosis in chronic hepatitis C infection. *Clin Chem* 2005; **51**: 1867-1873 [PMID: 16055434 DOI: 10.1373/clinchem.2005.048389]

93 **Kelleher TB**, Mehta SH, Bhaskar R, Sulkowski M, Astemborski J, Thomas DL, Moore RE, Afdhal NH. Prediction of hepatic fibrosis in HIV/HCV co-infected patients using serum fibrosis markers: the SHASTA index. *J Hepatol* 2005; **43**: 78-84 [PMID: 15894397 DOI: 10.1016/j.jhep.2005.02.025]

94 **Patel K**, Gordon SC, Jacobson I, Hézode C, Oh E, Smith KM, Pawlotsky JM, McHutchison JG. Evaluation of a panel of non-invasive serum markers to differentiate mild from moderate-to-advanced liver fibrosis in chronic hepatitis C patients. *J Hepatol* 2004; **41**: 935-942 [PMID: 15582126 DOI: 10.1016/j.jhep.2004.08.008]

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MINIREVIEWS

Imaging evaluation of the liver in oncology patients: A comparison of techniques

Patrícia S Freitas, Catarina Janicas, José Veiga, António P Matos, Vasco Herédia, Miguel Ramalho

ORCID number: Patrícia S Freitas 0000-0003-4448-1371; Catarina Janicas 0000-0002-3989-0931; José Veiga 0000-0001-9342-6526; António P Matos 0000-0001-9191-3742; Vasco Herédia 0000-0001-9803-1750; Miguel Ramalho 0000-0003-2522-1670.

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Patrícia S Freitas, José Veiga, Department of Radiology, Centro Hospitalar Universitário de Lisboa Central, Lisbon 1150-199, Portugal

Catarina Janicas, Department of Radiology, Centro Hospitalar de Lisboa Ocidental, Lisbon 1449-005, Portugal

António P Matos, Vasco Herédia, Miguel Ramalho, Department of Radiology, Hospital Garcia de Orta, EPE, Almada 2805-267, Portugal

António P Matos, Department of Radiology, Hospital CUF Tejo, Lisbon 1350-352, Portugal

Vasco Herédia, Department of Radiology, Hospital Espírito Santo de Évora-EPE, Évora 7000-811, Portugal

Miguel Ramalho, Department of Radiology, Hospital da Luz, Lisbon 1500-650, Portugal

Corresponding author: Miguel Ramalho, MD, Assistant Professor, Department of Radiology, Hospital Garcia de Orta, EPE, Av. Torrado da Silva, Almada 2805-267, Portugal. miguel-ramalho@netcabo.pt

Abstract

The liver is commonly affected by metastatic disease. Therefore, it is essential to detect and characterize liver metastases, assuming that patient management and prognosis rely on it. The imaging techniques that allow non-invasive assessment of liver metastases include ultrasonography, computed tomography (CT), magnetic resonance imaging (MRI), positron emission tomography (PET)/CT, and PET/MRI. In this paper, we review the imaging findings of liver metastases, focusing on each imaging modality's advantages and potential limitations. We also assess the importance of different imaging modalities for the management, follow-up, and therapy response of liver metastases. To date, both CT and MRI are the most appropriate imaging methods for initial lesion detection, follow-up, and assessment of treatment response. Multiparametric MRI is frequently used as a problem-solving technique for liver lesions and has evolved substantially over the past decade, including hardware and software developments and specific intravenous contrast agents. Several studies have shown that MRI performs better in small-sized metastases and moderate to severe liver steatosis cases. Although state-of-the-art MRI shows a greater sensitivity for detecting and characterizing liver metastases, CT remains the chosen method. We also present the controversial subject of the "economic implication" to use CT over MRI.

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Core Tip: Several imaging methods are clinically available to evaluate and characterize liver metastases. Both computed tomography and magnetic resonance imaging (MRI) are currently the techniques that show the highest diagnostic performance and are also the most suitable for assessing therapy response and follow-up. Several studies have shown that MRI has a higher sensitivity for detecting and characterizing liver metastases; therefore, it may be the ideal imaging method for treatment planning before and after neoadjuvant chemotherapy. The traditional paradigm for ordering imaging studies emphasizes diagnostic accuracy, which is why we believe that MRI should be favored when available, the first-line imaging for detecting liver metastases, and pre- and post-treatment follow-up.

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INTRODUCTION

The liver is one of the most common organs involved with metastatic disease. Secondary lesions are about 18-40 × more common than primary liver tumors[1,2]. Liver metastases are most often secondary to colorectal carcinoma (CRC) (40%), stomach (20%), pancreas (20%), lung (10%), and breast cancer (10%)[3]. Other less frequent primary tumors include neuroendocrine tumors (NETs), gastrointestinal stromal tumors (GISTs), and renal cell carcinomas[3].

The spectrum of presentation is broad. Liver metastases frequently present as multifocal and separate lesions; however, they can also be solitary or less frequently manifest as confluent masses[4]. The solitary mass form of presentation is most often associated with colon cancer. Meanwhile, breast cancer metastases may infrequently diffusely involve the liver in a pseudocirrhosis pattern (mimicking cirrhosis), particularly following chemotherapy[3].

Solid liver metastases are typically supplied by arterial blood flow; hence they can be classified as hypovascular or hypervascular[1]. The main group of hypovascular metastases includes CRC, gastric, breast, and lung cancer[5]. On the other hand, hypervascular liver metastases are more commonly seen in renal cell carcinoma (especially clear-cell type), NETs, melanoma, thyroid carcinoma, and GISTs. Breast cancer liver metastases may appear hypovascular and hypervascular. Additionally, liver metastases may be cystic, arising from cystic primaries, such as ovarian carcinoma or mucinous cystadenocarcinoma of the GI and pancreas. These may also arise from GIST, leiomyosarcoma, malignant melanoma, carcinoid, and pheochromocytoma[1]. Calcification may be present in mucinous adenocarcinomas from the gastrointestinal tract or the ovary and in breast, lung, renal, and medullary thyroid carcinoma[6,7].

In the current perspective of oncologic liver surgery or local ablation, imaging shows a vital role in the detection, characterization, and determination of metastases' exact location, on a per-patient and per-lesion basis, even in patients with stage IV disease. Surgery and a variety of interventional radiologic techniques are also performed in selected patients with oligometastatic disease.

Stage IV CRC is defined as distant metastasis that either is confined to one organ or site (stage IVa) or affects more than one organ or site or the peritoneum (stage IVb). The past decade has seen a paradigm shift in stage IV or metastatic CRC (mCRC) management, leading to a significant increase in overall survival for these patients, from less than 6 mo to nearly 2 years[6]. Much of this success is credited to the increased utilization of hepatectomy in patients with oligometastatic liver disease, the

development of newer chemotherapy regimens, and the identification of new molecular targets and their inhibitors. Imaging plays an essential role in the workup of patients with mCRC by helping enumerate the number and sites of metastases, determine resectability, assess response to systemic and liver-directed therapies, and detect drug toxicities and disease recurrences.

This paper aims to briefly review each imaging technique and subsequently evaluate them in assessing liver metastases, including detection, characterization, diagnosis, and treatment response evaluation.

IMAGING TECHNIQUES

Ultrasonography

Ultrasonography (US) is a safe, accessible, and inexpensive technique. Nevertheless, it has considerable limitations, including dependency on operator expertise, patient's body habitus, cooperation, and bowel gas interposition[8]. The lower performance of this technique is also explained by limited spatial resolution, and for this reason, small (< 3-5 mm), isoechoic, and deep-seated metastases can be missed[1,8]. The conventional US's general sensitivity for detecting liver metastases is approximately 69% (sensitivity of 50%-76% in series with a true gold standard – intraoperative US or resection)[1,9]. This sensitivity is probably lower in patients with subdiaphragmatic lesions, chronic hepatic disease, and severe hepatic steatosis, which may be induced by chemotherapy. Moreover, the ambiguity in segmental localization leads to a lack of reproducibility compared to computed tomography (CT) and magnetic resonance imaging (MRI).

The appearance of metastases on ultrasound is diverse, but most appear rounded with sharp or smooth margins. They show variable echogenicity (hypo-, iso, or hyperechoic relative to the surrounding parenchyma), with the hypoechoic pattern being the most common (65%)[7]. Sometimes a hypoechoic halo is noted (40%), especially if the lesion is iso- or hyperechoic (Figure 1)[7]. Hepatic metastases of CRC are typically well-defined, solid, hypoechoic lesions and hypovascular on Doppler ultrasound, and occasionally present a peripheral halo ("target" or "bulls-eye" appearance)[8,9]. This broad spectrum of appearance makes the distinction between benign and malignant lesions difficult, reducing its specificity[8].

Contrast-enhanced ultrasound (CEUS) has improved the sensitivity for the detection of liver metastases. A study by Kong *et al*[10], including 240 patients with liver metastases, showed that diffuse homogeneous hyperenhancement followed by rapid washout was the most common pattern on CEUS (55.4% and 96.2%, respectively).

Regarding CEUS, reports differ, mainly because they depend more on operator expertise and other technical factors. Bernatik *et al*[11] found that CEUS detected 97% of the lesions diagnosed by CT[8,11]. Piscaglia *et al*[12] examined 109 patients with colorectal and gastric cancer. They showed that CEUS improves sensitivity in the detection of liver metastases to 95.4% when compared to conventional US (76.9%) and CT (90.8%)[12]. Cantisani *et al*[8,13] showed that CEUS improved US sensitivity from 67.4%-71.6% to 93.4%-95.8%. On the other hand, Vialle *et al*[14] reported that the CEUS sensitivity was inferior to CT in detecting hepatic metastases from colorectal cancer (CEUS 64.5% *vs* CT 80.4%). Moreover, since metastatic liver disease frequently shows multiple lesions, the per-lesion evaluation would need multiple doses of ultrasound contrast agent[7].

The accuracy for the detection of hepatic lesions may differ with the US mode. Two-dimensional (2D) CEUS shows limitations in evaluating liver metastases since it is more prone to sampling errors, such as imaging caption of a single section and plane-to-plane perfusion variation. On the other hand, three-dimensional (3D) CEUS imaging techniques can image the tumor as a whole, provide spatial information, and allow volumetric images. El Kaffas *et al*[15] showed that 3D dynamic CEUS is superior to 2D dynamic CEUS imaging by reducing the sampling errors from heterogeneous tumor perfusion. Other studies have shown no significant differences between the two modes concerning sensitivity[16]. Nevertheless, the perception of the feeding arteries is improved with the 3D CEUS, which might be helpful for the treatment of hypervascular liver metastases[16].

Computed tomography

Cross-section imaging techniques, including CT, and positron emission tomography (PET)/CT, have advanced considerably, leading to early and accurate liver metastasis

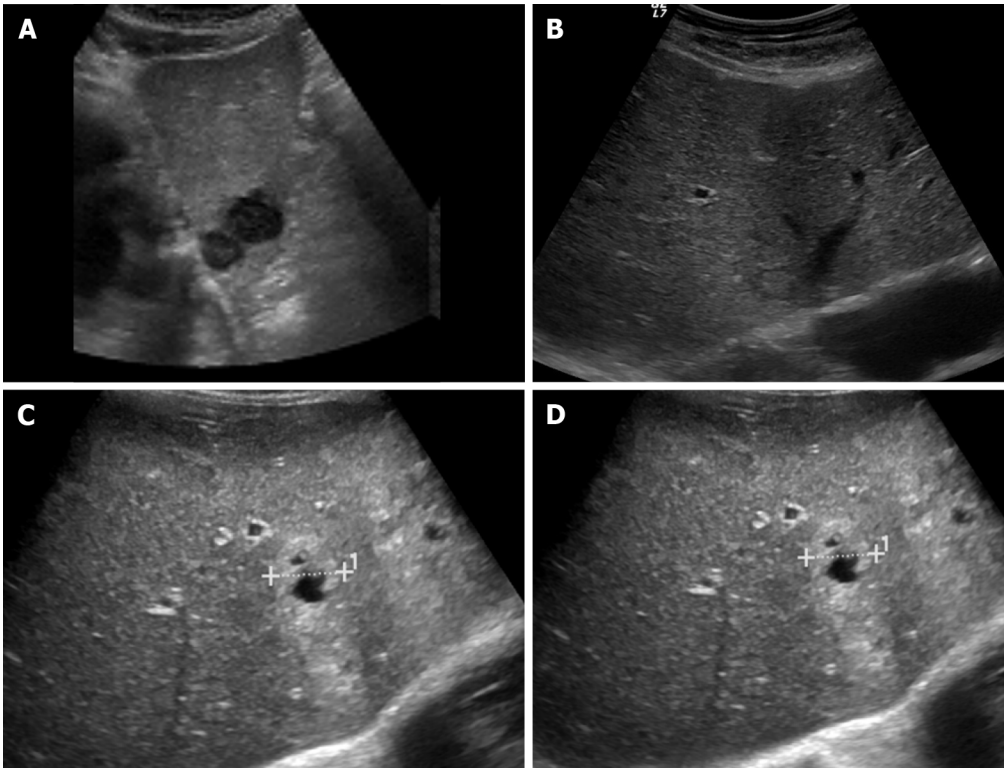

Figure 1 Ultrasound images showing variable echogenicity of liver metastases. A: Two hypoechoic lesions in the left liver lobe consistent with metastases in a patient with lung cancer; B: Isoechoic liver metastasis from lung cancer demonstrating a hypoechoic halo; C: Occult primary tumor with hepatic metastases, predominantly solid and hyperechoic; D: Occult primary tumor with hepatic metastases, showing central necrosis.

detection[17]. Multidetector CT is a reliable technique for detecting liver metastases and preoperative staging, allowing volumetric acquisition with high-quality multiplanar reformatted images, liver volume calculation, and 3D reconstructions preoperative tumor resection planning[3]. CT is fast and accessible, allows high-quality liver imaging and entire abdomen and chest coverage, and depicts extrahepatic disease[18]. CT shows a specificity of 77.3% and sensitivity up to 73.5% for the detection of liver metastases[19].

Liver metastases usually appear as hypo or iso-dense nodules on unenhanced CT. These nodules tend to be well-defined, but they can also be irregular, depending on size[6]. Necrosis and cystic transformation may be present, appearing as a central area of low attenuation. Besides, at times liver metastases may also show high attenuation due to hemorrhagic content[3].

Dynamic imaging is crucial, and its concept, perception, and evaluation are similar between CT and MRI (Figure 2). Most liver metastases are hypovascular and are best detected during the portal venous phase (PVP), which begins approximately 60-80 s after the initial injection. In this phase, the liver parenchyma enhances through the dominant blood supply by the portal vein. Hypovascular metastases appear as hypodense/hypoattenuating lesions compared to the background liver parenchyma (Figure 3)[1]. They usually show a peripheral rim enhancement in the late arterial phase (LAP), which fades centrally in the venous phase ("target appearance")[5,6]. On the other hand, hypervascular metastases enhance earlier in the LAP, which is demonstrated by contrast in the portal vein and absence in the hepatic veins. These lesions may fade and become isodense with the remaining liver parenchyma or show variable degrees of washout in the PVP and delayed acquisitions[5,6,20].

The PVP is considered the most critical phase, with a sensitivity of 91.5% for detecting hypovascular metastases[21]. However, the optimal number and choice of acquisition phases are still under debate, given the potential risks of higher radiation doses[1]. Honda *et al*[22] showed that adding a LAP improved liver metastases' detectability, particularly in lesions smaller than 10 mm. However, other studies, such as that from Ferlay *et al*[23], concluded that for evaluating CRC liver metastases, the addition of the LAP and delayed phases did not improve the performance compared to the PVP alone.

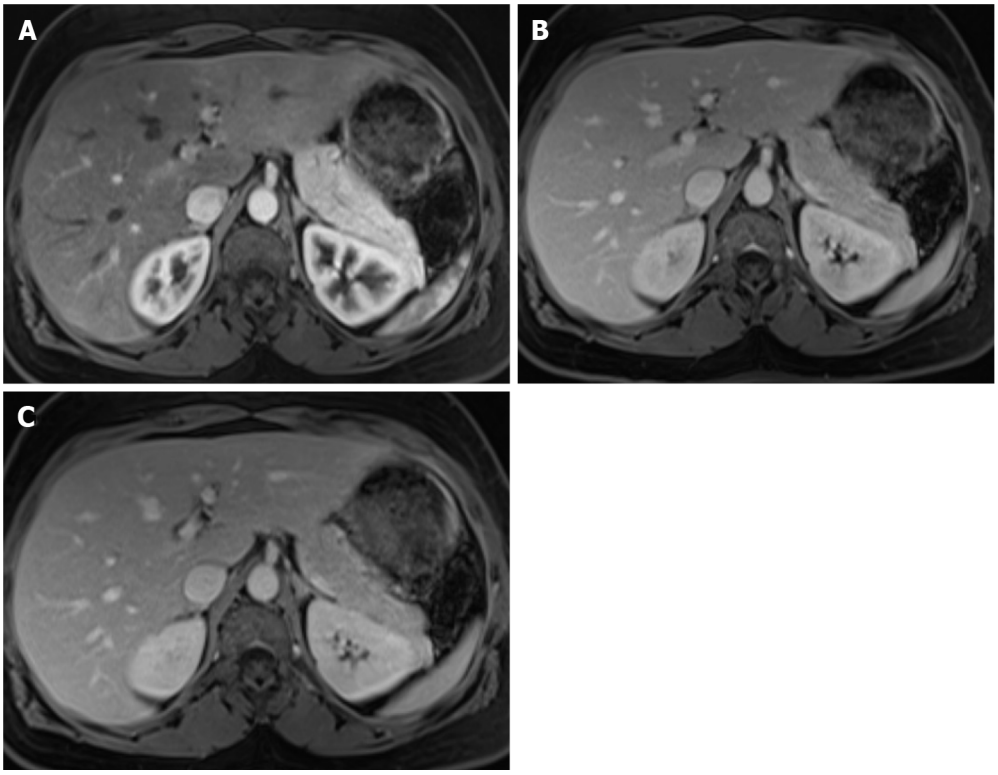

Figure 2 Dynamic phases of enhancement. A: Late hepatic arterial phase. It is characterized by contrast in hepatic arteries and portal veins, not in hepatic veins. It is helpful for hypervascular lesions and perfusional abnormalities. Note that the normal pancreas enhances greater than the liver; B: Portal venous phase. It is recognized by the contrast in the hepatic and portal veins. It is useful mainly for hypovascular lesion detection; C: Interstitial or delayed phase. It is helpful for lesion characterization, especially for late enhancement perception.

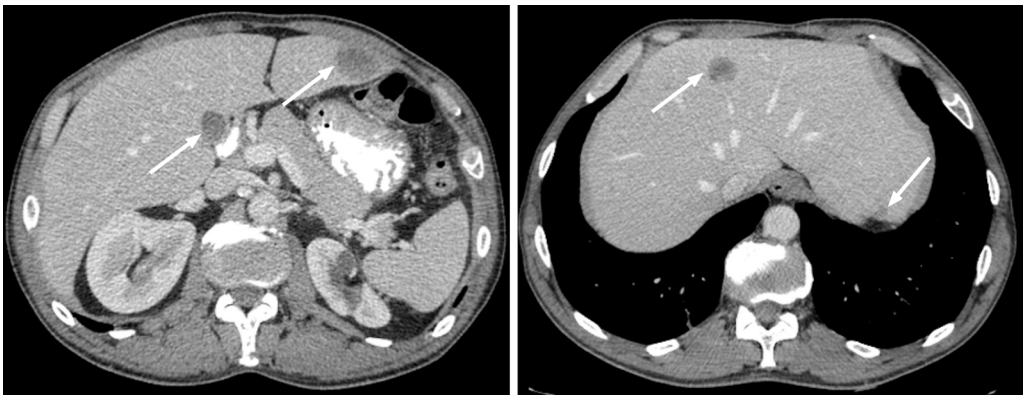

Figure 3 Metastatic lesions from lung cancer. Axial contrast-enhanced computed tomography in the portal-venous phase shows multiple hypodense and hypovascular lesions (arrows) consistent with metastatic lesions from lung cancer.

For hypervascular metastases, non-contrast-enhanced CT (NE-CT) only adds a small incremental value to contrast-enhanced CT (CE-CT) for their detection and characterization based on existing evidence. It seems that it is not worth adding further radiation exposure and the increased number of images for interpretation associated with NE-CT acquisition[24]. Still, NE-CT may be helpful as calcifications are present in up to 11% of liver metastases at initial presentation[25,26].

CT is the workhorse for abdominal imaging staging; however, liver metastases may be missed. The detection rate of lesions by CT declines as its diameter decreases, with a detection rate estimated at 72% for lesions measuring 10-20 mm and 16% for lesions smaller than 10 mm[19]. Benoist *et al*[27] showed that the rate of missed liver lesions after chemotherapy could be as high as 83%.

A recent study demonstrated that some liver metastases without sufficient contrast enhancement were more likely to be overlooked, as were subcapsular lesions, in case of liver steatosis or in cases of examination indication other than assessing malignant

tumors[17].

It has been shown that imaging during the exact correct vascular phase of contrast and an adequate iodine concentration (300-400 mg/mL) is essential for improving the detectability of hypoattenuating metastases[28]. However, it is known that higher contrast concentration may harm renal impaired patients and may also lead to contrast-induced nephropathy. As most patients will frequently need repeated examinations and extended follow-up periods, radiation exposure should also be kept in consideration, representing one of the most critical limitations of CT.

Dual-energy CT (DE-CT) scanners are getting progressively more available. It involves the acquisition of two or more CT measurements with distinct energy spectra. Using the differential attenuation of tissues and materials at different X-ray energies, DE-CT allows the distinction of tissues and materials beyond what is possible with conventional CT[29].

A study comparing DE-CT-driven low-keV virtual monoenergetic imaging to standard linearly blended images concluded that low-keV images improved quantitative size measurements and diagnostic accuracy of CRC liver metastases[30]. Also, this new technique improves the CT accuracy in differentiating liver abscesses from liver metastases in the context of hypovascular metastases, a common clinical dilemma. This technique may increase hypervascular and hypovascular liver lesions' conspicuity, improving CT performance in detecting metastases, especially in cases of concomitant liver steatosis[31].

Magnetic resonance imaging

Multiparametric MRI is frequently used as a problem-solving technique in the evaluation of liver lesions. MRI has evolved substantially over the past decade, including hardware and software developments and specific intravenous contrast agents[3]. Technological improvements also potentially allow better quality imaging in non-cooperative patients, one of the main challenges in MRI. Therefore, when reviewing this imaging technique's performance, one should be aware of these recent advances in the field of MRI, preferring the recent literature.

MRI allows anatomic and morphologic evaluation, as well as functional imaging. The diagnostic sensitivity in detecting hepatic metastases is approximately 87% and has increased with the introduction of diffusion-weighted imaging (WI) in routine protocols and the development of hepatocyte-specific contrast agents, reaching a sensitivity of 95%[21,26]. This technique significantly improves the diagnostic efficacy and accuracy in the approach of liver metastases. Several studies reported the superiority in detecting liver lesions compared to CT, especially if they are small[32, 33].

Contrary to CT, non-enhanced sequences in MRI are essential for the detection and characterization of liver metastases. Frequently, metastases are hypo- to isointense on T1-WI sequences and mildly hyperintense on T2-WI[1]. However, some liver metastases, such as those derived from NETs and sarcomas, may show moderately high signal on T2-WI. Moreover, cystic and necrotic metastases (such as from ovary tumors, NETs, melanoma, and sarcomas) may show moderately to markedly high T2 signal intensity[3]. Liver metastases may occasionally present intralesional hemorrhage, fat, or glycogen deposition and appear hyperintense on T1-WI. Also, melanoma and mucinous adenocarcinoma metastases may show high signal on T1-WI due to their high melanocytic and mucin content, respectively (Figure 4). Occasionally, they may appear as a target sign on T2-WI sequences, characterized by hyperintense central necrosis delimited by a lesser intense rim of viable tumor. On T1-WI, a hypointense rim surrounding a center of even lower signal intensity is known as the doughnut sign (Figure 5)[1,6].

Diffusion-WI (DWI)-MRI allows the interrogation of lesions' cellularity, taking advantage of water molecules' movement. Tissues with high cellularity (tumor, fibrosis, abscess, and cytotoxic edema) show restricted diffusion[1]. Diffusion may be quantified by the apparent diffusion coefficient (ADC), and low ADC values correspond to restriction. ADC values are reported to vary between 0.94-2.87; however, there may be an overlap between the ADC values for primary malignant hepatocellular lesions, such as hepatocellular carcinoma and benign hepatocellular lesions[34]. In clinical practice, the evaluation of DWI relies on subjective appreciation. DWI may also pose disadvantages due to the inherent low spatial resolution, low signal-to-noise ratio, and predisposition to artifacts, especially for subcapsular/subdiaphragmatic lesions.

Kim *et al*[35] reported a higher sensitivity for DWI when compared to CT (79% *vs* 50%) in the detection of small liver metastases (< 1 cm) (Figure 6). Other studies concluded that DWI is more sensitive than unenhanced T2-WI (88%-91% *vs* 45%-62%),

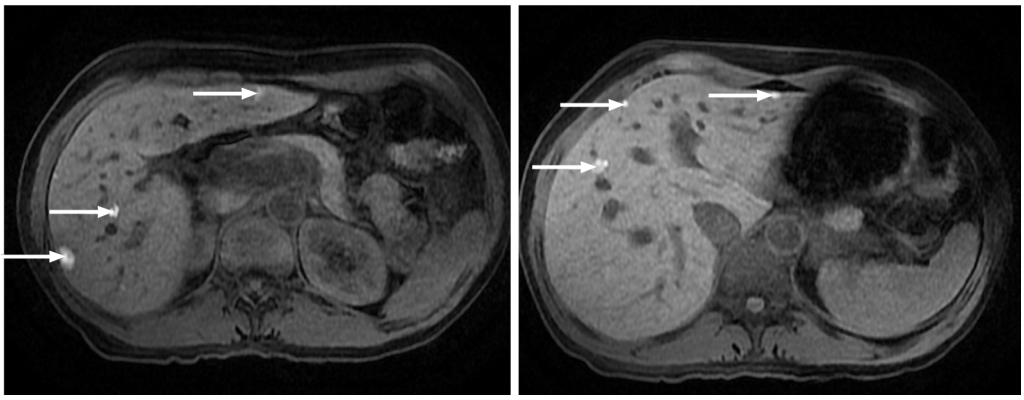

Figure 4 Multiple liver metastases from melanoma. Hepatic metastases showing a characteristic high signal on fat saturated T1-weighted imaging due to their melanocytic content (arrows).

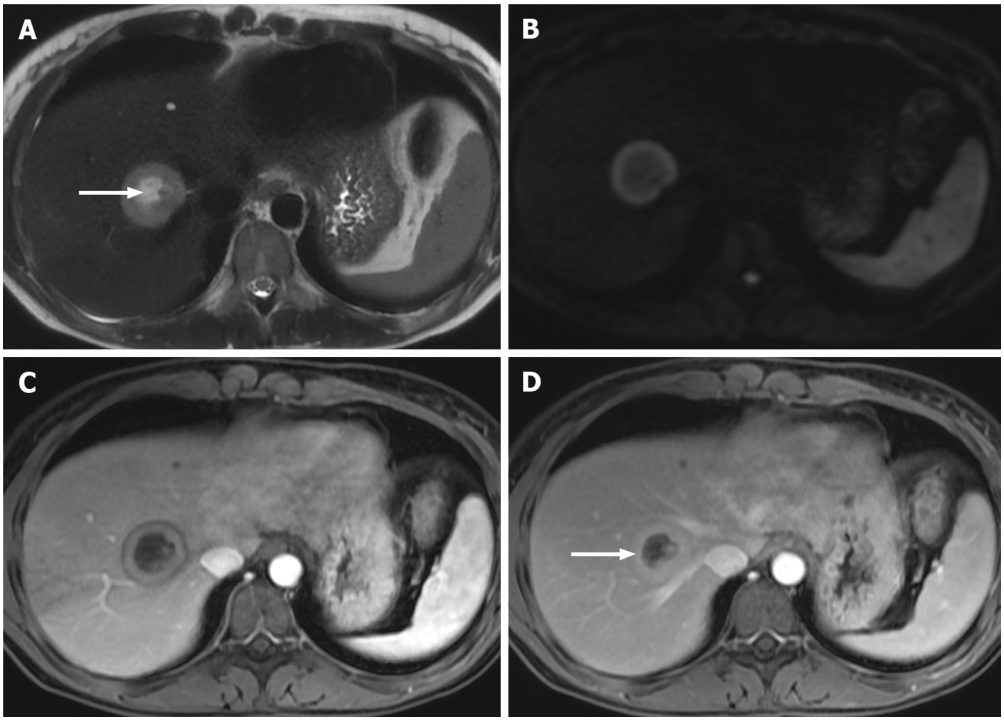

Figure 5 Right lobe liver metastasis from breast cancer. A: Axial T2-weighted imaging (WI) of the metastatic lesion shows a target sign characterized as a hyperintense center (arrow) - necrosis - marginated by a lesser intense rim of viable tumor; B: Diffusion WI shows viable tumor characterized by an increased signal; C: Axial fat sat (FS) contrast-enhanced magnetic resonance imaging (CE-MRI) T1-WI in the arterial phase shows a characteristic doughnut sign; D: Axial FS CE-MRI T1-WI in the interstitial phase reveals a mild progressive enhancement of the peripheral tumor (arrow).

and the difference is even more obvious when only small metastases are considered (85% *vs* 35%)[36,37].

For the characterization of liver metastases, it is crucial to combine pre- and post-contrast sequences as mentioned above. After entering the liver *via* the portal vein and hepatic artery, the extracellular gadolinium-based contrast agent (GBCA) is distributed through the extracellular interstitial space[1]. The desired effect is tissue enhancement on T1-WI, which is achieved by shortening the T1 and T2 relaxation times of adjacent hydrogen protons. The suggested dose for liver imaging is 0.1 mmol/kg, administered through a bolus injection at 2-3 mL/s[38]. Compared to iodine-based contrast agents (used on CT), a greater sensitivity and greater perception of enhancement are observed with GBCAs. GBCAs are considered safe, primarily because they are not nephrotoxic at the recommended doses and show fewer acute reactions than iodinated contrast agents. Although some centers still refrain from using GBCAs in renal impaired patients, one should know that class II contrast agents are rarely associated with nephrogenic systemic fibrosis. A risk-benefit analysis for every individual is required [39,40].

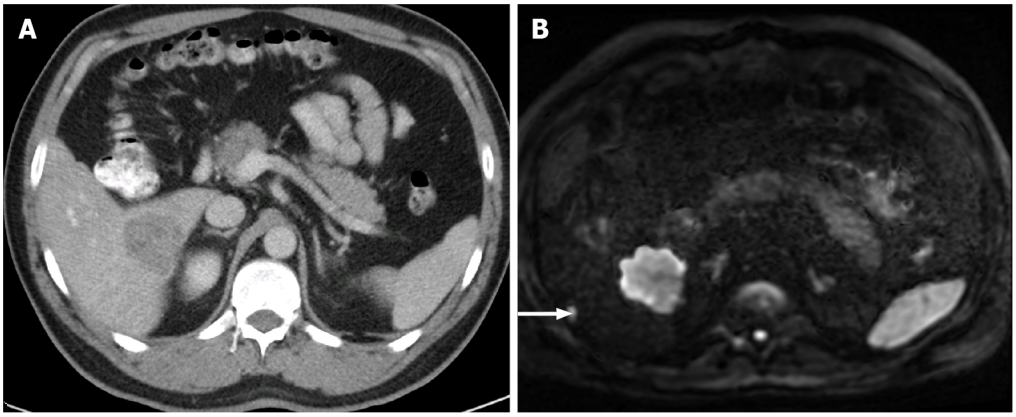

Figure 6 A 85-year-old man with a large hypovascular metastasis in the right lobe from pancreatic carcinoma proposed for tumorectomy.
A: Axial contrast-enhanced computed tomography (CE-CT) in the portal-venous phase shows a large hypodense and hypovascular metastasis; B: The patient underwent magnetic resonance imaging. An additional subcapsular small metastasis was depicted in diffusion-weighted imaging (DWI) (arrow). This example illustrates the higher sensitivity for lesion detection of DWI compared to CT.

As observed with CT, the characteristics of liver metastases vary with the primary tumor. Hypervascular metastases show a hyperintense signal in the LAP, and hypovascular metastases appear hypointense in the PVP (Figure 7). Hypovascular metastases tend to show a thin peripheral rim type enhancement in the LAP and PVP, with progressive central enhancement in interstitial phases (Figure 8)[3]. In the LAP, hypervascular metastases may show homogeneous enhancement (if smaller than 2 cm) or heterogeneous enhancement (if larger than 2 cm), demonstrating variable degrees of washout or in delayed phases (Figure 9). Isovascular metastases may be seen in breast cancer and avascular metastases on cystic metastases, such as ovarian cancer, and may demonstrate septal or wall enhancement (Figure 10). Chemotherapy-treated metastases may appear isovascular or avascular.

After being distributed in the vascular and extra-vascular space during the LAP, PVP, and delayed phases, hepatocyte-specific contrast agents are incorporated by functioning hepatocytes. The available hepatocyte-specific MRI contrast agents are gadobenate dimeglumine (Gd-BOPTA; MultiHance), with a recommended dose of 0.1 mmol/kg, and gadoxetic acid (Gb-EOB-DTPA; Primovist/Eovist), with a recommended dose of 0.025 mmol/kg[38]. The hepatobiliary phase is acquired after 90-150 min for MultiHance and 15-20 min for Primovist. These temporal differences for the hepatocyte phases are related to the degree of biliary excretion, estimated at 3%-5% for MultiHance and 50% for Primovist[1]. The kidneys excrete the remaining.

The normal functioning hepatocytes uptake the hepatocyte-specific MRI contrast agents and excrete them into the biliary system due to cellular membrane transporters. The contrast agent is responsible for shortening the T1 relaxation, which results in higher signal intensity of the healthy liver parenchyma on T1-WI in the hepatobiliary phase[1]. In the later (hepatobiliary) phase, there is also a subsequent excretion into the biliary canaliculi, allowing imaging of the biliary pathways. Therefore, the hepatobiliary phase is easily recognized because the normal liver parenchyma and bile ducts appear enhanced[41]. Non-hepatocellular lesions, as well as lesions with impaired hepatocytes, appear hypointense. In short, as liver metastases lack functioning hepatocytes and biliary ducts, they appear hypointense in the hepatobiliary phase. Allergic reactions are infrequent and comparable with those of extracellular GBCAs.

In a recent meta-analysis, Zhang *et al*[42] showed that the sensitivity of gadobenate (MultiHance) for detecting liver metastases on a per-lesion basis for pre-contrast and combined dynamic, delayed hepatobiliary phase imaging was 77.8%, 88.1%, and 95.1%, respectively. These results are comparable to those reported for gadoxetate (Primovist/Eovist).

Resembling only the MRI's specificities, a meta-analysis published in 2016 showed that the sensitivity of DWI and gadoxetic acid-enhanced MRI (GA-MRI) was 87.1% and 90.6%, respectively. When both sequences were combined, the sensitivity for detecting liver metastases on a per-lesion basis was the highest (95.5%)[43].

Therefore, MRI plays a crucial role in evaluating liver metastases and is considered the ideal imaging method for detection and follow-up in many university hospitals.

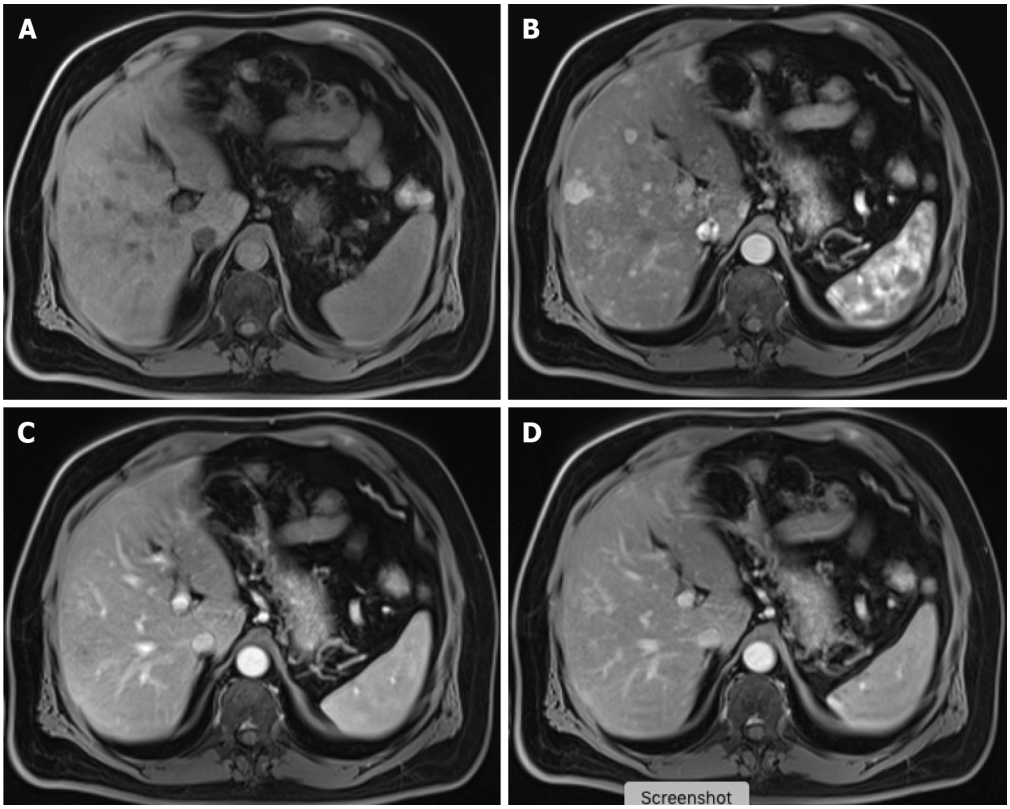

Figure 7 Carcinoid tumor with countless hypervascular liver metastases. A: Axial fat saturated (FS) non-contrast-enhanced magnetic resonance imaging (CE-MRI) T1-weighted imaging (WI) with barely imperceptible hypointense lesions; B: Axial FS CE-MRI T1-WI in the arterial phase detecting multiple hyperenhancing lesions compatible with hypervascular liver metastases; C: Axial FS CE-MRI T1-WI in the portal-venous phase shows fast fading of the lesions previously depicted; D: In the axial FS CE-MRI T1-WI in the delayed phase, the lesions become barely imperceptible. The arterial phase is crucial for the detection of hypervascular metastases.

Positron emission tomography/computed tomography

Liver metastases may have significant fluorine-18-labeled fluorodeoxyglucose (FDG) uptake. Previous investigations mentioned the impact of FDG-PET on the detection of such lesions (Figure 11). A meta-analysis published by Maffione *et al*[44] suggests that FDG-PET/CT is highly accurate in detecting liver metastases on a patient-based analysis, besides showing an added value in identifying extrahepatic disease. However, conventional PET proved to be less sensitive than MRI and CT in detecting CRLM, both on a patient-based (93% *vs* 100% *vs* 98%, respectively) and lesion-based analysis (66% *vs* 89% *vs* 79%, respectively). In addition to the detection of extrahepatic disease, PET/CT has the advantage of assessing treatment response (*i.e.*, chemotherapy) of liver metastases, demonstrated by a decrease in FDG uptake[1]. However, false negatives may arise immediately after completing a chemotherapy cycle due to residual metabolic inhibition. For this reason, PET/CT is not recommended to be performed earlier than 4 wk after finishing chemotherapy, and a negative result must not be fully trusted[45].

Positron emission tomography/magnetic resonance imaging

PET/MRI is a more recent technique that combines the advantages of metabolic imaging (FDG-PET) with MRI sensitivity to assess liver metastases. PET/MRI is a helpful diagnostic technique in detecting small hepatic lesions and may improve the evaluation of treatment response after radiation and chemotherapy. Beiderwellen *et al* [46] demonstrated that PET/MRI has a higher diagnostic accuracy for detecting liver metastases than PET/CT or multidetector CT. However, according to Lake *et al*[47], there is no significant difference in the diagnostic performance between PET/MRI and Gd-EOB-DTPA MRI. Moreover, PET/MRI also shows an incremental value for detecting additional extrahepatic metastases[47].

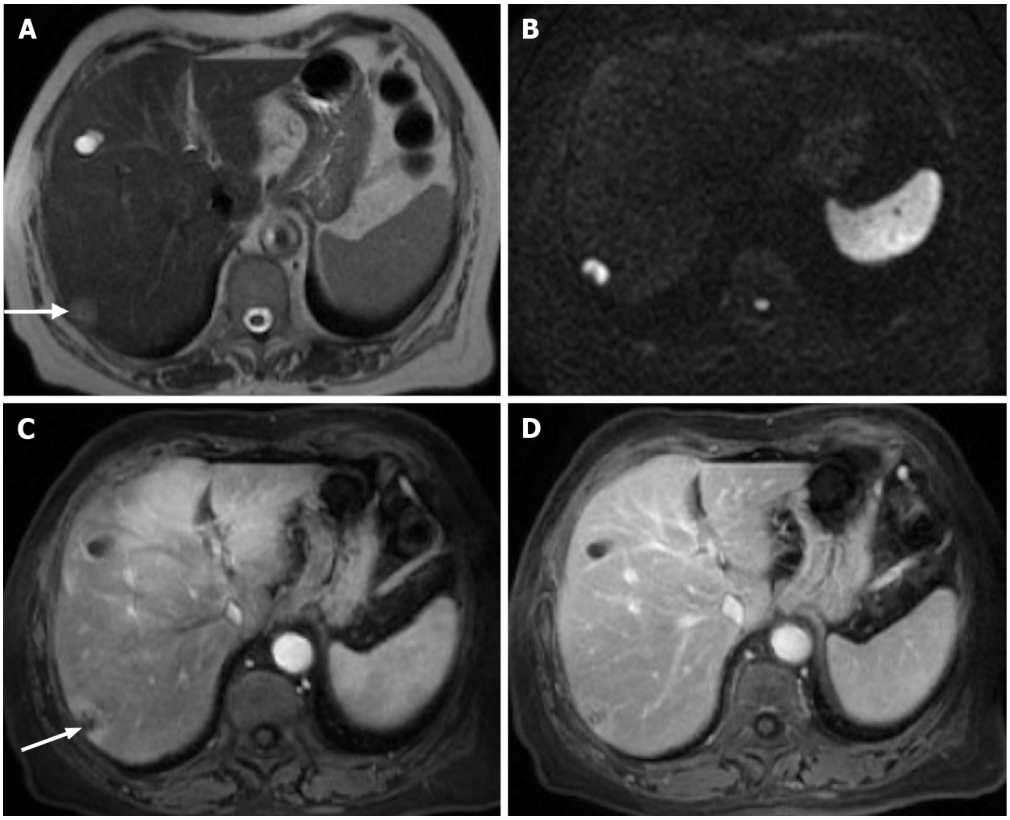

Figure 8 Pancreatic cancer liver metastasis is seen in the subcapsular region of segment VII. A: Axial T2- weighted imaging (WI) shows the pancreatic liver metastasis as a mildly hyperintense lesion (arrow); B: Note the very high signal intensity on high *b* value diffusion-weighted imaging; C: Axial fat saturated (FS) contrast-enhanced magnetic resonance imaging (CE-MRI) T1-WI in the arterial phase. Despite being hypovascular, it is common to find perilesional hyperenhancement in pancreatic cancer subcapsular metastases (arrow); D: Axial FS CE-MRI T1-WI interstitial phase - progressive central enhancement is appreciated in the interstitial phase.

DECIDING BETWEEN TECHNIQUES

It is crucial to detect hepatic metastases as accurately as possible in a per-patient and per-lesion manner to improve patient's clinical evolution, prognosis, and treatment planning. CT, MRI, and FDG-PET are historically the most accurate and precise imaging techniques for this purpose[45]. Below, we refer to various studies comparing these techniques, which will help choose the best option for evaluating liver metastases. Table 1 summarizes the *pros and cons* of cross sectional techniques.

Several studies reported that CE-MRI is more sensitive and specific than CE-CT for detecting liver metastases, mainly due to high intrinsic soft-tissue contrast, technical versatility, sensitivity to blood flow, and contrast enhancement and biochemical information[6]. Vreugdenburg *et al*[32] confirmed in their systematic meta-analysis that in terms of per-lesion diagnostic accuracy, GA-MRI is superior to CE-CT (sensitivity 86.9%-100% *vs* 51.8%-84.6% and specificity 80.2%-98% *vs* 77.2%-98%). This difference is more evident in lesions smaller than 10 mm, in which GA-MRI is notably more sensitive but less specific. Based on the reported sensitivity, an equivocal result will happen more frequently with CE-CT, which leads to a modest impact on patient prognosis and management. In 2017, similar results were reported by Choi *et al*[48], who compared MRI, CT, and PET/CT for the detection of CRC liver metastases, showing a sensitivity of 93.1% *vs* 82.1% *vs* 74.1% and specificity of 87.3%, 73.5%, and 93.9%, respectively (Figure 12). MRI showed a better accuracy than CT in detecting CRC liver metastases and presented an incremental value when added to CT alone to detect additional metastases[48]. In this study, the authors reported that neoadjuvant chemotherapy decreases the sensitivity of both CT and MRI; however, it does not significantly affect the sensitivity of PET/CT[48].

The superiority of MRI is self-evident in small metastases. It is supported by various studies, including that by Schulz *et al*[49], where they reported that the detection of CRLM should rely on MRI. Overall sensitivity/specificity for MRI, CT, and PET was 90%/87%, 68%/94%, and 61%/99%, respectively; and the sensitivity/specificity for lesions smaller than 10 mm for MRI, CT, and PET was 74%/88%, 16%/96% and

Table 1 Deciding between different imaging methods for liver metastasis diagnosis based on articles' analysis

Imaging methods	Critical details
MRI	*Pros:*
	Most accurate method, and superior to CT and PET-CT for the detection of liver metastases:
	Especially useful for smaller lesions (< 1 cm), characterization of hypervascular metastases, and in the setting of liver steatosis
	High grade of confidence in the distinction between malignant and benign lesions
	Anatomic and morphologic evaluation.
	Non-enhancing sequences play an important role
	Therapy response assessment
	Absence of ionizing radiation
	Less allergic reactions
	May be the most cost-effective option:
	Higher detection rate > more curative approach > avoids additional imaging examinations
	Cons:
	Lower availability
	Non-cooperative patients may result in suboptimal study
	Limited for pacemaker carriers
	Limited use if Glomerular filtration rate < 15 mL/min
CT	*Pros*:
	Low cost
	Higher availability
	Higher sensitivity compared to ultrasonography
	Whole-body evaluation
	Therapy response assessment
	Cons:
	Ionizing radiation
	Lower sensitivity for the detection of smaller metastases or in the setting of liver steatosis compared to MRI
	Low confidence in the distinction between malignant and benign lesions
	Not adequate for renal impaired patients
PET-CT	*Pros*:
	Accurate detection of extrahepatic disease
	Therapy response assessment
	Cons:
	False negatives after a chemotherapy cycle
	Lower sensitivity for small liver metastases
	Lower availability
	Highest ionizing radiation dose

MRI: Magnetic resonance imaging; CT: Computed tomography; PET-CT: Positron emission tomography-computed tomography.

9%/98%, respectively[49].

With the introduction of surgical removal of metastatic liver nodules, the overall survival rate has increased. Therefore, it is crucial to ensure the best imaging method to detect them, mainly the smaller ones, which can be easily missed. Ko *et al*[50]

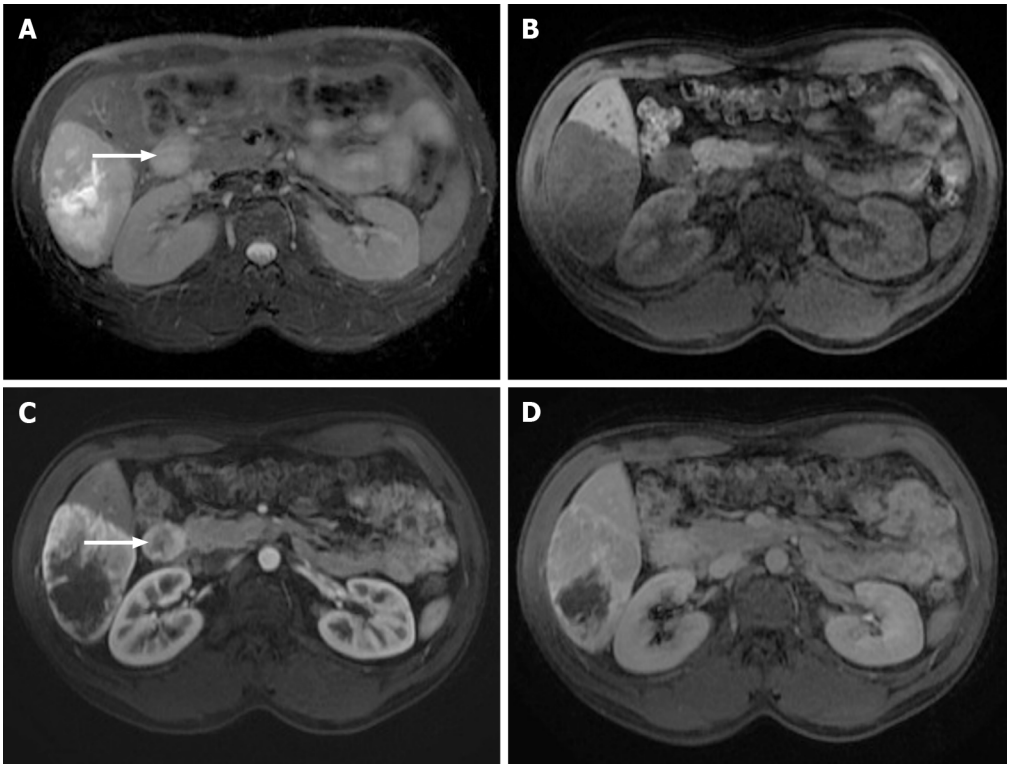

Figure 9 Images show a large liver metastasis from a duodenal neuroendocrine tumor. A: In the axial fat saturated (FS) T2-weighted imaging (WI), the liver metastasis is characterized by hyperintense central necrosis delimited by a lesser intense viable tumor. Note the duodenal neuroendocrine tumor (arrow); B: Axial FS non-contrast-enhanced magnetic resonance imaging (CE-MRI) T1-WI shows large hypointense liver metastasis; C: Axial FS CE-MRI T1-WI in the arterial phase demonstrates viable tumor with avid heterogeneous enhancement. The primary lesion is also hypervascular and depicted in the 2nd portion of the duodenum (arrow); D: Axial FS CE-MRI T1-WI in the interstitial phase reveals fading of the lesion.

showed that the sensitivity of CT was 8%, 55%, 91%, and 100% for nodules of 1-5 mm, 6-10 mm, 11-15 mm, and > 20 mm, respectively. Consequently, it appears obvious that in metastases that are "too small to characterize," CT has a limited role, particularly for those smaller than 5 mm[50]. However, GA-MRI and CE-CT seem equivalent for detecting lesions larger than 10 mm[21,26].

Maegerlein *et al*[51] confirmed that MRI was significantly superior (sensitivity of 87.4%) compared to PET/CT (sensitivity of 68.2%).

For metastases in a fatty liver background, the sensitivity of MRI is approximately 85%-88% (*vs* 65%-68.3% for CE-CT)[18,52]. In these conditions, Kulemann *et al*[18] found that MRI detects 66% of lesions up to 10 mm, while CT detects only 11%. Therefore, they determined that MRI is superior to CT in detecting CRLM in liver steatosis, especially the smaller ones[18,52].

MRI also showed to be significantly better than CE-CT in the detection and characterization of hypervascular liver metastases. For instance, according to Seemann *et al*[53], MRI presented a sensitivity of 98.2%, and CT showed a sensitivity of only 37.1% for detecting carcinoid metastases.

Nowadays, debate continues over whether MRI should be a first-line imaging technique for suspected liver metastases. The current European Society for Medical Oncology (ESMO) guidelines for rectal cancer diagnosis and follow-up (2017) consider that MRI is the imaging method of choice for loco-regional staging. However, CT is preferred for distant metastases[54]. Still, these recommendations are relatively poor (level V), and curiously that manuscript does not make any reference to the use of hepatospecific contrast agents[55]. The American College of Radiology in 2017 also stated that "the available evidence supports that both MRI and CT detect liver lesions with high accuracy."

The updated NCCN guidelines (March 2019) for colon and rectal cancer suggest chest, abdominal, and pelvic CT for metastatic disease's initial workup[21,24,56]. However, if surgical resection of hepatic metastases is considered, contrast-enhanced MRI (extracellular or hepatospecific contrast agent) is preferred over CT to assess their number and distribution[56]. Also, PET-CT may be pondered in selected cases with surgical curable M1 disease[21,26].

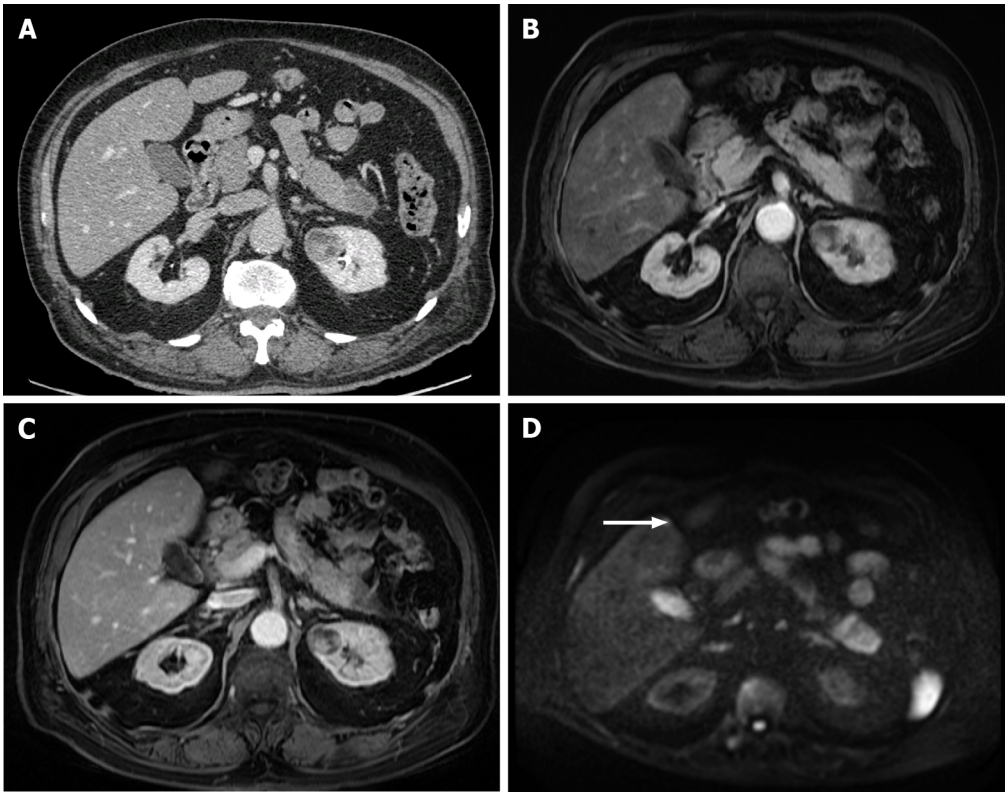

Figure 10 A 40-year-old woman with breast cancer showing a subcapsular millimetric iso-vascular metastasis only depicted in the diffusion-weighted imaging. Contrast-enhanced computed tomography (CE-CT) and dynamic magnetic resonance imaging (MRI) sequences could not detect the lesion. A: Axial CE-CT in the portal-venous phase; B: Axial fat saturated (FS) CE-MRI T1-weighted imaging (WI) in the arterial phase; C: Axial FS CE-MRI T1-WI in the portal-venous phase; D: Diffusion-weighted imaging showing a small lesion with high signal intensity on high *b* value corresponding to liver metastasis (arrow).

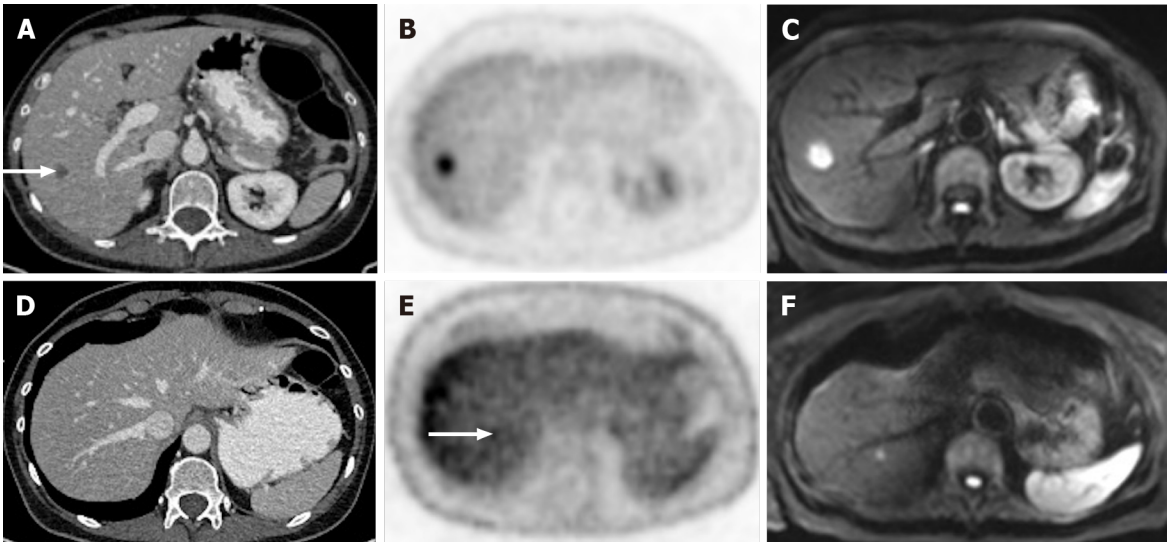

Figure 11 A 65-year-old woman with colorectal carcinoma shows liver metastasis in segment VII. A: Axial contrast-enhanced computed tomography (CE-CT) reveals a hypodense lesion corresponding to liver metastasis (arrow); B: Fluorodeoxyglucose (FDG) positron emission tomography (PET)-CT confirms metastatic origin; D: Axial CE-CT shows no apparent lesion; E: FDG PET-CT shows an additional barely visible nodule not seen in CT (arrow); C and F: Diffusion-weighted imaging confirmed that both lesions were secondary.

Many clinicians use the "economic implication" to use CT instead of MRI[55]. Patients often are referred to CT rather than MRI because of the perceived impression that money is being saved in the healthcare system. Zech *et al*[57] compared the three imaging techniques (GA-MRI, CE-MRI, and CE-CT), considering the diagnostic workup and surgery costs for patients with CRLM. The countries analyzed included Austria, Germany, Italy, Sweden, Switzerland, and Thailand and all of them showed

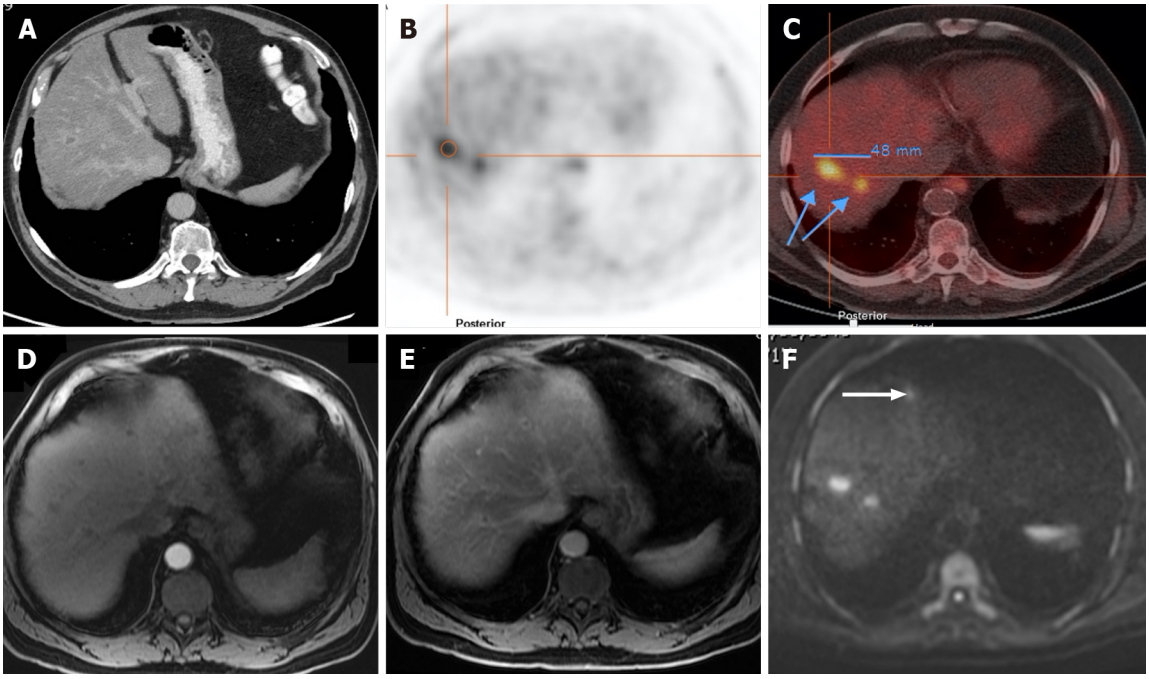

Figure 12 A 71-year-old man with colorectal carcinoma presenting with liver metastases. A: Axial contrast-enhanced computed tomography (CE-CT) in the portal-venous phase shows barely identified non-specific liver micronodules; B and C: Fluorodeoxyglucose positron emission tomography (PET)-CT shows two hypermetabolic lesions in the right lobe, consistent with viable neoplastic tissue; D: Axial fat saturated (FS) CE- magnetic resonance imaging (MRI) T1-weighted imaging (WI) in the arterial phase shows hypovascular liver lesions; E: Axial FS CE-MRI T1-WI in the venous phase confirms the liver metastases, showing hypointense nodules with venous ring enhancement; F: In the diffusion-weighted imaging study, these lesions are more conspicuous. Also, an additional metastasis (arrow) that was not detected either by CE-CT, CE-MRI, or PET-CT is shown.

an overall lower cost with GA-MRI compared to the other techniques[57]. The reason is that no patient needed any additional imaging technique to achieve a decision concerning the treatment in the group that used GA-MRI as the initial imaging method. However, in the group of patients submitted to extracellular CE-MRI and CE-CT as an initial approach, approximately 18.1% and 39.7%, respectively, performed an additional examination. Furthermore, it was also noted that the costs of surgery were higher in the GA-MRI group since more liver metastases were detected and consequently needed surgery for a curative approach.

According to these data, we concur that GA-MRI shows a superior sensitivity in detecting hepatic metastases, which leads to a more curative approach, avoids additional imaging examinations, and can be the most cost-effective option. Sadly, these studies did not significantly affect the current clinical guidelines, especially the latest consensus of ESMO, where MRI is still considered a second-line method[45,54].

In addition, according to a recent study, laparoscopic liver ultrasound might improve liver staging for CRLM compared to liver-specific contrast-enhanced MRI (sensitivity of 93.1% *vs* 85.6%)[58].

IMAGING TECHNIQUES FOR FOLLOW-UP

Approximately 80% of CRLM are unresectable at initial presentation, and chemotherapy is the treatment of choice (Figure 13). Some studies have reported that some of these lesions might respond to chemotherapy and become resectable, showing better long-term results than "conversion chemotherapy"[59]. As above-mentioned, these patients submitted to neoadjuvant chemotherapy may then appear with liver steatosis, especially after irinotecan and 5-FU or with sinusoidal obstruction (oxaliplatin), which may limit CT liver evaluation[21,60].

In follow-up studies of CRLM, CT may be used to evaluate response to systemic chemotherapy. In contrast, MRI (with hepatospecific contrast agent and DWI sequences) can be used to assess metastases after neoadjuvant chemotherapy, to assess resectability, and to estimate "disappearing" or "vanishing" metastases (DLM) (Figure 14)[21]. This term corresponds to complete radiologic response – treated metastases that are too small to be detected at follow-up imaging studies – ranging

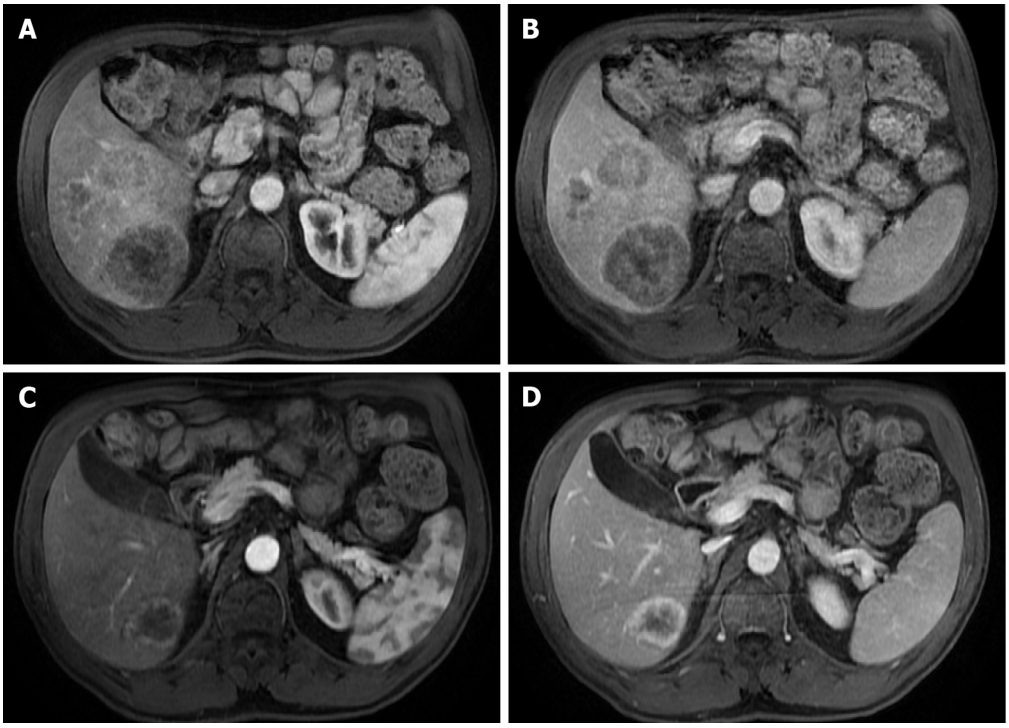

Figure 13 A 71-year-old man with unresectable CRLM. A and C: Axial fat saturated (FS) contrast-enhanced magnetic resonance imaging (CE-MRI) T1-weighted imaging (WI) in the arterial phase; B and D: Axial FS CE-MRI T1-WI in the portal-venous phase. Initial presentation of three heterogeneous hepatic lesions corresponding to unresectable CRLM before treatment (A and B). After chemotherapy (C and D), the patient presented partial response, with the disappearance of two lesions and reduced size of the larger lesion, which still presents viable peripheral tumor.

from 7%-24% in CRLM[21,61].

Barimani *et al*[61] showed that the combination of CE-CT, MRI, and intraoperative ultrasound (IOUS) showed promising results in detecting DLM in CRLM. Furthermore, it was suggested that when DLM remains undetectable by MRI and IOUS, it is a valid option to leave DLM *in situ* as an alternative approach to surgical resection.

According to Jhaveri *et al*[62], GA-MRI is superior to CE-CT for the detection of small CRLM (< 1 cm) in both categories of non-treated patients and those who underwent neoadjuvant chemotherapy.

In 2017, a study by Park *et al*[63] also concluded that MRI has a higher positive predictive value for the absence of tumors after chemotherapy than CT (78% *vs* 35.2%, respectively).

The RECIST criteria were developed to reach a standardized pattern of tumor response evaluation[64]. These criteria show limitations and appear inadequate for patients treated with immune checkpoint inhibitors due to the "pseudoprogression" phenomenon. Pseudoprogression may occur when molecular target agents diminish the tumor attenuation and enhancement to a lesser degree when compared to the surrounding liver, making the preexisting lesion now visible and mimicking disease progression. To assess this limitation, iRECIST criteria, based on RECIST-based measurements and immune-related response patterns, have been developed[55]. However, iRECIST criteria still need validation.

RECIST evaluation concerning CRLM often fails to identify clinically meaningful responses to bevacizumab-containing therapy. In this matter, Liu *et al*[65] created a developed-RECIST (D-RECIST) by combining CE-MRI and DWI-MRI. They showed that responders employing D-RECIST had a longer median disease-free survival than non-responders and that defined responses provided important prognostic information. It was concluded that D-RECIST might serve as a better response evaluation than RECIST in CRLM treated with bevacizumab-chemotherapy.

Some morphologic and dynamic features of liver metastases in MRI may predict the response before therapy[21,66]. For instance, a study showed that tumors with lower ADC values correlate with a better response to chemotherapy, while others report a poorer survival[67].

Besides chemotherapy, ablative therapies such as microwave ablation, transarterial chemoembolization, and radioembolization lead to a low-density lesion on CT and

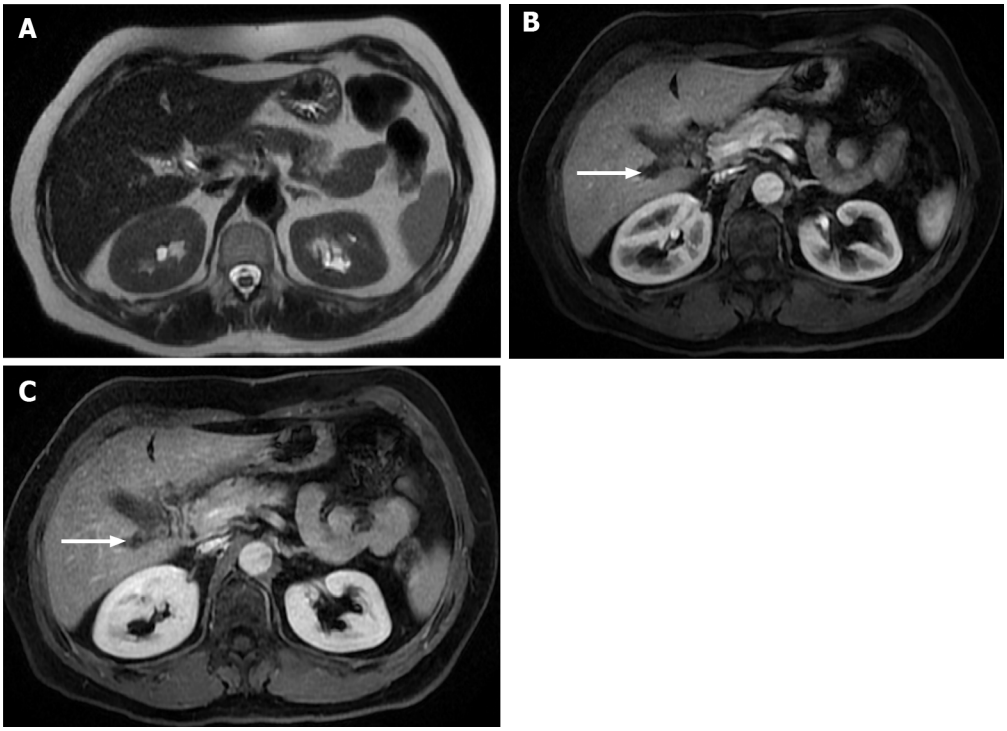

Figure 14 Follow-up of a 66-years-old woman with previous breast cancer liver metastases submitted to chemotherapy showing complete response in 2015. A: Axial T2-weighted imaging (WI) shows the liver metastasis characterized by an isointense lesion; B and C: Axial fat sat contrast-enhanced magnetic resonance imaging T1-WI in the arterial (B) and interstitial (C) phases present the liver metastasis without noticeable enhancement in the post-contrast dynamic study (arrow, B and C), which is consistent with treated metastasis (no viable tumor). To date, after 6 years, the patient is free of recurrent disease.

high T1 signal / low T2 signal on MRI due to coagulative necrosis[3]. These areas tend to shrink progressively with time. The existence of thick linear peripheral enhancement surrounding the lesion or nodular enhancement may suggest recurrence. Partial response is suggestive by a decrease in enhancement, and a complete response/successful embolization is shown by the absence of enhancement on CT/MRI and low T2 signal[3].

CONCLUSION

The liver is one of the most common organs involved with metastatic disease. Both CT and MRI are currently the techniques that show the highest diagnostic performance and are also the most suitable for assessing therapy response and follow-up. Studies have shown that MRI plays a crucial role and has a higher sensitivity in evaluating liver metastases. Therefore, it may be the ideal imaging method for treatment planning before and after neoadjuvant chemotherapy and is also considered the best technique for detection and follow-up in many university hospitals.

REFERENCES

1 **Lincke T**, Zech CJ. Liver metastases: Detection and staging. *Eur J Radiol* 2017; **97**: 76-82 [PMID: 29153371 DOI: 10.1016/j.ejrad.2017.10.016]
2 **Namasivayam S**, Martin DR, Saini S. Imaging of liver metastases: MRI. *Cancer Imaging* 2007; **7**: 2-9 [PMID: 17293303 DOI: 10.1102/1470-7330.2007.0002]
3 **Matos AP**, Altun E, Ramalho M, Velloni F, AlObaidy M, Semelka RC. An overview of imaging techniques for liver metastases management. *Expert Rev Gastroenterol Hepatol* 2015; **9**: 1561-1576 [PMID: 26414180 DOI: 10.1586/17474124.2015.1092873]
4 **Silva AC**, Evans JM, McCullough AE, Jatoi MA, Vargas HE, Hara AK. MR imaging of hypervascular liver masses: a review of current techniques. *Radiographics* 2009; **29**: 385-402 [PMID: 19325055 DOI: 10.1148/rg.292085123]
5 **Gore RM**, Thakrar KH, Wenzke DR, Newmark GM, Mehta UK, Berlin JW. That liver lesion on MDCT in the oncology patient: is it important? *Cancer Imaging* 2012; **12**: 373-384 [PMID: 23023318

DOI: 10.1102/1470-7330.2012.9028]
6 **Sica GT**, Ji H, Ros PR. CT and MR imaging of hepatic metastases. *AJR Am J Roentgenol* 2000; **174**: 691-698 [PMID: 10701611 DOI: 10.2214/ajr.174.3.1740691]
7 **Albrecht T**. Detection and Characterisation of Liver Metastases. In: Enhancing the Role of Ultrasound with Contrast Agents. 1st ed. Lencioni R editor. Milano: Springer, 2006: 53-67 [DOI: 10.1007/88-470-0476-4]
8 **Cantisani V**, Grazhdani H, Fioravanti C, Rosignuolo M, Calliada F, Messineo D, Bernieri MG, Redler A, Catalano C, D'Ambrosio F. Liver metastases: Contrast-enhanced ultrasound compared with computed tomography and magnetic resonance. *World J Gastroenterol* 2014; **20**: 9998-10007 [PMID: 25110428 DOI: 10.3748/wjg.v20.i29.9998]
9 **Tirumani SH**, Kim KW, Nishino M, Howard SA, Krajewski KM, Jagannathan JP, Cleary JM, Ramaiya NH, Shinagare AB. Update on the role of imaging in management of metastatic colorectal cancer. *Radiographics* 2014; **34**: 1908-1928 [PMID: 25384292 DOI: 10.1148/rg.347130090]
10 **Kong WT**, Ji ZB, Wang WP, Cai H, Huang BJ, Ding H. Evaluation of Liver Metastases Using Contrast-Enhanced Ultrasound: Enhancement Patterns and Influencing Factors. *Gut Liver* 2016; **10**: 283-287 [PMID: 26586554 DOI: 10.5009/gnl14324]
11 **Bernatik T**, Strobel D, Hahn EG, Becker D. Detection of liver metastases: comparison of contrast-enhanced wide-band harmonic imaging with conventional ultrasonography. *J Ultrasound Med* 2001; **20**: 509-515 [PMID: 11345108 DOI: 10.7863/jum.2001.20.5.509]
12 **Piscaglia F**, Lencioni R, Sagrini E, Pina CD, Cioni D, Vidili G, Bolondi L. Characterization of focal liver lesions with contrast-enhanced ultrasound. *Ultrasound Med Biol* 2010; **36**: 531-550 [PMID: 20350680 DOI: 10.1016/j.ultrasmedbio.2010.01.004]
13 **Cantisani V**, Ricci P, Erturk M, Pagliara E, Drudi F, Calliada F, Mortele K, D'Ambrosio U, Marigliano C, Catalano C, Marin D, Di Seri M, Longo F, Passariello R. Detection of hepatic metastases from colorectal cancer: prospective evaluation of gray scale US *vs* SonoVue® low mechanical index real time-enhanced US as compared with multidetector-CT or Gd-BOPTA-MRI. *Ultraschall Med* 2010; **31**: 500-505 [PMID: 20408122 DOI: 10.1055/s-0028-1109751]
14 **Vialle R**, Boucebci S, Richer JP, Velasco S, Herpe G, Vesselle G, Ingrand P, Tasu JP. Preoperative detection of hepatic metastases from colorectal cancer: Prospective comparison of contrast-enhanced ultrasound and multidetector-row computed tomography (MDCT). *Diagn Interv Imaging* 2016; **97**: 851-855 [PMID: 27132590 DOI: 10.1016/j.diii.2015.11.017]
15 **El Kaffas A**, Sigrist RMS, Fisher G, Bachawal S, Liau J, Wang H, Karanany A, Durot I, Rosenberg J, Hristov D, Willmann JK. Quantitative Three-Dimensional Dynamic Contrast-Enhanced Ultrasound Imaging: First-In-Human Pilot Study in Patients with Liver Metastases. *Theranostics* 2017; **7**: 3745-3758 [PMID: 29109773 DOI: 10.7150/thno.20329]
16 **Lee JC**, Yan K, Lee SK, Yang W, Chen MH. Focal Liver Lesions: Real-time 3-Dimensional Contrast-Enhanced Ultrasonography Compared With 2-Dimensional Contrast-Enhanced Ultrasonography and Magnetic Resonance Imaging. *J Ultrasound Med* 2017; **36**: 2015-2026 [PMID: 28646628 DOI: 10.1002/jum.14268]
17 **Nakai H**, Arizono S, Isoda H, Togashi K. Imaging Characteristics of Liver Metastases Overlooked at Contrast-Enhanced CT. *AJR Am J Roentgenol* 2019; **212**: 782-787 [PMID: 30779660 DOI: 10.2214/AJR.18.20526]
18 **Kulemann V**, Schima W, Tamandl D, Kaczirek K, Gruenberger T, Wrba F, Weber M, Ba-Ssalamah A. Preoperative detection of colorectal liver metastases in fatty liver: MDCT or MRI? *Eur J Radiol* 2011; **79**: e1-e6 [PMID: 20392584 DOI: 10.1016/j.ejrad.2010.03.004]
19 **Sahani DV**, Bajwa MA, Andrabi Y, Bajpai S, Cusack JC. Current status of imaging and emerging techniques to evaluate liver metastases from colorectal carcinoma. *Ann Surg* 2014; **259**: 861-872 [PMID: 24509207 DOI: 10.1097/SLA.0000000000000525]
20 **Kamaya A**, Maturen KE, Tye GA, Liu YI, Parti NN, Desser TS. Hypervascular liver lesions. *Semin Ultrasound CT MR* 2009; **30**: 387-407 [PMID: 19842564 DOI: 10.1053/j.sult.2009.06.001]
21 **Torre LA**, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A. Global cancer statistics, 2012. *CA Cancer J Clin* 2015; **65**: 87-108 [PMID: 25651787 DOI: 10.3322/caac.21262]
22 **Honda Y**, Higaki T, Higashihori H, Monzen Y, Tatsugami F, Date S, Awai K. Re-evaluation of detectability of liver metastases by contrast-enhanced CT: added value of hepatic arterial phase imaging. *Jpn J Radiol* 2014; **32**: 467-475 [PMID: 24854901 DOI: 10.1007/s11604-014-0331-z]
23 **Ferlay J**, Colombet M, Soerjomataram I, Dyba T, Randi G, Bettio M, Gavin A, Visser O, Bray F. Cancer incidence and mortality patterns in Europe: Estimates for 40 countries and 25 major cancers in 2018. *Eur J Cancer* 2018; **103**: 356-387 [PMID: 30100160 DOI: 10.1016/j.ejca.2018.07.005]
24 **Sadigh G**, Applegate KE, Baumgarten DA. Comparative accuracy of intravenous contrast-enhanced CT *vs* noncontrast CT plus intravenous contrast-enhanced CT in the detection and characterization of patients with hypervascular liver metastases: a critically appraised topic. *Acad Radiol* 2014; **21**: 113-125 [PMID: 24331274 DOI: 10.1016/j.acra.2013.08.023]
25 **Hale HL**, Husband JE, Gossios K, Norman AR, Cunningham D. CT of calcified liver metastases in colorectal carcinoma. *Clin Radiol* 1998; **53**: 735-741 [PMID: 9817090 DOI: 10.1016/s0009-9260(98)80315-2]
26 **Paulatto L**, Dioguardi Burgio M, Sartoris R, Beaufrère A, Cauchy F, Paradis V, Vilgrain V, Ronot M. Colorectal liver metastases: radiopathological correlation. *Insights Imaging* 2020; **11**: 99 [PMID: 32844319 DOI: 10.1186/s13244-020-00904-4]
27 **Benoist S**, Brouquet A, Penna C, Julié C, El Hajjam M, Chagnon S, Mitry E, Rougier P, Nordlinger

B. Complete response of colorectal liver metastases after chemotherapy: does it mean cure? *J Clin Oncol* 2006; **24**: 3939-3945 [PMID: 16921046 DOI: 10.1200/JCO.2006.05.8727]

28 **Jo BG**, Song YG, Shim SG, Kim YW. Comparison of enhancement and image quality: different iodine concentrations for liver on 128-slice multidetector computed tomography in the same chronic liver disease patients. *Korean J Intern Med* 2016; **31**: 461-469 [PMID: 26701234 DOI: 10.3904/kjim.2014.210]

29 **Rajiah P**, Parakh A, Kay F, Baruah D, Kambadakone AR, Leng S. Update on Multienergy CT: Physics, Principles, and Applications. *Radiographics* 2020; **40**: 1284-1308 [PMID: 32822281 DOI: 10.1148/rg.2020200038]

30 **Lenga L**, Lange M, Arendt CT, Booz C, Yel I, Bodelle B, D'Angelo T, Hammerstingl RM, Huizinga NA, Vogl TJ, Martin SS, Albrecht MH. Measurement Reliability and Diagnostic Accuracy of Virtual Monoenergetic Dual-Energy CT in Patients with Colorectal Liver Metastases. *Acad Radiol* 2020; **27**: e168-e175 [PMID: 31727567 DOI: 10.1016/j.acra.2019.09.020]

31 **Lenga L**, Lange M, Arendt CT, Yel I, Booz C, Durden J, Leithner D, Vogl TJ, Albrecht MH, Martin SS. Can Dual-energy CT-based Virtual Monoenergetic Imaging Improve the Assessment of Hypodense Liver Metastases in Patients With Hepatic Steatosis? *Acad Radiol* 2021; **28**: 769-777 [PMID: 32446765 DOI: 10.1016/j.acra.2020.03.044]

32 **Vreugdenburg TD**, Ma N, Duncan JK, Riitano D, Cameron AL, Maddern GJ. Comparative diagnostic accuracy of hepatocyte-specific gadoxetic acid (Gd-EOB-DTPA) enhanced MR imaging and contrast enhanced CT for the detection of liver metastases: a systematic review and meta-analysis. *Int J Colorectal Dis* 2016; **31**: 1739-1749 [PMID: 27682648 DOI: 10.1007/s00384-016-2664-9]

33 **Lee KH**, Lee JM, Park JH, Kim JH, Park HS, Yu MH, Yoon JH, Han JK, Choi BI. MR imaging in patients with suspected liver metastases: value of liver-specific contrast agent gadoxetic acid. *Korean J Radiol* 2013; **14**: 894-904 [PMID: 24265564 DOI: 10.3348/kjr.2013.14.6.894]

34 **Kanematsu M**, Kondo H, Goshima S, Kato H, Tsuge U, Hirose Y, Kim MJ, Moriyama N. Imaging liver metastases: review and update. *Eur J Radiol* 2006; **58**: 217-228 [PMID: 16406434 DOI: 10.1016/j.ejrad.2005.11.041]

35 **Kim HJ**, Lee SS, Byun JH, Kim JC, Yu CS, Park SH, Kim AY, Ha HK. Incremental value of liver MR imaging in patients with potentially curable colorectal hepatic metastasis detected at CT: a prospective comparison of diffusion-weighted imaging, gadoxetic acid-enhanced MR imaging, and a combination of both MR techniques. *Radiology* 2015; **274**: 712-722 [PMID: 25286324 DOI: 10.1148/radiol.14140390]

36 **Bruegel M**, Rummeny EJ. Hepatic metastases: use of diffusion-weighted echo-planar imaging. *Abdom Imaging* 2010; **35**: 454-461 [PMID: 19471997 DOI: 10.1007/s00261-009-9541-8]

37 **Ward J**. New MR techniques for the detection of liver metastases. *Cancer Imaging* 2006; **6**: 33-42 [PMID: 16766267 DOI: 10.1102/1470-7330.2006.0007]

38 **Giuga M**, De Gaetano AM, Guerra A, Infante A, Iezzi R, Spinelli I, Siciliano M, Grieco A, Rapaccini GL, Gasbarrini A, Pompili M, Bonomo L. An update on clinical applications of hepatospecific contrast media in magnetic resonance imaging of liver parenchyma. *Eur Rev Med Pharmacol Sci* 2016; **20**: 2515-2525 [PMID: 27383300]

39 **Schieda N**, Maralani PJ, Hurrell C, Tsampalieros AK, Hiremath S. Updated Clinical Practice Guideline on Use of Gadolinium-Based Contrast Agents in Kidney Disease Issued by the Canadian Association of Radiologists. *Can Assoc Radiol J* 2019; **70**: 226-232 [PMID: 31255393 DOI: 10.1016/j.carj.2019.04.001]

40 **Goldstein KM**, Lunyera J, Mohottige D, Amrhein TJ, Alexopoulos AS, Campbell H, Cameron CB, Sagalla N, Crowley MJ, Dietch JR, Gordon AM, Kosinski AS, Cantrell S, Williams Jr JW, Gierisch JM. Risk of Nephrogenic Systemic Fibrosis after Exposure to Newer Gadolinium Agents. Washington (DC): Department of Veterans Affairs, 2019 [PMID: 32687278]

41 **Hazhirkarzar B**, Khoshpouri P, Shaghaghi M, Ghasabeh MA, Pawlik TM, Kamel IR. Current state of the art imaging approaches for colorectal liver metastasis. *Hepatobiliary Surg Nutr* 2020; **9**: 35-48 [PMID: 32140477 DOI: 10.21037/hbsn.2019.05.11]

42 **Zhang L**, Yu X, Huo L, Lu L, Pan X, Jia N, Fan X, Morana G, Grazioli L, Schneider G. Detection of liver metastases on gadobenate dimeglumine-enhanced MRI: systematic review, meta-analysis, and similarities with gadoxetate-enhanced MRI. *Eur Radiol* 2019; **29**: 5205-5216 [PMID: 30915560 DOI: 10.1007/s00330-019-06110-1]

43 **Vilgrain V**, Esvan M, Ronot M, Caumont-Prim A, Aubé C, Chatellier G. A meta-analysis of diffusion-weighted and gadoxetic acid-enhanced MR imaging for the detection of liver metastases. *Eur Radiol* 2016; **26**: 4595-4615 [PMID: 26883327 DOI: 10.1007/s00330-016-4250-5]

44 **Maffione AM**, Lopci E, Bluemel C, Giammarile F, Herrmann K, Rubello D. Diagnostic accuracy and impact on management of (18)F-FDG PET and PET/CT in colorectal liver metastasis: a meta-analysis and systematic review. *Eur J Nucl Med Mol Imaging* 2015; **42**: 152-163 [PMID: 25319712 DOI: 10.1007/s00259-014-2930-4]

45 **Glazer ES**, Beaty K, Abdalla EK, Vauthey JN, Curley SA. Effectiveness of positron emission tomography for predicting chemotherapy response in colorectal cancer liver metastases. *Arch Surg* 2010; **145**: 340-5; discussion 345 [PMID: 20404283 DOI: 10.1001/archsurg.2010.41]

46 **Beiderwellen K**, Geraldo L, Ruhlmann V, Heusch P, Gomez B, Nensa F, Umutlu L, Lauenstein TC. Accuracy of [18F]FDG PET/MRI for the Detection of Liver Metastases. *PLoS One* 2015; **10**: e0137285 [PMID: 26335246 DOI: 10.1371/journal.pone.0137285]

47 **Lake ES**, Wadhwani S, Subar D, Kauser A, Harris C, Chang D, Lapsia S. The influence of FDG

PET-CT on the detection of extrahepatic disease in patients being considered for resection of colorectal liver metastasis. *Ann R Coll Surg Engl* 2014; **96**: 211-215 [PMID: 24780786 DOI: 10.1308/003588414X13814021679195]

48 **Choi SH**, Kim SY, Park SH, Kim KW, Lee JY, Lee SS, Lee MG. Diagnostic performance of CT, gadoxetate disodium-enhanced MRI, and PET/CT for the diagnosis of colorectal liver metastasis: Systematic review and meta-analysis. *J Magn Reson Imaging* 2018; **47**: 1237-1250 [PMID: 28901685 DOI: 10.1002/jmri.25852]

49 **Schulz A**, Viktil E, Godt JC, Johansen CK, Dormagen JB, Holtedahl JE, Labori KJ, Bach-Gansmo T, Kløw NE. Diagnostic performance of CT, MRI and PET/CT in patients with suspected colorectal liver metastases: the superiority of MRI. *Acta Radiol* 2016; **57**: 1040-1048 [PMID: 26622057 DOI: 10.1177/0284185115617349]

50 **Ko Y**, Kim J, Park JK, Kim H, Cho JY, Kang SB, Ahn S, Lee KJ, Lee KH. Limited detection of small (≤ 10 mm) colorectal liver metastasis at preoperative CT in patients undergoing liver resection. *PLoS One* 2017; **12**: e0189797 [PMID: 29244853 DOI: 10.1371/journal.pone.0189797]

51 **Maegerlein C**, Fingerle AA, Souvatzoglou M, Rummeny EJ, Holzapfel K. Detection of liver metastases in patients with adenocarcinomas of the gastrointestinal tract: comparison of (18)F-FDG PET/CT and MR imaging. *Abdom Imaging* 2015; **40**: 1213-1222 [PMID: 25348732 DOI: 10.1007/s00261-014-0283-x]

52 **Abdalla EK**, Vauthey JN, Ellis LM, Ellis V, Pollock R, Broglio KR, Hess K, Curley SA. Recurrence and outcomes following hepatic resection, radiofrequency ablation, and combined resection/ablation for colorectal liver metastases. *Ann Surg* 2004; **239**: 818-825 [PMID: 15166961 DOI: 10.1097/01.sla.0000128305.90650.71]

53 **Seemann MD**, Meisetschlaeger G, Gaa J, Rummeny EJ. Assessment of the extent of metastases of gastrointestinal carcinoid tumors using whole-body PET, CT, MRI, PET/CT and PET/MRI. *Eur J Med Res* 2006; **11**: 58-65 [PMID: 16504962 DOI: 10.1055/s-2005-867813]

54 **Glynne-Jones R**, Wyrwicz L, Tiret E, Brown G, Rödel C, Cervantes A, Arnold D; ESMO Guidelines Committee. Rectal cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann Oncol* 2017; **28**: iv22-iv40 [PMID: 28881920 DOI: 10.1093/annonc/mdx224]

55 **Renzulli M**, Clemente A, Ierardi AM, Pettinari I, Tovoli F, Brocchi S, Peta G, Cappabianca S, Carrafiello G, Golfieri R. Imaging of Colorectal Liver Metastases: New Developments and Pending Issues. *Cancers (Basel)* 2020; **12**: 151 [PMID: 31936319 DOI: 10.3390/cancers12010151]

56 **Benson AB**, Venook AP, Al-Hawary MM, Cederquist L, Chen YJ, Ciombor KK, Cohen S, Cooper HS, Deming D, Engstrom PF, Grem JL, Grothey A, Hochster HS, Hoffe S, Hunt S, Kamel A, Kirilcuk N, Krishnamurthi S, Messersmith WA, Meyerhardt J, Mulcahy MF, Murphy JD, Nurkin S, Saltz L, Sharma S, Shibata D, Skibber JM, Sofocleous CT, Stoffel EM, Stotsky-Himelfarb E, Willett CG, Wuthrick E, Gregory KM, Gurski L, Freedman-Cass DA. Rectal Cancer, Version 2.2018, NCCN Clinical Practice Guidelines in Oncology. *J Natl Compr Canc Netw* 2018; **16**: 874-901 [PMID: 30006429 DOI: 10.6004/jnccn.2018.0061]

57 **Zech CJ**, Ba-Ssalamah A, Berg T, Chandarana H, Chau GY, Grazioli L, Kim MJ, Lee JM, Merkle EM, Murakami T, Ricke J, B Sirlin C, Song B, Taouli B, Yoshimitsu K, Koh DM. Consensus report from the 8th International Forum for Liver Magnetic Resonance Imaging. *Eur Radiol* 2020; **30**: 370-382 [PMID: 31385048 DOI: 10.1007/s00330-019-06369-4]

58 **Russolillo N**, Borello A, Langella S, Casella M, Lo Tesoriere R, Ferrero A. Comparison of laparoscopic ultrasound and liver-specific magnetic resonance imaging for staging colorectal liver metastases. *Surg Endosc* 2021; **35**: 3547-3553 [PMID: 32710215 DOI: 10.1007/s00464-020-07817-9]

59 **Basso M**, Dadduzio V, Ardito F, Lombardi P, Strippoli A, Vellone M, Orlandi A, Rossi S, Cerchiaro E, Cassano A, Giuliante F, Barone C. Conversion Chemotherapy for Technically Unresectable Colorectal Liver Metastases: A Retrospective, STROBE-Compliant, Single-Center Study Comparing Chemotherapy Alone and Combination Chemotherapy With Cetuximab or Bevacizumab. *Medicine (Baltimore)* 2016; **95**: e3722 [PMID: 27196492 DOI: 10.1097/MD.0000000000003722]

60 **Rubbia-Brandt L**, Audard V, Sartoretti P, Roth AD, Brezault C, Le Charpentier M, Dousset B, Morel P, Soubrane O, Chaussade S, Mentha G, Terris B. Severe hepatic sinusoidal obstruction associated with oxaliplatin-based chemotherapy in patients with metastatic colorectal cancer. *Ann Oncol* 2004; **15**: 460-466 [PMID: 14998849 DOI: 10.1093/annonc/mdh095]

61 **Barimani D**, Kauppila JH, Sturesson C, Sparrelid E. Imaging in disappearing colorectal liver metastases and their accuracy: a systematic review. *World J Surg Oncol* 2020; **18**: 264 [PMID: 33032620 DOI: 10.1186/s12957-020-02037-w]

62 **Jhaveri K**, Cleary S, Audet P, Balaa F, Bhayana D, Burak K, Chang S, Dixon E, Haider M, Molinari M, Reinhold C, Sherman M. Consensus statements from a multidisciplinary expert panel on the utilization and application of a liver-specific MRI contrast agent (gadoxetic acid). *AJR Am J Roentgenol* 2015; **204**: 498-509 [PMID: 25714278 DOI: 10.2214/AJR.13.12399]

63 **Park MJ**, Hong N, Han K, Kim MJ, Lee YJ, Park YS, Rha SE, Park S, Lee WJ, Park SH, Lee CH, Nam CM, An C, Kim HJ, Kim H, Park MS. Use of Imaging to Predict Complete Response of Colorectal Liver Metastases after Chemotherapy: MR Imaging *vs* CT Imaging. *Radiology* 2017; **284**: 423-431 [PMID: 28327002 DOI: 10.1148/radiol.2017161619]

64 **Eisenhauer EA**, Therasse P, Bogaerts J, Schwartz LH, Sargent D, Ford R, Dancey J, Arbuck S, Gwyther S, Mooney M, Rubinstein L, Shankar L, Dodd L, Kaplan R, Lacombe D, Verweij J. New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). *Eur J Cancer* 2009; **45**: 228-247 [PMID: 19097774 DOI: 10.1016/j.ejca.2008.10.026]

65 **Liu LH**, Zhou GF, Lv H, Wang ZC, Rao SX, Zeng MS. Identifying response in colorectal liver metastases treated with bevacizumab: development of RECIST by combining contrast-enhanced and diffusion-weighted MRI. *Eur Radiol* 2021; **31**: 5640-5649 [PMID: 33449175 DOI: 10.1007/s00330-020-07647-2]

66 **Beckers RCJ**, Lambregts DMJ, Lahaye MJ, Rao SX, Kleinen K, Grootscholten C, Beets GL, Beets-Tan RGH, Maas M. Advanced imaging to predict response to chemotherapy in colorectal liver metastases - a systematic review. *HPB (Oxford)* 2018; **20**: 120-127 [PMID: 29196021 DOI: 10.1016/j.hpb.2017.10.013]

67 **Heijmen L**, ter Voert EE, Oyen WJ, Punt CJ, van Spronsen DJ, Heerschap A, de Geus-Oei LF, van Laarhoven HW. Multimodality imaging to predict response to systemic treatment in patients with advanced colorectal cancer. *PLoS One* 2015; **10**: e0120823 [PMID: 25831053 DOI: 10.1371/journal.pone.0120823]

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MINIREVIEWS

Liver manifestations and complications in inflammatory bowel disease: A review

Rui Gaspar, Catarina Castelo Branco, Guilherme Macedo

ORCID number: Rui Gaspar 0000-0003-0332-3844; Catarina Castelo Branco 0000-0002-8592-387X; Guilherme Macedo 0000-0002-9387-9872.

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Rui Gaspar, Guilherme Macedo, Department of Gastroenterology and Hepatology, Centro Hospitalar de São João, Porto 4200, Portugal

Catarina Castelo Branco, Internal Medicine Department, Centro Hospitalar e Universitário do Porto, Porto 4200, Portugal

Corresponding author: Rui Gaspar, MD, Doctor, Department of Gastroenterology and Hepatology, Centro Hospitalar de São João, Alameda Prof Hernani Monteiro, Porto 4200, Portugal. ruilopesgaspar@gmail.com

Abstract

Hepatobiliary manifestations are common in inflammatory bowel disease (IBD), with 30% of patients presenting abnormal liver tests and 5% developing chronic liver disease. They range from asymptomatic elevated liver tests to life-threatening disease and usually follow an independent course from IBD. The pathogenesis of liver manifestations or complications and IBD can be closely related by sharing a common auto-immune background (in primary sclerosing cholangitis, IgG4-related cholangitis, and autoimmune hepatitis), intestinal inflammation (in portal vein thrombosis and granulomatous hepatitis), metabolic impairment (in non-alcoholic fatty liver disease or cholelithiasis), or drug toxicity (in drug induced liver injury or hepatitis B virus infection reactivation). Their evaluation should prompt a full diagnostic workup to identify and readily treat all complications, improving management and outcome.

Key Words: Hepatobiliary manifestations; Inflammatory bowel disease; Drug induced liver injury; Primary sclerosing cholangitis; Viral hepatitis; Crohn's disease; Ulcerative colitis

Core Tip: Hepatobiliary manifestations are common in inflammatory bowel disease (IBD), ranging from incidental findings in asymptomatic patients to life-threatening liver failure. Their pathogenesis can be intrinsically linked to IBD (auto-immune background or metabolic abnormalities) or to its medication. Early recognition of these manifestations as well as a full diagnostic workup are mandatory to improve management and prognosis. In this review, we describe all hepatobiliary manifestations

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INTRODUCTION

Inflammatory bowel disease (IBD) is a group of chronic and recurrent gastrointestinal inflammatory conditions that result from the interaction of genetic, environmental, and immune factors. IBD is mainly divided into Crohn's disease (CD) and ulcerative colitis (UC), affecting equally men and women, with peak incidence between 20 and 30 and also from 50 to 60 years of age[1].

Extra-intestinal manifestations are described in up to 50% of patients, including arthropathy, metabolic bone disease, ocular, dermatological, hepatobiliary, neurologic, cardiovascular, pulmonary, and urological complications[2].

Hepatobiliary alterations are one of the most common extra-intestinal manifestations of IBD; up to 30% of patients have abnormal liver tests and 5% will develop chronic liver disease[3,4]. A wide diversity of hepatobiliary complications has been reported, ranging from incidental findings in asymptomatic patients to severe and life-threatening liver failure[5].

The pathogenesis of liver disease in IBD is not totally understood but multiple pathways may link them (Table 1)[2,5,6].

Inflammatory bowel disease related diseases

Diseases that share a common auto-immune background include primary sclerosing cholangitis (PSC), IgG4-related cholangitis, primary biliary cholangitis (PBC), auto-immune hepatitis, and overlap syndromes.

Diseases associated with intestinal inflammation include portal vein thrombosis, Budd-Chiari syndrome, granulomatous hepatitis, and liver abscesses.

Diseases associated with malabsorption or metabolic impairment are cholelithiasis, amyloidosis, and non-alcoholic fatty liver disease (NAFLD).

Inflammatory bowel disease related medications

Disorders associated with IBD treatment include direct hepatotoxicity with medications such as 5-aminosalicylic acid (5-ASA) compounds, methotrexate, azathioprine, or anti-TNF agents or hepatitis B reactivation due to immunosuppressants.

They can occur at any time during the natural history of disease and typically follow an independent course from the underlying intestinal disease activity. Granulomatous hepatitis, hepatic abscesses, cholelithiasis, and amyloidosis are more commonly observed in CD and PSC and auto-immune hepatitis in UC[6,7].

Moreover, these patients may present unrelated liver disease, making abnormal liver tests in IBD a challenging differential diagnosis.

Early recognition of these manifestations is of paramount importance to avoid liver injury and improve management of both diseases (Figure 1).

The aim of this paper is to review the hepatobiliary manifestations and complications found in IBD patients.

DISEASES SHARING A COMMON AUTO-IMMUNE BACKGROUND WITH INFLAMMATORY BOWEL DISEASE

Primary sclerosing cholangitis

PSC is a chronic and progressive bile duct disorder, characterized by multifocal intrahepatic and/or extrahepatic strictures and dilatations, that may result in cirrhosis and end-stage liver disease. The diagnosis is usually made by combination of clinical (jaundice, abdominal pain, and itching but it may also be asymptomatic), biochemical

Table 1 Inflammatory bowel disease related diseases and inflammatory bowel disease medication related diseases

IBD related diseases		IBD medication related diseases	
Ulcerative colitis	**Crohn's disease**	**Ulcerative colitis**	**Crohn's disease**
Primary sclerosing cholangitis	Granulomatous hepatitis	Drug-induced liver injury	Drug-induced liver injury
Auto-immune hepatitis	Liver abscesses	HBV reactivation	HBV reactivation
Overlap syndromes	Cholelithiasis		
Primary biliary cholangitis	Hepatic amyloidosis		
Portal vein thrombosis			
NAFLD	NAFLD		

IBD: Inflammatory bowel disease; HBV: Hepatitis B Virus; NAFLD: Non-alcoholic fatty liver disease.

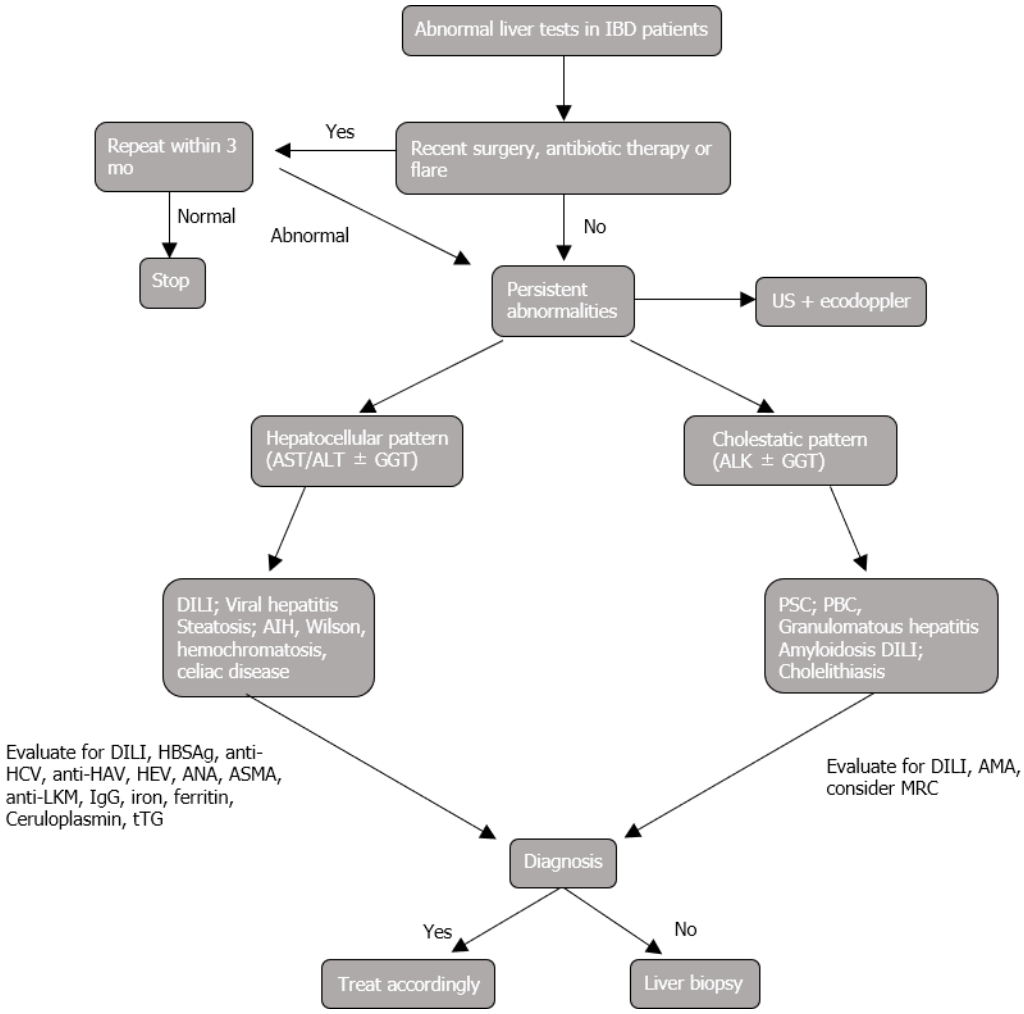

Figure 1 Management of abnormal liver tests. IBD: Inflammatory bowel disease; US: Ultrasonography; AST: Aspartate transaminase; ALT: Alanine transaminase; GGT: AIH: Autoimmune hepatitis; PSC: Primary sclerosing cholangitis; PBC: Primary biliary cholangitis; DILI: Drug induced liver injury; AMA: Anti-mitochondrial antibody; MRC: Magnetic resonance cholangiography.

(elevated cholestatic liver enzymes - alkaline phosphatase and/or GGT) and imagiological [magnetic resonance cholangiography (MRCP)] findings. The mean age at diagnosis is 30 to 40 years old and it has a male predominance[8,9].

PSC is closely linked to IBD, which occurs in 70% of patients, with a UC predominance (75%). On the other hand, only up to 3% of CD and 2%-8% of UC patients develop PSC[10]. Therefore, the presence of unexplained cholestasis should prompt an immediate investigation by MRCP in those with IBD and patients with PSC should

routinely undergo colonoscopy with biopsies, even in the absence of symptoms. If the index colonoscopy is negative, it should be repeated every 3 to 5 years[10,11]. The two disorders can occur at different times, but IBD diagnosis usually precedes that of PSC [12].

IBD in the setting of PSC is associated with a different clinical course, typically presenting extensive disease, rectal sparing (6% to 66% *vs* 2% to 25% in IBD without PSC), backwash ileitis (5% to 46% *vs* 3% to 24% in UC without PSC), and mild intestinal activity, as well as more frequent right colonic involvement[10,13]. Marelli *et al*[14] showed an inverse relationship between PSC severity and IBD activity. On the other hand, the effect of IBD in PSC prognosis is less established - higher rates of combined intrahepatic and extrahepatic involvement have been reported, although long-term outcomes of PSC do not seem to be changed[10,15,16].

PSC-IBD patients also present a greater risk of colorectal dysplasia and cancer, which supports the current recommendation of annual surveillance colonoscopy in this subset of patients. Although there are no specific recommendations, colectomy is suggested in case of indefinite or low-grade dysplasia, due to a high risk of colorectal cancer[10,17,18]. Similarly, prolonged duration of IBD was associated with an increased risk of cholangiocarcinoma, with a 33% higher risk per 10 years[19].

Small-duct primary sclerosing cholangitis

Small-duct PSC is very similar to large-duct PSC (close biochemical and histopathological findings) but presents a normal cholangiogram. The diagnosis requires liver biopsy and some patients may later develop the classic PSC (12%-23%)[6,20]. Almost all patients have IBD, mainly UC, and it affects females at greater rates than males. Small-duct PSC has a better prognosis and a negligible risk of cholangiocarcinoma[9, 21].

IgG4-associated cholangitis

IgG4-associated cholangitis, considered a secondary sclerosing cholangitis, is characterized by elevated serum levels of IgG4, dense infiltration of IgG4-positive plasma cells and lymphocytes, and fibrosis and obliterative phlebitis in the bile duct wall, being frequently associated with autoimmune pancreatitis[22]. The link between IgG4-associated cholangitis and IBD has been reported, but it is far less common than in PSC. Differential diagnosis is vital due to its responsiveness to corticosteroids[9].

Primary biliary cholangitis

PBC is an autoimmune liver disease that presents with chronic cholestasis and histological findings of nonsuppurative destructive cholangitis. The diagnosis is usually made by detection of anti-mitochondrial antibodies[23]. There are only few reports of PBC in patients with IBD, affecting mainly UC males and those at younger age[24,25].

Autoimmune hepatitis

Autoimmune hepatitis (AIH) is a rare and heterogeneous disease, affecting mostly middle-aged women. It is characterized by abnormal liver tests, hypergammaglobulinemia, circulating autoantibodies [mainly antinuclear antibody (ANA), smooth muscle antibody, and anti-liver-kidney muscle antibody], and interface hepatitis on liver histology[26].

A relationship between AIH and IBD has already been established in a study that demonstrated the presence of UC in 16% of patients with AIH[3,27].

More relevant is the fact that coexistent AIH and IBD can have a different course from either process alone - patients with UC and concurrent AIH are more likely to relapse, need proctocolectomy, have more extensive disease, and present right colon lesions[3,28]. Likewise, liver disease may also have distinct progression, developing at younger age, being more likely to be refractory to treatment, and determining higher risk of death and liver transplantation[3].

Overlap syndromes

Patients with AIH may also present features of other immune-mediated liver diseases. In patients with UC, AIH-PSC is the most common overlap syndrome, described in up to 10% of PSC patients with UC[3,29]. However, cases of overlap syndrome in CD have also been described[30]. AIH-PSC is more common in children and young adults, PSC features usually develop later, and it has a better prognosis than PSC alone[31,32].

DISEASES ASSOCIATED WITH INTESTINAL INFLAMMATION

Portal vein thrombosis and Budd-Chiari syndrome

IBD is associated with a pro-inflammatory hypercoagulable state that increases the risk of portal and mesenteric vein thrombosis, with an estimated incidence of 1% to 2% [33]. Several risk factors have been identified: elevated platelet count, high fibrinogen, high factors V and VIII levels, and acquired prothrombotic factors - surgery, extent of colon disease, immobilization, inflammation, corticosteroids, and smoking[6,31]. Portal vein thrombosis has been more frequently described in UC patients after proctocolectomy and Budd-Chiari syndrome has an eight-fold risk during acute flares[31,34,35]. Anticoagulation is the mainstay of treatment, even in cases with previous gastrointestinal bleeding. Pharmacological thromboprophylaxis is recommended during hospitalizations and suggested in cases of active disease after hospital discharge and after surgery[2].

Granulomatous hepatitis

Granulomatous hepatitis is a rare complication of IBD, with a prevalence lower than 1%, mainly affecting CD patients[31]. Clinical suspicion is raised by elevated alkaline phosphatase and it is diagnosed by identification of granulomas in liver biopsy. It is mainly asymptomatic and follows a benign course, rarely requiring treatment (corticosteroids and immunosuppressants)[6,31]. It has also been associated with sulfasalazine use but differential diagnosis includes infections (tuberculosis) and malignancies[6,36].

Liver abscesses

Liver abscesses are a rare complication of IBD, but can also be its first manifestation (mainly in CD)[31]. They can result either from direct extension of an intra-abdominal abscess or from portal pyemia secondary to increased intestinal permeability[6]. They are often multiple and more frequently located in the right lobe, presenting with fever, abdominal pain, jaundice, diarrhea, and hepatosplenomegaly, as well as elevated inflammatory markers and alkaline phosphatase[31,37].

In contrast with liver abscesses in the general population, isolated *Streptococcus* species are the most common isolated pathogens[9,37].

The treatment of choice is prolonged intravenous antibiotics, with percutaneous drainage in case of a large abscess or refractory disease[31,38].

DISEASES ASSOCIATED WITH MALABSORPTION OR METABOLIC IMPAIRMENT

Cholelithiasis

Cholelithiasis is a known complication of IBD, with CD patients presenting a two-fold risk of developing gallstones. On the contrary, UC is not associated with an increased risk of cholelithiasis[39]. The incidence of cholelithiasis in patients with ileal involvement or resection ranges from 13% to 34%. It is associated with malabsorption of bile salts, resulting in disruption and increased entero-hepatic circulation, which predisposes to formation of gallstones[40]. Many risk factors have been described, such as ileo-colonic localization, disease duration (> 15 years), extent of ileal resection (> 30 cm), longer hospital stay, higher number of hospitalizations (> 3), multiple total parenteral nutrition treatments, lifetime surgeries, and number of clinical recurrences (> 3)[39,40]. Complications of cholelithiasis may be an indication for cholecystectomy but systematic cholecystectomy following ileal resection is not recommended[31,40,41].

Hepatic amyloidosis

Hepatic amyloidosis is a rare complication of IBD, more frequent in CD (0.9%) than in UC (0.07%)[42]. There is a male predominance and prominent colonic involvement. It results from amyloid deposition due to chronic inflammation, presenting as asymptomatic disease or hepatomegaly. Treatment is focused on lowering systemic inflammation by controlling it in the gut[6,31,43].

Non-alcoholic fatty liver disease

NAFLD is one of the most common liver diseases with a prevalence of 25% worldwide [44]. IBD patients seem to have a higher susceptibility to NAFLD and its prevalence

reaches almost 40%[45,46].

The main risk factor for NAFLD in the general population is metabolic syndrome but IBD patients develop NAFLD with fewer metabolic risk factors. In turn, IBD-associated factors that increase the risk of NAFLD include small bowel surgery, disease activity and duration, parenteral nutrition, and use of high doses of corticosteroids[47]. The influence of anti-TNF therapy on NAFLD risk is controversial: Some studies reported the development of biopsy-proven NAFLD in patients under anti-TNF therapy while others suggested a protective effect of these treatments[48,49].

There are no current guidelines for screening or assessing for NAFLD in patients with IBD.

IBD RELATED MEDICATIONS - DRUG INDUCED LIVER INJURY

Most drugs used for IBD treatment have been reported to cause acute and/or chronic liver injury, although the incidence of serious complications is low. The mechanism of hepatotoxicity is complex and multifactorial; thus, causality may be difficult to establish[31,50,51].

Sulfasalazine and 5-aminosalicylic acid compounds

Sulfasalazine and 5-ASA compounds are used in mild-to-moderate UC. Sulfasalazine was the first aminosalicylate used for the treatment of IBD and can induce liver injury by several mechanisms[31]: (1) Hypersensitivity reaction that usually occurs within 2 mo of therapy initiation. A study revealed an incidence of 0.4% and symptoms include fever, rash, hepatomegaly, lymphadenophaty, atypical lymphocytosis, and eosinophilia. In most cases, stopping the medication is sufficient. In more severe cases, antipyretics, antihistamines, or corticosteroids may be considered[51-53]; (2) Sulfasalazine-induced granulomatous hepatitis, with elevated alkaline phosphatase and bilirubin and noncaseating granulomas on histology[51]; and (3) Cholestatic liver injury and, in rare cases, development of vanishing bile duct syndrome[54]. Mesalamine (5-ASA) is also associated with liver enzyme abnormalities in up to 2% of patients but, in most cases, it is not clinical significant[55].

Thiopurines

Azathioprine and its principal metabolite, 6-mercaptopurine, are immunomodulators used for maintenance or achievement of remission in patients with IBD.

Azathioprine is metabolized in mercaptopurine and then thiopurine methyltransferase (TPMT) will be responsible for its conversion to 6-methylmercaptopurine. Genetic polymorphisms of *TPMT* determine the level of enzyme activity and should be routinely tested before initiation of these medications. In cases of absent or low activity, thiopurines should be avoided due to high risk of toxicity, whereas in intermediate activity, a dose reduction should be applied[51,56].

The annual incidence of hepatotoxicity can reach 13% in prospective studies, although most resolve spontaneously or with dose adjustment, and need for discontinuation is rare (< 4%)[31,50,57].

Most cases of liver injury result in transient elevations of AST and ALT, but there are different types of hepatotoxicity[31,51,58-61]: (1) Allergic reaction, usually within the first month of treatment, which is not dose-dependent and should prompt immediate halt; (2) Non-allergic reactions, mainly associated with TPMT activity and dose-dependent, that can cause infections, bone marrow suppression, or hepatitis. Allopurinol has been suggested to alter metabolite levels and reduce hepatotoxicity; (3) Cholestatic liver injury, usually within the first 3 mo of therapy, requiring discontinuation; and (4) Hepatic endothelial injury that may present within 3 mo up to more than 4 years after therapy initiation. It can include sinusoidal dilatation, sinusoidal obstruction syndrome, peliosis, or nodular regenerative hyperplasia (NRH). NRH occurs due to endothelial injury and/or obliterative portal venopathy, with an estimated incidence of 0.8%, and can cause non-cirrhotic portal hypertension. It is dose-dependent and should prompt drug discontinuation.

Liver tests should be checked before starting thiopurines and repeated at weeks 2, 4 and 8, and every 3 mo thereafter. In the absence of previous liver disease, the prognosis of thiopurines-induced liver injury is good[51,56].

Methotrexate

Methotrexate is an immunosupressive and anti-proliferative agent used in the event of adverse effects or lack of efficacy of thiopurines for maintenance of clinical remission

in CD[6].

Myelossupression and liver toxicity are the most common side effects, with presence of abnormal aminotransferases levels in 24% of cases[62]. This liver injury is mainly associated with alcohol consumption, while folic acid supplementation seems to be protective[6].

There are also some reports of liver fibrosis and cirrhosis development, despite being more common in rheumatologic conditions, due to higher weekly dose use[6].

Most patients with liver injury due to methotrexate will have their liver function tests back to normal while on therapy and dose adjustment or discontinuation is rarely needed[62]. Regular liver function tests are recommended but liver biopsy is not routinely performed. Transient elastography is emerging as an interesting non-invasive tool to follow these patients[31,63].

Anti-TNF agents - infliximab and adalimumab

Infliximab and adalimumab are anti-TNF agents used for induction and maintenance of remission in moderate to severe CD and UC.

The main adverse effects are myelosuppression, opportunistic infections (namely tuberculosis), neurological diseases, and liver injury. There are reports of ALT increase in 39% of patients, although most (76%) of them were self-limited[64].

An auto-immune pattern of liver injury induced by anti-TNF agents with serological evidence (ANAs) has also been reported, which generally has a good prognosis as soon as the drug is stopped[51,65]. Cases of cholestatic liver injury and acute liver failure requiring liver transplant are very rare[66].

Liver functions tests should be checked in all patients before treatment institution [51].

Vedolizumab

Vedolizumab is an $\alpha_4\beta_7$ integrin inhibitor used in moderate to severe CD and UC.

In the premarketing trials, significant (≥ 3 ULN) elevations occurred in less than 2% of patients, similarly to those in the placebo arm[31]. Cholestatic and hepatocellular liver injuries have already been described in the post marketing analysis, which improved after drug discontinuation[67].

Naturally available anti-inflammatory compounds

Although less studied, there are several natural compounds that are tested for the treatment of IBD.

Curcumin, the main active compound of the plant *Curcuma longa*, has been shown to have anti-inflammatory, anti-oxidant, and antibacterial activities[68]. Kesharwani *et al* [69] showed that curcumin might have an important role in inhibiting IBD severity and colitis associated cancer. In addition, it has a good safety profile and is extremely well tolerated, besides some reports of its hepatoprotective effect[68,70-72].

Viral hepatitis and inflammatory bowel disease

Previous studies have suggested a higher prevalence of hepatitis B virus (HBV) and hepatitis C virus (HCV) infections in patients with IBD, due to blood transfusions and/or endoscopic procedures, which has not been demonstrated in more recent data [40,73,74].

HBV reactivation is one of the main concerns during IBD treatment, given the risk of fulminant hepatic failure and death[75]. Reactivation of HBV has already been described with high dose corticosteroids, thiopurines, and infliximab, though almost exclusively with concomitant use of other immunosuppressants[76-80]. Therefore, it is generally accepted that all patients with IBD should be screened for HBV exposure, preferably at diagnosis, which includes HBsAg and anti-HBs and anti-HBc antibodies [76,81]. According to the European Crohn's and Colitis Organisation (ECCO), IBD patients should follow these preventive measures[81]: Seronegative patients (HBsAg and anti-HBc negative) should be vaccinated and assessed for subsequent serological immune status; seropositive patients (HBsAg positive) should receive prophylactic treatment with nucleotide/nucleoside analogues for the time of treatment and at least 12 mo after stopping immunosuppressants; and HBsAg negative and anti-HBc positive patients should be monitored by HBV DNA quantification every 2-3 mo, since risk of HBV occult infection reactivation is low.

Regarding HCV infection, immunosuppressive therapy does not seem to have a detrimental effect on its course. Nevertheless, there are some reports of worsening liver function in the setting of concomitant HBV or HIV infection. Thus, the latest ECCO guidelines recommend systematic screening for HCV infection[81].

CONCLUSION

Hepatobiliary disease is one of the most common extra-intestinal manifestations in IBD patients, ranging from asymptomatic mild elevations of liver chemistries to life-threatening conditions.

Monitoring liver tests at regular intervals is crucial and must be routinely part of IBD management.

Abnormal liver tests in IBD patients may appear in the context of drug induced liver injury, common and easy to manage diseases such as NAFLD or cholelithiasis, as well as chronic and more complex diseases such as PSC or auto-immune hepatitis. As so, it should always prompt a structured and complete work-up and even benefit from a multidisciplinary approach, in order to improve patient management and outcomes.

REFERENCES

1 **Seyedian SS**, Nokhostin F, Malamir MD. A review of the diagnosis, prevention, and treatment methods of inflammatory bowel disease. *J Med Life* 2019; **12**: 113-122 [PMID: 31406511 DOI: 10.25122/jml-2018-0075]

2 **Harbord M**, Annese V, Vavricka SR, Allez M, Barreiro-de Acosta M, Boberg KM, Burisch J, De Vos M, De Vries AM, Dick AD, Juillerat P, Karlsen TH, Koutroubakis I, Lakatos PL, Orchard T, Papay P, Raine T, Reinshagen M, Thaci D, Tilg H, Carbonnel F; European Crohn's and Colitis Organisation. The First European Evidence-based Consensus on Extra-intestinal Manifestations in Inflammatory Bowel Disease. *J Crohns Colitis* 2016; **10**: 239-254 [PMID: 26614685 DOI: 10.1093/ecco-jcc/jjv213]

3 **DeFilippis EM**, Kumar S. Clinical Presentation and Outcomes of Autoimmune Hepatitis in Inflammatory Bowel Disease. *Dig Dis Sci* 2015; **60**: 2873-2880 [PMID: 25999245 DOI: 10.1007/s10620-015-3699-4]

4 **Yarur AJ**, Czul F, Levy C. Hepatobiliary manifestations of inflammatory bowel disease. *Inflamm Bowel Dis* 2014; **20**: 1655-1667 [PMID: 24874461 DOI: 10.1097/MIB.0000000000000065]

5 **Yaccob A**, Mari A. Practical clinical approach to the evaluation of hepatobiliary disorders in inflammatory bowel disease. *Frontline Gastroenterol* 2019; **10**: 309-315 [PMID: 31281626 DOI: 10.1136/flgastro-2018-101037]

6 **Rojas-Feria M**, Castro M, Suárez E, Ampuero J, Romero-Gómez M. Hepatobiliary manifestations in inflammatory bowel disease: the gut, the drugs and the liver. *World J Gastroenterol* 2013; **19**: 7327-7340 [PMID: 24259964 DOI: 10.3748/wjg.v19.i42.7327]

7 **Silva J**, Brito BS, Silva INN, Nóbrega VG, da Silva MCSM, Gomes HDN, Fortes FM, Pimentel AM, Mota J, Almeida N, Surlo VC, Lyra A, Rocha R, Santana GO. Frequency of Hepatobiliary Manifestations and Concomitant Liver Disease in Inflammatory Bowel Disease Patients. *Biomed Res Int* 2019; **2019**: 7604939 [PMID: 30834274 DOI: 10.1155/2019/7604939]

8 **European Society of Gastrointestinal Endoscopy**; European Association for the Study of the Liver. Role of endoscopy in primary sclerosing cholangitis: European Society of Gastrointestinal Endoscopy (ESGE) and European Association for the Study of the Liver (EASL) Clinical Guideline. *J Hepatol* 2017; **66**: 1265-1281 [PMID: 28427764 DOI: 10.1016/j.jhep.2017.02.013]

9 **Kummen M**, Schrumpf E, Boberg KM. Liver abnormalities in bowel diseases. *Best Pract Res Clin Gastroenterol* 2013; **27**: 531-542 [PMID: 24090940 DOI: 10.1016/j.bpg.2013.06.013]

10 **Palmela C**, Peerani F, Castaneda D, Torres J, Itzkowitz SH. Inflammatory Bowel Disease and Primary Sclerosing Cholangitis: A Review of the Phenotype and Associated Specific Features. *Gut Liver* 2018; **12**: 17-29 [PMID: 28376583 DOI: 10.5009/gnl16510]

11 **Loftus EV Jr**, Harewood GC, Loftus CG, Tremaine WJ, Harmsen WS, Zinsmeister AR, Jewell DA, Sandborn WJ. PSC-IBD: a unique form of inflammatory bowel disease associated with primary sclerosing cholangitis. *Gut* 2005; **54**: 91-96 [PMID: 15591511 DOI: 10.1136/gut.2004.046615]

12 **Nakazawa T**, Naitoh I, Hayashi K, Sano H, Miyabe K, Shimizu S, Joh T. Inflammatory bowel disease of primary sclerosing cholangitis: a distinct entity? *World J Gastroenterol* 2014; **20**: 3245-3254 [PMID: 24696608 DOI: 10.3748/wjg.v20.i12.3245]

13 **de Vries AB**, Janse M, Blokzijl H, Weersma RK. Distinctive inflammatory bowel disease phenotype in primary sclerosing cholangitis. *World J Gastroenterol* 2015; **21**: 1956-1971 [PMID: 25684965 DOI: 10.3748/wjg.v21.i6.1956]

14 **Marelli L**, Xirouchakis E, Kalambokis G, Cholongitas E, Hamilton MI, Burroughs AK. Does the severity of primary sclerosing cholangitis influence the clinical course of associated ulcerative colitis? *Gut* 2011; **60**: 1224-1228 [PMID: 21402617 DOI: 10.1136/gut.2010.235408]

15 **Rabinovitz M**, Gavaler JS, Schade RR, Dindzans VJ, Chien MC, Van Thiel DH. Does primary sclerosing cholangitis occurring in association with inflammatory bowel disease differ from that occurring in the absence of inflammatory bowel disease? *Hepatology* 1990; **11**: 7-11 [PMID: 2295474 DOI: 10.1002/hep.1840110103]

16 **Yanai H**, Matalon S, Rosenblatt A, Awadie H, Berdichevski T, Snir Y, Kopylov U, Katz L, Stein A, Mlynarsky L, Tulchinsky H, Konikoff FM, Horin SB, Braun M, Ben-Ari Z, Chowers Y, Baruch Y, Shibolet O, Dotan I. Prognosis of primary sclerosing cholangitis in israel is independent of coexisting

inflammatory bowel Disease. *J Crohns Colitis* 2015; **9**: 177-184 [PMID: 25518055 DOI: 10.1093/ecco-jcc/jju013]

17 **Zheng HH**, Jiang XL. Increased risk of colorectal neoplasia in patients with primary sclerosing cholangitis and inflammatory bowel disease: a meta-analysis of 16 observational studies. *Eur J Gastroenterol Hepatol* 2016; **28**: 383-390 [PMID: 26938805 DOI: 10.1097/MEG.0000000000000576]

18 **Farraye FA**, Odze RD, Eaden J, Itzkowitz SH, McCabe RP, Dassopoulos T, Lewis JD, Ullman TA, James T 3rd, McLeod R, Burgart LJ, Allen J, Brill JV; AGA Institute Medical Position Panel on Diagnosis and Management of Colorectal Neoplasia in Inflammatory Bowel Disease. AGA medical position statement on the diagnosis and management of colorectal neoplasia in inflammatory bowel disease. *Gastroenterology* 2010; **138**: 738-745 [PMID: 20141808 DOI: 10.1053/j.gastro.2009.12.037]

19 **Gulamhusein AF**, Eaton JE, Tabibian JH, Atkinson EJ, Juran BD, Lazaridis KN. Duration of Inflammatory Bowel Disease Is Associated With Increased Risk of Cholangiocarcinoma in Patients With Primary Sclerosing Cholangitis and IBD. *Am J Gastroenterol* 2016; **111**: 705-711 [PMID: 27002801 DOI: 10.1038/ajg.2016.55]

20 **Lindor KD**, Kowdley KV, Harrison ME; American College of Gastroenterology. ACG Clinical Guideline: Primary Sclerosing Cholangitis. *Am J Gastroenterol* 2015; **110**: 646-59; quiz 660 [PMID: 25869391 DOI: 10.1038/ajg.2015.112]

21 **Karlsen TH**, Boberg KM. Update on primary sclerosing cholangitis. *J Hepatol* 2013; **59**: 571-582 [PMID: 23603668 DOI: 10.1016/j.jhep.2013.03.015]

22 **Nakazawa T**, Naitoh I, Hayashi K, Okumura F, Miyabe K, Yoshida M, Yamashita H, Ohara H, Joh T. Diagnostic criteria for IgG4-related sclerosing cholangitis based on cholangiographic classification. *J Gastroenterol* 2012; **47**: 79-87 [PMID: 21947649 DOI: 10.1007/s00535-011-0465-z]

23 **Carey EJ**, Ali AH, Lindor KD. Primary biliary cirrhosis. *Lancet* 2015; **386**: 1565-1575 [PMID: 26364546 DOI: 10.1016/S0140-6736(15)00154-3]

24 **Xiao WB**, Liu YL. Primary biliary cirrhosis and ulcerative colitis: a case report and review of literature. *World J Gastroenterol* 2003; **9**: 878-880 [PMID: 12679954 DOI: 10.3748/wjg.v9.i4.878]

25 **Liberal R**, Gaspar R, Lopes S, Macedo G. Primary biliary cholangitis in patients with inflammatory bowel disease. *Clin Res Hepatol Gastroenterol* 2020; **44**: e5-e9 [PMID: 31171469 DOI: 10.1016/j.clinre.2019.05.002]

26 **European Association for the Study of the Liver**. EASL Clinical Practice Guidelines: Autoimmune hepatitis. *J Hepatol* 2015; **63**: 971-1004 [PMID: 26341719 DOI: 10.1016/j.jhep.2015.06.030]

27 **Saich R**, Chapman R. Primary sclerosing cholangitis, autoimmune hepatitis and overlap syndromes in inflammatory bowel disease. *World J Gastroenterol* 2008; **14**: 331-337 [PMID: 18200656 DOI: 10.3748/wjg.14.331]

28 **Ordonez F**, Lacaille F, Canioni D, Talbotec C, Fournet JC, Cerf-Bensussan N, Goulet O, Schmitz J, Ruemmele FM. Pediatric ulcerative colitis associated with autoimmune diseases: a distinct form of inflammatory bowel disease? *Inflamm Bowel Dis* 2012; **18**: 1809-1817 [PMID: 22238154 DOI: 10.1002/ibd.22864]

29 **Agrawal M**, Kim ES, Colombel JF. JAK Inhibitors Safety in Ulcerative Colitis: Practical Implications. *J Crohns Colitis* 2020; **14**: S755-S760 [PMID: 32006031 DOI: 10.1093/ecco-jcc/jjaa017]

30 **Malik TA**, Gutierrez AM, McGuire B, Zarzour JG, Mukhtar F, Bloomer J. Autoimmune hepatitis-primary sclerosing cholangitis overlap syndrome complicated by Crohn's disease. *Digestion* 2010; **82**: 24-26 [PMID: 20160443 DOI: 10.1159/000273735]

31 **Restellini S**, Chazouillères O, Frossard JL. Hepatic manifestations of inflammatory bowel diseases. *Liver Int* 2017; **37**: 475-489 [PMID: 27712010 DOI: 10.1111/liv.13265]

32 **Czaja AJ**. Diagnosis and management of the overlap syndromes of autoimmune hepatitis. *Can J Gastroenterol* 2013; **27**: 417-423 [PMID: 23862175 DOI: 10.1155/2013/198070]

33 **Sinagra E**, Aragona E, Romano C, Maisano S, Orlando A, Virdone R, Tesè L, Modesto I, Criscuoli V, Cottone M. The role of portal vein thrombosis in the clinical course of inflammatory bowel diseases: report on three cases and review of the literature. *Gastroenterol Res Pract* 2012; **2012**: 916428 [PMID: 23093957 DOI: 10.1155/2012/916428]

34 **Remzi FH**, Fazio VW, Oncel M, Baker ME, Church JM, Ooi BS, Connor JT, Preen M, Einstein D. Portal vein thrombi after restorative proctocolectomy. *Surgery* 2002; **132**: 655-61; discussion 661 [PMID: 12407350 DOI: 10.1067/msy.2002.127689]

35 **Spina L**, Saibeni S, Battaglioli T, Peyvandi F, de Franchis R, Vecchi M. Thrombosis in inflammatory bowel diseases: role of inherited thrombophilia. *Am J Gastroenterol* 2005; **100**: 2036-2041 [PMID: 16128949 DOI: 10.1111/j.1572-0241.2005.42029.x]

36 **Braun M**, Fraser GM, Kunin M, Salamon F, Tur-Kaspa R. Mesalamine-induced granulomatous hepatitis. *Am J Gastroenterol* 1999; **94**: 1973-1974 [PMID: 10406274 DOI: 10.1111/j.1572-0241.1999.01245.x]

37 **Margalit M**, Elinav H, Ilan Y, Shalit M. Liver abscess in inflammatory bowel disease: report of two cases and review of the literature. *J Gastroenterol Hepatol* 2004; **19**: 1338-1342 [PMID: 15610305 DOI: 10.1111/j.1440-1746.2004.03368.x]

38 **Albuquerque A**, Magro F, Rodrigues S, Lopes S, Pereira P, Melo RB, Madureira M, Macedo G. Liver abscess of the caudate lobe due to Staphylococcus aureus in an ulcerative colitis patient: First case report. *J Crohns Colitis* 2011; **5**: 360-363 [PMID: 21683308 DOI: 10.1016/j.crohns.2011.02.012]

39 **Parente F**, Pastore L, Bargiggia S, Cucino C, Greco S, Molteni M, Ardizzone S, Porro GB, Sampietro GM, Giorgi R, Moretti R, Gallus S. Incidence and risk factors for gallstones in patients with inflammatory bowel disease: a large case-control study. *Hepatology* 2007; **45**: 1267-1274 [PMID: 17464998 DOI: 10.1002/hep.21537]

40 **Fousekis FS**, Theopistos VI, Katsanos KH, Tsianos EV, Christodoulou DK. Hepatobiliary Manifestations and Complications in Inflammatory Bowel Disease: A Review. *Gastroenterology Res* 2018; **11**: 83-94 [PMID: 29707074 DOI: 10.14740/gr990w]

41 **Chew SS**, Ngo TQ, Douglas PR, Newstead GL, Selby W, Solomon MJ. Cholecystectomy in patients with Crohn's ileitis. *Dis Colon Rectum* 2003; **46**: 1484-1488 [PMID: 14605566 DOI: 10.1007/s10350-004-6798-4]

42 **Greenstein AJ**, Sachar DB, Panday AK, Dikman SH, Meyers S, Heimann T, Gumaste V, Werther JL, Janowitz HD. Amyloidosis and inflammatory bowel disease. A 50-year experience with 25 patients. *Medicine (Baltimore)* 1992; **71**: 261-270 [PMID: 1522802 DOI: 10.1097/00005792-199209000-00001]

43 **Kato T**, Komori A, Bae SK, Migita K, Ito M, Motoyoshi Y, Abiru S, Ishibashi H. Concurrent systemic AA amyloidosis can discriminate primary sclerosing cholangitis from IgG4-associated cholangitis. *World J Gastroenterol* 2012; **18**: 192-196 [PMID: 22253527 DOI: 10.3748/wjg.v18.i2.192]

44 **Hoffmann P**, Jung V, Behnisch R, Gauss A. Prevalence and risk factors of nonalcoholic fatty liver disease in patients with inflammatory bowel diseases: A cross-sectional and longitudinal analysis. *World J Gastroenterol* 2020; **26**: 7367-7381 [PMID: 33362390 DOI: 10.3748/wjg.v26.i46.7367]

45 **Likhitsup A**, Dundulis J, Ansari S, El-Halawany H, Michelson R, Hutton C, Kennedy K, Helzberg JH, Chhabra R. Prevalence of non-alcoholic fatty liver disease on computed tomography in patients with inflammatory bowel disease visiting an emergency department. *Ann Gastroenterol* 2019; **32**: 283-286 [PMID: 31040626 DOI: 10.20524/aog.2019.0371]

46 **Gaidos JKJ**, Fuchs M. Increased Prevalence of NAFLD in IBD Patients. *Dig Dis Sci* 2017; **62**: 1362 [PMID: 28357696 DOI: 10.1007/s10620-017-4552-8]

47 **Bessissow T**, Le NH, Rollet K, Afif W, Bitton A, Sebastiani G. Incidence and Predictors of Nonalcoholic Fatty Liver Disease by Serum Biomarkers in Patients with Inflammatory Bowel Disease. *Inflamm Bowel Dis* 2016; **22**: 1937-1944 [PMID: 27379445 DOI: 10.1097/MIB.0000000000000832]

48 **Chao CY**, Battat R, Al Khoury A, Restellini S, Sebastiani G, Bessissow T. Co-existence of non-alcoholic fatty liver disease and inflammatory bowel disease: A review article. *World J Gastroenterol* 2016; **22**: 7727-7734 [PMID: 27678354 DOI: 10.3748/wjg.v22.i34.7727]

49 **Barbuio R**, Milanski M, Bertolo MB, Saad MJ, Velloso LA. Infliximab reverses steatosis and improves insulin signal transduction in liver of rats fed a high-fat diet. *J Endocrinol* 2007; **194**: 539-550 [PMID: 17761893 DOI: 10.1677/JOE-07-0234]

50 **Gisbert JP**, Luna M, González-Lama Y, Pousa ID, Velasco M, Moreno-Otero R, Maté J. Liver injury in inflammatory bowel disease: long-term follow-up study of 786 patients. *Inflamm Bowel Dis* 2007; **13**: 1106-1114 [PMID: 17455203 DOI: 10.1002/ibd.20160]

51 **Shamberg L**, Vaziri H. Hepatotoxicity of Inflammatory Bowel Disease Medications. *J Clin Gastroenterol* 2018; **52**: 674-684 [PMID: 30036242 DOI: 10.1097/MCG.0000000000001084]

52 **Bashir RM**, Lewis JH. Hepatotoxicity of drugs used in the treatment of gastrointestinal disorders. *Gastroenterol Clin North Am* 1995; **24**: 937-967 [PMID: 8749906 DOI: 10.1016/s0889-8553(21)00235-1]

53 **Jobanputra P**, Amarasena R, Maggs F, Homer D, Bowman S, Rankin E, Filer A, Raza K, Jubb R. Hepatotoxicity associated with sulfasalazine in inflammatory arthritis: A case series from a local surveillance of serious adverse events. *BMC Musculoskelet Disord* 2008; **9**: 48 [PMID: 18405372 DOI: 10.1186/1471-2474-9-48]

54 **Khokhar OS**, Lewis JH. Hepatotoxicity of agents used in the management of inflammatory bowel disease. *Dig Dis* 2010; **28**: 508-518 [PMID: 20926880 DOI: 10.1159/000320410]

55 **Loftus EV Jr**, Kane SV, Bjorkman D. Systematic review: short-term adverse effects of 5-aminosalicylic acid agents in the treatment of ulcerative colitis. *Aliment Pharmacol Ther* 2004; **19**: 179-189 [PMID: 14723609 DOI: 10.1111/j.0269-2813.2004.01827.x]

56 **Kopylov U**, Ben-Horin S, Seidman E. Therapeutic drug monitoring in inflammatory bowel disease. *Ann Gastroenterol* 2014; **27**: 304-312 [PMID: 25331715 DOI: 10.1097/mib.0000000000000497]

57 **Gisbert JP**, González-Lama Y, Maté J. Thiopurine-induced liver injury in patients with inflammatory bowel disease: a systematic review. *Am J Gastroenterol* 2007; **102**: 1518-1527 [PMID: 17391318 DOI: 10.1111/j.1572-0241.2007.01187.x]

58 **Björnsson ES**, Gu J, Kleiner DE, Chalasani N, Hayashi PH, Hoofnagle JH; DILIN Investigators. Azathioprine and 6-Mercaptopurine-induced Liver Injury: Clinical Features and Outcomes. *J Clin Gastroenterol* 2017; **51**: 63-69 [PMID: 27648552 DOI: 10.1097/MCG.0000000000000568]

59 **Morris JM**, Oien KA, McMahon M, Forrest EH, Morris J, Stanley AJ, Campbell S. Nodular regenerative hyperplasia of the liver: survival and associated features in a UK case series. *Eur J Gastroenterol Hepatol* 2010; **22**: 1001-1005 [PMID: 20075739 DOI: 10.1097/MEG.0b013e3283360021]

60 **Vernier-Massouille G**, Cosnes J, Lemann M, Marteau P, Reinisch W, Laharie D, Cadiot G, Bouhnik Y, De Vos M, Boureille A, Duclos B, Seksik P, Mary JY, Colombel JF. Nodular regenerative hyperplasia in patients with inflammatory bowel disease treated with azathioprine. *Gut* 2007; **56**:

1404-1409 [PMID: 17504943 DOI: 10.1136/gut.2006.114363]
61 **Calabrese E**, Hanauer SB. Assessment of non-cirrhotic portal hypertension associated with thiopurine therapy in inflammatory bowel disease. *J Crohns Colitis* 2011; **5**: 48-53 [PMID: 21272804 DOI: 10.1016/j.crohns.2010.08.007]
62 **Fournier MR**, Klein J, Minuk GY, Bernstein CN. Changes in liver biochemistry during methotrexate use for inflammatory bowel disease. *Am J Gastroenterol* 2010; **105**: 1620-1626 [PMID: 20160715 DOI: 10.1038/ajg.2010.21]
63 **Laharie D**, Zerbib F, Adhoute X, Boué-Lahorgue X, Foucher J, Castéra L, Rullier A, Bertet J, Couzigou P, Amouretti M, de Lédinghen V. Diagnosis of liver fibrosis by transient elastography (FibroScan) and non-invasive methods in Crohn's disease patients treated with methotrexate. *Aliment Pharmacol Ther* 2006; **23**: 1621-1628 [PMID: 16696812 DOI: 10.1111/j.1365-2036.2006.02929.x]
64 **Rossi RE**, Parisi I, Despott EJ, Burroughs AK, O'Beirne J, Conte D, Hamilton MI, Murray CD. Anti-tumour necrosis factor agent and liver injury: literature review, recommendations for management. *World J Gastroenterol* 2014; **20**: 17352-17359 [PMID: 25516646 DOI: 10.3748/wjg.v20.i46.17352]
65 **Ghabril M**, Bonkovsky HL, Kum C, Davern T, Hayashi PH, Kleiner DE, Serrano J, Rochon J, Fontana RJ, Bonacini M; US Drug-Induced Liver Injury Network. Liver injury from tumor necrosis factor-α antagonists: analysis of thirty-four cases. *Clin Gastroenterol Hepatol* 2013; **11**: 558-564. e3 [PMID: 23333219 DOI: 10.1016/j.cgh.2012.12.025]
66 **Tobon GJ**, Cañas C, Jaller JJ, Restrepo JC, Anaya JM. Serious liver disease induced by infliximab. *Clin Rheumatol* 2007; **26**: 578-581 [PMID: 16547695 DOI: 10.1007/s10067-005-0169-y]
67 **Stine JG**, Wang J, Behm BW. Chronic Cholestatic Liver Injury Attributable to Vedolizumab. *J Clin Transl Hepatol* 2016; **4**: 277-280 [PMID: 27777897 DOI: 10.14218/JCTH.2016.00018]
68 **Rivera-Espinoza Y**, Muriel P. Pharmacological actions of curcumin in liver diseases or damage. *Liver Int* 2009; **29**: 1457-1466 [PMID: 19811613 DOI: 10.1111/j.1478-3231.2009.02086.x]
69 **Kesharwani SS**, Ahmad R, Bakkari MA, Rajput MKS, Dachineni R, Valiveti CK, Kapur S, Jayarama Bhat G, Singh AB, Tummala H. Site-directed non-covalent polymer-drug complexes for inflammatory bowel disease (IBD): Formulation development, characterization and pharmacological evaluation. *J Control Release* 2018; **290**: 165-179 [PMID: 30142410 DOI: 10.1016/j.jconrel.2018.08.004]
70 **Kyung EJ**, Kim HB, Hwang ES, Lee S, Choi BK, Kim JW, Kim HJ, Lim SM, Kwon OI, Woo EJ. Evaluation of Hepatoprotective Effect of Curcumin on Liver Cirrhosis Using a Combination of Biochemical Analysis and Magnetic Resonance-Based Electrical Conductivity Imaging. *Mediators Inflamm* 2018; **2018**: 5491797 [PMID: 29887757 DOI: 10.1155/2018/5491797]
71 **Granados-Castro LF**, Rodríguez-Rangel DS, Fernández-Rojas B, León-Contreras JC, Hernández-Pando R, Medina-Campos ON, Eugenio-Pérez D, Pinzón E, Pedraza-Chaverri J. Curcumin prevents paracetamol-induced liver mitochondrial alterations. *J Pharm Pharmacol* 2016; **68**: 245-256 [PMID: 26773315 DOI: 10.1111/jphp.12501]
72 **Wang X**, Chang X, Zhan H, Zhang Q, Li C, Gao Q, Yang M, Luo Z, Li S, Sun Y. Curcumin and Baicalin ameliorate ethanol-induced liver oxidative damage *via* the Nrf2/HO-1 pathway. *J Food Biochem* 2020; e13425 [PMID: 32770697 DOI: 10.1111/jfbc.13425]
73 **Biancone L**, Pavia M, Del Vecchio Blanco G, D'Incà R, Castiglione F, De Nigris F, Doldo P, Cosco F, Vavassori P, Bresci GP, Arrigoni A, Cadau G, Monteleone I, Rispo A, Fries W, Mallardi B, Sturniolo GC, Pallone F; Italian Group for the Study of the Colon and Rectum (GISC). Hepatitis B and C virus infection in Crohn's disease. *Inflamm Bowel Dis* 2001; **7**: 287-294 [PMID: 11720317 DOI: 10.1097/00054725-200111000-00002]
74 **Mahfouz M**, Martin P, Carrion AF. Hepatic Complications of Inflammatory Bowel Disease. *Clin Liver Dis* 2019; **23**: 191-208 [PMID: 30947871 DOI: 10.1016/j.cld.2018.12.003]
75 **Hou JK**, Velayos F, Terrault N, Mahadevan U. Viral hepatitis and inflammatory bowel disease. *Inflamm Bowel Dis* 2010; **16**: 925-932 [PMID: 20480515 DOI: 10.1002/ibd.21284]
76 **Gisbert JP**, Chaparro M, Esteve M. Review article: prevention and management of hepatitis B and C infection in patients with inflammatory bowel disease. *Aliment Pharmacol Ther* 2011; **33**: 619-633 [PMID: 21416659 DOI: 10.1111/j.1365-2036.2010.04570.x]
77 **Cheng AL**, Hsiung CA, Su IJ, Chen PJ, Chang MC, Tsao CJ, Kao WY, Uen WC, Hsu CH, Tien HF, Chao TY, Chen LT, Whang-Peng J; Lymphoma Committee of Taiwan Cooperative Oncology Group. Steroid-free chemotherapy decreases risk of hepatitis B virus (HBV) reactivation in HBV-carriers with lymphoma. *Hepatology* 2003; **37**: 1320-1328 [PMID: 12774010 DOI: 10.1053/jhep.2003.50220]
78 **Sacco R**, Bertini M, Bresci G, Romano A, Altomare E, Capria A. Entecavir for hepatitis B virus flare treatment in patients with Crohn's disease. *Hepatogastroenterology* 2010; **57**: 242-245 [PMID: 20583421 DOI: 10.1016/j.gcb.2010.09.009]
79 **Ostuni P**, Botsios C, Punzi L, Sfriso P, Todesco S. Hepatitis B reactivation in a chronic hepatitis B surface antigen carrier with rheumatoid arthritis treated with infliximab and low dose methotrexate. *Ann Rheum Dis* 2003; **62**: 686-687 [PMID: 12810441 DOI: 10.1136/ard.62.7.686]
80 **Michel M**, Duvoux C, Hezode C, Cherqui D. Fulminant hepatitis after infliximab in a patient with hepatitis B virus treated for an adult onset still's disease. *J Rheumatol* 2003; **30**: 1624-1625 [PMID: 12858469]
81 **Rahier JF**, Magro F, Abreu C, Armuzzi A, Ben-Horin S, Chowers Y, Cottone M, de Ridder L, Doherty G, Ehehalt R, Esteve M, Katsanos K, Lees CW, Macmahon E, Moreels T, Reinisch W, Tilg H, Tremblay L, Veereman-Wauters G, Viget N, Yazdanpanah Y, Eliakim R, Colombel JF; European Crohn's and Colitis Organisation (ECCO). Second European evidence-based consensus on the

prevention, diagnosis and management of opportunistic infections in inflammatory bowel disease. *J Crohns Colitis* 2014; **8**: 443-468 [PMID: 24613021 DOI: 10.1016/j.crohns.2013.12.013]

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MINIREVIEWS

Dengue hemorrhagic fever and the liver

Wattana Leowattana, Tawithep Leowattana

ORCID number: Wattana Leowattana 0000-0003-4257-2480; Tawithep Leowattana 0000-0003-2316-3585.

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Wattana Leowattana, Department of Clinical Tropical Medicine, Faculty of Tropical Medicine, Mahidol University, Bangkok 10400, Bangkok, Thailand

Tawithep Leowattana, Department of Medicine, Faculty of Medicine, Srinakharinwirot University, Bangkok 10110, Bangkok, Thailand

Corresponding author: Wattana Leowattana, BSc, MD, MSc, PhD, Associate Professor, Senior Researcher, Staff Physician, Department of Clinical Tropical Medicine, Faculty of Tropical Medicine, Mahidol University, 420/6 Rajavithi Road, Rachatawee, Bangkok 10400, Bangkok, Thailand. wattana.leo@mahidol.ac.th

Abstract

Dengue hemorrhagic fever (DHF) is one of the most rapidly emerging infections of tropical and subtropical regions worldwide. It affects more rural and urban areas due to many factors, including climate change. Although most people with dengue viral infection are asymptomatic, approximately 25% experience a self-limited febrile illness with mild to moderate biochemical abnormalities. Severe dengue diseases develop in a small proportion of these patients, and the common organ involvement is the liver. The hepatocellular injury was found in 60%-90% of DHF patients manifested as hepatomegaly, jaundice, elevated aminotransferase enzymes, and critical condition as an acute liver failure (ALF). Even the incidence of ALF in DHF is very low (0.31%-1.1%), but it is associated with a relatively high mortality rate (20%-68.3%). The pathophysiology of liver injury in DHF included the direct cytopathic effect of the DENV causing hepatocytes apoptosis, immune-mediated hepatocyte injury induced hepatitis, and cytokine storm. Hepatic hypoperfusion is another contributing factor in dengue shock syndrome. The reduction of morbidity and mortality in DHF with liver involvement is dependent on the early detection of warning signs before the development of ALF.

Key Words: Dengue hemorrhagic fever; Dengue viral infection; Liver involvement; Liver injury; Acute liver failure; Hepatocyte apoptosis; Cytokine storm; Severe dengue disease

Core Tip: The liver is the most common organ involvement in dengue hemorrhagic fever (DHF) patients with ranges from mild subclinical biochemical changes to severe liver disease as an acute liver failure (ALF). However, the low incidence of ALF in

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DHF with liver injury is associated with a high fatality rate. The hepatocyte injury is caused by direct viral cytopathic, immune-mediated, and poor hepatic perfusion. Early detection of severe hepatocellular injury development may reduce the morbidity and mortality in DHF patients with liver involvement.

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INTRODUCTION

Dengue virus (DENV) is a mosquito-borne flavivirus that consists of four serotypes (1–4) circulating in endemic areas. Most DENV infections are asymptomatic. However, the clinical manifestation of DENV infections could be dengue fever (DF), dengue hemorrhagic fever (DHF), or dengue shock syndrome (DSS). Dengue is one of the most rapidly evolving vector-borne infections, affecting 129 countries, 70% of the actual burden is in Asia, causing nearly 390 million affected patients each year, of which 96 million manifests clinically. The number of dengue cases reported to World Health Organization increased over eightfold during the last two decades, from 505430 cases in 2000 to over 2.4 million in 2010 and 4.2 million in 2019[1]. It is predicted that the transmission of dengue will be more strengthened in dengue-endemic countries, and due to climate change and increases in international traveling, the infection may spread to countries in Europe and the US that are currently not significantly affected by DENV[2,3]. Liver injury associated with DENV infection was first reported in 1967 [4]. The liver is one of the common organs involved in dengue infection. Hepatic complications were found in 60%-90% of infected patients included hepatomegaly, jaundice, elevated aspartate aminotransferase (AST), elevated alanine aminotransferase (ALT), and acute liver failure (ALF). All four serotypes have been associated with dengue-related liver injury, but DENV-1 and DENV-3 have more significant injuries[5]. Abnormal liver function in DENV infections resulted from the direct viral effect on hepatocytes or a dysregulated immunologic injury against the virus[6]. Moreover, underlying chronic diseases common among adults in several tropical and sub-tropical countries potentially compound the effects of acute dengue-related liver injury. However, the evidence to date is still conflicting and needs to be elucidated. We review the current evidence on liver injury in DHF patients and discuss the association between clinical manifestations, laboratory findings, pathological findings, and molecular evidence with the pathophysiology of a derangement of the liver in DHF.

GENOMIC ORGANIZATION OF THE DENGUE VIRUS

DENV genome is a linear, single-stranded, positive-sense RNA which translated as a single open reading frame. It was bordered by 50 and 30 untranslated regions on each side. DENV particle was a spherical 50 nm virion. The ssRNA genome was encapsulated by multiple copies of the capsid (C) protein to form a nucleocapsid core. This core is covered by a lipid bilayer forming an outer glycoprotein envelop (E) protective casing. When DENV enters the host cell, the positive ssRNA genome is released from the capsid and translated to a polyprotein of 3400 amino acids. The polyprotein is subsequently cleaved by viral and host proteases to 10 kinds of protein. These proteins are three structural proteins [C, E, pre-membrane (prM)] and seven nonstructural (NS) proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5)[7,8]. The structural proteins are essential in virion assembly, release, maturation, and infectivity. In comparison, viral replication and eluding a host cell's immune response are the NS proteins' primary functions. DENV has four serotypes (DEN 1-4), each sharing 60%-70% amino acid sequence homology.

DENGUE HEMORRHAGIC FEVER AND LIVER INVOLVEMENT

Clinical manifestations and laboratory findings

The spectrum of symptoms in DHF patients is very diverse, ranging from mild to severe dengue disease (SDD). DENV infection (DVI) has an incubation period of 3-14 d with the same symptom as a common cold and gastroenteritis. The patients usually have an abrupt fever, retro-orbital pain, headache, muscle ache, arthralgia, nausea, vomiting, diarrhea, and rashes. Less than 5% of DVI patients progress to severe life-threatening manifestations, particularly those previously infected with different serotypes. DHF has 3 distinct phases comprise of febrile, critical, and recovery. The patient has a biphasic fever commonly over 40°C with retro-orbital pain and headache ranging 2-7 d for the febrile phase. Fifty to eighty percent of the patients exhibit rashes or petechiae. The critical phase is characterized by plasma leakage with or without bleeding, which starts abruptly after defervescence. During this phase, an increase in capillary permeability with the rising of hematocrit can occur[9,10]. Moreover, the accumulation of fluids in the abdominal cavities and thoracic could be detected, leading to hypovolemic shock resulting in multiple organ dysfunctions, metabolic acidosis, disseminated intravascular coagulation (DIC), and severe bleeding. The mortality rate of SDD is relatively high at 20%, while early and appropriate treatment with intravenous fluid can decrease mortality to less than 1%. The recovery phase lasts for a few days with rash and a fluid overload, affecting the brain as a reduced level of consciousness or seizures[11,12].

Hepatic injury in DVI is more common in DHF than DF. Moreover, it is more severe in children patients, especially in previous dengue infection (primary infection), high hematocrit values, low platelet counts, and vascular leakage[13-15]. The clinical manifestations of DHF with hepatic involvement were from mild biochemical changes without symptoms to ALF. It manifests as right subcostal pain, hepatomegaly with tenderness, elevated aminotransferase enzymes, hyper-bilirubinemia, hypoalbuminemia, or ALF. The prevalence of liver involvement in DHF has many variations across different investigators (Table 1). This variation probably from the difference in DENV serotypes, case definition, age group, host susceptibilities, pre-existing diseases, especially chronic liver diseases (CLD). The most common symptoms associated with liver involvement in DHF are anorexia, nausea, vomiting, and abdominal pain[16-19,23,25-27,29]. The most common physical sign is hepatomegaly, with a wide range from several studies between 10.0 to 80.8% of the patients. The smaller number of DHF patients are clinically jaundiced (3.6%-48%)[16,21,26,28,29,31]. The hepatomegaly demonstrated an increased risk for SDD with an odds ratio of 4.75 (95%CI: 1.76-12.57)[32].

The elevation of AST and ALT is the commonest finding of DHF with liver involvement[16-31]. The elevated AST is usually modest and greater than ALT. The greater elevation in AST than ALT is partly due to AST release from muscles damaged. Mean AST and ALT concentrations ranged from 2-fold to 5-fold rises, which demonstrated mild hepatitis with self-limited. The 10-fold elevation of AST and ALT was reported in 4%-15% of the patients associated with SDD and may deteriorate to be ALF[33,34]. The physical sign of hepatomegaly with hepatic tenderness did not predict the rising of AST and ALT[16]. The highest level of AST and ALT occurs approximately day 7 of fever and should return to the normal level within 21 d of illness. The elevation of AST and ALT appears to correlate with SDD[30,35]. Hypoalbuminemia has been reported in broad ranges from 35.3%-76.0% in several studies due to the population heterogeneity and the disease severity[16,20,27-29]. The meta-analysis conducted by Huy and colleagues revealed that hypoalbuminemia was significantly associated with DSS[35]. Abnormal coagulation has been found in many studies with 34.0%-42.5% of prolonged prothrombin time (PT) and partial thromboplastin time (PTT)[16,21,26]. Notably, consumptive coagulopathy may also contribute to DSS.

Pathological findings

Pathological studies in humans DHF are uncommon and limited as the liver biopsy is invasive and hazardous. The human hepatocytes are an essential site for replication of DENV[36]. In 2014, Aye and colleagues reported an autopsy study of 13 patients who died of severe DHF. They found that the liver had significant levels of DENV RNA and histopathological changes consisting of microvesicular and macrovesicular steatosis, Councilman bodies, hepatocellular necrosis, and lack of inflammatory cell infiltrates[37]. In the liver, DENV infection occurred in hepatocytes and Kupffer cells but not in endothelial cells. Other studies reported the same pathological findings[34,38,39]. Recently, Win and colleagues reported that the prominent findings of the ultrastructure features of human liver specimens from patients who died of DHF were

Table 1 Clinical and laboratory findings of Dengue hemorrhagic fever with liver involvement

Investigators	No. of patients	Hepatomegaly (%)	Elevated AST (%)	Elevated ALT (%)	Hyper-bilirubinemia (%)	Low albumin (%)
Bandyopadhyay *et al* [16]	110	79.1	92.7	78.2	4.5	66.4
Kittitrakul *et al*[17]	127	34.6	88.2	69.3	N/A	N/A
Saha *et al*[18]	570	28.6	N/A	N/A	N/A	N/A
Roy *et al*[19]	120	80.8	94.2	89.2	N/A	N/A
Nascimento *et al*[20]	68	N/A	83.8	73.5	N/A	35.3
Karoli *et al*[21]	138	N/A	N/A	92.0	48.0	N/A
Lee *et al*[22]	690	N/A	86.0	46.0	N/A	N/A
Jagadishkumar *et al*[23]	110	79.0	93.6	78.2	N/A	N/A
Parkash *et al*[24]	699	N/A	95.0	86.0	N/A	N/A
Trung *et al*[25]	644	34.8	97.0	97.0	N/A	N/A
Wong and Shen [26]	127	11.8	90.6	71.7	13.4	N/A
Uehara *et al*[27]	41	10.0	80.5	61.0	N/A	48.4
Itha *et al*[28]	45	N/A	96.0	96.0	30.0	76.0
Fernando *et al*[29]	55	36.4	90.1	81.8	3.6	72.7
Souza *et al*[30]	1585	N/A	63.4	45.0	N/A	N/A
Kuo *et al*[31]	270	N/A	93.3	82.2	7.2	N/A

AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; N/A: Not applicable.

extensive cellular damage and steatosis. Moreover, no virus-induced endoplasmic replicating structures have been identified in the hepatocytes. They postulated that DENV in the hepatocytes and Kupffer cells might not be the key contributor to hepatic steatosis[40]. Hepatic steatosis was the significant pathologic finding in acute alcoholic and non-alcoholic steatohepatitis[41]. The hypotheses on the mechanism of hepatic steatosis were the breakdown of the intestinal barrier, allowing bacterial pathogens to reach the liver (microbial translocation). Recent studies demonstrated that elevated lipopolysaccharide (LPS) levels during DVIs correlated with disease severity, primarily when determined in plasma leakage[42,43].

DHF AND ACUTE LIVER FAILURE

ALF is a rare condition in DHF patients. Kye Mon and colleagues conducted a retrospective cohort study to evaluate the incidence and clinical outcome in 1926 patients with DHF. They reported the 0.31% incidence of ALF associated with DHF. It was most common among young adults with the median duration from onset of fever to ALF development was 7.5 d. The patients with the severe stage of dengue had a higher risk of developing ALF. They concluded that although the development of ALF is relatively rare in patients with DHF, it is associated with a high mortality rate (66.7%) (Table 2)[44]. In 2010, Trung and colleagues conducted a study to evaluate the liver involvement associated with DVI in 644 adults and found that ALF was 0.77% with a 20.0% mortality rate. They concluded that clinically severe liver involvement was infrequent but usually resulted in severe clinical outcomes[25]. In 2016, Laoprasopwattana and colleagues reported the study of clinical course and outcomes of liver functions in children with dengue viral infection-caused ALF. They found that 41 patients (1.1%) of 3630 DHF children had ALF. The fatality rate of DVI-caused ALF in this study was 28 of 41 (68.3%) compared with 2 of 197 (1.0%) in severe dengue patients without ALF. They concluded that the DHF patients with ALF had the major cause from the profound shock, which induced microcirculatory abnormality in the liver cells[45]. In 2020, Devarbhavi and colleagues conducted the study to determine the incidence and clinical outcome in 10108 DHF patients. They found that 36 patients

Table 2 The incidence and mortality rate of acute liver failure in Dengue hemorrhagic fever patients with liver involvement

Investigators	Countries	Study population	Incidence rate (%)	Mortality rate (%)
Teerasarntipan *et al*[46]	Thailand	2311 adults	0.71	58.82
Devarbhavi *et al*[34]	Qatar	10108 adults	0.35	58.30
Laoprasopwattana *et al*[45]	Thailand	3630 children	1.10	68.30
Trung *et al*[25]	Vietnam	644 adults	0.77	20.00
Kye Mon *et al*[44]	Thailand	1926 age ≥ 15 yr	0.31	66.70

(0.35%) developed ALF with a 58.3% mortality rate. They concluded that dengue hepatitis progressing to ALF is rare and were seen in only 0.35%. However, the development of ALF is associated with a very high mortality rate. Lactate levels, pH, and model for end-stage liver disease (MELD) score at admission were the only predictors of mortality[34]. Recently, Teerasarntipan and colleagues conducted a retrospective study of 2311 serologically confirmed adult dengue patients to evaluate ALF and fatality rate incidence. They found that ALF incidence in their study was 17 of 2396 DHF patients (0.71%). The mortality rate of ALF was 10 of 17 SDD patients (58.82%). They concluded that the MELD score is the best predictor of ALF in dengue-induced severe hepatitis (DISH) patients[46].

PATHOPHYSIOLOGY OF LIVER DAMAGE IN DHF

The mechanism of hepatocellular injury in DHF is poorly understood. Several findings include the direct cytopathic effect of the DENV causing hepatocytes apoptosis, immune-mediated hepatocyte injury by CD4 lymphocyte induced hepatitis, and cytokine storm. Poor hepatic perfusion is also a potential contributing factor in SDD patients.

Direct cytopathic effect

There have been very few studies reporting the presence of DENV in hepatocytes of DHF patients. Moreover, the association between DENV replication and hepatocellular damage has never been concluded. In 1989, Rosen and colleagues firstly demonstrated the recovery of DENV from 5 of 17 livers of children who died from DHF[47]. In 1995, Kangwanpong and colleagues detected DENV RNA in hepatocytes located in the mid-zonal region of the DHF patients' liver by in situ PCR method[48]. In 1999, Couvelard and colleagues confirmed that DENV RNA was found in liver specimens of DHF patient. They concluded that nested PCR was the most sensitive method to identify the DENV RNA in clinical specimens[49]. Furthermore, Huerre and colleagues identified dengue antigens in formalin-fixed paraffin-embedded human liver by immunohistochemical analysis in 2001[50]. Several studies could demonstrate the cytopathic effects of DENV, which induced hepatocytes apoptosis[51-54]. Therefore, the exact effect of DENV in direct cytopathic effect and caused hepatocytes apoptosis is be confirmed. Although hepatocyte apoptosis could contribute to liver injury in DHF patients, it probably has a beneficial effect in inhibiting DENV replication and spread.

Immune mediated hepatocyte injury and cytokine storm

Macrophages and Kupffer cells recognize DENV particles and release cytokines and chemokines, which activated the inflammatory cells and act as antigen-presenting cells. Furthermore, Th1 cells released pro-inflammatory cytokines, which induce parenchymal cell damage and vascular vasodilatation. Moreover, NK cells induced TNF-related apoptosis-inducing ligand (TRAIL) expression and contribute to hepatocytes apoptosis[55,56]. Cells involved in the immune response for DVI include CD8+ cells, NK cells, and Th1 cells. The different immune cells caused hepatocyte damage at different stages of the disease. CD8+ cells are attracted to hepatocytes by regulated inactivation, and normal T cell expressed and secreted have been shown to recognize the $NS4B_{99-17}$ epitope expressed on infected hepatocytes[57]. NK cell infiltration correlated with a rise in cleaved caspase 3 in liver tissue, meaning that it could induce hepatocytes apoptosis. Although the exact mechanisms of NK cell-mediated apoptosis are not well understood, up-regulation of TRAIL maybe a significant role

[56]. During a secondary DVI, memory T cells from the previous infection were rapidly stimulated, leading to a potent inflammatory response. However, the cross-reactive memory T cells have less specificity to the new DENV strain. Hence, the T cell activation would be insufficient to inhibit the virus but potent enough to cause immunopathogenesis[58]. Monocytes have been recognized as important targets of DVI and amplification, particularly in low concentrations of dengue-specific antibodies. The dramatic enhancement by dengue antibody of DENV replication in monocytes and other cells is known as antibody-dependent enhancement (ADE). During a secondary DVI, ADE contributes to severe manifestations caused by IgG antibodies from the primary infection. It fails to neutralize the different strains of DENV, but it could opsonize the viral particles and facilitate the viral uptake into the immune cells. DENV infection of monocytes stimulates the release of numerous immunological factors, some of which modulate the function of other cells, particularly vascular endothelial cells. TNF released by antibody-enhanced DENV-infected monocytes activates endothelial cells. Circulating TNF levels are altered in severely afflicted dengue patients, and TNF is a crucial factor in DENV-induced hemorrhage. This phenomenon could promote a severe inflammatory response with numerous cytokines released as cytokine storms[59,60].

Poor hepatic perfusion

ALF frequently occurs in SDD with shock. Poor hepatic perfusion has been considered a causative factor. However, extensive research regarding the role of microcirculatory injury resulting in hepatocyte ischemia has not been adequately studied[29,61].

In 2019, Kulkarni and colleagues conducted a study to compare the manifestations of DVI in 95 patients with and without the liver disease [group A (without liver disease) = 71, group B (chronic hepatitis) = 12, and group C (cirrhosis = 12)]. They found that one patient in group A had ALF with renal failure and shock. Another one in group A had DHF with multiorgan failure and ARDS. A total of 3 patients expired in group C compared to 1 in group A and none in group B. Moreover, patients in group C required prolonged hospital stay compared to those in group A and group B. They concluded that DVI could have varied manifestations, ranging from simple fever to acute-on-chronic liver failure (ACLF) and ALF[62]. In 2013, Jha *et al*[63] conducted a prospective study to evaluate the etiology, clinical profile, and in-hospital mortality of ACLF in 52 ACLF patients. They found 46.1% hepatitis virus infection and 36.5% bacterial infection were the most common acute infection. The other acute injuries were drugs, autoimmune disease, surgery, malaria, and dengue. The mortality rate was higher in patients with dual insults than single insult (66.6% *vs* 51.1%). They concluded that dual acute insult is not uncommon and may increase mortality in these patients. DVI may be associated with ACLF[63]. In 2019, Galante and colleagues reported the first case in the world of liver transplantation performed in a patient with severe ALF due to DF. Liver transplantation may be considered as a treatment option for patients presenting with acute ALF secondary to DVI[64].

CONCLUSION

The clinical manifestations, laboratory, and pathological findings suggest that liver involvement is very common in DHF. The extent of liver damage may range from asymptomatic with slightly elevated AST and ALT to ALF. Hepatic injury in DHF could be from the direct cytopathic effects of DENV and caused hepatocytes apoptosis. Moreover, the immune-mediated hepatocytes injury by CD4 lymphocyte induced hepatitis and cytokine storm are also crucial factors. Notably, poor hepatic perfusion in SDD with shock is another co-factor in hepatocellular damage. Host defense mechanisms may overcome DVI with a less virulent strain and low viral loads. Infection with a more virulent DENV serotype with high viral loads would lead to extensive hepatocyte damage. Although ALF is a rare condition in DHF patients, the mortality rate in these patients is very high. The early detection of warning signs before the development of ALF in DHF is a critical issue, reducing the fatality rate.

REFERENCES

1 **World Health Organization**. Dengue. [cited 25 February 2021]. Available from: https://www.who.int/news-room/fact-sheets/detail/dengue-and-severe-dengue

2 **Bhatt S**, Gething PW, Brady OJ, Messina JP, Farlow AW, Moyes CL, Drake JM, Brownstein JS,

Hoen AG, Sankoh O, Myers MF, George DB, Jaenisch T, Wint GR, Simmons CP, Scott TW, Farrar JJ, Hay SI. The global distribution and burden of dengue. *Nature* 2013; **496**: 504-507 [PMID: 23563266 DOI: 10.1038/nature12060]

3 **Messina JP**, Brady OJ, Pigott DM, Golding N, Kraemer MU, Scott TW, Wint GR, Smith DL, Hay SI. The many projected futures of dengue. *Nat Rev Microbiol* 2015; **13**: 230-239 [PMID: 25730702 DOI: 10.1038/nrmicro3430]

4 **Munasinghe DR**, Rajasuriya K. Hepatitis in dengue-fever. *Ceylon Med J* 1967; **12**: 222-223 [PMID: 5594773]

5 **Ling LM**, Wilder-Smith A, Leo YS. Fulminant hepatitis in dengue haemorrhagic fever. *J Clin Virol* 2007; **38**: 265-268 [PMID: 17306619 DOI: 10.1016/j.jcv.2006.12.011]

6 **Samanta J**, Sharma V. Dengue and its effects on liver. *World J Clin Cases* 2015; **3**: 125-131 [PMID: 25685758 DOI: 10.12998/wjcc.v3.i2.125]

7 **Perera R**, Kuhn RJ. Structural proteomics of dengue virus. *Curr Opin Microbiol* 2008; **11**: 369-377 [PMID: 18644250 DOI: 10.1016/j.mib.2008.06.004]

8 **Zhang X**, Ge P, Yu X, Brannan JM, Bi G, Zhang Q, Schein S, Zhou ZH. Cryo-EM structure of the mature dengue virus at 3.5-Å resolution. *Nat Struct Mol Biol* 2013; **20**: 105-110 [PMID: 23241927 DOI: 10.1038/nsmb.2463]

9 **Muller DA**, Depelsenaire AC, Young PR. Clinical and Laboratory Diagnosis of Dengue Virus Infection. *J Infect Dis* 2017; **215**: S89-S95 [PMID: 28403441 DOI: 10.1093/infdis/jiw649]

10 **Wilder-Smith A**, Ooi EE, Horstick O, Wills B. Dengue. *Lancet* 2019; **393**: 350-363 [PMID: 30696575 DOI: 10.1016/S0140-6736(18)32560-1]

11 **Halstead S**. Recent advances in understanding dengue. *F1000Res* 2019; **8** [PMID: 31448083 DOI: 10.12688/f1000research.19197.1]

12 **Lee TH**, Lee LK, Lye DC, Leo YS. Current management of severe dengue infection. *Expert Rev Anti Infect Ther* 2017; **15**: 67-78 [PMID: 27786589 DOI: 10.1080/14787210.2017.1248405]

13 **Estofolete CF**, de Oliveira Mota MT, Bernardes Terzian AC, de Aguiar Milhim BHG, Ribeiro MR, Nunes DV, Mourão MP, Rossi SL, Nogueira ML, Vasilakis N. Unusual clinical manifestations of dengue disease - Real or imagined? *Acta Trop* 2019; **199**: 105134 [PMID: 31415737 DOI: 10.1016/j.actatropica.2019.105134]

14 **Mohan B**, Patwari AK, Anand VK. Hepatic dysfunction in childhood dengue infection. *J Trop Pediatr* 2000; **46**: 40-43 [PMID: 10730040 DOI: 10.1093/tropej/46.1.40]

15 **Wahid SF**, Sanusi S, Zawawi MM, Ali RA. A comparison of the pattern of liver involvement in dengue hemorrhagic fever with classic dengue fever. *Southeast Asian J Trop Med Public Health* 2000; **31**: 259-263 [PMID: 11127322]

16 **Bandyopadhyay D**, Chattaraj S, Hajra A, Mukhopadhyay S, Ganesan V. A Study on Spectrum of Hepatobiliary Dysfunctions and Pattern of Liver Involvement in Dengue Infection. *J Clin Diagn Res* 2016; **10**: OC21-OC26 [PMID: 27437266 DOI: 10.7860/JCDR/2016/16946.7784]

17 **Kittitrakul C**, Silachamroon U, Phumratanaprapin W, Krudsood S, Wilairatana P, Treeprasertsuk S. Liver function tests abnormality and clinical severity of dengue infection in adult patients. *J Med Assoc Thai* 2015; **98** Suppl 1: S1-S8 [PMID: 25764606]

18 **Saha AK**, Maitra S, Hazra SCh. Spectrum of hepatic dysfunction in 2012 dengue epidemic in Kolkata, West Bengal. *Indian J Gastroenterol* 2013; **32**: 400-403 [PMID: 24037764 DOI: 10.1007/s12664-013-0382-6]

19 **Roy A**, Sarkar D, Chakraborty S, Chaudhuri J, Ghosh P. Profile of hepatic involvement by dengue virus in dengue infected children. *N Am J Med Sci* 2013; **5**: 480-485 [PMID: 24083224 DOI: 10.4103/1947-2714.117313]

20 **Nascimento Dd**, Castro AR, Froes ÍB, Bigaton G, Oliveira ÉC, Dal Fabbro MF, Cunha RV, Costa IP. Clinical and laboratory findings in patients with dengue associated with hepatopathy. *Rev Soc Bras Med Trop* 2011; **44**: 674-677 [PMID: 22234356 DOI: 10.1590/s0037-86822011005000061]

21 **Karoli R**, Fatima J, Siddiqi Z, Kazmi KI, Sultania AR. Clinical profile of dengue infection at a teaching hospital in North India. *J Infect Dev Ctries* 2012; **6**: 551-554 [PMID: 22842941 DOI: 10.3855/jidc.2010]

22 **Lee LK**, Gan VC, Lee VJ, Tan AS, Leo YS, Lye DC. Clinical relevance and discriminatory value of elevated liver aminotransferase levels for dengue severity. *PLoS Negl Trop Dis* 2012; **6**: e1676 [PMID: 22679523 DOI: 10.1371/journal.pntd.0001676]

23 **Jagadishkumar K**, Jain P, Manjunath VG, Umesh L. Hepatic involvement in dengue Fever in children. *Iran J Pediatr* 2012; **22**: 231-236 [PMID: 23056891]

24 **Parkash O**, Almas A, Jafri SM, Hamid S, Akhtar J, Alishah H. Severity of acute hepatitis and its outcome in patients with dengue fever in a tertiary care hospital Karachi, Pakistan (South Asia). *BMC Gastroenterol* 2010; **10**: 43 [PMID: 20459677 DOI: 10.1186/1471-230X-10-43]

25 **Trung DT**, Thao le TT, Hien TT, Hung NT, Vinh NN, Hien PT, Chinh NT, Simmons C, Wills B. Liver involvement associated with dengue infection in adults in Vietnam. *Am J Trop Med Hyg* 2010; **83**: 774-780 [PMID: 20889864 DOI: 10.4269/ajtmh.2010.10-0090]

26 **Wong M**, Shen E. The utility of liver function tests in dengue. *Ann Acad Med Singap* 2008; **37**: 82-83 [PMID: 18265906]

27 **Uehara PM**, da Cunha RV, Pereira GR, de Oliveira PA. [Liver involvement in patients with dengue hemorrhagic fever: a rare phenomenon? *Rev Soc Bras Med Trop* 2006; **39**: 544-547 [PMID: 17308699 DOI: 10.1590/s0037-86822006000600006]

28 **Itha S**, Kashyap R, Krishnani N, Saraswat VA, Choudhuri G, Aggarwal R. Profile of liver

involvement in dengue virus infection. *Natl Med J India* 2005; **18**: 127-130 [PMID: 16130612]
29 **Fernando S**, Wijewickrama A, Gomes L, Punchihewa CT, Madusanka SD, Dissanayake H, Jeewandara C, Peiris H, Ogg GS, Malavige GN. Patterns and causes of liver involvement in acute dengue infection. *BMC Infect Dis* 2016; **16**: 319 [PMID: 27391896 DOI: 10.1186/s12879-016-1656-2]
30 **Souza LJ**, Alves JG, Nogueira RM, Gicovate Neto C, Bastos DA, Siqueira EW, Souto Filho JT, Cezário Tde A, Soares CE, Carneiro Rda C. Aminotransferase changes and acute hepatitis in patients with dengue fever: analysis of 1,585 cases. *Braz J Infect Dis* 2004; **8**: 156-163 [PMID: 15361994 DOI: 10.1590/s1413-86702004000200006]
31 **Kuo CH**, Tai DI, Chang-Chien CS, Lan CK, Chiou SS, Liaw YF. Liver biochemical tests and dengue fever. *Am J Trop Med Hyg* 1992; **47**: 265-270 [PMID: 1355950 DOI: 10.4269/ajtmh.1992.47.265]
32 **Zhang H**, Zhou YP, Peng HJ, Zhang XH, Zhou FY, Liu ZH, Chen XG. Predictive symptoms and signs of severe dengue disease for patients with dengue fever: a meta-analysis. *Biomed Res Int* 2014; **2014**: 359308 [PMID: 25097856 DOI: 10.1155/2014/359308]
33 **Treeprasertsuk S**, Kittitrakul C. Liver complications in adult dengue and current management. *Southeast Asian J Trop Med Public Health* 2015; **46** Suppl 1: 99-107 [PMID: 26506735]
34 **Devarbhavi H**, Ganga D, Menon M, Kothari K, Singh R. Dengue hepatitis with acute liver failure: Clinical, biochemical, histopathological characteristics and predictors of outcome. *J Gastroenterol Hepatol* 2020; **35**: 1223-1228 [PMID: 31749188 DOI: 10.1111/jgh.14927]
35 **Huy NT**, Van Giang T, Thuy DH, Kikuchi M, Hien TT, Zamora J, Hirayama K. Factors associated with dengue shock syndrome: a systematic review and meta-analysis. *PLoS Negl Trop Dis* 2013; **7**: e2412 [PMID: 24086778 DOI: 10.1371/journal.pntd.0002412]
36 **Chatel-Chaix L**, Fischl W, Scaturro P, Cortese M, Kallis S, Bartenschlager M, Fischer B, Bartenschlager R. A Combined Genetic-Proteomic Approach Identifies Residues within Dengue Virus NS4B Critical for Interaction with NS3 and Viral Replication. *J Virol* 2015; **89**: 7170-7186 [PMID: 25926641 DOI: 10.1128/JVI.00867-15]
37 **Aye KS**, Charngkaew K, Win N, Wai KZ, Moe K, Punyadee N, Thiemmeca S, Suttitheptumrong A, Sukpanichnant S, Prida M, Halstead SB. Pathologic highlights of dengue hemorrhagic fever in 13 autopsy cases from Myanmar. *Hum Pathol* 2014; **45**: 1221-1233 [PMID: 24767772 DOI: 10.1016/j.humpath.2014.01.022]
38 **Pagliari C**, Quaresma JA, Fernandes ER, Stegun FW, Brasil RA, de Andrade HF Jr, Barros V, Vasconcelos PF, Duarte MI. Immunopathogenesis of dengue hemorrhagic fever: contribution to the study of human liver lesions. *J Med Virol* 2014; **86**: 1193-1197 [PMID: 24114877 DOI: 10.1002/jmv.23758]
39 **Vinodh B N**, Bammigatti C, Kumar A, Mittal V. Dengue fever with acute liver failure. *J Postgrad Med* 2005; **51**: 322-323 [PMID: 16388178]
40 **Win MM**, Charngkaew K, Punyadee N, Aye KS, Win N, Chaisri U, Chomanee N, Avirutnan P, Yoksan S, Malasit P. Ultrastructural Features of Human Liver Specimens from Patients Who Died of Dengue Hemorrhagic Fever. *Trop Med Infect Dis* 2019; **4** [PMID: 31013708 DOI: 10.3390/tropicalmed4020063]
41 **Alpert L**, Hart J. The Pathology of Alcoholic Liver Disease. *Clin Liver Dis* 2016; **20**: 473-489 [PMID: 27373610 DOI: 10.1016/j.cld.2016.02.006]
42 **Douglas KO**, Samuels TA, Gittens-St Hilaire M. Serum LPS Associated with Hantavirus and Dengue Disease Severity in Barbados. *Viruses* 2019; **11** [PMID: 31505806 DOI: 10.3390/v11090838]
43 **van de Weg CA**, Koraka P, van Gorp EC, Mairuhu AT, Supriatna M, Soemantri A, van de Vijver DA, Osterhaus AD, Martina BE. Lipopolysaccharide levels are elevated in dengue virus infected patients and correlate with disease severity. *J Clin Virol* 2012; **53**: 38-42 [PMID: 22014848 DOI: 10.1016/j.jcv.2011.09.028]
44 **Kye Mon K**, Nontprasert A, Kittitrakul C, Tangkijvanich P, Leowattana W, Poovorawan K. Incidence and Clinical Outcome of Acute Liver Failure Caused by Dengue in a Hospital for Tropical Diseases, Thailand. *Am J Trop Med Hyg* 2016; **95**: 1338-1344 [PMID: 27928082 DOI: 10.4269/ajtmh.16-0374]
45 **Laoprasopwattana K**, Jundee P, Pruekprasert P, Geater A. Outcome of Severe Dengue Viral Infection-caused Acute Liver Failure in Thai Children. *J Trop Pediatr* 2016; **62**: 200-205 [PMID: 26851434 DOI: 10.1093/tropej/fmv099]
46 **Teerasarntipan T**, Chaiteerakij R, Komolmit P, Tangkijvanich P, Treeprasertsuk S. Acute liver failure and death predictors in patients with dengue-induced severe hepatitis. *World J Gastroenterol* 2020; **26**: 4983-4995 [PMID: 32952344 DOI: 10.3748/wjg.v26.i33.4983]
47 **Rosen L**, Khin MM, U T. Recovery of virus from the liver of children with fatal dengue: reflections on the pathogenesis of the disease and its possible analogy with that of yellow fever. *Res Virol* 1989; **140**: 351-360 [PMID: 2772416 DOI: 10.1016/s0923-2516(89)80115-3]
48 **Kangwanpong D**, Bhamarapravati N, Lucia HL. Diagnosing dengue virus infection in archived autopsy tissues by means of the in situ PCR method: a case report. *Clin Diagn Virol* 1995; **3**: 165-172 [PMID: 15566798 DOI: 10.1016/0928-0197(94)00032-p]
49 **Couvelard A**, Marianneau P, Bedel C, Drouet MT, Vachon F, Hénin D, Deubel V. Report of a fatal case of dengue infection with hepatitis: demonstration of dengue antigens in hepatocytes and liver apoptosis. *Hum Pathol* 1999; **30**: 1106-1110 [PMID: 10492047 DOI: 10.1016/s0046-8177(99)90230-7]
50 **Huerre MR**, Lan NT, Marianneau P, Hue NB, Khun H, Hung NT, Khen NT, Drouet MT, Huong VT,

Ha DQ, Buisson Y, Deubel V. Liver histopathology and biological correlates in five cases of fatal dengue fever in Vietnamese children. *Virchows Arch* 2001; **438**: 107-115 [PMID: 11253111 DOI: 10.1007/s004280000329]

51 **Thepparit C**, Khakpoor A, Khongwichit S, Wikan N, Fongsaran C, Chingsuwanrote P, Panraksa P, Smith DR. Dengue 2 infection of HepG2 Liver cells results in endoplasmic reticulum stress and induction of multiple pathways of cell death. *BMC Res Notes* 2013; **6**: 372 [PMID: 24034452 DOI: 10.1186/1756-0500-6-372]

52 **Courageot MP**, Catteau A, Desprès P. Mechanisms of dengue virus-induced cell death. *Adv Virus Res* 2003; **60**: 157-186 [PMID: 14689694 DOI: 10.1016/s0065-3527(03)60005-9]

53 **Matsuda T**, Almasan A, Tomita M, Tamaki K, Saito M, Tadano M, Yagita H, Ohta T, Mori N. Dengue virus-induced apoptosis in hepatic cells is partly mediated by Apo2 Ligand/tumour necrosis factor-related apoptosis-inducing ligand. *J Gen Virol* 2010; **91**: 2658 [PMID: 20847365 DOI: 10.1099/vir.0.84530-0]

54 **Nasirudeen AM**, Wang L, Liu DX. Induction of p53-dependent and mitochondria-mediated cell death pathway by dengue virus infection of human and animal cells. *Microbes Infect* 2008; **10**: 1124-1132 [PMID: 18606243 DOI: 10.1016/j.micinf.2008.06.005]

55 **Zellweger RM**, Prestwood TR, Shresta S. Enhanced infection of liver sinusoidal endothelial cells in a mouse model of antibody-induced severe dengue disease. *Cell Host Microbe* 2010; **7**: 128-139 [PMID: 20153282 DOI: 10.1016/j.chom.2010.01.004]

56 **Sung JM**, Lee CK, Wu-Hsieh BA. Intrahepatic infiltrating NK and CD8 T cells cause liver cell death in different phases of dengue virus infection. *PLoS One* 2012; **7**: e46292 [PMID: 23050007 DOI: 10.1371/journal.pone.0046292]

57 **Lei HY**, Yeh TM, Liu HS, Lin YS, Chen SH, Liu CC. Immunopathogenesis of dengue virus infection. *J Biomed Sci* 2001; **8**: 377-388 [PMID: 11549879 DOI: 10.1007/BF02255946]

58 **Manh DH**, Weiss LN, Thuong NV, Mizukami S, Dumre SP, Luong QC, Thanh LC, Thang CM, Huu PT, Phuc LH, Nhung CTH, Mai NT, Truong NQ, Ngu VTT, Quoc DK, Ha TTN, Ton T, An TV, Halhouli O, Quynh LN, Kamel MG, Karbwang J, Huong VTQ, Huy NT, Hirayama K. Kinetics of $CD4^+$ T Helper and $CD8^+$ Effector T Cell Responses in Acute Dengue Patients. *Front Immunol* 2020; **11**: 1980 [PMID: 33072068 DOI: 10.3389/fimmu.2020.01980]

59 **Wan SW**, Wu-Hsieh BA, Lin YS, Chen WY, Huang Y, Anderson R. The monocyte-macrophage-mast cell axis in dengue pathogenesis. *J Biomed Sci* 2018; **25**: 77 [PMID: 30409217 DOI: 10.1186/s12929-018-0482-9]

60 **Kuczera D**, Assolini JP, Tomiotto-Pellissier F, Pavanelli WR, Silveira GF. Highlights for Dengue Immunopathogenesis: Antibody-Dependent Enhancement, Cytokine Storm, and Beyond. *J Interferon Cytokine Res* 2018; **38**: 69-80 [PMID: 29443656 DOI: 10.1089/jir.2017.0037]

61 **Khongphatthanayothin A**, Mahayosnond A, Poovorawan Y. Possible cause of liver failure in patient with dengue shock syndrome. *Emerg Infect Dis* 2013; **19**: 1161-1163 [PMID: 23763890 DOI: 10.3201/eid1907.121820]

62 **Kulkarni AV**, Choudhury AK, Premkumar M, Jain P, Gupta E, Sarin SK. Spectrum, Manifestations and Outcomes of Dengue Infection in Individuals with and without Liver Disease. *J Clin Transl Hepatol* 2019; **7**: 106-111 [PMID: 31293909 DOI: 10.14218/JCTH.2018.00047]

63 **Jha AK**, Nijhawan S, Rai RR, Nepalia S, Jain P, Suchismita A. Etiology, clinical profile, and inhospital mortality of acute-on-chronic liver failure: a prospective study. *Indian J Gastroenterol* 2013; **32**: 108-114 [PMID: 23526372 DOI: 10.1007/s12664-012-0295-9]

64 **Galante A**, Adeyi O, Lau L, Humar A, Galvin Z, Selzner N, Lilly L, Sapisochin G, Bhat M. Liver Transplantation for Acute Liver Failure Due to Dengue Fever. *Hepatology* 2019; **70**: 1863-1865 [PMID: 31162847 DOI: 10.1002/hep.30803]

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MINIREVIEWS

Artificial Intelligence in hepatology, liver surgery and transplantation: Emerging applications and frontiers of research

Fadl H Veerankutty, Govind Jayan, Manish Kumar Yadav, Krishnan Sarojam Manoj, Abhishek Yadav, Sindhu Radha Sadasivan Nair, T U Shabeerali, Varghese Yeldho, Madhu Sasidharan, Shiraz Ahmad Rather

ORCID number: Fadl H Veerankutty 0000-0003-3167-0405; Govind Jayan 0000-0001-6318-8299; Manish Kumar Yadav 0000-0002-7561-562X; Krishnan Sarojam Manoj 0000-0002-8394-0828; Abhishek Yadav 0000-0002-1137-8389; Sindhu Radha Sadasivan Nair 0000-0003-3167-0007; T U Shabeerali 0000-0001-8917-1292; Varghese Yeldho 0000-0003-3167-0009; Madhu Sasidharan 0000-0003-4086-0753; Shiraz Ahmad Rather 0000-0002-7169-3882.

Author contributions: Veerankutty FH conceptualized the study; Jayan G, Rather SA, Yadav A, Nair SRS, Yeldho V and Sasidharan M collected the data and contributed to manuscript preparation; Veerankutty FH, Jayan G, Shabeerali TU, Yadav MK, Manoj KS and Rather SA drafted and edited the manuscript.

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Fadl H Veerankutty, Abhishek Yadav, Comprehensive Liver Care, VPS Lakeshore Hospital, Cochin 682040, Kerala, India

Govind Jayan, Sindhu Radha Sadasivan Nair, T U Shabeerali, Varghese Yeldho, Shiraz Ahmad Rather, Hepatobiliary Pancreatic and Liver Transplant Surgery, Kerala Institute of Medical Sciences, Trivandrum 695029, Kerala, India

Manish Kumar Yadav, Krishnan Sarojam Manoj, Department of Radiodiagnosis, Kerala Institute of Medical Sciences, Trivandrum 695029, Kerala, India

Madhu Sasidharan, Gastroenterology and Hepatology, Kerala Institute of Medical Sciences, Thiruvananthapuram 695029, India

Corresponding author: Fadl H Veerankutty, MBBS, MS, DNB (GI SURGERY), Consultant, Comprehensive Liver Care, VPS Lakeshore Hospital, NH-66 Bypass, Cochin 682040, Kerala, India. fadl_05@yahoo.com

Abstract

The integration of artificial intelligence (AI) and augmented realities into the medical field is being attempted by various researchers across the globe. As a matter of fact, most of the advanced technologies utilized by medical providers today have been borrowed and extrapolated from other industries. The introduction of AI into the field of hepatology and liver surgery is relatively a recent phenomenon. The purpose of this narrative review is to highlight the different AI concepts which are currently being tried to improve the care of patients with liver diseases. We end with summarizing emerging trends and major challenges in the future development of AI in hepatology and liver surgery.

Key Words: Liver disease; Machine learning; Deep learning; Artificial neural networks; Transplantation; Hepatectomy

Core Tip: Much of the advanced technologies utilized by medical providers today have

quality classification
Grade A (Excellent): 0
Grade B (Very good): B
Grade C (Good): 0
Grade D (Fair): 0
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INTRODUCTION

Artificial intelligence (AI) is gradually changing the way that medicine is being practiced across the world, with technological advancements in the field of imaging, navigation and robotic intervention. It is increasingly being used for risk stratification, genomics, imaging and diagnosis, precision medicine, and drug discovery. The introduction of AI in hepatology and liver surgery is more recent and it has a strong root in machine learning (ML)-based algorithms, imaging and navigation, with early techniques focused on feature detection and computer-assisted intervention for both pre-operative planning and intra-operative guidance. AI-based solutions can assist in timely detection of liver tumors, more precise diagnosis and predicting disease course as well as outcomes. Diseases affecting the liver are heterogeneous and complex in nature, caused by various etiological factors, such as genetics, sex, ethnicity, body mass index (commonly known as BMI), environmental exposures to toxins, and comorbid conditions like diabetes mellitus. AI-based approaches could be highly useful in analyzing these various types of complex data in hepatology practice and research.

Components of AI systems can be broadly classified into expert system, search algorithm, ML, and deep learning (DL)[1]. Among them, ML is the most commonly used term, which can be considered as a branch of AI in which computers learn from data, with emphasis on computational algorithms, and analyze tons of data within no time[1]. ML can be of supervised or unsupervised learning. Supervised learning can be defined as a kind of ML which helps in predicting a known outcome, based on inputs, in the presence of an expert 'supervisor'[2]. While unsupervised learning is another type of ML, which can discover naturally occurring patterns without a pre-defined outcome, in the absence of an expert 'supervisor'[2]. The artificial neural network (ANN) is a type of statistical system used to derive outputs, based on interactions of weighted inputs and outputs and it mimics the intricate architecture of neuronal networks in the brain[3]. One other subset of ML is DL, which uses automatic discovery of representations from raw data (representation learning) for detection or classification[4]. Convolutional neural network (CNN) is a kind of DL ANN which utilizes multiple building blocks, such as pooling layers and convolution layers, and performs feature extraction to yield final output[5]. CNNs can be considered as one of the most successful DL models, due to their exceptional capability for processing spatial information[6]. Another type of neural network, known as recurrent neural network, utilizes feedback connections and displays great accuracy in labelling and forecasting sequential data[7]. Radiomics is another method in AI that extracts innumerable features from radiographic images by using data-characterization algorithms[8]. These radiomic features have the potential to unearth many characteristics of a disease that fail to be appreciated by the naked eye examination of a clinician. Radiomics can be coupled with AI, as it is capable of handling a massive amount of data in contrast to the traditional statistical methods[9]. Almost all AI techniques require a large dataset comprising laboratory and radiological findings, and outcome data. In the future, AI will definitely be useful in supporting clinical decisions, minimizing medical errors, and forecasting clinical outcomes. In this article, we will review the emerging role of AI in the management liver diseases, liver surgery

and liver transplantation.

AI IN LIVER DISEASES

Non-alcoholic fatty liver disease

Non-alcoholic fatty liver disease (NAFLD) is a growing epidemic globally, in part attributable to the increasing incidence of obesity and insulin resistance resulting in liver accumulation of free fatty acids and triglycerides. NAFLD patients are at higher risk of liver-related as well as cardiovascular-related mortality, and it is rapidly becoming the chief indication for liver transplantation[10,11]. Besides, NAFLD has been identified as a major risk factor for hepatocellular carcinoma (HCC)[12]. ML has been explored extensively for pattern recognition in NAFLD (Table 1). Timely identification of patients with NAFLD is paramount to arrest the disease progression to cirrhosis and related complications. Liver biopsy remains the gold standard for definitive diagnosis but it is invasive and inappropriate for screening. The development of non-invasive advanced imaging, biochemical and genetic tests as well as AI techniques will undoubtedly offer clinicians a great deal of information in the near future that can be utilized for early diagnosis and targeted treatment options.

Imaging of liver with ultrasound (US) is considered as a keystone for the initial diagnosis of NAFLD as it is widely available and image acquisition is easy. Magnetic resonance imaging (MRI) with proton density fat fraction (PDFF) has been considered as the reference standard in the quantification of hepatic steatosis; however, this technique has its own limitations, like cost and limited availability[13]. Methods exist for sonographic diagnosis of NAFLD, but these are often qualitative. Han *et al*[14] attempted to develop and evaluate DL algorithms that use radiofrequency data for NAFLD assessment, with MRI-derived PDFF as the reference. The investigators analyzed data of 204 prospectively enrolled adult research participants. The image acquisition was conducted *via* a typical right intercostal approach, with a 1–4 MHz curved probe and time-gain compensation, with the addition of 10 radiofrequency frames acquired during a breath-hold in shallow expiration. They found that DL algorithms with radiofrequency US data are very precise for diagnosis of NAFLD and hepatic fat fraction quantification with fairly good correlation (Pearson r = 0.85) with MRI PDFF when other causes of steatosis are excluded[14]. In another study, Byra *et al*[15] used CNN to automatically detect the amount of fat in liver from US images and showed high accuracy [area under the curve (AUC) of 0.98] compared to gold-standard liver biopsy, thus showing that ML can help in overcoming the issue of inter-operator variability as well.

ML-based algorithms were also used for early identification of patients with high risk for development of hepatic steatosis. Perveen *et al*[16] used a systematic ML-based decision-tree method to analyze data from electronic medical records in four Canadian populations and accurately predicted risk of development and progression of NAFLD. A similar application of ML to predict and screen for NAFLD in a Chinese population was carried out by Ma *et al*[17] and showed high accuracy, sensitivity and specificity. In a comparison study of different ML-based algorithms, the investigators found that all ML-based algorithms were found to be more efficient than the hepatic steatosis index (commonly known as HSI; F-measure 0.524) and the Fatty Liver Index (commonly known as FLI; F-measure, 0.318) and the Bayesian network model performed the best of 11 ML-based algorithms in the classification of patients with NAFLD (F-measure, 0.655).

ML-based algorithms have been deployed to analyze images from liver biopsy by using 47 unique liver biopsy images with manual annotations, performed by two pathologists. Vanderbeck *et al*[18] devised a classification algorithm. By utilizing a color analysis protocol, the algorithm was able to find out key features in biopsy specimens (macrosteatosis, portal veins, sinusoids and bile ducts) with good precision and high recall (> 82%)[18]. Similarly, Gawrieh *et al*[19] developed an AI-based tool to accurately quantify hepatic fibrosis and architectural pattern in liver biopsy specimens. These examples show that various ML tools may be chosen for application in appropriate situations for a specific problem.

Viral hepatitis

Progression to cirrhosis is an important event to be monitored in patients with hepatitis B virus (HBV) as well as hepatitis C virus (HCV) infections. Rates of progression to cirrhosis vary dramatically across individuals and not all patients progress to cirrhosis. Accurate risk stratification is essential to avoid excess monitoring

Table 1 Review of articles where artificial intelligence has been studied in the context of non-alcoholic liver disease

Ref.	Dataset	Number	ML algorithms	Problem	Performance measures
Byra *et al*[15], 2018	Department of Internal Medicine, Hypertension and Vascular Diseases, Medical University of Warsaw, Poland	55	Deep CNN	Automatically diagnose the amount of fat in the liver from US images	AUROC, Delong statistical test, lasso regression method, Spearman correlation coefficient, Meng test
Perveen *et al*[16], 2018	CPCSSN	667907	Decision tree	Classification, NAFLD progression risk	Micro- and Macro-average of Precision, Recall and F-measure, MCC, AUROC
Ma *et al*[17], 2018	First Affiliated Hospital, College of Medicine, Zhejiang University, China	10508	Several, Weka open source software	Classification, feature selection	Accuracy, specificity, precision, recall (*i.e.* sensitivity), and the F-measure
Vanderbeck *et al*[18], 2014	Medical College of Wisconsin, Milwaukee, United States	59	SVM	Automated assessment of histological features of NAFLD	Precision rate, recall rate, and AUROC
Meffert *et al*[68], 2014	SHIP	4222	Boosting algorithm, discrimination and calibration plots	Scoring system for hepatic steatosis risk	Discrimination (AUROC) and calibration
Sowa *et al*[69], 2014	University Hospital Essen	82	Logistic regression, decision trees, SVM, RF	Distinguish NAFLD from ALD	Sensitivity, specificity, and accuracy
Kuppili *et al*[70], 2017	Instituto Superior Tecnico, University of Lisbon, Portugal	63	Extreme Learning Machine-SLFFNN	Stratification of FLD disease in US liver images	AUROC, reliability and stability analysis
Sorino *et al*[71], 2020	MICOL cohort	2970	SVM	Stratify NAFLD risk to reduce need for imaging	Accuracy, variance, calculated confidence limits (95%), the weight of each model (as a %) and the number of ultrasound examinations it could avoid
Wu *et al*[72], 2019	New Taipei City Municipal Hospital Banqiao Branch	577	ANN, NB, RF, LR	Diagnosis and risk stratification in NAFLD	Accuracy, sensitivity, specificity

ALD: Alcoholic liver disease; ANN: Artificial neural network; AUROC: Area under the receiver operating characteristic; CNN: Convolutional neural network; CPCSSN: Canadian Primary Care Sentinel Surveillance Network; FLD: Fatty liver disease; LR: Logistic regression; MCC: Matthews correlation coefficient; MICOL: Multi-centre Italian study on cholelithiasis; ML: Machine learning; NAFLD: Non-alcoholic fatty liver disease; NB: Naïve Bayes; RF: Random forest; SHIP: Study of Health in Pomerania; SLFFNN: Single-layer feed-forward neural network; SVM: Support vector machine; US: Ultrasound.

of slow progressors as well as for appropriate monitoring of rapid progressors, for timely treatment. Availability of highly accurate risk prediction models would facilitate proactive identification of patients in need of more intensive monitoring and management. ML methods were used for genetic analyses of various HCV strains and was then applied to recognize relevant genetic markers related to fibrosis progression in HCV[20]. Shousha *et al*[21] combined data-mining strategies and ML algorithms (NN algorithms) using IL28B genotype and biochemical markers to predict advanced fibrosis in HCV patients, yielding a higher performance than both aspartate aminotransferase-to-platelet ratio index (commonly known as APRI) and fibrosis-4 (commonly known as FIB-4).

Primary sclerosing cholangitis

ML has been useful in patients with primary sclerosing cholangitis (PSC) throughout the disease course, from diagnosis to prediction of liver decompensation risk and post-transplant survival. Ringe *et al*[22] showed that PSC-compatible cholangiographic changes on 3D-magnetic resonance cholangiopancreatography (commonly known as MRCP) can be detected by DL algorithms with high sensitivity (95%) and low mean absolute error (7%). The PSC Risk Estimation Tool (referred to as PREsTo), which was developed by Eaton *et al*[23] using a gradient boosting machine (commonly known as GBM) algorithm, has been validated in an international multicenter cohort to accurately predict risk of liver decompensation in these patients and has also been shown to be far more accurate than existing prediction systems. LT in PSC patients is a contentious issue in view of the association with inflammatory bowel disease and risk of colorectal neoplasia and cholangiocarcinoma. Due to limited organ availability, identifying individuals who are most likely to benefit from the procedure is of paramount importance in patient selection. Andres *et al*[24] analyzed data of 2769 PSC patients from the Scientific Registry of Transplant Recipients (referred to as SRTR) database using a novel multitime-point calibrated model for the prediction of individual survival after LT. The accuracy of the model in predicting long-term survival was shown to surpass the traditional Cox regression analysis, which completely fails at 10 years.

Liver space occupying lesions and underlying liver disease

The application of ML toward image recognition has evolved into facial recognition software programs which are commonly used in smartphones. Employing this feature in healthcare, Park *et al*[7] were able to create an algorithm based on recurrent neural network to accurately predict visual field examination, thereby aiding in the diagnosis of optic neuropathies. Others have utilized similar ML tools in detection of lung nodules and cerebral aneurysms[25]. Recently, such computer-aided diagnosis/detection has been used in hepatology as well. Hassan *et al*[26] used a stacked sparse auto encode system based on support vector machines to differentiate HCC, hemangioma and liver cysts from US images. This method was shown to have 97.2% accuracy, outperforming software based on other DL algorithms. A DL system was developed by Schmauch *et al*[27] to diagnose and categorize space occupying lesions in the liver into malignant or benign tumors. By means of a supervised training using a database of 367 US images together with the radiological reports, the resulting algorithm could detect and characterize the lesions with a mean receiver operating characteristic of 0.93 and 0.916, respectively[27]. Although this model needs validation, it could warn of possible malignant lesions and boost the diagnostic yield of US for liver lesions. Another study used the patient's clinical data along with MRI sequences to devise an automated classification system cataloguing such hepatic lesions as cyst, adenoma, hemangioma, HCC and metastasis, with acceptable sensitivity and specificity rates[28]. A retrospective study analyzed the yield of an ANN, composed of three layers, for classifications of liver lesions by means of contrast-enhanced CT into five groups (A, classic HCC; B, malignant tumors apart from HCC; C, indeterminate masses, dysplastic nodules or early HCC and benign masses other than cysts or hemangiomas; D, hemangiomas; E, cysts)[29]. They obtained a high accuracy for the classification of hepatic lesions after supervised training using data from more than 55000 images, particularly for the distinction between groups A-B and C-D[30].

Diagnosis of HCC is currently based on imaging, tumor markers and sometimes biopsy. However, several other routine tests, such as biomarkers of liver inflammation, liver function test and viral markers, can help in prediction of HCC risk. The contribution of each variable toward accurate HCC prediction could be identified by data mining analysis of large volumes of data of patients with HCC and this in turn could help in the formation of a prediction model. This was attempted by Sato *et al*[31] when they analyzed data from 4242 patients at the University of Tokyo's hospital liver clinic. The patients were divided into those who had HCC diagnosed at first presentation (who formed the HCC-positive group of 539 patients) and others who developed HCC in follow-up (who formed the HCC-negative group of 1043 patients) after eliminating those with insufficient data. The available data was analyzed, and the gradient boosting provided the highest predictive accuracy for the presence of HCC (87.34%) and produced an AUC of 0.940. By using a cut-off of 200 ng/mL for alpha-fetoprotein (AFP), 40 mAu/mL for Des-gamma carboxyprothrombin (DCP), and 15% for AFP-L3, the accuracies of AFP, DCP, and AFP-L3 for predicting HCC were 70.67% (AUC: 0.766), 74.91% (AUC: 0.644), and 71.05% (AUC: 0.683), respectively[31]. Furthermore, an innovative model devised by Książek *et al*[31], used patient information, such as

viral status, occurrence of comorbidities and laboratory results to forecast the development of HCC. This is based on 23 quantitative and 26 qualitative features and has attained an 88.5% accuracy for this prediction model. When analyzing large data sets, ML models have proven superior over the classical statistical regression models. This framework of identifying optimal classifiers is the path towards fine-tuning personalized medicine.

Another important arena in the management of HCC is risk stratification for recurrence, which has been facilitated by the ability to digitize pathology slides. Saillard *et al*[32] showed that DL algorithms based on digitized slides were more accurate in predicting survival of HCC patients after liver resection compared to scores formed using various clinical, biological and pathological factors. Another DL model by Chaudhary *et al*[33] used data from The Cancer Genome Atlas to identify a subgroup of HCC patients with inactivation mutations in *TP53* genes, frequent BIRC5 expressions and stemness markers (KRT19 and EPCAM), and a high proportion of activated Akt and Wnt signaling pathways associated with aggressive tumors[33].

After HCC resection, vascular microinvasion (VMI) is considered as one of the major predictive factors of recurrence. In a recent publication by Dong *et al*[34], radiomic algorithms based on US images were used to elaborate radiomic signatures with the potential to aid in the preoperative prediction of VMI and to classify patients with VMI into low risk (≤ 5 MVI in adjacent liver tissue and ≤ 1 cm from the tumor) and high-risk groups (> 5 MVI or MVI in liver tissue and > 1 cm from the tumor) with promising results. Moreover, researchers have validated CT-based ANN and deep CNN to predict survival of HCC patients[35,36]. Ji *et al*[35] designed a novel three-feature radiomic signature of the contrast-enhanced CT image, where performance was enhanced by combining it with clinical features [concordance-index (c-index): 0.63–0.69 *vs* 0.73–0.801]. Wang and colleagues[36] employed multiphase CT radiomics features along with clinical models to yield a combined model (AUC: 0.82).

Tsilimigras *et al*[37] attempted to identify the most important prognostic factors in the pre- and postoperative setting for each Barcelona Clinic Liver Cancer (BCLC) stage by using a ML method. The investigators used a Classification and Regression Tree (CART) model to analyze data drawn from an international multi-institutional database. The preoperative CART model selected AFP and Charlson comorbidity score as the first and second most important preoperative factors of overall survival among BCLC-0/A patients, whereas radiologic tumor burden score was the best predictor of overall survival among BCLC-B patients. The postoperative CART model showed the lymphovascular invasion as the best postoperative predictor of long-term survival among BCLC-0/A patients, whereas tumor burden score remained the best predictor of long-term outcomes among BCLC-B patients in the postoperative setting[37].

AI algorithms were also successfully employed to predict response to transarterial chemoembolization (commonly known as TACE) and radiofrequency ablation (commonly known as RFA)[38-42]. A fully automated ML algorithm was proposed by Morshid *et al*[38] using the clinical information and features of CT images and to forecast the response to the treatment by TACE. Using the combination of BCLC stage and quantitative imaging features, the investigators attained a prediction accuracy of 74.2% against using just the BCLC stage alone. Liu *et al*[41] validated three AI-based predictive models (one deep and two ML), using radiomic features of contrast-enhance US scans. In that study, the DL model was found to be superior to the two other methods in assigning patients in the validation cohort to either objective-response to TACE or non-response, with a decent accuracy (AUC: 0.93)[41]. Wu *et al*[42] developed an ANN-based on 15 clinical features to predict 1-year and 2-year disease-free survival of patients who underwent CT-guided percutaneous RFA in early stages of HCC. The accuracy of the model was better when predicting 1-year disease-free survival than 2-year disease-free survival, with an accuracy of 85.0% and 67.9%, respectively[42].

AI IN LIVER SURGERY

Surgery offers the best chance of cure for patients with liver tumors. However, surgical removal of liver tumors is challenging because of its complex anatomy and concerns about functional liver remnant. Accurate knowledge of liver anatomy is thus a key point for any successful hepatic resection or living donor LT (LDLT). Even a minor change in the surgical plan can have a dramatic impact on the surgical outcome. The anatomy is so complex that it is often difficult to reconstruct it mentally based on CT or MRI images alone. Over decades, intraoperative visualization of preoperative image data in hepatic surgery has been a hot research topic for computer scientists and

clinicians. The introduction of AI in liver surgery is more recent and it mainly focuses on imaging and navigation that make pre-operative planning and intra-operative guidance easier. 3D visualization techniques and 3D printing technology can significantly benefit the understanding and display of surgical anatomy. ML has been applied in various aspects of the 3D printing technique to improve the whole design and manufacturing workflow[43]. Virtual liver resection can be performed before actual surgery using 3D visualization techniques to assess the resectability of the lesion and calculate future liver remnant (FLR)[44]. In LDLT, 3D imaging can predict the requirement for vascular reconstruction based on the vascular anatomy of the donor liver, resulting in improved safety and outcome of LDLT[44]. The application of 3D printing technology in liver surgery has been evaluated in a few studies. In pediatric LDLT, 3D-printed liver models have been found useful in evaluating discrepancies in size between small pediatric recipients and adult liver grafts[45]. Nevertheless, there are still many issues (like cost and time of manufacturing) that must be addressed before 3D printing can become more accepted and widespread. ML could be exploited to solve these problems by streamlining the 3D modelling process through rapid medical image segmentation and improved patient selection and image acquisition [46].

Automated hepatic volumetry

It is widely accepted that accurate assessment of volume of FLR can reduce post-hepatectomy liver failure. Hepatocytes in the remnant liver after resection must overcome necrosis and regenerate sufficiently to preserve synthetic function which requires an adequate volume of functional FLR. Widely followed limits of FLR for safe resection range between 20% and 30% for normal liver and 30% and 40% in those with underlying liver disease. Several imaging modalities have been experimented in liver volume assessment, including even conventional US and 3D US[47,48]. However, contrast-enhanced CT scan is globally accepted for FLR assessment, pre-transplant LD evaluation and for assessment of response to FLR volume induction. The first described method of liver volume assessment based on manually tracing the entire liver was time-consuming but precise. Recently, semi-automatic and automatic segmentation techniques using mathematical model,s such as the ones reported by Suzuki *et al*[49] and Nakayama *et al*[50], have shown good accuracy. A CNN-based algorithm has been developed by Wang *et al*[51] to fully automate liver volume assessment from CT as well as MRI. A similar algorithm developed by Winkel *et al*[52] has shown good accuracy, speed and good agreement with manual segmentation. The criticism of fully automatic segmentation is that it often can be unsuccessful for some CT images that are low in contrast or have missing edges due to similar intensity of adjacent organs or machine artifact.

Surgical navigation systems

Surgical navigation systems have been playing a crucial role in neurosurgery and spinal surgery for many years; yet, they have not become established as standard in liver surgery. This is largely due to the technical challenge of navigating a moving organ. The surgical navigation system must be able to measure the intraoperative alterations in position and shape of the liver due to respiration and surgical manipulation, in order to adapt the preoperative navigation data to the current situation. Techniques like augmented virtuality (referred to as AV), augmented reality (referred to as AR) and mixed reality can be used to synchronize 3D reconstructed images with real-time surgery and can offer a safe and reliable surgical navigation method. Accurate surgical navigation can better guide laparoscopic surgeons to perform hepatectomy and improve the safety of surgery. In a preliminary trial, Phutane *et al* [53] demonstrated that AR-based hepatectomy for HCC could help detect intrahepatic tumors, decide the transection plane, and locate the hepatic veins, which can result in improved safety of operation by reducing bleeding and duration of surgery. The laparoscopic hepatectomy navigation system (LHNS) is a multimodal assistant system presented by Zhang *et al*[54] which consists of a fusion model of CT-based 3D models with indocyanine green (commonly known as ICG) fluorescence images. LHNS was used for real-time visualization of the relationship between liver lesions and intrahepatic anatomical structures. Using LHNS, the optimal cutting plane for the liver resection can be planned preoperatively. The system consisted of preoperative model segmentation, intraoperative laparoscopic stereo surface reconstruction, intraoperative laparoscopic posture tracking modules and intraoperative registration. Authors retrospectively compared the clinical outcomes of patients who underwent the laparoscopic hepatectomy using the LHNS (LHNS group) with patients who underwent the procedure without LHNS guidance (non-LHNS group). They found that the LHNS

group had significantly less blood loss, less intraoperative blood transfusion rate and a shorter postoperative hospital stay than the non-LHNS group. There was no significant difference in operative time and the overall complication rate between the two groups. The LHNS system was also helpful to clearly delineate the liver transection line in most cases[54]. Ntourakis *et al*[55] reported in a pilot study that AR helped in detecting missing lesions after chemotherapy for CRLM and obtaining a margin negative resection status without any local recurrence at a median follow-up of 22 mo. Application of AR in robotic hepatectomy can enhance the ability of the surgeon to achieve a safe tumor resection with adequate peritumoral margin[56,57].

AI to predict postoperative morbidity

AI algorithms are also being used to predict postoperative morbidity and recurrence of tumor after surgery. Post-hepatectomy liver failure is a worrisome complication after major liver resection for HCC and is the chief cause of postoperative mortality. Early identification and timely intervention are vital to avoid the mortality associated with it. Mai *et al*[58] attempted to validate an ANN model to forecast severe post-hepatectomy liver failure in patients with HCC who underwent partial hepatectomy (353 patients). They found that the predictive performance of the ANN model for severe post-hepatectomy liver failure surpassed the traditional logistic regression model and normally used scoring systems[58].

AI IN LIVER TRANSPLANTATION

Liver transplantation is a complex process that involves analysis of numerous variables related to both donor and recipient and expert decisions that are essential for long-term graft and patient survival. The high number of variables involved often makes the decision-making process difficult. In such a circumstance, ML techniques play an important role, with the ability to build accurate models for liver graft survival.

Organ allocation and donor-recipient matching

In a liver transplantation program, the major bottleneck in delivery of care now is organ availability. The United Network for Organ Sharing (commonly known as UNOS) survey has identified about a 20% drop-out of patients listed for liver transplantation[59]. Attempts to reduce this dropout rate by utilization of extended criteria donors (older donors, donors with fatty liver, donation after cardiac death donors) have resulted in inferior post-transplant outcomes and decreased utilization due to an increase in discarded grafts. This problem is expected to worsen in the coming years as growth in the general population is projected to overtake growth in the donor pool, thus potentially exacerbating the organ shortage and further increasing the waiting time for transplant. Such insights demonstrate the precious nature of each liver graft and the paramount importance of appropriate organ allocation to reduce waiting list mortality as well as to promote efficient utilization of available organs. A first attempt at guiding organ allocation using donor information was the quantitative donor risk index by Feng *et al*[60], which used a Cox regression model to predict graft failure using donor characteristics alone. The widely validated model for end-stage liver disease (MELD) score, which is the keystone of current allocation policy in the United States and worldwide, is based on the "sickest-first" principle, utilizing recipient information alone. Undoubtedly, a method which utilizes donor as well as recipient characteristics for appropriate pairing would ideally reduce waiting list mortality and organ wastage with good post-transplant survival. Many strategies, including ML, are being tried to reduce the discrepancy between the number of potential liver graft recipients and the number of organs available. This was attempted by Pérez-Ortiz *et al*[61] using ordinal regression and the support vector machine to arrive at a model that could be used in conjunction with the MELD score to allocate the organ to one of the first patients on the waiting list (according to MELD score) who would have a higher survival possibility. This can circumvent flaws in MELD score-based allocation and also eliminates futile transplants. The Optimized Prediction of Mortality (commonly known as OPOM) model developed by Bertsimas *et al*[62] employing ML optimal classification tree model in comparison with MELD-based allocation using Liver Simulated Allocation Model (commonly known as LSAM) has been shown to reduce waiting list mortality on average by 417.96 deaths every year. OPOM has been found to adhere more accurately to the "sickest-first" principle and utilizes more variables than the MELD and MELD-Na scores. Another neural

network-derived algorithm is the MPENSGA 2 developed by Cruz-Ramírez *et al*[63] which seeks to complement MELD-based allocation and improve its efficiency.

In 2014, a donor–recipient matching model was presented by Briceño *et al*[64] which can make the clinical decision-making easier in liver transplantation. The investigators used two ANN models: One was to enhance the probability of graft survival, and the other was to reduce the probability of graft loss. They analyzed variables of 64 donors and recipients from a set of 1003 LTs from a multicenter study. The chief aim was to devise an innovative decision-making system that can optimize the principles of fairness, efficiency and equity in allocating liver graft. They found that ANN models were significantly more accurate than already validated scores of graft survival [MELD, Delta MELD, donor-risk index (DRI), Survival Outcomes Following Liver Transplant (SOFT), the preallocation (P)-SOFT and balance-of-risk (BAR)][64]. Wingfield *et al*[65], from the United Kingdom, published the first ever systematic review of AI computing techniques being used in liver transplantation to predict individual patient graft survival. They concluded that AI techniques can provide high accuracy in predicting graft survival based on donors and recipient variables; additionally, compared with the standard techniques, AI methods had the benefits of being dynamic and able to be trained and validated within every population. Table 2 provides a concise review of recently published studies where AI-based algorithms have been applied to liver transplantation.

Challenges and prospects

It is evident from the above-mentioned studies that ML is going to be a powerful weapon in the armamentarium of the hepatologist and liver surgeon, with applications ranging from screening to postoperative follow-up. Given the recent advances in AI and the lack of any precedence, the Hippocratic philosophy of 'do no harm' should be at the forefront of any decision to integrate it into the clinical practice. There are some ethical and legal issues to be addressed before widespread adoption of AI into clinical practice. Data privacy and cyber security are the main ethical concerns. Next is the issue of accountability. For example, if a ML tool gives a wrong diagnosis or incorrectly assesses the hepatic volume, resulting in post-hepatectomy liver failure, whom should be held responsible?

AI is going to be a major player in organ allocation, donor-recipient matching, and even in optimizing immunosuppressant doses[66,67]. AI can be employed *via* smartphones to remotely monitor patient health. However, like any other evolving technology, AI is not without shortcomings. The ability of ML to analyze large volumes of data is responsible for its most important handicap. Quality of the output is inexorably linked to the quality of input data. This is the case with conventional biostatistical methods as well. Hence, high-quality data collection is essential for the development of AI systems as data sets are the lifeblood of algorithms and statistical modelling on which AI systems are trained. So, it is the duty of all physicians to come forward to help drive these innovations rather than passively waiting for the technology to become useful in their practice. Hepatologists and liver surgeons should seek opportunities to partner with data scientists to capture novel forms of clinical data and help generate meaningful interpretations of that data. Moreover, the accuracy of any AI system can be affected by factors such as study design, data integration strategy, selection of ML model and the relevance of the selected ML model to the particular study setting. Hence, physicians must have clearly defined, clinically relevant questions that require AI technology as the analysis tool. Early work in ML has focused on individual areas, such as radiomics or genomics, but future work should be aimed more towards amalgamating these to form a comprehensive care plan of the patient.

CONCLUSION

To conclude, as the incorporation of AI into the management of liver diseases seems inevitable, training of clinicians in interpreting and applying it into the routine practice is of paramount importance. If appropriately designed and implemented, AI has the potential to revolutionize the way hepatology and liver surgery is taught and practiced, with the promise of a future optimized for high-quality patient care.

Table 2 Review of recently published studies where artificial intelligence-based algorithms have been applied to liver transplantation

Ref.	Dataset	Number	ML algorithms	Problem	Performance measures
Bertsimas *et al* [62], 2019	STAR dataset	-	OCT	Predict 3 mo waitlist mortality-OPOM	ROC curve
Cruz-Ramírez *et al* [63], 2013	Spanish multi-center study	-	Radial basis function NN	Improve donor-recipient matching using rule-based allocation—MPENSGA 2 algorithm	Accuracy, minimum sensitivity, ROC curve, RMSE, Cohen's kappa
Briceño *et al*[64], 2014	Spanish multi-center study	1003	Neural Net Evolutionary Programming	Improve equity in donor-recipient matching	Multiple regression analysis, simple logistic regression analysis, ROC curve
Ayllón *et al*[73], 2018	King's College Hospital,United Kingdom + MADR-E, Spain	1437	ANN	Classification, end-point (3 mo, 1 yr)	ROC curve
Wadhwani *et al* [74], 2019	UNOS	1482	RF	Classification, end-point (3 yr)	Chi-square test, *t*-test, Wilcoxon rank sum test
Dorado-Moreno *et al*[75], 2017	King's College Hospital, United Kingdom + MADR-E, Spain	1492	Ordinal ANN	Ordinal classification, fourclasses	MAE and the MZE, accuracy, GMS, AMAE
Guijo-Rubio *et al* [76], 2019	UNOS	39095	Cox, SVM, GB	Survival time	C-index, ROC curve, concordance index ipcw
Lee *et al*[77], 2018	Seoul National University Hospital	1211	Several ML methods compared, GBM found to be best	Prediction of AKI after liver transplant	ROC curve, accuracy
Lau *et al*[78], 2017	Austin Hospital, Melbourne, Australia	180	RF, ANN, logistic regression	Predict 30-d risk of graft failure	ROC curve

AKI: Acute kidney injury; AMAE: Average mean absolute error; ANN: Artificial neural network; c-index: Concordance index; GB: Gradient boosting; GBM: Gradient boosting machine; GMS: Geometric mean of the sensitivities; MADR-E: Model for Allocation of Donor and Recipient in España; MAE: Mean absolute error; MPENSG-A: Memetic Pareto evolutionary non-dominated sorting genetic algorithm; ML: Machine learning; MZE: Mean zero-one error; NN: Neural network; OCT: Optimal classification tree; OPOM: Optimized prediction of mortality; RF: Random forest; RMSE: Root mean squared error; ROC: Receiver operating characteristic; STAR: Standard Transplant Analysis and Research; SVM: Support vector machine; UNOS: United Network for Organ Sharing.

REFERENCES

1 **Yang YJ**, Bang CS. Application of artificial intelligence in gastroenterology. *World J Gastroenterol* 2019; **25**: 1666-1683 [PMID: 31011253 DOI: 10.3748/wjg.v25.i14.1666]

2 **Sidey-Gibbons JAM**, Sidey-Gibbons CJ. Machine learning in medicine: a practical introduction. *BMC Med Res Methodol* 2019; **19**: 64 [PMID: 30890124 DOI: 10.1186/s12874-019-0681-4]

3 **Agatonovic-Kustrin S**, Beresford R. Basic concepts of artificial neural network (ANN) modeling and its application in pharmaceutical research. *J Pharm Biomed Anal* 2000; **22**: 717-727 [PMID: 10815714 DOI: 10.1016/S0731-7085(99)00272-1]

4 **LeCun Y**, Bengio Y, Hinton G. Deep learning. *Nature* 2015; **521**: 436-444 [PMID: 26017442 DOI: 10.1038/nature14539]

5 **Yamashita R**, Nishio M, Do RKG, Togashi K. Convolutional neural networks: an overview and application in radiology. *Insights Imaging* 2018; **9**: 611-629 [PMID: 29934920 DOI: 10.1007/s13244-018-0639-9]

6 **Min S**, Lee B, Yoon S. Deep learning in bioinformatics. *Brief Bioinform* 2017; **18**: 851-869 [PMID: 27473064 DOI: 10.1093/bib/bbw068]

7 **Park K**, Kim J, Lee J. Visual Field Prediction using Recurrent Neural Network. *Sci Rep* 2019; **9**: 8385 [PMID: 31182763 DOI: 10.1038/s41598-019-44852-6]

8 **Hu W**, Yang H, Xu H, Mao Y. Radiomics based on artificial intelligence in liver diseases: where we are? *Gastroenterol Rep (Oxf)* 2020; **8**: 90-97 [PMID: 32280468 DOI: 10.1093/gastro/goaa011]

9 **Koçak B**, Durmaz EŞ, Ateş E, Kılıçkesmez Ö. Radiomics with artificial intelligence: a practical guide for beginners. *Diagn Interv Radiol* 2019; **25**: 485-495 [PMID: 31650960 DOI: 10.5152/dir.2019.19321]

10 **Benedict M**, Zhang X. Non-alcoholic fatty liver disease: An expanded review. *World J Hepatol* 2017; **9**: 715-732 [PMID: 28652891 DOI: 10.4254/wjh.v9.i16.715]

11 **Chalasani N**, Younossi Z, Lavine JE, Charlton M, Cusi K, Rinella M, Harrison SA, Brunt EM, Sanyal AJ. The diagnosis and management of nonalcoholic fatty liver disease: Practice guidance from

the American Association for the Study of Liver Diseases. *Hepatology* 2018; **67**: 328-357 [PMID: 28714183 DOI: 10.1002/hep.29367]

12 **Tobari M**, Hashimoto E, Taniai M, Kodama K, Kogiso T, Tokushige K, Yamamoto M, Takayoshi N, Satoshi K, Tatsuo A. The characteristics and risk factors of hepatocellular carcinoma in nonalcoholic fatty liver disease without cirrhosis. *J Gastroenterol Hepatol* 2020; **35**: 862-869 [PMID: 31597206 DOI: 10.1111/jgh.14867]

13 **Greenhill C**. MRI versus biopsy to assess NAFLD. *Nat Rev Gastroenterol Hepatol* 2012; **9**: 366-366 [DOI: 10.1038/nrgastro.2012.102]

14 **Han A**, Byra M, Heba E, Andre MP, Erdman JW Jr, Loomba R, Sirlin CB, O'Brien WD Jr. Noninvasive Diagnosis of Nonalcoholic Fatty Liver Disease and Quantification of Liver Fat with Radiofrequency Ultrasound Data Using One-dimensional Convolutional Neural Networks. *Radiology* 2020; **295**: 342-350 [PMID: 32096706 DOI: 10.1148/radiol.2020191160]

15 **Byra M**, Styczynski G, Szmigielski C, Kalinowski P, Michałowski Ł, Paluszkiewicz R, Ziarkiewicz-Wróblewska B, Zieniewicz K, Sobieraj P, Nowicki A. Transfer learning with deep convolutional neural network for liver steatosis assessment in ultrasound images. *Int J Comput Assist Radiol Surg* 2018; **13**: 1895-1903 [PMID: 30094778 DOI: 10.1007/s11548-018-1843-2]

16 **Perveen S**, Shahbaz M, Keshavjee K, Guergachi A. A Systematic Machine Learning Based Approach for the Diagnosis of Non-Alcoholic Fatty Liver Disease Risk and Progression. *Sci Rep* 2018; **8**: 2112 [PMID: 29391513 DOI: 10.1038/s41598-018-20166-x]

17 **Ma H**, Xu CF, Shen Z, Yu CH, Li YM. Application of Machine Learning Techniques for Clinical Predictive Modeling: A Cross-Sectional Study on Nonalcoholic Fatty Liver Disease in China. *Biomed Res Int* 2018; **2018**: 4304376 [PMID: 30402478 DOI: 10.1155/2018/4304376]

18 **Vanderbeck S**, Bockhorst J, Komorowski R, Kleiner DE, Gawrieh S. Automatic classification of white regions in liver biopsies by supervised machine learning. *Hum Pathol* 2014; **45**: 785-792 [PMID: 24565203 DOI: 10.1016/j.humpath.2013.11.011]

19 **Gawrieh S**, Sethunath D, Cummings OW, Kleiner DE, Vuppalanchi R, Chalasani N, Tuceryan M. Automated quantification and architectural pattern detection of hepatic fibrosis in NAFLD. *Ann Diagn Pathol* 2020; **47**: 151518 [PMID: 32531442 DOI: 10.1016/j.anndiagpath.2020.151518]

20 **Lara J**, López-Labrador F, González-Candelas F, Berenguer M, Khudyakov YE. Computational models of liver fibrosis progression for hepatitis C virus chronic infection. *BMC Bioinformatics* 2014; **15** Suppl 8: S5 [PMID: 25081062 DOI: 10.1186/1471-2105-15-S8-S5]

21 **Shousha HI**, Awad AH, Omran DA, Elnegouly MM, Mabrouk M. Data Mining and Machine Learning Algorithms Using IL28B Genotype and Biochemical Markers Best Predicted Advanced Liver Fibrosis in Chronic Hepatitis C. *Jpn J Infect Dis* 2018; **71**: 51-57 [PMID: 29279441 DOI: 10.7883/yoken.JJID.2017.089]

22 **Ringe KI**, Vo Chieu VD, Wacker F, Lenzen H, Manns MP, Hundt C, Schmidt B, Winther HB. Fully automated detection of primary sclerosing cholangitis (PSC)-compatible bile duct changes based on 3D magnetic resonance cholangiopancreatography using machine learning. *Eur Radiol* 2021; **31**: 2482-2489 [PMID: 32974688 DOI: 10.1007/s00330-020-07323-5]

23 **Eaton JE**, Vesterhus M, McCauley BM, Atkinson EJ, Schlicht EM, Juran BD, Gossard AA, LaRusso NF, Gores GJ, Karlsen TH, Lazaridis KN. Primary Sclerosing Cholangitis Risk Estimate Tool (PREsTo) Predicts Outcomes of the Disease: A Derivation and Validation Study Using Machine Learning. *Hepatology* 2020; **71**: 214-224 [PMID: 29742811 DOI: 10.1002/hep.30085]

24 **Andres A**, Montano-Loza A, Greiner R, Uhlich M, Jin P, Hoehn B, Bigam D, Shapiro JAM, Kneteman NM. A novel learning algorithm to predict individual survival after liver transplantation for primary sclerosing cholangitis. *PLoS One* 2018; **13**: e0193523 [PMID: 29543895 DOI: 10.1371/journal.pone.0193523]

25 **Lee SM**, Seo JB, Yun J, Cho YH, Vogel-Claussen J, Schiebler ML, Gefter WB, van Beek EJR, Goo JM, Lee KS, Hatabu H, Gee J, Kim N. Deep Learning Applications in Chest Radiography and Computed Tomography: Current State of the Art. *J Thorac Imaging* 2019; **34**: 75-85 [PMID: 30802231 DOI: 10.1097/RTI.0000000000000387]

26 **Hassan TM**, Elmogy M, Sallam E-S. Diagnosis of Focal Liver Diseases Based on Deep Learning Technique for Ultrasound Images. *Arab J Sci Eng* 2017; **42**: 3127-3140 [DOI: 10.1007/s13369-016-2387-9]

27 **Schmauch B**, Herent P, Jehanno P, Dehaene O, Saillard C, Aubé C, Luciani A, Lassau N, Jégou S. Diagnosis of focal liver lesions from ultrasound using deep learning. *Diagn Interv Imaging* 2019; **100**: 227-233 [PMID: 30926443 DOI: 10.1016/j.diii.2019.02.009]

28 **Acharya UR**, Koh JEW, Hagiwara Y, Tan JH, Gertych A, Vijayananthan A, Yaakup NA, Abdullah BJJ, Bin Mohd Fabell MK, Yeong CH. Automated diagnosis of focal liver lesions using bidirectional empirical mode decomposition features. *Comput Biol Med* 2018; **94**: 11-18 [DOI: 10.1016/j.compbiomed.2017.12.024]

29 **Yasaka K**, Akai H, Abe O, Kiryu S. Deep Learning with Convolutional Neural Network for Differentiation of Liver Masses at Dynamic Contrast-enhanced CT: A Preliminary Study. *Radiology* 2018; **286**: 887-896 [PMID: 29059036 DOI: 10.1148/radiol.2017170706]

30 **Sato M**, Morimoto K, Kajihara S, Tateishi R, Shiina S, Koike K, Yatomi Y. Machine-learning Approach for the Development of a Novel Predictive Model for the Diagnosis of Hepatocellular Carcinoma. *Sci Rep* 2019; **9**: 7704 [PMID: 31147560 DOI: 10.1038/s41598-019-44022-8]

31 **Książek W**, Abdar M, Acharya UR, Pławiak P. A novel machine learning approach for early detection of hepatocellular carcinoma patients. *Cogn Syst Res* 2019; **54**: 116-127 [DOI:

10.1016/j.cogsys.2018.12.001]

32 **Saillard C**, Schmauch B, Laifa O, Moarii M, Toldo S, Zaslavskiy M, Pronier E, Laurent A, Amaddeo G, Regnault H, Sommacale D, Ziol M, Pawlotsky JM, Mulé S, Luciani A, Wainrib G, Clozel T, Courtiol P, Calderaro J. Predicting Survival After Hepatocellular Carcinoma Resection Using Deep Learning on Histological Slides. *Hepatology* 2020; **72**: 2000-2013 [PMID: 32108950 DOI: 10.1002/hep.31207]

33 **Chaudhary K**, Poirion OB, Lu L, Garmire LX. Deep Learning-Based Multi-Omics Integration Robustly Predicts Survival in Liver Cancer. *Clin Cancer Res* 2018; **24**: 1248-1259 [PMID: 28982688 DOI: 10.1158/1078-0432.CCR-17-0853]

34 **Dong Y**, Zhou L, Xia W, Zhao XY, Zhang Q, Jian JM, Gao X, Wang WP. Preoperative Prediction of Microvascular Invasion in Hepatocellular Carcinoma: Initial Application of a Radiomic Algorithm Based on Grayscale Ultrasound Images. *Front Oncol* 2020; **10**: 353 [PMID: 32266138 DOI: 10.3389/fonc.2020.00353]

35 **Ji GW**, Zhu FP, Xu Q, Wang K, Wu MY, Tang WW, Li XC, Wang XH. Machine-learning analysis of contrast-enhanced CT radiomics predicts recurrence of hepatocellular carcinoma after resection: A multi-institutional study. *EBioMedicine* 2019; **50**: 156-165 [PMID: 31735556 DOI: 10.1016/j.ebiom.2019.10.057]

36 **Wang W**, Chen Q, Iwamoto Y, Han X, Zhang Q, Hu H, Lin L, Chen YW. Deep Learning-Based Radiomics Models for Early Recurrence Prediction of Hepatocellular Carcinoma with Multi-phase CT Images and Clinical Data. In: 2019 41st Annual International Conference of the IEEE Engineering in Medicine and Biology Society (EMBC). *IEEE* 2019; 4881-4884

37 **Tsilimigras DI**, Mehta R, Moris D, Sahara K, Bagante F, Paredes AZ, Farooq A, Ratti F, Marques HP, Silva S, Soubrane O, Lam V, Poultsides GA, Popescu I, Grigorie R, Alexandrescu S, Martel G, Workneh A, Guglielmi A, Hugh T, Aldrighetti L, Endo I, Pawlik TM. Utilizing Machine Learning for Pre- and Postoperative Assessment of Patients Undergoing Resection for BCLC-0, A and B Hepatocellular Carcinoma: Implications for Resection Beyond the BCLC Guidelines. *Ann Surg Oncol* 2020; **27**: 866-874 [PMID: 31696396 DOI: 10.1245/s10434-019-08025-z]

38 **Morshid A**, Elsayes KM, Khalaf AM, Elmohr MM, Yu J, Kaseb AO, Hassan M, Mahvash A, Wang Z, Hazle JD, Fuentes D. A machine learning model to predict hepatocellular carcinoma response to transcatheter arterial chemoembolization. *Radiol Artif Intell* 2019; **1** [PMID: 31858078 DOI: 10.1148/ryai.2019180021]

39 **Mähringer-Kunz A**, Wagner F, Hahn F, Weinmann A, Brodehl S, Schotten S, Hinrichs JB, Düber C, Galle PR, Pinto Dos Santos D, Kloeckner R. Predicting survival after transarterial chemoembolization for hepatocellular carcinoma using a neural network: A Pilot Study. *Liver Int* 2020; **40**: 694-703 [PMID: 31943703 DOI: 10.1111/liv.14380]

40 **Abajian A**, Murali N, Savic LJ, Laage-Gaupp FM, Nezami N, Duncan JS, Schlachter T, Lin M, Geschwind JF, Chapiro J. Predicting Treatment Response to Intra-arterial Therapies for Hepatocellular Carcinoma with the Use of Supervised Machine Learning-An Artificial Intelligence Concept. *J Vasc Interv Radiol* 2018; **29**: 850-857.e1 [PMID: 29548875 DOI: 10.1016/j.jvir.2018.01.769]

41 **Liu D**, Liu F, Xie X, Su L, Liu M, Kuang M, Huang G, Wang Y, Zhou H, Wang K, Lin M, Tian J. Accurate prediction of responses to transarterial chemoembolization for patients with hepatocellular carcinoma by using artificial intelligence in contrast-enhanced ultrasound. *Eur Radiol* 2020; **30**: 2365-2376 [PMID: 31900703 DOI: 10.1007/s00330-019-06553-6]

42 **Wu CF**, Wu YJ, Liang PC, Wu CH, Peng SF, Chiu HW. Disease-free survival assessment by artificial neural networks for hepatocellular carcinoma patients after radiofrequency ablation. *J Formos Med Assoc* 2017; **116**: 765-773 [PMID: 28117199 DOI: 10.1016/j.jfma.2016.12.006]

43 **Goh GD**, Sing SL, Yeong WY. A review on machine learning in 3D printing: applications, potential, and challenges. *Artif Intell Rev* 2021; **54**: 63-94 [DOI: 10.1007/s10462-020-09876-9]

44 **Mise Y**, Hasegawa K, Satou S, Shindoh J, Miki K, Akamatsu N, Arita J, Kaneko J, Sakamoto Y, Kokudo N. How Has Virtual Hepatectomy Changed the Practice of Liver Surgery? *Ann Surg* 2018; **268**: 127-133 [PMID: 28288065 DOI: 10.1097/SLA.0000000000002213]

45 **Soejima Y**, Taguchi T, Sugimoto M, Hayashida M, Yoshizumi T, Ikegami T, Uchiyama H, Shirabe K, Maehara Y. Three-dimensional printing and biotexture modeling for preoperative simulation in living donor liver transplantation for small infants. *Liver Transpl* 2016; **22**: 1610-1614 [PMID: 27434755 DOI: 10.1002/lt.24516]

46 **Huff TJ**, Ludwig PE, Zuniga JM. The potential for machine learning algorithms to improve and reduce the cost of 3-dimensional printing for surgical planning. *Expert Rev Med Devices* 2018; **15**: 349-356 [PMID: 29723481 DOI: 10.1080/17434440.2018.1473033]

47 **Kitajima K**, Taboury J, Boleslawski E, Savier E, Vaillant JC, Hannoun L. Sonographic preoperative assessment of liver volume before major liver resection. *Gastroenterol Clin Biol* 2008; **32**: 382-389 [PMID: 18403156 DOI: 10.1016/j.gcb.2008.02.007]

48 **Xu HX**, Yin XY, Lu MD, Liu GJ, Xu ZF. Estimation of liver tumor volume using a three-dimensional ultrasound volumetric system. *Ultrasound Med Biol* 2003; **29**: 839-846 [PMID: 12837499 DOI: 10.1016/s0301-5629(02)00775-5]

49 **Suzuki K**, Huynh HT, Liu YP, Calabrese D, Zhou K, Oto A, Hori M. Computerized segmentation of liver in hepatic CT and MRI by means of level-set geodesic active contouring. In: 2013 35th Annual International Conference of the IEEE Engineering in Medicine and Biology Society (EMBC). IEEE, 2013: 2984–2987

50 **Nakayama Y**, Li Q, Katsuragawa S, Ikeda R, Hiai Y, Awai K, Kusunoki S, Yamashita Y, Okajima H, Inomata Y, Doi K. Automated hepatic volumetry for living related liver transplantation at multisection CT. *Radiology* 2006; **240**: 743-748 [PMID: 16857979 DOI: 10.1148/radiol.2403050850]

51 **Wang K**, Mamidipalli A, Retson T, Bahrami N, Hasenstab K, Blansit K, Bass E, Delgado T, Cunha G, Middleton MS, Loomba R, Neuschwander-Tetri BA, Sirlin CB, Hsiao A; members of the NASH Clinical Research Network. Automated CT and MRI Liver Segmentation and Biometry Using a Generalized Convolutional Neural Network. *Radiol Artif Intell* 2019; **1** [PMID: 32582883 DOI: 10.1148/ryai.2019180022]

52 **Winkel DJ**, Weikert TJ, Breit HC, Chabin G, Gibson E, Heye TJ, Comaniciu D, Boll DT. Validation of a fully automated liver segmentation algorithm using multi-scale deep reinforcement learning and comparison *vs* manual segmentation. *Eur J Radiol* 2020; **126**: 108918 [PMID: 32171914 DOI: 10.1016/j.ejrad.2020.108918]

53 **Phutane P**, Buc E, Poirot K, Ozgur E, Pezet D, Bartoli A, Le Roy B. Preliminary trial of augmented reality performed on a laparoscopic left hepatectomy. *Surg Endosc* 2018; **32**: 514-515 [PMID: 28791423 DOI: 10.1007/s00464-017-5733-4]

54 **Zhang P**, Luo H, Zhu W, Yang J, Zeng N, Fan Y, Wen S, Xiang N, Jia F, Fang C. Real-time navigation for laparoscopic hepatectomy using image fusion of preoperative 3D surgical plan and intraoperative indocyanine green fluorescence imaging. *Surg Endosc* 2020; **34**: 3449-3459 [PMID: 31705286 DOI: 10.1007/s00464-019-07121-1]

55 **Ntourakis D**, Memeo R, Soler L, Marescaux J, Mutter D, Pessaux P. Augmented Reality Guidance for the Resection of Missing Colorectal Liver Metastases: An Initial Experience. *World J Surg* 2016; **40**: 419-426 [PMID: 26316112 DOI: 10.1007/s00268-015-3229-8]

56 **Pessaux P**, Diana M, Soler L, Piardi T, Mutter D, Marescaux J. Towards cybernetic surgery: robotic and augmented reality-assisted liver segmentectomy. *Langenbecks Arch Surg* 2015; **400**: 381-385 [PMID: 25392120 DOI: 10.1007/s00423-014-1256-9]

57 **Buchs NC**, Volonte F, Pugin F, Toso C, Fusaglia M, Gavaghan K, Majno PE, Peterhans M, Weber S, Morel P. Augmented environments for the targeting of hepatic lesions during image-guided robotic liver surgery. *J Surg Res* 2013; **184**: 825-831 [PMID: 23684617 DOI: 10.1016/j.jss.2013.04.032]

58 **Mai RY**, Lu HZ, Bai T, Liang R, Lin Y, Ma L, Xiang BD, Wu GB, Li LQ, Ye JZ. Artificial neural network model for preoperative prediction of severe liver failure after hemihepatectomy in patients with hepatocellular carcinoma. *Surgery* 2020; **168**: 643-652 [PMID: 32792098 DOI: 10.1016/j.surg.2020.06.031]

59 **Mehta N**, Dodge JL, Hirose R, Roberts JP, Yao FY. Predictors of low risk for dropout from the liver transplant waiting list for hepatocellular carcinoma in long wait time regions: Implications for organ allocation. *Am J Transplant* 2019; **19**: 2210-2218 [PMID: 30861298 DOI: 10.1111/ajt.15353]

60 **Feng S**, Goodrich NP, Bragg-Gresham JL, Dykstra DM, Punch JD, DebRoy MA, Greenstein SM, Merion RM. Characteristics associated with liver graft failure: the concept of a donor risk index. *Am J Transplant* 2006; **6**: 783-790 [PMID: 16539636 DOI: 10.1111/j.1600-6143.2006.01242.x]

61 **Pérez-Ortiz M**, Cruz-Ramírez M, Ayllón-Terán MD, Heaton N, Ciria R, Hervás-Martínez C. An organ allocation system for liver transplantation based on ordinal regression. *Appl Soft Comput* 2014; **14**: 88-98 [DOI: 10.1016/j.asoc.2013.07.017]

62 **Bertsimas D**, Kung J, Trichakis N, Wang Y, Hirose R, Vagefi PA. Development and validation of an optimized prediction of mortality for candidates awaiting liver transplantation. *Am J Transplant* 2019; **19**: 1109-1118 [PMID: 30411495 DOI: 10.1111/ajt.15172]

63 **Cruz-Ramírez M**, Hervás-Martínez C, Fernández JC, Briceño J, de la Mata M. Predicting patient survival after liver transplantation using evolutionary multi-objective artificial neural networks. *Artif Intell Med* 2013; **58**: 37-49 [PMID: 23489761 DOI: 10.1016/j.artmed.2013.02.004]

64 **Briceño J**, Cruz-Ramírez M, Prieto M, Navasa M, Ortiz de Urbina J, Orti R, Gómez-Bravo MÁ, Otero A, Varo E, Tomé S, Clemente G, Bañares R, Bárcena R, Cuervas-Mons V, Solórzano G, Vinaixa C, Rubín A, Colmenero J, Valdivieso A, Ciria R, Hervás-Martínez C, de la Mata M. Use of artificial intelligence as an innovative donor-recipient matching model for liver transplantation: results from a multicenter Spanish study. *J Hepatol* 2014; **61**: 1020-1028 [PMID: 24905493 DOI: 10.1016/j.jhep.2014.05.039]

65 **Wingfield LR**, Ceresa C, Thorogood S, Fleuriot J, Knight S. Using Artificial Intelligence for Predicting Survival of Individual Grafts in Liver Transplantation: A Systematic Review. *Liver Transpl* 2020; **26**: 922-934 [PMID: 32274856 DOI: 10.1002/lt.25772]

66 **Naushad SM**, Kutala VK. Artificial neural network and bioavailability of the immunosuppression drug. *Curr Opin Organ Transplant* 2020; **25**: 435-441 [PMID: 32452906 DOI: 10.1097/MOT.0000000000000770]

67 **Briceño J**, Ayllón MD, Ciria R. Machine-learning algorithms for predicting results in liver transplantation: the problem of donor-recipient matching. *Curr Opin Organ Transplant* 2020; **25**: 406-411 [PMID: 32487891 DOI: 10.1097/MOT.0000000000000781]

68 **Meffert PJ**, Baumeister SE, Lerch MM, Mayerle J, Kratzer W, Völzke H. Development, external validation, and comparative assessment of a new diagnostic score for hepatic steatosis. *Am J Gastroenterol* 2014; **109**: 1404-1414 [PMID: 24957156 DOI: 10.1038/ajg.2014.155]

69 **Sowa JP**, Atmaca Ö, Kahraman A, Schlattjan M, Lindner M, Sydor S, Scherbaum N, Lackner K, Gerken G, Heider D, Arteel GE, Erim Y, Canbay A. Non-invasive separation of alcoholic and non-alcoholic liver disease with predictive modeling. *PLoS One* 2014; **9**: e101444 [PMID: 24988316 DOI: 10.1371/journal.pone.0101444]

70 **Kuppili V**, Biswas M, Sreekumar A, Suri HS, Saba L, Edla DR, Marinho RT, Sanches JM, Suri JS. Extreme Learning Machine Framework for Risk Stratification of Fatty Liver Disease Using Ultrasound Tissue Characterization. *J Med Syst* 2017; **41**: 152 [PMID: 28836045 DOI: 10.1007/s10916-017-0797-1]

71 **Sorino P**, Caruso MG, Misciagna G, Bonfiglio C, Campanella A, Mirizzi A, Franco I, Bianco A, Buongiorno C, Liuzzi R, Cisternino AM, Notarnicola M, Chiloiro M, Pascoschi G, Osella AR; MICOL Group. Selecting the best machine learning algorithm to support the diagnosis of Non-Alcoholic Fatty Liver Disease: A meta learner study. *PLoS One* 2020; **15**: e0240867 [PMID: 33079971 DOI: 10.1371/journal.pone.0240867]

72 **Wu CC**, Yeh WC, Hsu WD, Islam MM, Nguyen PAA, Poly TN, Wang YC, Yang HC, Jack Li YC. Prediction of fatty liver disease using machine learning algorithms. *Comput Methods Programs Biomed* 2019; **170**: 23-29 [PMID: 30712601 DOI: 10.1016/j.cmpb.2018.12.032]

73 **Ayllón MD**, Ciria R, Cruz-Ramírez M, Pérez-Ortiz M, Gómez I, Valente R, O'Grady J, de la Mata M, Hervás-Martínez C, Heaton ND, Briceño J. Validation of artificial neural networks as a methodology for donor-recipient matching for liver transplantation. *Liver Transpl* 2018; **24**: 192-203 [PMID: 28921876 DOI: 10.1002/lt.24870]

74 **Wadhwani SI**, Hsu EK, Shaffer ML, Anand R, Ng VL, Bucuvalas JC. Predicting ideal outcome after pediatric liver transplantation: An exploratory study using machine learning analyses to leverage Studies of Pediatric Liver Transplantation Data. *Pediatr Transplant* 2019; **23**: e13554 [PMID: 31328849 DOI: 10.1111/petr.13554]

75 **Dorado-Moreno M**, Pérez-Ortiz M, Gutiérrez PA, Ciria R, Briceño J, Hervás-Martínez C. Dynamically weighted evolutionary ordinal neural network for solving an imbalanced liver transplantation problem. *Artif Intell Med* 2017; **77**: 1-11 [PMID: 28545607 DOI: 10.1016/j.artmed.2017.02.004]

76 **Guijo-Rubio D**, Villalón-Vaquero PJ, Gutiérrez PA, Ayllón MD, Briceño J, Hervás-Martínez C. Modelling Survival by Machine Learning Methods in Liver Transplantation: Application to the UNOS Dataset. 2019: 97–104

77 **Lee HC**, Yoon SB, Yang SM, Kim WH, Ryu HG, Jung CW, Suh KS, Lee KH. Prediction of Acute Kidney Injury after Liver Transplantation: Machine Learning Approaches vs. Logistic Regression Model. *J Clin Med* 2018; **7** [PMID: 30413107 DOI: 10.3390/jcm7110428]

78 **Lau L**, Kankanige Y, Rubinstein B, Jones R, Christophi C, Muralidharan V, Bailey J. Machine-Learning Algorithms Predict Graft Failure After Liver Transplantation. *Transplantation* 2017; **101**: e125-e132 [PMID: 27941428 DOI: 10.1097/TP.0000000000001600]

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MINIREVIEWS

De novo and recurrence of metabolic dysfunction-associated fatty liver disease after liver transplantation

Ma Ai Thanda Han, Raquel Olivo, Catherine J Choi, Nikolaos Pyrsopoulos

ORCID number: Ma Ai Thanda Han 0000-0002-2740-2486; Raquel Olivo 0000-0003-3845-5900; Catherine J Choi 000-0003-4310-8575; Nikolaos Pyrsopoulos 0000-0002-6950-8174.

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Ma Ai Thanda Han, Nikolaos Pyrsopoulos, Department of Gastroenterology and Hepatology, Rutgers New Jersey Medical School, Newark, NJ 07103, United States

Raquel Olivo, Department of Gastroenterology and Hepatology, Rutgers University, New Jersey Medical School, Newark, NJ 07103, United States

Catherine J Choi, Department of Medicine, Rutgers New Jersey Medical School, Newark, NJ 07101, United States

Corresponding author: Nikolaos Pyrsopoulos, FAASLD, AGAF, FACG, MD, PhD, Director, Professor, Department of Gastroenterology and Hepatology, Rutgers New Jersey Medical School, 185 South Orange Avenue, H-536, Newark, NJ 07103, United States.
pyrsopni@njms.rutgers.edu

Abstract

Metabolic dysfunction-associated fatty liver disease (MAFLD) is a new acronym adopted from the consensus of international experts. Given the increasing prevalence of MAFLD in pre-transplant settings, *de novo* and recurrent graft steatosis/MAFLD are common in post-transplant settings. The impact of graft steatosis on long-term outcomes is unclear. The current knowledge of incidence rate, risk factors, diagnosis, long-term outcomes, and management of graft steatosis (both *de novo* and recurrent) is discussed in this review.

Key Words: Metabolic dysfunction-associated fatty liver disease; Metabolic dysfunction-associated steatohepatitis; *De novo*; Recurrent; Graft steatosis; Fibrosis; Survival

Core Tip: Metabolic dysfunction-associated fatty liver disease (MAFLD) is common after liver transplantation. Post transplant metabolic dysfunction, obesity and consequences of immunosuppressant contribute to the development of either *de novo* or recurrent graft steatosis. Post liver transplant MAFLD impact on cardiovascular outcome without significant impact on graft and patient survival. Weight control and tailoring of immunosuppression are the main strategies to prevent post liver transplant MAFLD.

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INTRODUCTION

Metabolic dysfunction-associated fatty liver disease (MAFLD) is a new acronym adopted from the consensus of international experts. MAFLD is defined by the evidence of hepatic steatosis and one of the following criteria: Overweight or obesity, presence of type 2 diabetes mellitus (DM), or evidence of metabolic dysfunction[1,2] Given the increasing prevalence of obesity, nonalcoholic fatty liver disease (NAFLD) has become one of the leading causes of liver transplantation in the United States[3]. The utilization of immunosuppressants in post liver transplant (LT) patients significantly impacts metabolic dysfunction through the development of insulin resistance (IR), DM, hypertension, obesity, and hyperlipidemia[4-7]. Either *de novo* or recurrent graft steatosis can occur after liver transplantation[8]. Most of the studies showed an association between metabolic dysfunction and the occurrence of either *de novo* or recurrent graft steatosis[9-12]. Therefore, the graft steatosis can be referred to as post LT MAFLD. The ongoing injury from graft steatosis can progress to the different stages of hepatic fibrosis and eventually cirrhosis which may develop further complications. In this review, we are going to discuss epidemiology, risk factors or predictors, diagnostic techniques, natural history, outcomes, and management of *de novo* and recurrent graft steatosis.

EPIDEMIOLOGY

Hepatic steatosis has been recognized as the hepatic manifestation of metabolic syndrome (MetS). LT resolves the complications of cirrhosis due to metabolic-associated steatohepatitis (MASH), but the metabolic risks persist and often can get aggravated by exposure to immunosuppressive therapy after LT[13]. Therefore, it is not surprising to expect a higher rate of recurrent graft steatosis after LT compared to that of *de novo* graft steatosis due to the underlying MetS and IR that initially led to cirrhosis[14]. Recurrent or *de novo* graft steatosis after LT poses potential threats to the viability and survival of allografts, and therefore it is critical to characterize and identify the prevalence of recurrent and *de novo* graft steatosis after LT, and identify the risk factors for post-LT MAFLD to improve the overall clinical outcomes in the transplant recipients.

The true incidence of recurrent and *de novo* graft steatosis after LT remains uncertain as previously published studies were from single-center, retrospective studies with heterogeneous definitions of the diseases and methodologies[11,15]. Despite these limitations, we aim to describe the rates of recurrence and occurrence of steatosis in allografts, mainly abstracted from systematic reviews and meta-analyses by Saeed *et al* [11] and Losurdo *et al*[12]. In the review by Saeed *et al*[11] 17 studies representing 2378 patients primarily from North American and Europe were included, and they were categorized into three groups based on the nature of included studies: Recurrent, *de novo*, and combined graft steatosis among LT recipients at 1, 3, and ≥ 5-year follow-ups after LT. The estimated incidence rates of recurrent graft steatosis are 59% (range: 8%-100%), 57% (24%-100%), 82.1% (59%-100%) at 1, 3, and ≥ 5-year after LT respectively while those of recurrent steatohepatitis are 53% (24%-82%), 57.4% (31%-100%), and 38% (4%-71%)[11]. Recurrent graft steatosis was very common after LT, recurring in more than half of the recipients as early as 1 year after LT[11]. The studies assessing both recurrent and *de novo* graft steatosis and steatohepatitis reported 1, 3, and ≥ 5 year incidence rates as 42% (30%-65%), 34% (23%-52%), and 33% (26%-33%) for graft steatosis while 10% (5%-15%), 11% (6%-17%), and 19% (10%-27%) for steatohepatitis [11]. One of the largest studies with 275 subjects assessing recurrent graft steatosis and steatohepatitis has reported the recurrence of graft steatosis in 31% of patients and the recurrence of graft steatohepatitis in 4% of patients after LT[16].

The study by Dumortier *et al*[17] reported *de novo* graft steatosis in 31% and graft steatohepatitis in 3.8% of 421 recipients at 3.3 years after LT. In the systematic review

and meta-analysis by Saeed *et al*[11], incidence rates for *de novo* graft steatosis at 1, 3, and ≥ 5 years after LT were 67%, 40%, and 78% while 13%, 16%, and 17% for *de novo* graft steatohepatitis. These incidence rates were varied depending on the different follow-up periods, but *de novo* graft steatosis was overall very common in post-transplant patients[11]. Also, these incidence rates noted in the review by Saeed *et al* [11] were higher compared to another systematic review and meta-analysis by Losurdo *et al*[12], which reported summarized weighted prevalence of *de novo* graft steatosis as 26% [95% Confidence interval (CI): 20%-31%] and *de novo* graft steatohepatitis as 2% (95%CI: 0-3%). Larger, prospective future studies with clear, consistent inclusion and diagnosis criteria are warranted to better characterize the incidence of recurrent and *de novo* MAFLD and MASH, but existing studies consistently demonstrated very high rates of recurrence and occurrence of graft steatosis among LT recipients.

RISK FACTORS/PREDICTORS

The development of graft steatosis after LT is related to different factors: Recipient, environmental, genetic, and immunosuppressive factors[13]. A retrospective study by El Altrache *et al*[18] reported the association of recurrent graft steatosis with the occurrence of metabolic abnormalities after LT. Similarly, another study by Dureja *et al* [19] described the risk factors for the development of recurrent graft steatosis including an increased body mass index (BMI), post-transplant hypertriglyceridemia, steroid use, MetS, and insulin use. A retrospective study by Galvin *et al*[20], identified risk factors for *de novo* graft steatosis in a post-LT cohort included diabetes, weight gain, BMI, hepatitis C virus (HCV) infection, sirolimus-based immunosuppressant therapy. If none of these factors existed, *de novo* graft steatosis occurred in only 5.4% of patients, but if all 5 factors were present, it would occur in 100% of patients[20]. All these risk factors are associated with IR, and therefore it was suggested that IR might be at the root of the development of *de novo* graft steatosis[20] In a study by Vallin *et al* [10] in comparing recurrent and *de novo* graft steatosis, the prevalence of DM was significantly higher in the recurrent graft steatosis group compared to the *de novo* graft steatosis group (100% *vs* 37.5%, $P < 0.01$)

Among patients with pre-transplant NAFLD, hepatic and peripheral IR leads to insufficient inhibition of hepatic gluconeogenesis, increased lipid accumulation, and reduced glycogen synthesis[21]. Increased circulating free fatty acids from the above-mentioned process further promote inflammation and endoplasmic reticulum stress, which aggravates IR more, leading to a vicious cycle[22]. The immunosuppressive regimen used after LT also plays a critical role in MetS as corticosteroids decrease peripheral glucose absorption, increase hepatic glucose production, and therefore increases the risk of developing post-LT diabetes[13]. Calcineurin inhibitors (CNIs) that are often used as a part of immunosuppressive therapy also are diabetogenic in nature[23]. The chronic use of sirolimus, which inhibits mammalian target of rapamycin (mTOR) multiprotein complexes, has also been shown to lead to hepatic IR [24].

Despite these proposed risk factors for developing graft steatosis after LT, there were inconsistencies among previous studies, likely related to the relatively small sample sizes, and therefore further studies with larger sample sizes are required to better elucidate the heterogeneous findings[25]. In the multivariate analysis with 9 related studies, the most consistent predictors of post-LT graft steatosis and steatohepatitis were post-LT BMI, hyperlipidemia, and history of alcohol use[11]. However, a subsequent meta-analysis showed that post-LT BMI was the only risk factor with a significant impact, a summarized odds ratio of 1.27 (1.19-1.35, $P < 0.001$)[11]. Pre-transplant variables did not have a consistent independent impact on the risk of post-LT graft steatosis and steatohepatitis in the meta-analysis, and immunosuppressive regimens did not show consistent effects[11]. Although post-LT BMI was identified as the consistent predictor, given inconsistent findings of pre-LT variables as a significant risk factor for post-LT graft steatosis and steatohepatitis, immunosuppressive regimen, and hyperlipidemia as risk factors, targeting post-LT obesity may not be sufficient for effective risk factor reduction.

In another meta-analysis assessing *de novo* graft steatosis and steatohepatitis in liver-transplanted patients, alcoholic and cryptogenic cirrhosis was related to the highest prevalence of *de novo* graft steatosis, 37%, and 35% respectively[12]. Ethanol consumption can cause excessive reactive oxygen species, hepatic lipid peroxidation [26], and cryptogenic cirrhosis is often thought to be "burnt-out" steatohepatitis, and

underlying steatohepatitis may be under-recognized. Therefore, such association of the highest prevalence of *de novo* graft steatosis in alcoholic and cryptogenic cirrhosis aligns with existing literature findings[12].

Dumortier *et al*[17] reported steatosis in donors as an important predictor of *de novo* NAFLD, and therefore the interaction between donor and recipient genetics may also affect disease recurrence[13]. Previous genomic studies have reported genetic variation in the patatin-like phospholipase domain as conferring susceptibility for the risk of fibrosis and steatosis[27]. The clinical implication of utilizing steatotic graft is uncertain, and therefore it is not clear if graft steatosis itself is a risk factor for post-LT graft steatosis[28]. Detecting recurrent or *de novo* graft steatosis/steatohepatitis is critical for better clinical outcomes in transplant recipients, and therefore further studies assessing optimal follow-up methodology such as specific diagnostic modalities and timing of follow-ups are warranted to quality care in this vulnerable population. Overall risk factors are summarized in Figure 1.

DIAGNOSIS

Liver biopsy is the gold standard to diagnose hepatic steatosis, hepatic fibrosis, and cirrhosis[29]. Although it has limitations of invasiveness, a small risk of complications, and potential sampling errors[30,31], liver biopsy is shown to be a safe and adequate diagnostic tool in post LT patients. It provides an ability to exclude or detect the presence and/or severity of the coexisting chronic liver disease[29,32]. The approach to diagnose graft steatosis and fibrosis is summarized in Figure 1.

Steatosis

The sensitivity of ultrasound to detect hepatic steatosis is poor when the liver occupies less than 20% of steatosis[33]. Computed tomography-based liver to spleen attenuation ratio can identify only if hepatic macrovesicular steatosis is more than 30%[34]. Biomarker panels such as the fatty liver index and the hepatic steatosis index can enhance the result of ultrasound in identifying hepatic steatosis[35,36]. However, there is limited literature regarding the roles of biomarkers in diagnosing hepatic steatosis in post-transplant settings. Transient elastography (TE) with controlled attenuation parameter (CAP) can predict the degree of hepatic steatosis in pre-transplant settings [37,38]. One study showed detecting graft steatosis with CAP in post LT patients but there is no histologic validation in the study[39]. Magnetic resonance imaging (MRI) based techniques such as MR spectroscopy and MRI-proton density fat fraction (MRI-PDFF) has been shown to accurately detect different degrees of hepatic steatosis[37, 38]. Further studies of MRI-based techniques in diagnosis post-transplant graft steatosis are warranted.

Fibrosis

Both ultrasound and computed tomography are unable to detect different stages of hepatic fibrosis unless the patients have the late stage of cirrhosis with portal hypertension[40]. Ultrasound based shear wave elastography (SWE), using acoustic radiation force impulse (ARFI) techniques, detect fibrosis in fatty liver patients. Studies showed point SWE and two-dimensional SWE accurately detect advanced fibrosis with good sensitivity and specificity in pre-LT setting[38]. Liver stiffness measured by TE also provides good performance in identifying advanced fibrosis. However, obesity, significant ascites, postprandial state, and significant hepatic inflammation or congestion can influence the interpretation. MR elastography (MRE) has also provided a useful and accurate way to identify advanced hepatic fibrosis[37, 38]. Noninvasive serum biomarker especially NAFLD fibrosis score (NFS), aspartate aminotransferase (AST) to platelet ratio index (APRI), and FIB4-score, AST, alanine aminotransferase (ALT) ratio (AAR), BARD, and fibrospect test have been shown to provide good performances in identifying advanced fibrosis in pretransplant NAFLD patients. However, the accuracy of MRE is outperformed compared to that of simple serum biomarkers to predict advanced fibrosis[41]. The major limitations of MRI-based techniques are availability, technical complexity, high cost, and contraindication in claustrophobic patients[37].

In post LT patients, quantifying the degree of liver stiffness or graft fibrosis is challenging. It can be due to preservation injury, fibrosis present before the transplantation. Fibrosis can be heterogeneous across the graft[42]. The acute cellular rejection or any inflammatory conditions overestimates liver stiffness measurement [43]. Given thrombocytopenia persists after liver transplantation despite the resolution

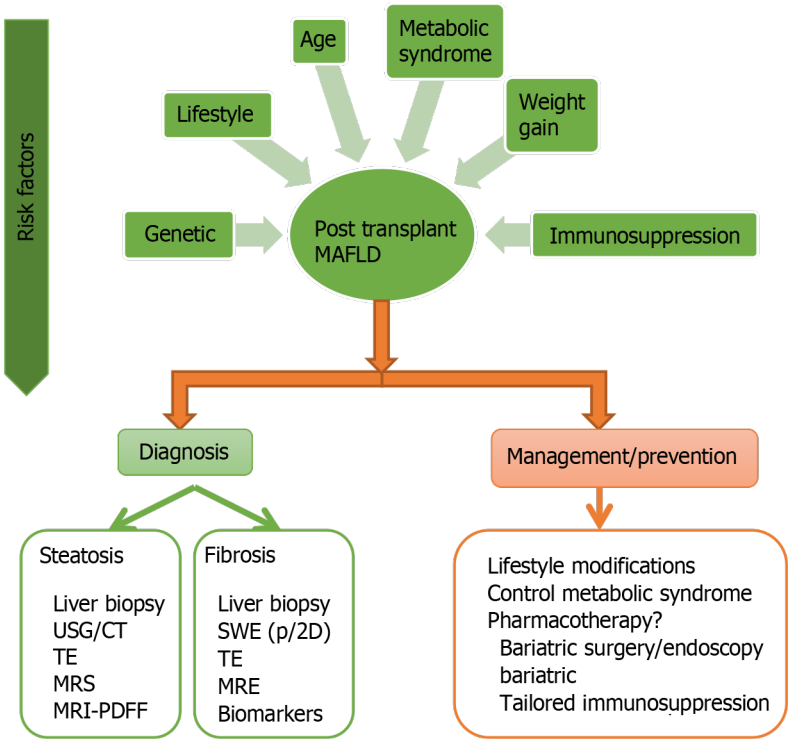

Figure 1 Overview of approach and management of post liver transplant metabolic dysfunction-associated fatty liver disease patients.
USG: Ultrasound; CT: Computed tomography; TE: Transient elastography; MRS: Magnetic resonance spectroscopy; MRI-PDFF: Magnetic resonance imaging-proton density fat fraction; SWE: Shear wave elastography; MRE: Magnetic resonance elastography.

of portal hypertension, serum biomarkers such as APRI or FIB4 that rely on platelet count may overestimate fibrosis[42]. There are a few studies regarding different noninvasive fibrosis tests (NITs) in post LT patients to identify recurrent fibrosis in different types of liver disease conditions. The meta-analysis suggests TE performs better than APRI and FIB4-score to diagnose significant fibrosis. The summary odds ratio was significantly higher for TE (21.27, 95%CI: 14.10-31.77, $P = 1 \times 10^{-30}$) compared to APRI (9.02, 94%CI: 5.79-14.07; $P = 1 \times 10^{-30}$) and FIB-4 (7.08, 95%CI: 4.00-12.55; $P = 1.93 \times 10^{-11}$). However, the majority of the studies are HCV patients[44]. Liver stiffness measured by TE at 3-mo post LT also predicts survival in LT recipients[45]. In a prospective study using ARFI to correlate histologic fibrosis score in 58 post-LT patients of mixed etiologies, the result demonstrated that SWE accurately detect advanced fibrosis (F ≥ 3) and cirrhosis (F4) with AUROC of 93 % and 80%, respectively. However, authors did not provide data on graft steatosis in these populations[46]. In a study of 32 post LT patients, the accuracy of both MRE and fibrospect test is high (AUROC of 0.87 and 0.84, respectively) in detecting fibrosis due to recurrent HCV[47]. In another study of 31 patients who underwent living donor liver transplantation with recurrent HCV infection to compare the accuracy of MRE, TE, and serum biomarkers (APRI and fibro α score to identify advanced fibrosis defined by Metavir stage ≥ 3, it showed MRE and fibro α score can accurately diagnose advanced fibrosis with AUROC of 0.708 and 0.833, respectively. The correlation of TE and APRI was not statistically significant to detect advanced fibrosis[48]. In a pooled analysis of MRE in LT recipients, AUROCs of MRE in detecting advanced fibrosis (stage ≥ 3) using a cut-off of 4.10 kPa and cirrhosis using a cut-off of 5.91 kPa were 0.83 and 0.96 respectively, suggesting high diagnostic accuracy[49].

However, there is limited literature in identifying different stages of hepatic fibrosis with NITs in post LT patients with either *de novo* or recurrent graft steatosis. A study by Galvin *et al*[20] of 430 post LT patients who developed *de novo* graft steatosis showed that the modest accuracy of FIB-4 and NFS to identify advanced fibrosis (F3-4) with AUROCs of 0.75 and 0.74, respectively. AAR with the optimal threshold of > 1.625 was found to have high specificity and accuracy with AUROC of 0.99 to identify cirrhosis (F4). However, only 9 (6%) of patients in the cohort had cirrhosis[20].

More studies are necessary to explore the accuracy of NITs in the diagnosis and assessment of steatosis and fibrosis in the post LT patients with either *de novo* or recurrent MAFLD.

NATURAL HISTORY AND LIVER OUTCOMES

Time-dependent relationships of either *de novo* or recurrent graft steatosis in the post LT patients were found in a few studies. Recurrent graft steatosis was diagnosed by TE in 87.5% of 56 post LT patients at a median time of 75 mo from liver transplantation. Advanced fibrosis was found in 26.8% whereas clinically compensated cirrhosis was found in 5.4% of patients. Recurrent graft steatosis was diagnosed by liver biopsy in 88.2% of 34 post LT patients at a median time of 47 mo from liver transplantation. Recurrent graft steatohepatitis was found in 41.2% of patients and bridging fibrosis was also found in 20.6% of patients who underwent liver biopsy[50]. Another study also showed that a time-dependent increase in the risk of recurrent graft steatosis approached 100% by 5 years compared to approximately 25% incidence of *de novo* graft steatosis in weight-matched controls who were being transplanted for primary biliary cirrhosis/primary sclerosing cholangitis or alcoholic liver cirrhosis[51]. *De novo* graft steatosis was found in 36.11% of 252 post LT patients after 5 years of liver transplantation in a study by Tejedor-Tejada *et al*[52]. Among the patients with *de novo* graft steatosis, significant fibrosis (F ≥ 2) was found in 85.6% with NFS, 81.9% with FIB4, 57.9% with APRI, 61.7% with AAR, and 83% with BARD after 5 years post LT. Similarly, 33.3% of 430 post LT liver biopsies from all causes were found to have *de novo* graft steatosis or steatohepatitis at a median of 3 years after liver transplantation. The significant risk factor for the development of significant fibrosis is age (OR 1.092, 95%CI: 1.02-1.17) on logistic regression analysis. The annual progression of fibrosis in patients with *de novo* graft steatosis was estimated to be 0.4 (interquartile range: 0.2-0.7) per year based on an approximation of fibrosis stage in relation to the number of years after liver transplantation. Insulin use is the only modifiable factor associated with the development of significant fibrosis (F ≥ 2)[20]. In a study by Vallin *et al*[10] that compared the natural history of *de novo* graft steatosis to recurrent graft steatosis, *de novo* graft steatosis was found in 67% and recurrent graft steatosis was found in 100% after 1 year. The prevalence of *de novo* graft steatosis increased to 69% after 3 years and 78% after 5 years. Steatosis disappeared in 22.5% of patients with *de novo* graft steatosis but none of the patients with recurrent graft steatosis disappeared graft steatosis. Recurrent graft steatosis developed advanced fibrosis (stage ≥ 3) in 71.4% of patients whereas *de novo* graft steatosis developed advanced fibrosis in only 12.5% of patients after 5 years post LT. Similarly, more frequent graft steatohepatitis was found in the recurrent graft steatosis group compared to the *de novo* graft steatosis group (71.4% *vs* 17.2%, $P < 0.01$).

Studies have shown worse outcomes in patients being transplanted from steatohepatitis with HCC as well as patients being re-transplanted for graft steatohepatitis[53,54]. *De novo* neoplasms were generally increased in patients with *de novo* graft steatosis compared to controls[52]. However, there is no literature showed an increase in the incidence of recurrent HCC in post LT patients with either *de novo* or recurrent graft steatosis.

PATIENT AND GRAFT SURVIVAL

In a large *de novo* graft steatosis cohort studied by Galvin *et al*[20], there is no significant difference in the short term (1 year) or long-term survival up to 15 years of patients with *de novo* graft steatosis ($n = 143$) compared to those without graft steatosis ($n = 287$) (log-rank 0.54). In another study by Narayanan *et al*[9], neither graft steatosis nor steatohepatitis (regardless of *de novo* or recurrent) was associated with patient mortality at 1 year after adjusting other patient characteristics ($P = 0.25$). De novo steatosis did not statistically significant impact patient survival (time-dependent HR 1.36, 95%CI: 0.99-1.87, $P = 0.057$) or graft survival (time-dependent HR 1.26, 95%CI: 0.92-1.72, $P = 0.15$) after excluding patients with pretransplant hepatic steatosis. Graft survival was not affected by time-dependent graft steatosis nor pre-transplant steatohepatitis. None of the cohorts required re-transplantation due to recurrent steatohepatitis. The study did not show any significant difference in death and fibrosis progression between patients with biopsy-proven *de novo vs* recurrent steatohepatitis [9]. In a study of 252 post LT patients by Tejedor-Tejada *et al*[52], there is no significant difference in the medium and long-term survival between patients with *de novo* graft steatosis and controls[52].

EXTRAHEPATIC OUTCOMES

MAFLD, by definition, is associated with obesity, IR, dyslipidemia, and hypertension, and those conditions have an important impact on transplanted patient outcomes. MAFLD and MetS are intertwined, and this is evident in post-transplant patients that develop MAFLD, either *de novo* or recurrent. In recurrent MAFLD, the MetS risk factors that exist before transplant will persist. In *de novo* MALFD, those risk factors are triggered by immunosuppression (IS) or rapid weight gain after transplant. In both cases, patients carry the same metabolic profile: IR, dyslipidemia, hypertension, and obesity. Indeed, one-third of patients develop DM and obesity in 3 years post-transplant[55]. Another common element between *de novo* and recurrent MAFLD is the use of IS after transplant. Steroids, CNIs are known to cause hypertension, hyperglycemia. mTOR inhibitors often triggers hyperlipidemia in post-transplant patients.

The evidence shows that transplanted patients with recurrent graft steatosis have an increased rate of DM, dyslipidemia, and weight gain[56]. There is reciprocity between MAFLD and MetS. Transplanted patients with *de novo* graft steatosis are five times more likely to be obese and two times more likely to have DM[57]. On the other hand, Sprinzl *et al*[58] reported that almost one-third of patients who underwent a LT in his cohort developed MetS, linked to graft steatosis. Indeed, obesity and dyslipidemia were predictors for the development of *de novo* graft steatosis within one year post LT [58].

The most common cause of death in the population with steatohepatitis are cardiovascular (CV) disease and malignancies[9]. It is easy to extrapolate that the CV and malignancies are also a significant cause of mobility and mortality in post-transplant patients who develop MASH, either *de novo* or recurrent. CV events included myocardial infarction, angina, ischemic stroke, sudden death, and peripheral artery disease. Extrahepatic malignancy included urology, head and neck, skin, lung, hematological, gynecological, gastrointestinal, and brain cancer. Bhati *et al*[50] showed that mortality was attributed to cancer in 25%, infections in 25%, and CV complications in 21% in post LT patients with recurrent graft steatosis[50]. Gitto *et al*[57] demonstrated that post LT patients with *de novo* graft steatosis had an increased risk for CV disease and extrahepatic cancers. Specific factors associated with CV disease in the post-transplant setting are age > 55 years old, male sex, DM, and kidney failure [59]. In a study by Tejedor-Tejada *et al*[52], CV events were found more frequently in patients with post LT *de novo* graft steatosis than controls (23.08% *vs* 19.88%). Similarly, *de novo* malignancies were found more in *de novo* graft steatosis group compared to control (24.18% *vs* 19.25%)[52]

MANAGEMENT

There is very scarce data about post LT *de novo* and recurrent MAFLD management, but recommendations can be drawn from the treatment of MAFLD in the general population. In general, prevention of MetS and gaining weight is the best approach in post-transplant patients. Overall management is summarized in Table 1 and Figure 1.

Lifestyle modifications

Lifestyle modifications are the backbone of the treatment of MAFLD. This approach can target specific components of MetS and is the recommended first treatment for hepatic steatosis[29,60]. Fussner *et al*[61] showed that an increase in BMI was a concrete risk factor for MetS at one-year post-transplant. Hence, avoiding excessive weight gain in the immediate post-transplant setting can help decrease the incidence of MetS. Lifestyle modifications include various and multidisciplinary strategies like physical activity, personalized diet, and behavioral interventions to hold weight gain. Loss of 3%-5% of the body weight showed improved steatosis, and loss of 7%-10% of body weight improved steatohepatitis on a report by Vilar-Gomez *et al*[62]. Evidence shows that decreasing the caloric intake by 750-1000 kcal/d or by 30% resulted in improved IR and hepatic steatosis[63,64]. The literature also shows that high cholesterol diets can trigger steatohepatitis in a mice model[65]. Additionally, the European Association for the Study of the Liver (EASL) recommends avoiding fructose intake since it is associated with hepatic steatosis[60]. The American Association for the Study of Liver Diseases recommends abstinence of heavy alcohol drinking (more than four standard drinks on any day or more than 14 drinks per week in men or more than three drinks on any day or seven drinks per week in women)[29].

Table 1 Summary management strategies

Lifestyle modifications	Dietary modification
	Exercise/ physical activity
	Avoid heavy alcohol consumption
	Benefit with coffee consumption
Pharmacotherapy	No approved drug for MAFLD in post liver transplants patients
Bariatric treatment	Surgery
	Endoscopic
Tailored Immunosuppression	Early taper of steroids
	Decreasing CNIs as possible
	Avoid/cautious use of mTOR inhibitors

CNIs: Calcineurin inhibitors; MAFLD: Metabolic dysfunction-associated fatty liver disease.

In comparison, EASL recommends keeping the alcohol consumption below 30 g in men and 20 g in women since there is evidence of a decrease in the prevalence of hepatic steatosis with moderate alcohol[60]. Interestingly, coffee consumption has been associated with fibrosis risk reduction[66].

In terms of exercise, Kistler *et al*[67] reported that vigorous physical activity held fibrosis progression in hepatic steatosis. The combination of caloric restriction and exercise resulted in weight loss associated with histological improvement of steatohepatitis[62]. However, a trial of dietary counseling and exercise *vs* standard of care after liver transplantation reported only a moderate benefit; still, adhesion to the program was achieved on only 37% of the patients[68]. Therefore, the recommendation for post LT patients with MAFLD is weight loss through diet and exercise.

Pharmacotherapy

It is essential to acknowledge that there is no approved drug for the specific treatment of MAFLD. Nevertheless, there is a significant number of drugs under investigation for hepatic steatosis and steatohepatitis. Pharmacotherapy in patients with hepatic steatosis is used in two ways: to achieve control goals in diabetes, dyslipidemia, and hypertension and to target the progression of the hepatic steatosis. In both cases, caution with drug interaction in post-transplant patients is recommended[69]. MAFLD patients with MetS comorbidities need to have reasonable control of their sugars, lipids, and blood pressure, and they should be referred to a specialist in those areas if necessary. Although not recommended for the treatment of MAFLD *per se*, statins should not be held for those patients meeting lipid profile criteria for statin use[29,70]. The same can be said for diabetic agents; none of them are approved for MALFD treatment but may be used in diabetic patients with steatosis as some have shown some benefits such as pioglitazone and empagliflozin.

In the PIVENS trial, both pioglitazone and vitamin E improved biopsy-proven NASH, although the histological improvement with vitamin E was better[71]. Vitamin E should be used only in diabetic patients. Interestingly, pioglitazone was associated with weight gain. Liraglutide, a glucagon-like peptide-1, was associated in a randomized trial with the resolution of steatohepatitis, minor progression of fibrosis, and weight loss in patients with biopsy-proven NASH[72]. More recently, empagliflozin, a sodium-glucose cotransporter-2 inhibitor, has been shown to reduce steatosis and improve ALT in NAFLD diabetic patients[73]. Orlistat, a medication used for weight loss, has been associated with steatosis improvement, though this effect can be attributed to the weight loss in itself[74].

Metformin, ursodeoxycholic acid, and pentoxifylline have been tried with poor outcomes. Nevertheless, many other drugs as obeticholic acid and elafibranor, are under investigation with promising results. There is no clinical trial of an investigational drug in post LT patients with either *de novo* or recurrent MALFLD.

Bariatric surgery

Maintaining an adequate weight proves to be challenging. Although weight loss of > 7% was associated with improvement in steatohepatitis, only half of the patients

Table 2 Summary of clinical significances and outcomes of *de novo* and recurrent metabolic dysfunction-associated fatty liver disease in post liver transplant patients

	De novo MAFLD	Recurrent MAFLD
Risk factors/Predictors for post LT MAFLD	Post LT weight gain	Post LT weight gain
	HCV	Post-transplant hypertriglyceridemia
	Sirolimus-based immunosuppressant therapy	Steroid
	Insulin resistance/diabetes mellitus	Post LT Metabolic syndrome
		Insulin use
		Insulin resistance/ diabetes mellitus
Progression to steatohepatitis and advanced fibrosis	Less common	More common
Cardiovascular events	Common	Common
Patient and graft survival	No significant impact	No significant impact

LT: Liver transplant; HCV: Hepatitis C virus; MAFLD: Metabolic dysfunction-associated fatty liver disease.

achieved this goal[62]. Bariatric surgery improves long-term mortality from CV disease and cancer in the general population[75]. In a study with steatohepatitis patients who underwent bariatric surgery, 85% had resolution of steatohepatitis with improved fibrosis in 33% of the patients[76]. There are some case reports of bariatric surgery in transplanted patients; Al-Nowaylati *et al*[77] described improvement in weight, glycemia, and HDL in seven patients. Diwan *et al*[78] reported similar findings, but with a high rate of complications and mortality of 20%. Endoscopic bariatric approaches are also on the rise; those techniques demonstrate to be effective weight loss leading to improvement in steatohepatitis[79]. Endoscopy bariatric treatment can be a very feasible option in the post-transplant setting for patients with MAFLD.

Tailored IS

It is known that IS is a contributing factor in the development of MetS after LT. IS can exacerbate preexisting risk factors and contribute to recurrent MAFLD. Similarly, IS can create the conditions to develop *de novo* MAFLD in patients transplanted for other causes requiring higher IS, such as autoimmune hepatitis or rejection. Alas, IS is essential in the post-transplant period. Consequently, a tailored approach looking to reduce the risk factors for MetS and hence MAFLD should be used. Early taper of steroids and decreasing as possible CNIs by adding other agents can add to the glycemic control in transplanted patients with diabetes. Everolimus plus a low dose of tacrolimus has shown a moderate decrease in weight in post-transplant patients[80]; this strategy, along with a rapid decrease in steroids, can be helpful in obese patients. CNIs can also contribute to hypertension and dyslipidemia. Approaches to minimize those side effects can be helpful. mTOR inhibitors are associated with elevated triglycerides; thus, they should be avoided in patients is MAFLD. In summary, protocols with early tapering of steroids and minimal use of CI:N should be considered in post-transplant patients with already risk factors for MAFLD and to minimize the development of those.

CONCLUSION

Given MAFLD is the fastest growing indication for liver transplantation; both *de novo* and recurrent graft steatosis in the context of MetS or MAFLD are common in the post-transplant settings. The role of noninvasive tests in detecting graft steatosis and fibrosis is challenging. Given the performance of image-based techniques is promising, larger cohort studies with histologic validation are necessary. Liver biopsy remains the gold standard for detecting graft steatosis and different degree of graft fibrosis. Although *de novo* and recurrent MAFLD after transplant have common pathways, it appears that recurrent MASH is more severe than *de novo*. Recurrent graft steatosis with the progression of fibrosis is found to be more frequent in patients being transplanted for hepatic steatosis compared to those with *de novo* graft steatosis. Even

though graft steatosis has an impact on CV events and incidence of *de novo* neoplasms, the patient and graft survival seem to be not affected by either *de novo* or recurrent graft steatosis. Management is mainly focused on weight control and tailoring of immunosuppressive therapy. The clinical significances and outcomes of both *de novo* and recurrent MAFLD in post LT population is summarized in Table 2. There are many knowledge gaps in the field of post LT MAFLD and MASH. Further studies are required for long-term outcomes of post LT MAFLD and MASH population and management strategies.

REFERENCES

1 **Eslam M**, Newsome PN, Sarin SK, Anstee QM, Targher G, Romero-Gomez M, Zelber-Sagi S, Wai-Sun Wong V, Dufour JF, Schattenberg JM, Kawaguchi T, Arrese M, Valenti L, Shiha G, Tiribelli C, Yki-Järvinen H, Fan JG, Grønbæk H, Yilmaz Y, Cortez-Pinto H, Oliveira CP, Bedossa P, Adams LA, Zheng MH, Fouad Y, Chan WK, Mendez-Sanchez N, Ahn SH, Castera L, Bugianesi E, Ratziu V, George J. A new definition for metabolic dysfunction-associated fatty liver disease: An international expert consensus statement. *J Hepatol* 2020; **73**: 202-209 [PMID: 32278004 DOI: 10.1016/j.jhep.2020.03.039]

2 **Eslam M**, Sanyal AJ, George J, Sanyal A, Neuschwander-tetri B, Tiribelli C, Kleiner DE, Brunt E, Bugianesi E, Yki-järvinen H, Grønbæk H, Cortez-pinto H, Fan J, Valenti L, Abdelmalek M, Romero-gomez M, Rinella M, Arrese M, Eslam M, Bedossa P, Newsome PN, Anstee QM, Jalan R, Bataller R, Loomba R, Sookoian S, Sarin SK, Harrison S, Kawaguchi T, Wong VW, Ratziu V, Yilmaz Y, Younossi Z. MAFLD: A Consensus-Driven Proposed Nomenclature for Metabolic Associated Fatty Liver Disease. *Gastroenterology* 2020; **158**: 1999-2014.e1 [PMID: 32044314 DOI: 10.1053/j.gastro.2019.11.312]

3 **Younossi ZM**, Stepanova M, Ong J, Trimble G, AlQahtani S, Younossi I, Ahmed A, Racila A, Henry L. Nonalcoholic Steatohepatitis Is the Most Rapidly Increasing Indication for Liver Transplantation in the United States. *Clin Gastroenterol Hepatol* 2021; **19**: 580-589.e5 [PMID: 32531342 DOI: 10.1016/j.cgh.2020.05.064]

4 **Davidson JA**, Wilkinson A; International Expert Panel on New-Onset Diabetes after Transplantation. New-Onset Diabetes After Transplantation 2003 International Consensus Guidelines: an endocrinologist's view. Diabetes Care. 2004; 27: 805-812 [PMID: 14988309 DOI: 10.2337/diacare.27.3.805]

5 **Pham PT**, Pham PM, Pham SV, Pham PA, Pham PC. New onset diabetes after transplantation [NODAT]: an overview. *Diabetes Metab Syndr Obes* 2011; **4**: 175-186 [PMID: 21760734 DOI: 10.2147/DMSO.S19027]

6 **Charco R**, Cantarell C, Vargas V, Capdevila L, Lázaro JL, Hidalgo E, Murio E, Margarit C. Serum cholesterol changes in long-term survivors of liver transplantation: a comparison between cyclosporine and tacrolimus therapy. *Liver Transpl Surg* 1999; **5**: 204-208 [PMID: 10226111 DOI: 10.1002/Lt.500050303]

7 **Watt KD**, Pedersen RA, Kremers WK, Heimbach JK, Charlton MR. Evolution of causes and risk factors for mortality post-liver transplant: results of the NIDDK long-term follow-up study. *Am J Transplant* 2010; **10**: 1420-1427 [PMID: 20486907 DOI: 10.1111/j.1600-6143.2010.03126.x]

8 **Bhagat V**, Mindikoglu AL, Nudo CG, Schiff ER, Tzakis A, Regev A. Outcomes of liver transplantation in patients with cirrhosis due to nonalcoholic steatohepatitis *vs* patients with cirrhosis due to alcoholic liver disease. *Liver Transpl* 2009; **15**: 1814-1820 [PMID: 19938128 DOI: 10.1002/lt.21927]

9 **Narayanan P**, Mara K, Izzy M, Dierkhising R, Heimbach J, Allen AM, Watt KD. Recurrent or De Novo Allograft Steatosis and Long-term Outcomes After Liver Transplantation. *Transplantation* 2019; **103**: e14-e21 [PMID: 29994981 DOI: 10.1097/TP.0000000000002317]

10 **Vallin M**, Guillaud O, Boillot O, Hervieu V, Scoazec JY, Dumortier J. Recurrent or de novo nonalcoholic fatty liver disease after liver transplantation: natural history based on liver biopsy analysis. Liver Transpl. 2014; 20: 1064-1071 [PMID: 24961607 DOI: 10.1002/lt.23936]

11 **Saeed N**, Glass L, Sharma P, Shannon C, Sonnenday CJ, Tincopa MA. Incidence and Risks for Nonalcoholic Fatty Liver Disease and Steatohepatitis Post-liver Transplant: Systematic Review and Meta-analysis. *Transplantation* 2019; **103**: e345-e354 [PMID: 31415032 DOI: 10.1097/TP.0000000000002916]

12 **Losurdo G**, Castellaneta A, Rendina M, Carparelli S, Leandro G, Di Leo A. Systematic review with meta-analysis: de novo non-alcoholic fatty liver disease in liver-transplanted patients. *Aliment Pharmacol Ther* 2018; **47**: 704-714 [PMID: 29359341 DOI: 10.1111/apt.14521]

13 **Sharma P**, Arora A. Approach to prevention of non-alcoholic fatty liver disease after liver transplantation. *Transl Gastroenterol Hepatol* 2020; **5**: 51 [PMID: 33073046 DOI: 10.21037/tgh.2020.03.02]

14 **Pais R**, Barritt AS 4th, Calmus Y, Scatton O, Runge T, Lebray P, Poynard T, Ratziu V, Conti F. NAFLD and liver transplantation: Current burden and expected challenges. *J Hepatol* 2016; **65**: 1245-1257 [PMID: 27486010 DOI: 10.1016/j.jhep.2016.07.033]

15 **Burra P**, Becchetti C, Germani G. NAFLD and liver transplantation: Disease burden, current

management and future challenges. *JHEP Rep* 2020; **2**: 100192 [PMID: 33163950 DOI: 10.1016/j.jhepr.2020.100192]

16 **Yalamanchili K**, Saadeh S, Klintmalm GB, Jennings LW, Davis GL. Nonalcoholic fatty liver disease after liver transplantation for cryptogenic cirrhosis or nonalcoholic fatty liver disease. *Liver Transpl* 2010; **16**: 431-439 [PMID: 20373454 DOI: 10.1002/lt.22004]

17 **Dumortier J**, Giostra E, Belbouab S, Morard I, Guillaud O, Spahr L, Boillot O, Rubbia-Brandt L, Scoazec JY, Hadengue A. Non-alcoholic fatty liver disease in liver transplant recipients: another story of "seed and soil". *Am J Gastroenterol* 2010; **105**: 613-620 [PMID: 20040915 DOI: 10.1038/ajg.2009.717]

18 **El Atrache MM**, Abouljoud MS, Divine G, Yoshida A, Kim DY, Kazimi MM, Moonka D, Huang MA, Brown K. Recurrence of non-alcoholic steatohepatitis and cryptogenic cirrhosis following orthotopic liver transplantation in the context of the metabolic syndrome. *Clin Transplant* 2012; **26**: E505-E512 [PMID: 23061759 DOI: 10.1111/ctr.12014]

19 **Dureja P**, Mellinger J, Agni R, Chang F, Avey G, Lucey M, Said A. NAFLD recurrence in liver transplant recipients. *Transplantation* 2011; **91**: 684-689 [PMID: 21248661 DOI: 10.1097/TP.0b013e31820b6b84]

20 **Galvin Z**, Rajakumar R, Chen E, Adeyi O, Selzner M, Grant D, Sapisochin G, Greig P, Cattral M, McGilvray I, Ghanekar A, Selzner N, Lilly L, Patel K, Bhat M. Predictors of De Novo Nonalcoholic Fatty Liver Disease After Liver Transplantation and Associated Fibrosis. *Liver Transpl* 2019; **25**: 56-67 [PMID: 30609189 DOI: 10.1002/lt.25338]

21 **Tilg H**, Moschen AR, Roden M. NAFLD and diabetes mellitus. *Nat Rev Gastroenterol Hepatol* 2017; **14**: 32-42 [PMID: 27729660 DOI: 10.1038/nrgastro.2016.147]

22 **Asrih M**, Jornayvaz FR. Metabolic syndrome and nonalcoholic fatty liver disease: Is insulin resistance the link? *Mol Cell Endocrinol* 2015; **418** Pt 1: 55-65 [PMID: 25724480 DOI: 10.1016/j.mce.2015.02.018]

23 **Zimmermann A**, Zobeley C, Weber MM, Lang H, Galle PR, Zimmermann T. Changes in lipid and carbohydrate metabolism under mTOR- and calcineurin-based immunosuppressive regimen in adult patients after liver transplantation. *Eur J Intern Med* 2016; **29**: 104-109 [PMID: 26775180 DOI: 10.1016/j.ejim.2015.12.022]

24 **Bhat M**, Sonenberg N, Gores GJ. The mTOR pathway in hepatic malignancies. *Hepatology* 2013; **58**: 810-818 [PMID: 23408390 DOI: 10.1002/hep.26323]

25 **Tokodai K**, Karadagi A, Kjaernet F, Romano A, Ericzon BG, Nowak G. Characteristics and risk factors for recurrence of nonalcoholic steatohepatitis following liver transplantation. *Scand J Gastroenterol* 2019; **54**: 233-239 [PMID: 30999770 DOI: 10.1080/00365521.2019.1577484]

26 **Chauhan SS**, Mahmood A, Ojha S. Ethanol and age enhances fluoride toxicity through oxidative stress and mitochondrial dysfunctions in rat intestine. *Mol Cell Biochem* 2013; **384**: 251-262 [PMID: 24022343 DOI: 10.1007/s11010-013-1804-6]

27 **Romeo S**, Kozlitina J, Xing C, Pertsemlidis A, Cox D, Pennacchio LA, Boerwinkle E, Cohen JC, Hobbs HH. Genetic variation in PNPLA3 confers susceptibility to nonalcoholic fatty liver disease. *Nat Genet* 2008; **40**: 1461-1465 [PMID: 18820647 DOI: 10.1038/ng.257]

28 **Deroose JP**, Kazemier G, Zondervan P, Ijzermans JN, Metselaar HJ, Alwayn IP. Hepatic steatosis is not always a contraindication for cadaveric liver transplantation. *HPB (Oxford)* 2011; **13**: 417-425 [PMID: 21609375 DOI: 10.1111/j.1477-2574.2011.00310.x]

29 **Chalasani N**, Younossi Z, Lavine JE, Charlton M, Cusi K, Rinella M, Harrison SA, Brunt EM, Sanyal AJ. The diagnosis and management of nonalcoholic fatty liver disease: Practice guidance from the American Association for the Study of Liver Diseases. *Hepatology* 2018; **67**: 328-357 [PMID: 28714183 DOI: 10.1002/hep.29367]

30 **Bedossa P**, Dargère D, Paradis V. Sampling variability of liver fibrosis in chronic hepatitis C. *Hepatology* 2003; **38**: 1449-1457 [PMID: 14647056 DOI: 10.1016/j.hep.2003.09.022]

31 **Takyar V**, Etzion O, Heller T, Kleiner DE, Rotman Y, Ghany MG, Fryzek N, Williams VH, Rivera E, Auh S, Liang TJ, Hoofnagle JH, Koh C. Complications of percutaneous liver biopsy with Klatskin needles: a 36-year single-centre experience. *Aliment Pharmacol Ther* 2017; **45**: 744-753 [PMID: 28074540 DOI: 10.1111/apt.13939]

32 **Alten TA**, Negm AA, Voigtländer T, Jaeckel E, Lehner F, Brauner C, Wedemeyer H, Manns MP, Lankisch TO. Safety and performance of liver biopsies in liver transplant recipients. *Clin Transplant* 2014; **28**: 585-589 [PMID: 24628078 DOI: 10.1111/ctr.12352]

33 **Dasarathy S**, Dasarathy J, Khiyami A, Joseph R, Lopez R, McCullough AJ. Validity of real time ultrasound in the diagnosis of hepatic steatosis: a prospective study. *J Hepatol* 2009; **51**: 1061-1067 [PMID: 19846234 DOI: 10.1016/j.jhep.2009.09.001]

34 **Rogier J**, Roullet S, Cornélis F, Biais M, Quinart A, Revel P, Bioulac-Sage P, Le Bail B. Noninvasive assessment of macrovesicular liver steatosis in cadaveric donors based on computed tomography liver-to-spleen attenuation ratio. *Liver Transpl* 2015; **21**: 690-695 [PMID: 25761371 DOI: 10.1002/lt.24105]

35 **Bedogni G**, Bellentani S, Miglioli L, Masutti F, Passalacqua M, Castiglione A, Tiribelli C. The Fatty Liver Index: a simple and accurate predictor of hepatic steatosis in the general population. *BMC Gastroenterol* 2006; **6**: 33 [PMID: 17081293 DOI: 10.1186/1471-230X-6-33]

36 **Lee JH**, Kim D, Kim HJ, Lee CH, Yang JI, Kim W, Kim YJ, Yoon JH, Cho SH, Sung MW, Lee HS. Hepatic steatosis index: a simple screening tool reflecting nonalcoholic fatty liver disease. *Dig Liver Dis* 2010; **42**: 503-508 [PMID: 19766548 DOI: 10.1016/j.dld.2009.08.002]

37 **Han MA**, Saouaf R, Ayoub W, Todo T, Mena E, Noureddin M. Magnetic resonance imaging and transient elastography in the management of Nonalcoholic Fatty Liver Disease [NAFLD]. *Expert Rev Clin Pharmacol* 2017; **10**: 379-390 [PMID: 28277807 DOI: 10.1080/17512433.2017.1299573]

38 **Han MAT**. Noninvasive Tests [NITs] for Hepatic Fibrosis in Fatty Liver Syndrome. *Life [Basel]* 2020; **10**: 198 [PMID: 32933184 DOI: 10.3390/Life10090198]

39 **Karlas T**, Kollmeier J, Böhm S, Müller J, Kovacs P, Tröltzsch M, Weimann A, Bartels M, Rosendahl J, Mössner J, Berg T, Keim V, Wiegand J. Noninvasive characterization of graft steatosis after liver transplantation. *Scand J Gastroenterol* 2015; **50**: 224-232 [PMID: 25429378 DOI: 10.3109/00365521.2014.983156]

40 **Lee SS**, Park SH. Radiologic evaluation of nonalcoholic fatty liver disease. *World J Gastroenterol* 2014; **20**: 7392-7402 [PMID: 24966609 DOI: 10.3748/wjg.v20.i23.7392]

41 **Cui J**, Ang B, Haufe W, Hernandez C, Verna EC, Sirlin CB, Loomba R. Comparative diagnostic accuracy of magnetic resonance elastography vs. eight clinical prediction rules for non-invasive diagnosis of advanced fibrosis in biopsy-proven non-alcoholic fatty liver disease: a prospective study. *Aliment Pharmacol Ther* 2015; **41**: 1271-1280 [PMID: 25873207 DOI: 10.1111/apt.13196]

42 **Germani G**, Laryea M, Rubbia-Brandt L, Egawa H, Burra P, O'Grady J, Watt KD. Management of Recurrent and De Novo NAFLD/NASH After Liver Transplantation. *Transplantation* 2019; **103**: 57-67 [PMID: 30335694 DOI: 10.1097/TP.0000000000002485]

43 **Crespo G**, Castro-Narro G, García-Juárez I, Benítez C, Ruiz P, Sastre L, Colmenero J, Miquel R, Sánchez-Fueyo A, Forns X, Navasa M. Usefulness of liver stiffness measurement during acute cellular rejection in liver transplantation. *Liver Transpl* 2016; **22**: 298-304 [PMID: 26609794 DOI: 10.1002/lt.24376]

44 **Bhat M**, Tazari M, Sebastiani G. Performance of transient elastography and serum fibrosis biomarkers for non-invasive evaluation of recurrent fibrosis after liver transplantation: A meta-analysis. *PLoS One* 2017; **12**: e0185192 [PMID: 28953939 DOI: 10.1371/journal.pone.0185192]

45 **Pfeiffenberger J**, Hornuss D, Houben P, Wehling C, Von Haken R, Lozanovski V, Mieth M, Mehrabi A, Weiss KH, Friedrich K. Routine Liver Elastography Could Predict Actuarial Survival after Liver Transplantation. *J Gastrointestin Liver Dis* 2019; **28**: 271-277 [PMID: 31517323 DOI: 10.15403/jgld-218]

46 **Wildner D**, Strobel D, Konturek PC, Görtz RS, Croner RS, Neurath MF, Zopf S. Impact of acoustic radiation force impulse imaging in clinical practice of patients after orthotopic liver transplantation. *Med Sci Monit* 2014; **20**: 2027-2035 [PMID: 25342166 DOI: 10.12659/MSM.891126]

47 **Lee VS**, Miller FH, Omary RA, Wang Y, Ganger DR, Wang E, Rao S, Levitsky J. Magnetic resonance elastography and biomarkers to assess fibrosis from recurrent hepatitis C in liver transplant recipients. *Transplantation* 2011; **92**: 581-586 [PMID: 21822174 DOI: 10.1097/TP.0b013e31822805fa]

48 **El-Meteini M**, Sakr M, Eldorry A, Mohran Z, Abdelkader NA, Dabbous H, Montasser I, Refaie R, Salah M, Aly M. Non-Invasive Assessment of Graft Fibrosis After Living Donor Liver Transplantation: Is There Still a Role for Liver Biopsy? *Transplant Proc* 2019; **51**: 2451-2456 [PMID: 31358454 DOI: 10.1016/j.transproceed.2019.01.197]

49 **Singh S**, Venkatesh SK, Keaveny A, Adam S, Miller FH, Asbach P, Godfrey EM, Silva AC, Wang Z, Murad MH, Asrani SK, Lomas DJ, Ehman RL. Diagnostic accuracy of magnetic resonance elastography in liver transplant recipients: A pooled analysis. *Ann Hepatol* 2016; **15**: 363-376 [PMID: 27049490 DOI: 10.5604/16652681.1198808]

50 **Bhati C**, Idowu MO, Sanyal AJ, Rivera M, Driscoll C, Stravitz RT, Kohli DR, Matherly S, Puri P, Gilles H, Cotterell A, Levy M, Sterling RK, Luketic VA, Lee H, Sharma A, Siddiqui MS. Long-term Outcomes in Patients Undergoing Liver Transplantation for Nonalcoholic Steatohepatitis-Related Cirrhosis. *Transplantation* 2017; **101**: 1867-1874 [PMID: 28296807 DOI: 10.1097/TP.0000000000001709]

51 **Contos MJ**, Cales W, Sterling RK, Luketic VA, Shiffman ML, Mills AS, Fisher RA, Ham J, Sanyal AJ. Development of nonalcoholic fatty liver disease after orthotopic liver transplantation for cryptogenic cirrhosis. *Liver Transpl* 2001; **7**: 363-373 [PMID: 11303298 DOI: 10.1053/jlts.2001.23011]

52 **Tejedor-Tejada J**, Valenzuela EF, Muñoz RN, Gómez LH, García-Pajares F, Álvarez CA, Sánchez-Martín F, Alonso-Martín C, Sánchez-Antolín G. De-novo nonalcoholic fatty liver disease at 5 years after liver transplantation: prevalence and predictive factors. *Eur J Gastroenterol Hepatol* 2021; **33**: 399-406 [PMID: 32317584 DOI: 10.1097/MEG.0000000000001736]

53 **Thuluvath AJ**, Chen PH, Thuluvath PJ, Kantsevoy S, Savva Y. Poor Survival After Retransplantation in NASH Cirrhosis. *Transplantation* 2019; **103**: 101-108 [PMID: 29470354 DOI: 10.1097/TP.0000000000002135]

54 **Kern B**, Feurstein B, Fritz J, Fabritius C, Sucher R, Graziadei I, Bale R, Tilg H, Zoller H, Newsome P, Eschertzhuber S, Margreiter R, Öfner D, Schneeberger S. High incidence of hepatocellular carcinoma and postoperative complications in patients with nonalcoholic steatohepatitis as a primary indication for deceased liver transplantation. *Eur J Gastroenterol Hepatol* 2019; **31**: 205-210 [PMID: 30320609 DOI: 10.1097/MEG.0000000000001270]

55 **Watt KD**. Metabolic syndrome: is immunosuppression to blame? *Liver Transpl* 2011; **17** Suppl 3: S38-S42 [PMID: 21761552 DOI: 10.1002/lt.22386]

56 **Malik SM**, Devera ME, Fontes P, Shaikh O, Sasatomi E, Ahmad J. Recurrent disease following liver transplantation for nonalcoholic steatohepatitis cirrhosis. *Liver Transpl* 2009; **15**: 1843-1851 [PMID:

19938117 DOI: 10.1002/lt.21943]
57 **Gitto S**, de Maria N, di Benedetto F, Tarantino G, Serra V, Maroni L, Cescon M, Pinna AD, Schepis F, Andreone P, Villa E. De-novo nonalcoholic steatohepatitis is associated with long-term increased mortality in liver transplant recipients. *Eur J Gastroenterol Hepatol* 2018; **30**: 766-773 [PMID: 29505475 DOI: 10.1097/MEG.0000000000001105]
58 **Sprinzl MF**, Weinmann A, Lohse N, Tönissen H, Koch S, Schattenberg J, Hoppe-Lotichius M, Zimmermann T, Galle PR, Hansen T, Otto G, Schuchmann M. Metabolic syndrome and its association with fatty liver disease after orthotopic liver transplantation. *Transpl Int* 2013; **26**: 67-74 [PMID: 23126674 DOI: 10.1111/j.1432-2277.2012.01576.x]
59 **Vanwagner LB**, Bhave M, Te HS, Feinglass J, Alvarez L, Rinella ME. Patients transplanted for nonalcoholic steatohepatitis are at increased risk for postoperative cardiovascular events. *Hepatology* 2012; **56**: 1741-1750 [PMID: 22611040 DOI: 10.1002/hep.25855]
60 **European Association for the Study of the Liver (EASL)**; European Association for the Study of Diabetes (EASD); European Association for the Study of Obesity (EASO). EASL-EASD-EASO Clinical Practice Guidelines for the management of non-alcoholic fatty liver disease. *J Hepatol* 2016; **64**: 1388-1402 [PMID: 27062661 DOI: 10.1016/j.jhep.2015.11.004]
61 **Fussner LA**, Heimbach JK, Fan C, Dierkhising R, Coss E, Leise MD, Watt KD. Cardiovascular disease after liver transplantation: When, What, and Who Is at Risk. *Liver Transpl* 2015; **21**: 889-896 [PMID: 25880971 DOI: 10.1002/lt.24137]
62 **Vilar-Gomez E**, Martinez-Perez Y, Calzadilla-Bertot L, Torres-Gonzalez A, Gra-Oramas B, Gonzalez-Fabian L, Friedman SL, Diago M, Romero-Gomez M. Weight Loss Through Lifestyle Modification Significantly Reduces Features of Nonalcoholic Steatohepatitis. *Gastroenterology* 2015; **149**: 367-78.e5; quiz e14 [PMID: 25865049 DOI: 10.1053/j.gastro.2015.04.005]
63 **Kirk E**, Reeds DN, Finck BN, Mayurranjan SM, Patterson BW, Klein S. Dietary fat and carbohydrates differentially alter insulin sensitivity during caloric restriction. *Gastroenterology* 2009; **136**: 1552-1560 [PMID: 19208352 DOI: 10.1053/j.gastro.2009.01.048]
64 **Haufe S**, Engeli S, Kast P, Böhnke J, Utz W, Haas V, Hermsdorf M, Mähler A, Wiesner S, Birkenfeld AL, Sell H, Otto C, Mehling H, Luft FC, Eckel J, Schulz-Menger J, Boschmann M, Jordan J. Randomized comparison of reduced fat and reduced carbohydrate hypocaloric diets on intrahepatic fat in overweight and obese human subjects. *Hepatology* 2011; **53**: 1504-1514 [PMID: 21400557 DOI: 10.1002/hep.24242]
65 **Marí M**, Caballero F, Colell A, Morales A, Caballeria J, Fernandez A, Enrich C, Fernandez-Checa JC, García-Ruiz C. Mitochondrial free cholesterol loading sensitizes to TNF- and Fas-mediated steatohepatitis. *Cell Metab* 2006; **4**: 185-198 [PMID: 16950136 DOI: 10.1016/j.cmet.2006.07.006]
66 **Molloy JW**, Calcagno CJ, Williams CD, Jones FJ, Torres DM, Harrison SA. Association of coffee and caffeine consumption with fatty liver disease, nonalcoholic steatohepatitis, and degree of hepatic fibrosis. *Hepatology* 2012; **55**: 429-436 [PMID: 21987293 DOI: 10.1002/hep.24731]
67 **Kistler KD**, Brunt EM, Clark JM, Diehl AM, Sallis JF, Schwimmer JB; NASH CRN Research Group. Physical activity recommendations, exercise intensity, and histological severity of nonalcoholic fatty liver disease. *Am J Gastroenterol* 2011; **106**: 460-8; quiz 469 [PMID: 21206486 DOI: 10.1038/ajg.2010.488]
68 **Krasnoff JB**, Vintro AQ, Ascher NL, Bass NM, Paul SM, Dodd MJ, Painter PL. A randomized trial of exercise and dietary counseling after liver transplantation. *Am J Transplant* 2006; **6**: 1896-1905 [PMID: 16889545 DOI: 10.1111/j.1600-6143.2006.01391.x]
69 **Charlton M**. Evolving aspects of liver transplantation for nonalcoholic steatohepatitis. *Curr Opin Organ Transplant* 2013; **18**: 251-258 [PMID: 23652610 DOI: 10.1097/MOT.0b013e3283615d30]
70 **Foster T**, Budoff MJ, Saab S, Ahmadi N, Gordon C, Guerci AD. Atorvastatin and antioxidants for the treatment of nonalcoholic fatty liver disease: the St Francis Heart Study randomized clinical trial. *Am J Gastroenterol* 2011; **106**: 71-77 [PMID: 20842109 DOI: 10.1038/ajg.2010.299]
71 **Sanyal AJ**, Chalasani N, Kowdley KV, McCullough A, Diehl AM, Bass NM, Neuschwander-Tetri BA, Lavine JE, Tonascia J, Unalp A, Van Natta M, Clark J, Brunt EM, Kleiner DE, Hoofnagle JH, Robuck PR; NASH CRN. Pioglitazone, vitamin E, or placebo for nonalcoholic steatohepatitis. *N Engl J Med* 2010; **362**: 1675-1685 [PMID: 20427778 DOI: 10.1056/NEJMoa0907929]
72 **Armstrong MJ**, Gaunt P, Aithal GP, Barton D, Hull D, Parker R, Hazlehurst JM, Guo K; LEAN trial team, Abouda G, Aldersley MA, Stocken D, Gough SC, Tomlinson JW, Brown RM, Hübscher SG, Newsome PN. Liraglutide safety and efficacy in patients with non-alcoholic steatohepatitis [LEAN]: a multicentre, double-blind, randomised, placebo-controlled phase 2 study. *Lancet* 2016; **387**: 679-690 [PMID: 26608256 DOI: 10.1016/S0140-6736(15)00803-X]
73 **Kuchay MS**, Krishan S, Mishra SK, Farooqui KJ, Singh MK, Wasir JS, Bansal B, Kaur P, Jevalikar G, Gill HK, Choudhary NS, Mithal A. Effect of Empagliflozin on Liver Fat in Patients With Type 2 Diabetes and Nonalcoholic Fatty Liver Disease: A Randomized Controlled Trial [E-LIFT Trial]. *Diabetes Care* 2018; **41**: 1801-1808 [PMID: 29895557 DOI: 10.2337/dc18-0165]
74 **Harrison SA**, Fecht W, Brunt EM, Neuschwander-Tetri BA. Orlistat for overweight subjects with nonalcoholic steatohepatitis: A randomized, prospective trial. *Hepatology* 2009; **49**: 80-86 [PMID: 19053049 DOI: 10.1002/hep.22575]
75 **Sjöström L**, Peltonen M, Jacobson P, Sjöström CD, Karason K, Wedel H, Ahlin S, Anveden Å, Bengtsson C, Bergmark G, Bouchard C, Carlsson B, Dahlgren S, Karlsson J, Lindroos AK, Lönroth H, Narbro K, Näslund I, Olbers T, Svensson PA, Carlsson LM. Bariatric surgery and long-term

cardiovascular events. *JAMA* 2012; **307**: 56-65 [PMID: 22215166 DOI: 10.1001/jama.2011.1914]

76 **Lassailly G**, Caiazzo R, Buob D, Pigeyre M, Verkindt H, Labreuche J, Raverdy V, Leteurtre E, Dharancy S, Louvet A, Romon M, Duhamel A, Pattou F, Mathurin P. Bariatric Surgery Reduces Features of Nonalcoholic Steatohepatitis in Morbidly Obese Patients. *Gastroenterology* 2015; **149**: 379-388; quiz e15 [PMID: 25917783 DOI: 10.1053/j.gastro.2015.04.014]

77 **Al-Nowaylati AR**, Al-Haddad BJ, Dorman RB, Alsaied OA, Lake JR, Chinnakotla S, Slusarek BM, Sampson BK, Ikramuddin S, Buchwald H, Leslie DB. Gastric bypass after liver transplantation. *Liver Transpl* 2013; **19**: 1324-1329 [PMID: 24039124 DOI: 10.1002/lt.23734]

78 **Diwan TS**, Rice TC, Heimbach JK, Schauer DP. Liver Transplantation and Bariatric Surgery: Timing and Outcomes. *Liver Transpl* 2018; **24**: 1280-1287 [PMID: 30080949 DOI: 10.1002/lt.25303]

79 **Salomone F**, Sharaiha RZ, Boškoski I. Endoscopic bariatric and metabolic therapies for non-alcoholic fatty liver disease: Evidence and perspectives. *Liver Int* 2020; **40**: 1262-1268 [PMID: 32181573 DOI: 10.1111/liv.14441]

80 **Charlton M**, Rinella M, Patel D, McCague K, Heimbach J, Watt K. Everolimus Is Associated With Less Weight Gain Than Tacrolimus 2 Years After Liver Transplantation: Results of a Randomized Multicenter Study. *Transplantation* 2017; **101**: 2873-2882 [PMID: 28817434 DOI: 10.1097/TP.0000000000001913]

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MINIREVIEWS

Liver dysfunction as a cytokine storm manifestation and prognostic factor for severe COVID-19

Gergana Taneva, Dimitar Dimitrov, Tsvetelina Velikova

ORCID number: Gergana Taneva 0000-0002-8916-6415; Dimitar Dimitrov 0000-0002-72146434; Tsvetelina Velikova 0000-0002-0593-1272.

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Gergana Taneva, Dimitar Dimitrov, Department of Gastroenterology, Sveta Sofia Hospital, Sofia 1618, Bulgaria

Tsvetelina Velikova, Department of Clinical Immunology, University Hospital Lozenetz, Sofia 1407, Bulgaria

Tsvetelina Velikova, Medical Faculty, Sofia University St. Kliment Ohridski, Sofia 1407, Bulgaria

Corresponding author: Tsvetelina Velikova, MD, PhD, Assistant Professor, Department of Clinical Immunology, University Hospital Lozenetz, Kozyak 1 Str., Sofia 1407, Bulgaria. tsvelikova@medfac.mu-sofia.bg

Abstract

Liver damage in severe acute respiratory coronavirus 2 infection occurs in patients with or without preexisting liver disorders, posing a significant complication and mortality risk. During coronavirus disease 2019 (COVID-19), abnormal liver function is typically observed. However, liver injury may occur because of the treatment as well. Ischemia, cytokine storm, and hypoxia were identified as the three major factors contributing to liver damage during COVID-19. Indeed, raised liver enzymes during hospitalizations may be attributed to medications used, as well as sepsis and shock. As a result, the proportion of hospitalized patients afflicted with COVID-19 and pathological liver biomarkers varies from 14% to 53%. Aminotransferases and bilirubin are found most often elevated. Usually, increased gamma-glutamyltransferase, alkaline phosphatase, and decreased serum albumin levels are demonstrated. Additionally, although there is no specific treatment for COVID-19, many of the drugs used to treat the infection are hepatotoxic. In this mini-review, we focus on how liver dysfunction can be one of the features associated with the COVID-19 cytokine storm. Furthermore, data show that liver injury can be an independent predictor of severe COVID-19, the need for hospitalization, and death.

Key Words: Liver dysfunction; Liver damage; Cytokine storm; Prognostic factor; COVID-19; Severe COVID-19; SARS-CoV-2; Aspartate aminotransferase; Alanine aminotransferase; Bilirubin; Interleukin-6

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Core Tip: Looking at the liver tests in patients with severe coronavirus disease 2019 (COVID-19), C-reactive protein (CRP) showed a strong correlation with the aspartate aminotransferase (AST) levels. This was observed in both intensive care units (ICU) and non-ICU patients. However, CRP levels were higher in non-ICU patients with liver damage, whereas alanine aminotransferase (ALT) was higher in ICU COVID-19 patients. Thus, like interleukin-6 (IL-6), ferritin, and CRP correlated directly with AST and ALT levels in non-ICU patients, there is a direct correlation of IL-6 and acute phase proteins with AST in severe COVID-19 cases. These observations confirm the critical impact of systemic inflammation and specifically elevated IL-6 during severe acute respiratory coronavirus 2 cytokine storm on liver injury.

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INTRODUCTION

The newly emerged severe acute respiratory coronavirus 2 (SARS-CoV-2) and the disease that causes coronaviral disease 2019 (COVID-19) are still unclear regarding all virulence factors, immunological effects and deteriorations of human organs during infection[1]. However, it is assumed that the interaction between the SARS-CoV-2 virus and the individual's immune system substantially influences the disease's onset and progression and the pathological effects on many organs. Both humoral and cell-mediated immune mechanisms participate in the immune response to a viral infection [2].

However, in some patients, these antiviral immunological mechanisms escape the regulatory control and eventually contribute to the multiorgan failure caused by the virus, including liver failure. Furthermore, an overreaction of the host immune system triggers a systemic inflammatory state that causes significant tissue and organs damage due to high cytokine release. The latter phenomenon is known as cytokine storm, leading to extreme tissue damage[2]. Therefore, the mortality rate and the COVID-19 complications in the elderly and patients with preexisting medical comorbidities, such as diabetes, asthma and cardiovascular disease, are even higher. Furthermore, the risk of severe COVID-19 might be increased by the underlying liver disease. In addition, it can cause direct or indirect damage to the liver by creating a multisystem inflammation[3].

Liver damage in SARS-CoV-2 infection occurring during disease progression in patients with or without preexisting liver diseases is a substantial challenge for clinical practice. Abnormal liver function is expected during COVID-19 infection because of SARS-CoV-2 direct and indirect impact on the liver. Additionally, certain hepatotoxic medications, especially for COVID-19 treatment, are connected with drug-induced liver damage. However, liver injury is defined as any liver damage occurring during disease and treatment. Therefore, hospitalized patients infected with COVID-19 with abnormal liver biomarkers range from 14% to 53%; this is most often observed for aminotransferase and bilirubin[1]. In addition, increased levels of gamma-glutamyl transferase (GGT), alkaline phosphatase, and decreasing serum albumin levels are also observed[4].

As significant liver biomarkers changes are observed in patients with severe COVID-19, more frequent in adults in the intensive care unit, studies documented that elevation of liver enzymes is associated with severity of COVID-19. Additionally, male sex and CRP were demonstrated as independent risk factors of COVID-19 complicated by liver injury[5].

This mini-review discusses how liver dysfunction can be one of the manifestations of the COVID-19-associated cytokine storm. Furthermore, liver damage might be an independent prognostic factor for severe COVID-19 and hospitalization and death.

LIVER DYSFUNCTION AS A MANIFESTATION OF THE CYTOKINE STORM

Cytokine storm syndrome occurring in some of the COVID-19 infected patients involved many organs, such as lungs, kidneys, heart, and liver[2]. COVID-19 may also lead to multiorgan failure and severe consequences owing to systemic inflammatory conditions caused by a cytokine cascade with pulmonary, cardiac, and hepatic involvement, as described above[6].

Three main factors are associated with liver damage during COVID-19: ischemia, cytokine storm, and hypoxia. Other influential contributors are the direct cytopathic effect of the virus on cholangiocytes (*via* ACE2 receptors), preexisting liver disease (*i.e.*, steatosis, hepatitis, cholangitis, thrombosis, Kupfer cell proliferation, liver impairment), severe inflammatory responses/sepsis[6].

Direct or indirect effects of SARS-CoV-2 on other organs are described beyond the respiratory system. In addition, it was shown that additional receptors might facilitate the virus to enter and infect the human cells *via* spike protein, including the liver. This suggests that there might be additional receptor pathways for infection with COVID-19 that can be targeted with specific treatment.

SARS-CoV-2 caused dysfunction and inducing a systemic inflammatory response leading to severe liver injury by binding to ACE2 receptors on cholangiocytes. In detail, spike protein binds the asialoglycoprotein receptor located on human hepatocytes. It was recently published that in vitro, SARS-CoV-2 spike protein can bind the asialoglycoprotein receptor 1 Located on primary human hepatocytes and hepatocyte-like cells[7]. In line with this, the serum GGT as a diagnostic marker for cholangiocyte injury has been found at elevated levels in up to 72% of severe COVID-19 patients[8].

Hypoxic liver injury (HLI) is not rare in patients with severe COVID-19 and has a high mortality. Its leading causes are lung and cardiac failure and may be associated with the immune-mediated inflammatory response. Patients with HLI have high mortality as a result of the deterioration of multiple organ failures. Levels of total bilirubin (TBIL), C-reactive protein (CRP), procalcitonin, and interleukin-6 (IL-6) show a statistically significant elevation in HLI cases compared with that in non-HLI cases. Besides, the median survival time of patients with HLI is significantly shorter than that of those not developing HLI[9].

Massive cytokine release causes a cytokine storm (also known as cytokine release syndrome) and is characterized by elevated CRP, IL-6, lactate dehydrogenase (LDH), and ferritin concentrations[10]. Furthermore, the subsequent organ dysfunction (*i.e.*, acute respiratory distress syndrome, progressive liver damage, and liver failure). As a result, systemic pro-inflammatory cytokine release appears to be a driver of disease progression in COVID-19[11-13].

Notably, COVID-19 patients had hepatic lymphocyte infiltration, centrilobular sinusoidal dilation, and patchy necrosis following the SARS-CoV-2 directly binding to ACE2-expressing cholangiocytes. However, the cause of the liver damage is unknown and may be due to systemic inflammation, SARS-CoV-2 infection, or drug administration[14].

Effenberger *et al*[10] discovered a clear link between systemic inflammation (as measured by IL-6, CRP, and ferritin) and liver damage. IL-6 development can be attributed to immune cells, fibroblasts, endothelial cells, and hepatocytes, orchestrating an acute phase response in the liver. Though IL-6 signaling impacts hepatic regeneration, clinical trials (for example, testing the effect of IL-6 administration in cancer patients) have shown that this pathway is essential in hepatic injury and hepatotoxicity[10]. The authors also found a strong association between acute-phase proteins and IL-6 in the serum of COVID-19 patients with elevated aspartate aminotransferase (AST), which is consistent with the importance of systemic inflammation and, in particular, IL-6 on liver injury.

The main sources of IL-6, which is the chief stimulator of the production of most acute phase proteins, are macrophages and monocytes at inflammatory sites. It has been shown that macrophages and monocytes produce high amounts of IL-6 in response to SARS-CoV-2 proteins[15].

COVID-19 patients with gastrointestinal complaints (nausea, vomiting, diarrhea, *etc.*) had higher AST and alanine aminotransferase (ALT) levels. Furthermore, there was a significant increase in enzymes among COVID-19 patients, primarily in the intensive care unit (ICU) facilities[16]. A relationship between liver enzyme elevation and disease activity has been also demonstrated[17].

Furthermore, the incidence of elevated AST levels was found to be greater than that of ALT levels and significantly higher in patients with severe COVID-19 (45.5%) relative to non-severe cases (15.0%). Thus, Lei *et al*[18] established a link between liver

injury and inpatient mortality in COVID-19 patients. They also found a correlation between AST abnormality and mortality risk compared to other liver injury measures during hospitalization[18].

Liver biopsies revealed moderate microvesicular steatosis with slight lobular and portal inflammation, indicating either direct viral or drug-induced liver damage[19]. It is proposed that a direct virus-mediated cytopathic effect exists. The latter can result after triggered immunological reactions and inflammatory cytokines, leading to liver injury[20,21]. Monocyte and macrophage dysfunction contribute to the progression of liver damage. Activation of liver-resident macrophages (Kupffer cells) and damage-associated molecular patterns result in recruitment of effector cells to the injured liver. Early monocyte infiltration is a major factor in the progression of local tissue destruction. Furthermore, the local inflammation results in the secretion of more and more pro-inflammatory cytokines that drive systemic inflammatory response syndrome[22].

Additionally, predominated parenchymal liver damage according to the elevated AST (23.2%) and ALT (21.2%), rather than bile duct injury, as shown by GGT (9.7%) and ALP (4.0%) levels in COVID-19 patients[16]. Patients with mild COVID-19 also have liver damage which resolves without any specific treatment. Most of the patients with liver failure during hospitalization, associated with severe COVID-19, are due to several drugs' hepatotoxicity.

Different drugs can impair liver function. However, the hepatotoxicity of medications varies on race, sex, and age of the patients[23]. Thus, the knowledge on the potential contributors to liver failure is significant. In addition, some medications can induce asymptomatic elevations of liver enzymes, acute hepatitis.

Many of the patients required treatment with antibiotics, anti-inflammatory, and antiviral agents. Antibiotics, anti-inflammatory, and antiviral medications used to treat COVID-19 patients are among the medicines that can induce liver harm[24,25]. Some of them cause asymptomatic elevation of the liver enzymes, while others lead to acute hepatitis. In some cases (*e.g.*, acetaminophen), these effects are dose-dependent. In contrast, in other medications, liver damage occurs independently of the drug dosage [24].

Hydroxychloroquine alone or in combination with azithromycin, lopinavir / ritonavir, remdesivir, darunavir, umifenovir, interferon beta, baricitinib, imatinib exert hepatotoxicity. Their immediate availability has led to off-label use for COVID-19 treatment in many countries[26].

There is currently no specific antiviral medication for SARS-CoV-2. Still, many COVID-19 patients are given antivirals approved for different uses (*i.e.*, remdesivir, lopinavir, or ritonavir, and other medications[27], all of which have been linked to hepatotoxicity and liver impairment[26].

Incorrect liver metabolization may also result in COVID 19-induced liver impairment which increases the risk of poisoning. However, a combination of patient records and thorough laboratory tests is carried out to diagnose drug-induced liver impairment to exclude other hepatic diseases and identify the relationship between hepatic injuries and probable causative medications.

More COVID 19 individuals suffer from fever, and hepatotoxicity can be triggered by antipyretics and analgesics (*i.e.*, paracetamol). This is associated with liver injuries, resulting in a potentially deadly combination, generally in the most severe phases of COVID-19. Furthermore, some antiviral drugs - remdesivir, lopinavir, ritonavir, IL-6 inhibitors (*i.e.*, tocilizumab), antibiotics - azithromycin, may cause idiosyncratic drug-induced liver failure[26].

Mechanisms involved in liver injury during COVID-19 infection and cytokine storm are presented on Figure 1.

LIVER FAILURE AS A PROGNOSTIC FACTOR IN SEVERE COVID-19 PATIENTS

Different risk factors can be associated with severe liver injury. Specifically, preexisting liver diseases - obesity with non-alcoholic fatty liver disease (NAFLD), alcoholic liver disease, cirrhosis - all of them correlate with Child-Pugh class and model for end-stage liver disease score. Moreover, autoimmune liver diseases, chronic hepatitis B infections could be reactivated and contribute to high levels of AST/ALT [28,29].

Patients with cirrhosis have a high risk of mortality from respiratory failure following severe SARS-CoV-2 infection. This risk might occur through multiple

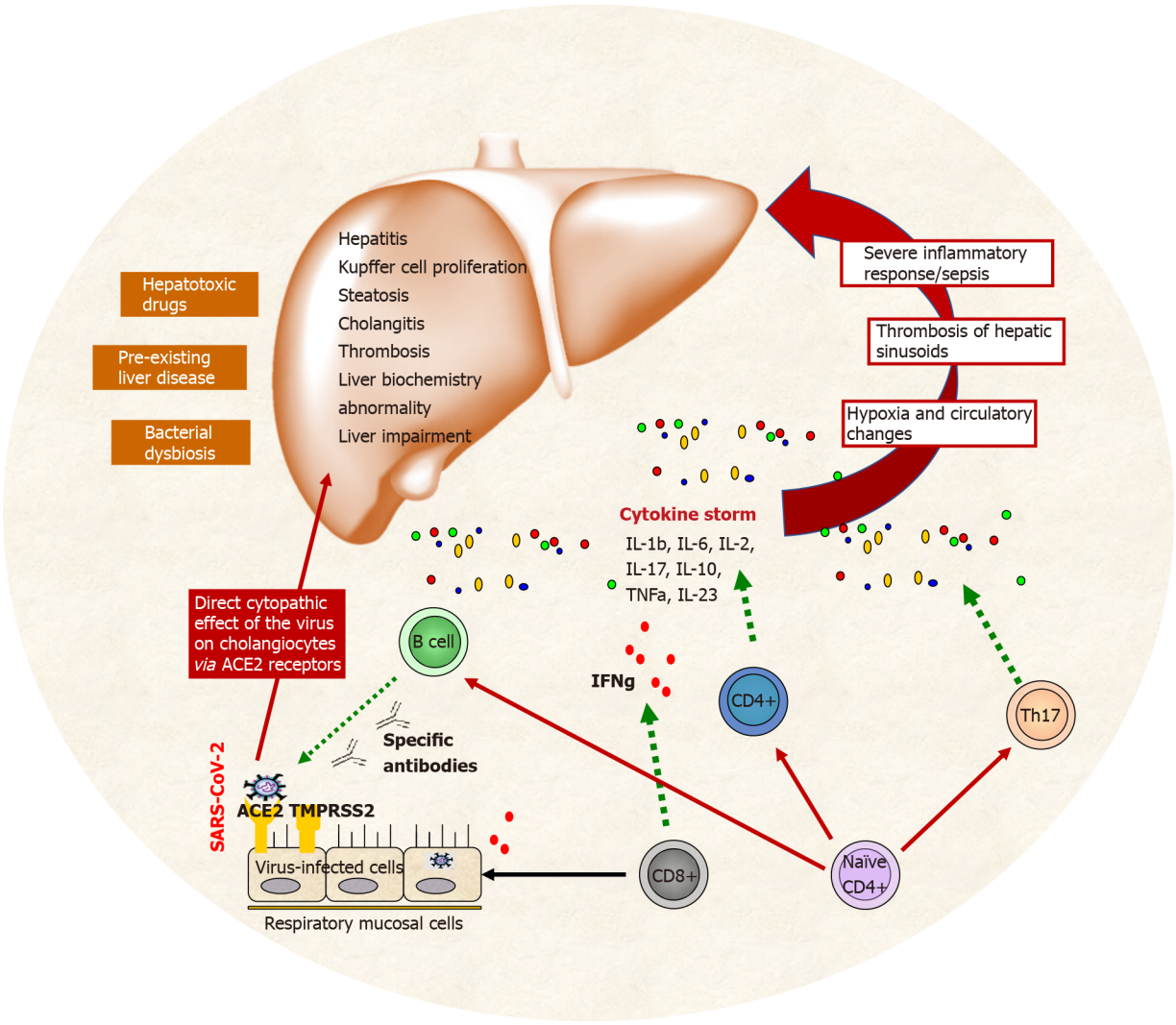

Figure 1 Liver dysfunction defined by the negative effects of cytokine storm (severe inflammation, thrombosis, hypoxia, *etc.*) during coronavirus disease 2019 infection. Other contributing factors for liver injury are also presented – preexisting liver condition, direct cytopathic action of severe acute respiratory coronavirus 2 and treatment with hepatotoxic drugs.

converging pathways, including contributions from cirrhosis-associated immune dysfunction, acute hepatic decompensation, and systemic inflammatory response. Cirrhosis-associated immune dysfunction could also lead to defective immune responses following future SARS-CoV-2 vaccination[20]. Patient with abnormal liver tests had a higher mortality rate (28.9% *vs* 9.0%, $P < 0.001$) and higher chance to develop systemic inflammatory response[30,31].

Interestingly, abnormal liver tests and liver injury can be associated with the progression of severe pneumonia[12]. The abnormalities can be hepatocellular, cholesteric, or mixed. Some clinical research studies show that patients with abnormal liver test results, especially in hepatocyte or mixed type ALT/AST and ALP/GGT at admission or during hospitalization, had significantly higher odds of progressing to severe COVID-19[28].

As we mentioned above, the pattern of liver injury is predominantly hepatocellular rather than cholestatic, although elevations in TBIL and ALT may be more common than reported in earlier studies. Since the ACE2 receptor is predominantly expressed in cholangiocytes than in hepatocytes, it is suggested that the most prevalent mechanism of liver impairment is not due to a direct cytopathic effect of the SARS-CoV-2 virus[32].

Raised liver enzymes during hospitalizations could be partly due to drugs used for treatment and might be due to sepsis and shock[28]. Looking at the liver tests, CRP showed a strong correlation with the AST levels, especially in hospitalized patients. Additionally, for both ICU and non-ICU patients, where this association was demonstrated at admission. However, CRP levels were higher in non-ICU patients with liver damage, whereas ALT was higher in ICU COVID-19 patients[33]. IL-6,

ferritin, and CRP correlated directly with AST and ALT levels in non-ICU patients.

Further analysis revealed a direct correlation of IL-6 and acute phase proteins with AST. In severe COVID-19 cases. To sum up, these observations confirm the critical impact of systemic inflammation and specifically IL-6 on liver injury. Furthermore, these observations led to the establishment of abnormal AST and direct bilirubin (DBil) at hospital admission as independent risk factors for increased COVID-19 mortality [33].

We can emphasize that the pathological examination of liver tissues from deceased patients with COVID-19 confirmed that liver involvement of COVID-19 was characterized by microvesicular steatosis, focal necrosis with lymphocytes infiltration, and micro thrombosis in the portal area[34]. Furthermore, pathological levels DBil were often found during the hospitalization of deceased COVID-19 patients. Both baseline and higher AST and DBil levels were independently associated with in-hospital death in patients with COVID-19. While liver anomalies are typical in COVID-19, these findings indicate that the liver is unlikely to be the primary organ driving COVID-19 mortality.

Since the number of people who develop severe and fatal COVID-19 is increased in elderly patients and those with liver failure and NAFLD, it is typically advised that older COVID-9 patients on hepatotoxic medication be closely followed up. Moreover, NAFLD can make the liver more sensitive to the most recommended and widespread antipyretic medication treatment for symptomatic diseases, such as acetaminophen[35, 36]. However, while the association of the COVID-19 with the liver steatosis disease is still unknown, a recent histological study of a COVID-19 patient's liver revealed microvesicular liver steatosis[19,37].

CONCLUSION

We can conclude that the pathological mechanisms of liver damage during COVID-19 confirmed that liver involvement was often observed with an increased risk for complications and death. Furthermore, the incidence of abnormal liver enzymes, significantly elevated AST and ALT levels were observed in patients with severe COVID-19 than non-severe cases. Additionally, a link between liver injury and inpatient mortality in COVID-19 patients was established. Moreover, recent studies confirmed that if liver dysfunction, preexisting or acquired during COVID-19 treatment, is a prognostic factor for severe COVID-19, development of complications and death.

REFERENCES

1 **Sun J**, Aghemo A, Forner A, Valenti L. COVID-19 and liver disease. *Liver Int* 2020; **40**: 1278-1281 [PMID: 32251539 DOI: 10.1111/liv.14470]

2 **Velikova TV**, Kotsev SV, Georgiev DS, Batselova HM. Immunological aspects of COVID-19: What do we know? *World J Biol Chem* 2020; **11**: 14-29 [PMID: 33024515 DOI: 10.4331/wjbc.v11.i2.14]

3 **Boeckmans J**, Rodrigues RM, Demuyser T, Piérard D, Vanhaecke T, Rogiers V. COVID-19 and drug-induced liver injury: a problem of plenty or a petty point? *Arch Toxicol* 2020; **94**: 1367-1369 [PMID: 32266419 DOI: 10.1007/s00204-020-02734-1]

4 **Yadav DK**, Singh A, Zhang Q, Bai X, Zhang W, Yadav RK, Zhiwei L, Adhikari VP, Liang T. Involvement of liver in COVID-19: systematic review and meta-analysis. *Gut* 2021; **70**: 807-809 [PMID: 32669289 DOI: 10.1136/gutjnl-2020-322072]

5 **Shen JX**, Zhuang ZH, Zhang QX, Huang JF, Chen GP, Fang YY, Cheng AG. Risk Factors and Prognosis in Patients with COVID-19 and Liver Injury: A Retrospective Analysis. *J Multidiscip Healthc* 2021; **14**: 629-637 [PMID: 33731999 DOI: 10.2147/JMDH.S293378]

6 **Vitiello A**, La Porta R, D'Aiuto V, Ferrara F. The risks of liver injury in COVID-19 patients and pharmacological management to reduce or prevent the damage induced. *Egypt Liver J* 2021; **11**: 11 [DOI: 10.1186/s43066-021-00082-y]

7 **Collins DP**, Steer CJ. Binding of the SARS-CoV-2 Spike Protein to the Asialoglycoprotein Receptor on Human Primary Hepatocytes and Immortalized Hepatocyte-Like Cells by Confocal Analysis. *Hepat Med* 2021; **13**: 37-44 [PMID: 33883951 DOI: 10.2147/HMER.S301979]

8 **Lagana SM**, Kudose S, Iuga AC, Lee MJ, Fazlollahi L, Remotti HE, Del Portillo A, De Michele S, de Gonzalez AK, Saqi A, Khairallah P, Chong AM, Park H, Uhlemann AC, Lefkowitch JH, Verna EC. Hepatic pathology in patients dying of COVID-19: a series of 40 cases including clinical, histologic, and virologic data. *Mod Pathol* 2020; **33**: 2147-2155 [PMID: 32792598 DOI: 10.1038/s41379-020-00649-x]

9 **Huang H**, Li H, Chen S, Zhou X, Dai X, Wu J, Zhang J, Shao L, Yan R, Wang M, Wang J, Tu Y, Ge

M. Prevalence and Characteristics of Hypoxic Hepatitis in COVID-19 Patients in the Intensive Care Unit: A First Retrospective Study. *Front Med (Lausanne)* 2020; **7**: 607206 [PMID: 33681238 DOI: 10.3389/fmed.2020.607206]

10 **Effenberger M**, Grander C, Grabherr F, Griesmacher A, Ploner T, Hartig F, Bellmann-Weiler R, Joannidis M, Zoller H, Weiss G, Adolph TE, Tilg H. Systemic inflammation as fuel for acute liver injury in COVID-19. *Dig Liver Dis* 2021; **53**: 158-165 [PMID: 32873520 DOI: 10.1016/j.dld.2020.08.004]

11 **Mehta P**, McAuley DF, Brown M, Sanchez E, Tattersall RS, Manson JJ; HLH Across Speciality Collaboration, UK. COVID-19: consider cytokine storm syndromes and immunosuppression. *Lancet* 2020; **395**: 1033-1034 [PMID: 32192578 DOI: 10.1016/S0140-6736(20)30628-0]

12 **Moore JB**, June CH. Cytokine release syndrome in severe COVID-19. *Science* 2020; **368**: 473-474 [PMID: 32303591 DOI: 10.1126/science.abb8925]

13 **Giamarellos-Bourboulis EJ**, Netea MG, Rovina N, Akinosoglou K, Antoniadou A, Antonakos N, Damoraki G, Gkavogianni T, Adami ME, Katsaounou P, Ntaganou M, Kyriakopoulou M, Dimopoulos G, Koutsodimitropoulos I, Velissaris D, Koufargyris P, Karageorgos A, Katrini K, Lekakis V, Lupse M, Kotsaki A, Renieris G, Theodoulou D, Panou V, Koukaki E, Koulouris N, Gogos C, Koutsoukou A. Complex Immune Dysregulation in COVID-19 Patients with Severe Respiratory Failure. *Cell Host Microbe* 2020; **27**: 992-1000.e3 [PMID: 32320677 DOI: 10.1016/j.chom.2020.04.009]

14 **Zhang C**, Shi L, Wang FS. Liver injury in COVID-19: management and challenges. *Lancet Gastroenterol Hepatol* 2020; **5**: 428-430 [PMID: 32145190 DOI: 10.1016/S2468-1253(20)30057-1]

15 **Karwaciak I**, Sałkowska A, Karaś K, Dastych J, Ratajewski M. Nucleocapsid and Spike Proteins of the Coronavirus SARS-CoV-2 Induce *IL6* in Monocytes and Macrophages-Potential Implications for Cytokine Storm Syndrome. *Vaccines (Basel)* 2021; **9** [PMID: 33467724 DOI: 10.3390/vaccines9010054]

16 **Wijarnpreecha K**, Ungprasert P, Panjawatanan P, Harnois DM, Zaver HB, Ahmed A, Kim D. COVID-19 and liver injury: a meta-analysis. *Eur J Gastroenterol Hepatol* 2021; **33**: 990-995 [PMID: 32639420 DOI: 10.1097/MEG.0000000000001817]

17 **Chen H**, Guo J, Wang C, Luo F, Yu X, Zhang W, Li J, Zhao D, Xu D, Gong Q, Liao J, Yang H, Hou W, Zhang Y. Clinical characteristics and intrauterine vertical transmission potential of COVID-19 infection in nine pregnant women: a retrospective review of medical records. *Lancet* 2020; **395**: 809-815 [PMID: 32151335 DOI: 10.1016/S0140-6736(20)30360-3]

18 **Lei F**, Liu YM, Zhou F, Qin JJ, Zhang P, Zhu L, Zhang XJ, Cai J, Lin L, Ouyang S, Wang X, Yang C, Cheng X, Liu W, Li H, Xie J, Wu B, Luo H, Xiao F, Chen J, Tao L, Cheng G, She ZG, Zhou J, Wang H, Lin J, Luo P, Fu S, Ye P, Xiao B, Mao W, Liu L, Yan Y, Chen G, Huang X, Zhang BH, Yuan Y. Longitudinal Association Between Markers of Liver Injury and Mortality in COVID-19 in China. *Hepatology* 2020; **72**: 389-398 [PMID: 32359177 DOI: 10.1002/hep.31301]

19 **Xu Z**, Shi L, Wang Y, Zhang J, Huang L, Zhang C, Liu S, Zhao P, Liu H, Zhu L, Tai Y, Bai C, Gao T, Song J, Xia P, Dong J, Zhao J, Wang FS. Pathological findings of COVID-19 associated with acute respiratory distress syndrome. *Lancet Respir Med* 2020; **8**: 420-422 [PMID: 32085846 DOI: 10.1016/S2213-2600(20)30076-X]

20 **Fan Z**, Chen L, Li J, Cheng X, Yang J, Tian C, Zhang Y, Huang S, Liu Z, Cheng J. Clinical Features of COVID-19-Related Liver Functional Abnormality. *Clin Gastroenterol Hepatol* 2020; **18**: 1561-1566 [PMID: 32283325 DOI: 10.1016/j.cgh.2020.04.002]

21 **Gu J**, Han B, Wang J. COVID-19: Gastrointestinal Manifestations and Potential Fecal-Oral Transmission. *Gastroenterology* 2020; **158**: 1518-1519 [PMID: 32142785 DOI: 10.1053/j.gastro.2020.02.054]

22 **Triantafyllou E**, Woollard KJ, McPhail MJW, Antoniades CG, Possamai LA. The Role of Monocytes and Macrophages in Acute and Acute-on-Chronic Liver Failure. *Front Immunol* 2018; **9**: 2948 [PMID: 30619308 DOI: 10.3389/fimmu.2018.02948]

23 **Björnsson ES**. Hepatotoxicity by Drugs: The Most Common Implicated Agents. *Int J Mol Sci* 2016; **17**: 224 [PMID: 26861310 DOI: 10.3390/ijms17020224]

24 **Cascella M**, Rajnik M, Cuom, A, Dulebohn SC, Di Napoli R. Features, Evaluation, and Treatment of Coronavirus (COVID-19). In: StatPearls. Treasure Island: StatPearls Publishing, 2020 [PMID: 32150360]

25 **Cao R**, Hu Y, Wang Y, Gurley EC, Studer EJ, Wang X, Hylemon PB, Pandak WM, Sanyal AJ, Zhang L, Zhou H. Prevention of HIV protease inhibitor-induced dysregulation of hepatic lipid metabolism by raltegravir *via* endoplasmic reticulum stress signaling pathways. *J Pharmacol Exp Ther* 2010; **334**: 530-539 [PMID: 20472667 DOI: 10.1124/jpet.110.168484]

26 **Olry A**, Meunier L, Délire B, Larrey D, Horsmans Y, Le Louët H. Drug-Induced Liver Injury and COVID-19 Infection: The Rules Remain the Same. *Drug Saf* 2020; **43**: 615-617 [PMID: 32514859 DOI: 10.1007/s40264-020-00954-z]

27 **Zha BS**, Wan X, Zhang X, Zha W, Zhou J, Wabitsch M, Wang G, Lyall V, Hylemon PB, Zhou H. HIV protease inhibitors disrupt lipid metabolism by activating endoplasmic reticulum stress and inhibiting autophagy activity in adipocytes. *PLoS One* 2013; **8**: e59514 [PMID: 23533630 DOI: 10.1371/journal.pone.0059514]

28 **Cai Q**, Huang D, Yu H, Zhu Z, Xia Z, Su Y, Li Z, Zhou G, Gou J, Qu J, Sun Y, Liu Y, He Q, Chen J, Liu L, Xu L. COVID-19: Abnormal liver function tests. *J Hepatol* 2020; **73**: 566-574 [PMID: 32298767 DOI: 10.1016/j.jhep.2020.04.006]

29 **Wu ZH**, Yang DL. A meta-analysis of the impact of COVID-19 on liver dysfunction. *Eur J Med Res* 2020; **25**: 54 [PMID: 33148326 DOI: 10.1186/s40001-020-00454-x]

30 **Marjot T**, Webb GJ, Barritt AS 4th, Moon AM, Stamataki Z, Wong VW, Barnes E. COVID-19 and liver disease: mechanistic and clinical perspectives. *Nat Rev Gastroenterol Hepatol* 2021; **18**: 348-364 [PMID: 33692570 DOI: 10.1038/s41575-021-00426-4]

31 **Chen LY**, Chu HK, Bai T, Tu SJ, Wei Y, Li ZL, Hu LL, Zhu R, Zhang L, Han CQ, Xiao L, He Q, Song J, Liu WH, Zhu QJ, Chen H, Yang L, Hou XH. Liver damage at admission is an independent prognostic factor for COVID-19. *J Dig Dis* 2020; **21**: 512-518 [PMID: 32713118 DOI: 10.1111/1751-2980.12925]

32 **Hundt MA**, Deng Y, Ciarleglio MM, Nathanson MH, Lim JK. Abnormal Liver Tests in COVID-19: A Retrospective Observational Cohort Study of 1,827 Patients in a Major U.S. Hospital Network. *Hepatology* 2020; **72**: 1169-1176 [PMID: 32725890 DOI: 10.1002/hep.31487]

33 **Ding ZY**, Li GX, Chen L, Shu C, Song J, Wang W, Wang YW, Chen Q, Jin GN, Liu TT, Liang JN, Zhu P, Zhu W, Li Y, Zhang BH, Feng H, Zhang WG, Yin ZY, Yu WK, Yang Y, Zhang HQ, Tang ZP, Wang H, Hu JB, Liu JH, Yin P, Chen XP, Zhang B; Tongji Multidisciplinary Team for Treating COVID-19 (TTTC). Association of liver abnormalities with in-hospital mortality in patients with COVID-19. *J Hepatol* 2021; **74**: 1295-1302 [PMID: 33347952 DOI: 10.1016/j.jhep.2020.12.012]

34 **National Health Commission of China**. Guidance for COVID-19: Prevention, Control, Diagnosis and Management. Version 7.0. 2020. [accessed 2021 Nov 5]. Available from: https://www.yoifos.com/sites/default/files/covid_19_guideline_chn.pdf.

35 **Massart J**, Begriche K, Moreau C, Fromenty B. Role of nonalcoholic fatty liver disease as risk factor for drug-induced hepatotoxicity. *J Clin Transl Res* 2017; **3**: 212-232 [PMID: 28691103 DOI: 10.18053/jctres.03.2017S1.006]

36 **Michaut A**, Moreau C, Robin MA, Fromenty B. Acetaminophen-induced liver injury in obesity and nonalcoholic fatty liver disease. *Liver Int* 2014; **34**: e171-e179 [PMID: 24575957 DOI: 10.1111/liv.12514]

37 **Xu L**, Liu J, Lu M, Yang D, Zheng X. Liver injury during highly pathogenic human coronavirus infections. *Liver Int* 2020; **40**: 998-1004 [PMID: 32170806 DOI: 10.1111/liv.14435]

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MINIREVIEWS

COVID-19 and the liver: A brief and core review

Bircan Kayaaslan, Rahmet Guner

ORCID number: Bircan Kayaaslan 0000-0001-5225-8319; Rahmet Guner 0000-0002-1029-1185.

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Bircan Kayaaslan, Rahmet Guner, Department of Infectious Disease and Clinical Microbiology, Ankara City Hospital, Ankara Yildirim Beyazit University, Ankara 06800, Turkey

Corresponding author: Bircan Kayaaslan, MD, Associate Professor, Department of Infectious Disease and Clinical Microbiology, Ankara City Hospital, Ankara Yildirim Beyazit University, No. 1 Bilkent street, Çankaya District, Ankara 06800, Turkey. drbican@gmail.com

Abstract

Coronavirus disease 2019 has a wide range of clinical spectrum from asymptomatic infection to severe infection resulting in death within a short time. Currently, it is known that severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) does not only cause a respiratory tract infection but a more complicated disease that can lead to multiple system involvement including the liver. Herein, we evaluate the epidemiology, the impact of liver injury/dysfunction on disease prognosis, the pathophysiological mechanisms and management of liver injury. More than one-fourth of the patients have abnormal liver function tests, mostly a mild-to-moderate liver dysfunction. Liver injury is significantly associated with a poor clinical outcome. Direct cytotoxic effect of SARS-CoV-2, the immune response ("cytokine storm"), the complications related to the disease, and drugs used in the treatments are the pathophysiological mechanisms responsible for liver injury. However, the exact mechanism is not yet clearly explained. The binding of SARS-CoV-2 to the angiotensin-converting enzyme 2 receptors and entering the hepatocyte and cholangiocytes can cause cytotoxic effects on the liver. Excessive immune response has an important role in disease progression and causes acute respiratory distress syndrome and multi-organ failures accompanied by liver injury. Treatment drugs, particularly lopinavir/ritonavir, remdesivir and antibiotics are a frequent reason for liver injury. The possible reasons should be meticulously investigated and resolved.

Key Words: COVID-19; SARS-CoV-2; Liver injury; Liver dysfunction; Chronic liver disease; Pathophysiology

Core Tip: The study evaluated the incidence of liver injury in coronavirus disease 2019 (COVID-19) patients and its impact on clinical outcomes and pathophysiological mechanism of liver injury. More than one-fourth of COVID-19 patients had suffered

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from liver injury, mostly a mild-to-moderate liver dysfunction. Liver involvement is independently associated with adverse clinical outcomes. Direct viral cytotoxic effect, complications of the disease, and drugs used in the treatments are the pathophysiological mechanisms suggested for liver injury. However, the exact mechanism was not clearly explained. The actual cause should be carefully investigated in the presence of abnormal liver function tests, and appropriate treatments provided for possible factors.

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INTRODUCTION

The emergence of the novel coronavirus disease 2019 (COVID-19) pandemic was a breaking point that deeply affected the whole world and changed medical priorities in daily practice. From the early time of the pandemic, it has been understood that severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is not only a respiratory system virus that causes severe lung disease but a systemic disease agent that can affect all systems. Numerous studies from around the world have shown that the liver is damaged in varying degrees in patients with SARS-CoV-2 infection[1]. Recent studies have shown that a considerable part of the COVID-19 patients showed abnormality in liver function tests[2-5]. Liver injury causes a poorer outcome in affected patients, however, its effect on the disease may be more profound than it appears. Herein, we aimed to evaluate the epidemiological characteristics and impact of the liver injury on the clinical outcome, the interaction between pre-existing chronic liver diseases (CLDs) and COVID-19, the pathophysiology of liver involvement and hepatic histopathological findings, and management of liver injury.

DEFINITION

The liver is a vital organ that is mainly responsible for protein synthesis, storage of glycogen and regulation of blood glucose levels, metabolism of toxic substances, and many other physiological processes[1]. A great majority of studies revealed that a mild-to-moderate liver involvement was present in a considerable part of COVID-19 patients. However, what liver damage means has not been clearly defined. Zheng *et al*[6] pointed out that there is no clarity on what liver damage means in their letter to the editors. There are no standardized diagnostic criteria to be considered as a liver injury. The cut-off value of liver function tests varies among studies. The World Health Organization defined the severity of acute COVID-19 as mild, moderate, severe, and critical illness based on respiratory and other systemic findings using technical guidelines[7]. However, the degree of liver and other organ involvement has not been defined yet. There is no standard for cut-off values of liver function tests established by the consensus of researchers. Researchers usually have used different cut-off values, as Zheng Ye *et al*[6] emphasized. Most of them defined any elevated value above the upper limit of normal (ULN) as liver injury, others preferred values 2-3 times higher than UNL[6,8]. Cai *et al*[8] defined liver test abnormalities as two groups, elevations of liver enzymes (higher than ULN) and liver injury. Aspartate transaminase (AST)/alanine transaminase (ALT) values above 3 times ULN, or alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), and total bilirubin values above 2 times ULN were accepted as liver injury.

Lv *et al*[9] stated concern about the possible misinterpretation of AST data. Determining liver injury incidences based on AST may have led to overestimation. It is believed that ALT is more specific for liver disease and reflects the real hepatic injury. AST is a less specific marker for the liver due to being produced by other tissue such as kidneys, cardiac, and skeletal muscles rather than the liver. Therefore, to be sure of the source of AST, isoform analysis should be done that is not available in routine practice. In addition, antibiotics and antivirals used during the disease also contribute

frequently to the elevation of the AST value[5]. A recent study showed that the first rising enzyme is AST followed by ALT[10]. These raise the question of whether the increase in AST may have been caused by other tissues or causes. On the other hand, the studies reported the association between AST level and the disease severity regardless of its source.

In addition, previous diagnosed or undiagnosed CLDs such as chronic viral hepatitis, alcoholic liver disease, non-alcoholic fatty liver disease (NAFLD), may also result in abnormal liver tests. The use of an established set of standards for liver dysfunction/liver injury by researchers is essential in terms of comparability of study results. Therefore, there is an urgent need to define clearly what liver dysfunction/injury means.

EPIDEMIOLOGY AND PROGNOSIS

Incidence of liver dysfunction

Numerous studies have reported liver injury and varying levels of liver dysfunctions in SARS-CoV-2 infection[3,5]. Most infections manifested as mild to moderate liver disorders presented with abnormal liver function tests [AST/ALT elevations, GGT/ALP elevations, and in some cases hypoproteinemia and prolonged prothrombin time (PT)][2-4,11-15]. In their meta-analysis, Kulkarni *et al*[5] reported liver function test abnormality in 19% of 1290 non-severe COVID-19 patients from nine articles. Cai *et al*[8] reported liver injury in 24.9% of non-severe cases. Emerging data from cohort studies have pointed out that liver dysfunction is a commonly encountered entity, usually in more than usually in more than one-third of hospitalized COVID-19 patients[11,16,17]. However, as pointed out above, the incidence of liver injury varies between cohorts, sometimes due to reasons such as differences among study and patient populations, the variety of the drug treatments, and their usage rates. Herein, we mostly addressed several meta-analyses and reviews which evaluated and summarized liver involvements in SARS-CoV-2 infections. A meta-analysis reported the pooled incidence of liver dysfunction as 23.1% at early presentation and 24.1% through the disease course among 15407 patients[5]. The incidence of abnormal levels of liver function was also reported as 29% in another meta-analysis evaluating a total of 38 studies with 3062 COVID-19 patients[17].

In a review, Alqahtani *et al*[18] analyzed more than thirty published, ahead of print and preprint reports which consisted of mostly case series. They summarized the details of the study types, patients' numbers, hepatobiliary function markers, inflammatory markers, and proposed possible mechanisms of liver injury. More than 20 publications included in the review had reported abnormal levels of aminotransferase, up to 61.1% of cases. Almost all cases had a modest liver injury except one who had an AST reaching a maximum of 1263 U/L and ALT reaching 2093 U/L. Another retrospective study by Chen *et al*[19], included in the review, reported that one case had experienced severe hepatitis with an AST of 1445 and ALT of 7590 U/L. A negligible part of patients had pre-existing liver disease. COVID-19 causes usually mild-to-moderate liver injury presented with modest abnormality in liver function tests, and it occasionally resulted in severe hepatitis.

In a comprehensive review evaluating the incidence of hepatic abnormalities in SARS, the Middle East respiratory syndrome, and SARS-CoV-2, Kukla *et al*[20] analyzed 2541 patients infected with SARS CoV-2 in 11 studies reported from China and reported that liver involvement had occurred with predominantly mild to moderately high transaminases, hypoalbuminemia, and prolongation of PT. A large-scale study of 5700 patients hospitalized with COVID-19 reported elevations of ALT and AST in 39.0% and 58.4% of the patient population, respectively[21]. Cai *et al*[8] reported 76.3% abnormal liver function tests (higher than ULN) and 21.5% liver injury (defined higher than 3 × AST/ALT or 2 × ALP/GGT/total bilirubin) at admission.

A slight hyperbilirubinemia is accompanied by elevated transaminase in COVID-19. Its incidence was reported as 13.4% in Kulkarni *et al*[3]'s study. The studies also reported the increase in other liver function tests (ALP, GGT), prolonged PT and decrease in albumin level. Cai *et al*[8] reported GGT elevation in more than 15% of the patients at admission and in approximately half of the patients during hospitalization. The pooled incidence of prolonged PT was 9.7% in adults with a meta-analysis[5]. As a result, although the incidence rates are in a wide range in studies, the incidence of liver injury was present in at least one-fourth of patients or more.

Liver dysfunction and clinical outcomes

Accumulated data since the beginning of the pandemic shows that liver dysfunction is significantly associated with a poor outcome in SARS-CoV-2 infection[3,8,11,16,17,22]. Cai *et al*[8] reported that patients with liver injury had a 9-fold-greater risk of severe COVID-19. A meta-analysis involving 3722 cases in 13 studies revealed that mortality and clinical severity were associated with liver injury in COVID-19 patients[3]. Fu *et al*[16] reported a higher mortality rate in patients with abnormal liver function tests compared to those with normal liver function tests (29.6% *vs* 6.5%, $P < 0.001$), especially AST elevation and total bilirubin elevation groups. Serum AST level was higher in deceased patients and severe COVID-19 cases than in surviving patients and non-severe cases [odds ratio (OR) = 4.48, 95% confidence interval (CI): 3.24-7.21, $P < 0.001$][3]. A comprehensive meta-analysis investigating the incidence of elevated liver functions, and the association of the patients' outcomes with liver dysfunction and CLDs upon 15407 patients revealed that COVID-19 patients with elevated liver functions had an increased risk for mortality (OR = 3.46, 95%CI: 2.42-4.95, P <0.001) and severe disease (OR = 2.87, 95%CI: 2.29-3.6, P <0.001) compared to patients without elevated liver functions[5]. In another meta-analysis, a higher level of AST, ALT, and bilirubin values, prolonged PT, and a lower level of serum albumin value were found to be associated with severe COVID-19[23]. In consequence, the elevated transaminase and abnormality of other liver function tests were common in COVID-19 patients and independently associated with adverse clinical outcomes.

PATHOPHYSIOLOGY OF LIVER INJURY

Although much has been learned about SARS-CoV-2 in the elapsed time since the beginning of the pandemic, there remain many points that need to be clarified, particularly its pathogenesis. There is still a dilemma about whether SARS-CoV-2 increases transaminases directly by viral cytotoxic effect or by the consequences of the disease such as hyperinflammation, sepsis, and drugs[24]. Although not yet fully clarified, the pathogenesis of COVID-19 associated liver injury appears to be related to direct viral hepatitis, or the disease-induced complications such as severe respiratory involvement related to hypoxia [*e.g.*, acute respiratory distress syndrome (ARDS)], sepsis, cytokine storm, or drug-related liver enzyme elevations during the infection[9,20,25]. Possible mechanism of liver injury is given in Figure 1.

Direct cytopathic effect of SARS-CoV-2 on the liver

Recent studies show that SARS-CoV-2 uses angiotensin-converting enzyme 2 (ACE2) receptors, mainly expressed in type 2 alveolar cells of the lungs, to enter the body[26,27]. ACE2 receptors are also mainly localized in the heart, kidney, testes, and other tissues[8]. The liver is a potential target organ for the virus due to its containing high levels of ACE2 receptors[28]. The direct cytotoxic effect and/or inflammatory response of the body to SARS-CoV-2 may be responsible for liver injury. It has been suggested that the binding of SARS-CoV-2 to the ACE2 receptors and entering the hepatocyte and cholangiocytes can cause a direct viral cytotoxic effect on the liver[5], a suggestion that is supported by the findings of a previous study where SARS-CoV-2 RNA was detected in a liver sample[29]. Nardo *et al*[30] reviewed the pathological findings of COVID-19 patients and proposed that the pathological findings of COVID-19 might be caused by hepatocellular infection with direct cytopathic effect of SARS-CoV-2 and cytokine storm, hypoxic conditions due to ARDS and drug-induced liver injury (DILI) may contribute to these findings. Previous studies had extensively investigated the cell entry mechanism of SARS-CoV-2, and reported that viral entry is triggered by the binding of receptor-binding domain of ACE receptors to the target cells such as alveolar type 2 cells, hepatocytes or cholangiocytes and activated by human proteases such as TMPRSS2[31-33]. However, more data is required to assess the relevance between virus and liver damage. Interestingly, ACE2 expression in cholangiocytes is at similar levels to the lungs, and higher than in the hepatocytes[28]. This may explain the increase in ALP, GGT, and total bilirubin levels. However, COVID-19 patients do not commonly denote a cholestatic pattern of hepatic dysfunction; increased transaminase levels are more predominant. This can be explained by the possibility that hepatic dysfunction predominantly results from secondary causes such as hypoxia and cytokine storm than the direct viral cytopathic effect of the virus[28,34]. Further studies are required to explain why serum transaminases are elevated more than ALP and bilirubin, and to assess the relevance between virus and liver injury.

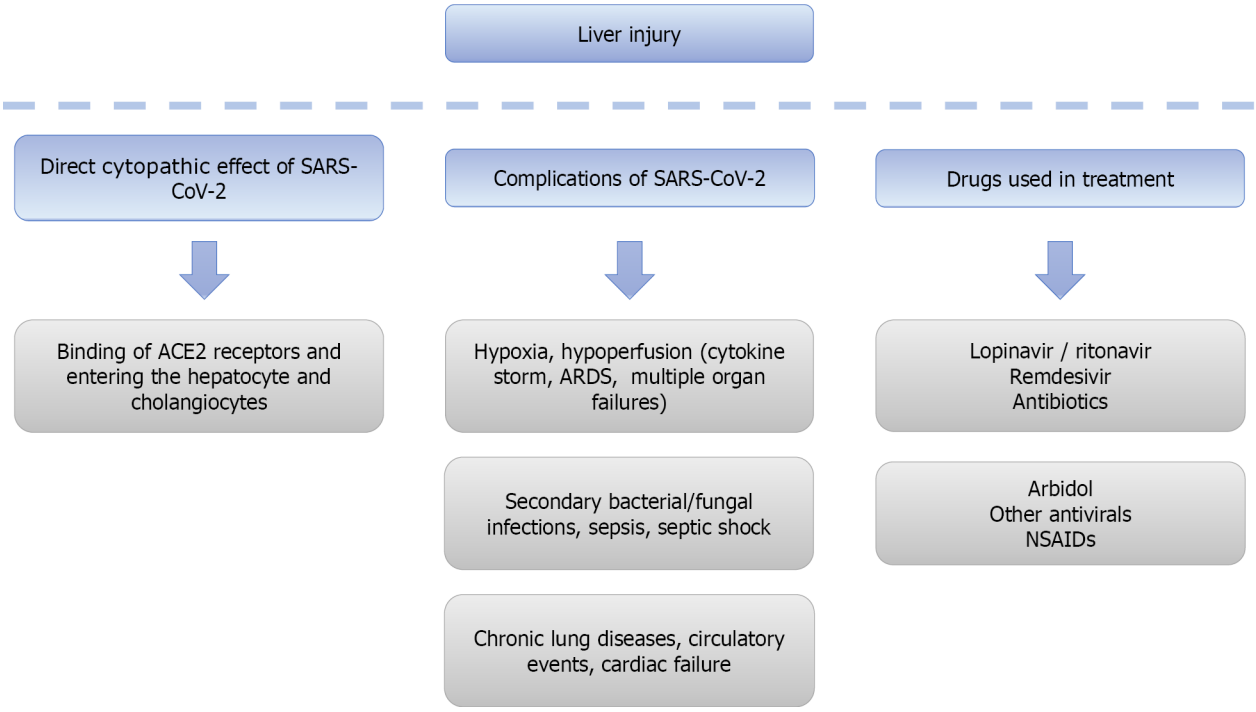

Figure 1 Possible mechanisms of liver injury in coronavirus disease 2019. ACE2: Angiotensin-converting enzyme 2; ARDS: Acute respiratory distress syndrome; NSAIDs: Non-steroidal anti-inflammatory drugs; SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2.

Complications of SARS-CoV-2

COVID-19 has a wide range of clinical spectrum from asymptomatic infection to severe infection resulting in death within a short time. COVID-19 patients particularly with severe illness suffer from various degrees of respiratory system involvement and multiple organ failure. Its pathogenesis is complicated and mainly based on immune system dysfunction, at local and systemic levels[35]. Accumulated data on COVID-19 pathogenesis indicates that SARS-CoV-2 induces an excessive cytokine release, known as cytokine storm in some patients, and causes ARDS and multiple organ failures including heart, liver, and kidney[35-37]. Cytokine storm is the life-threatening overactivation of immune cells and dysregulated inflammatory cytokine and chemical production in relation to a triggering factor such as bacterial, fungal and viral pathogens, and is accepted as the main cause of multiple organ injury. It was confirmed that a high level of inflammatory markers such as C-reactive protein, cytokines [interleukin (IL) 1, IL-6, IL-18, tumor necrosis factor, granulocyte-colony stimulating factor], and chemokines are associated with severe infection[11,34,35,38-43]. Cytokines and chemokines stimulate both the innate and adoptive immune system resulting in apoptosis of the infected cells and immune cell hyperstimulation. Therefore, cytokine storm may play a role in the appearance of abnormal liver function tests.

Thromboembolic events are frequent in COVID-19 patients, and another possible explanation of liver involvement is endothelial injury and hyper-coagulability[44]. In a preliminary study, the signs of acute (thrombosis, luminal ectasia) and chronic (fibrous thickening of the vascular wall or phlebosclerosis, presence of abnormal portal intrahepatic system) hepatic vascular involvement was found in all specimens in varying degrees among the main pathological findings[45].

Multiple organ dysfunction induced by other COVID-19-related complications probably contribute to elevated liver function tests. COVID-19 patients, particularly with a severe and critical illness, are at risk for secondary bacterial and fungal infections[46]. Sepsis is a common condition in COVID-19 patients, especially those who are followed up in the intensive care unit and can cause multiple organ dysfunction, including the liver. Besides, the development of septic shock increases the risk of hepatotoxicity through hypoperfusion[47]. Hypoxia and cardiac failure in affected COVID-19 patients can lead to liver injury[34]. Circulatory events, underlying CLD disorders are other secondary reasons for liver injury[11,28,34].

Therapeutic drugs

Liver injury may be partially attributed to the drugs used in COVID-19 treatment[5,11]. Liver damage has been reported with the use of lopinavir/ritonavir as an antiviral in SARS-CoV-2 infection[5,8,11]. Cai *et al*[8] did not detect any significant evidence for increased risk for liver injury in patients using suspected drugs (including antibiotics, non-steroidal anti-inflammatory drugs (NSAIDs), ribavirin, herbal medication used in Chinese medicine, and interferon), except for lopinavir/ritonavir. Patients who used lopinavir/ritonavir had a higher GGT and ALP level. Similarly, Cichoż-Lach *et al*[11] reported that they did not find any association between the use of antibiotics, NSAIDs, ribavirin, and interferon, and hepatic complications. Only lopinavir/ritonavir had provoked the deterioration of liver function. In a study, the rate of lopinavir/ritonavir use had been detected higher in the patients with hepatic dysfunction than in those without hepatic dysfunction[48]. Kulkarni *et al*[5] also reported that drug-induced liver injury due to the use of lopinavir/ritonavir, remdesivir, and arbidol is common, but not resulting in life-threatening conditions. The incidence of abnormal liver function tests with lopinavir/ritonavir ranges from 22.7% to 54.6%. Remdesivir is another drug that causes frequent increases (15.2%) in liver function tests. Elevated liver function tests were reported at a rate of 18.7% with the use of arbidol.

Hydroxychloroquine, an antimalarial drug, is one of the most used and studied as immunomodulatory drugs in the treatment of COVID-19[49,50]. Although there is conflicting information about its effectiveness in COVID-19, hepatotoxicity is not a common side effect of hydroxychloroquine. Hydroxychloroquine has been used in the treatment of systemic lupus erythematosus, rheumatoid arthritis, and related diseases for over 70 years[51]. There are only a few case reports of hepatoxicity with hydroxychloroquine[34,52].

Interpreting the data on whether antibiotics, NSAIDs, and other drugs used to treat COVID-19 patients cause hepatotoxic effects is a complicated issue. As discussed above, elevated AST and ALT levels are seen in severe cases or occur during the disease course even if it is normal on admission. These cases stay longer in hospital and combat unfavorable conditions such as secondary bacterial and fungal infections, sepsis, and cytokine storm which require the administration of certain other medications. Rather than thinking that liver enzyme elevation is related solely to the drugs used, it seems more plausible to account that all factors contribute.

HISTOPATHOLOGICAL FINDINGS OF THE LIVER

Understanding histopathological findings of COVID-19 has an important role in elucidating the pathogenesis of the disease and how liver damage develops. The most common finding in histopathology is steatosis. In a review that involved 9 biopsies and 226 autopsies, histopathology findings of COVID-19 cases in the published studies were evaluated and the most important histopathological findings of lung, heart, liver, and kidney were summarized[53]. Although a limited number of samples was performed in biopsy/autopsy, the most remarkable findings have been detected as steatosis and inflammation. Similarly, Díaz *et al*[24] reported detecting hepatic steatosis and vascular thrombosis as major and prevalent histological liver findings. Portal and lobular inflammation and Kupffer cell hyperplasia or proliferation were other frequent findings. Steatosis was higher than the normal population. It should be noted that these findings may lead to a bias since patients with more severe illnesses are included in the autopsy or biopsy studies. Besides, it can also be explained by the co-existence of other common causes of steatosis (*e.g.*, diabetes, obesity, NAFLD, hypertension, and heart diseases) in severe COVID-19 patients[9,24].

PRE-EXISTING LIVER DISEASES

The prevalence of CLDs among COVID-19 patients is low. Kulkarni *et al*[5] reported the pooled prevalence of underlying CLDs as 3.6% (95%CI: 2.5-5.1) among 15407 patients in 50 articles, and as 3.9% among 1587 severely ill patients in 15 articles that reported it. However, there are higher rates of its prevalence in different studies. Oyelade *et al*[54] reported its prevalence as 3%-11% in their meta-analysis. Fu *et al*[16] reported the prevalence of CLDs as 19.9% (viral hepatitis 8.9% and NAFLD 1%) in their study population and did not find any significant associations between CLDs and elevated liver function tests. Certain studies reported that underlying CLDs are

associated with higher mortality[55-57]. Contrary to this, in the comprehensive meta-analysis by Kulkarni *et al*[5], the presence of CLDs was not associated with severe COVID-19 (OR = 0.8, 95%CI: 0.31-2.09, P = 0.67). Similar to Kulkarni, Lippi *et al*[58] could not find any association between CLDs and COVID-19 severity (OR = 0.96, 95%CI: 0.36–2.52) and its mortality (OR = 2.33, 95%CI: 0.77–7.04). Conflicting results in the literature about the relation between SARS-CoV-2 infection and pre-existing liver disease may be associated with the heterogeneity of the study populations and the type (*e.g.*, alcoholic liver disease, NAFLD, viral hepatitis) and severity of the underlying liver diseases (*e.g.*, cirrhosis, decompensated disease or hepatocellular carcinoma), and further investigation is needed to clearly understand.

An observational study found the presence of alcohol-related liver disease, decompensated cirrhosis, and hepatocellular carcinoma as independent risk factors for higher mortality in patients with CLDs[55]. In APCOLIS study (APASL COVID-19 Liver Injury Spectrum Study), patients with obesity (in cirrhotic) and diabetes mellitus (in non-cirrhotic) were vulnerable to liver injury[59]. In fact, it appears that chronic liver patients in advanced stages, rather than all chronic liver patients, have a higher risk of severe infection and mortality[56].

The individual risk to being infected with COVID-19 in patients with CLDs depends on several factors including comorbidity, etiology of chronic disease, and baseline liver disease stage[56,60]. Controlled viral hepatitis B and C was not accepted as an exact predisposing factor to SARS-CoV-2 infection[25]. Patients with cirrhosis or hepatocellular carcinoma may be more vulnerable to SARS-CoV-2 infection because of the impairment of patients' immune systems[61]. However, many more studies are needed to clarify the issue of whether chronic viral hepatitis creates a predisposition to SARS-CoV-2 infection.

MANAGEMENT OF LIVER INJURY

In mild cases of COVID-19, liver injury usually resolves spontaneously[61]. If liver injury develops during the COVID-19 clinical course, it should first be investigated whether the abnormal liver function tests are related to the drugs including antivirals, antibiotics, NSAIDs used in the treatment, and if necessary, the drug held responsible for liver damage should be discontinued[34]. However, severe liver injury may require a more meticulous evaluation and careful treatment. The actual cause of liver injury should be investigated, and appropriate treatment provided for possible factors. If present, hypoxia and hypoperfusion should be regulated. Timely control of immune-mediated systemic inflammation and cytokine storm improve the prognosis and reduce respiratory cell infiltration and hypoxia. Anti-inflammatory treatments such as dexamethasone or other corticosteroids that have been found to reduce mortality by suppression of inflammation are used. Dexamethasone 6 mg IV or orally for 10 d (or until discharge if earlier), is recommended in severe cases of COVID-19 particularly with end organ dysfunction. Alternatively, methylprednisolone 32 mg and prednisone 40 mg which are equivalent doses to dexamethasone 6 mg can also be used[62-64]. Corticosteroids are also one of the treatment options in hemophagocytic lymphohistiocytosis, a type of cytokine storm associated with deepening laboratory abnormalities including elevated liver function tests and seen in COVID-19 patients[35]. Other immunomodulatory and cytokine antagonists can be used in the treatment[35]. Adding tocilizumab to standard of care is recommended for progressive severe and critical cases of COVID-19 who have elevated markers of systemic inflammation[62]. Thus, liver damage due to hypoxia or hyperinflammation can be reduced with appropriate and on-time treatment.

To prevent the risks that may arise with COVID-19 infection, EASL recommends SARS-CoV-2 vaccination as early as possible in patients with CLDs, hepatocellular carcinoma, and candidates for liver transplantations as the potential benefits of the vaccine outweigh the risks associated with the vaccine. In transplanted patients, the optimal time of vaccination is 3-6 mo after transplantation[60].

CONCLUSION

In conclusion, we summarized the epidemiological characteristics of liver involvement in COVID-19 infection and the effects of liver dysfunction on the COVID-19 prognosis. We also evaluated the data on the pathophysiology of liver injury. Abnormal liver function tests have been detected in more than one-fourth of patients with COVID-19

and were associated with poorer outcomes. Abnormal liver function tests in COVID-19 need to be carefully investigated. The detection of real mechanisms on liver injury is a complicated and concurrent condition. Direct viral cytotoxic effect, the disease-induced complications and drugs used in COVID-19 treatment can cause singular or joined liver injury. Appropriate treatment should be provided for the possible reasons of liver injury.

REFERENCES

1 **Trefts E**, Gannon M, Wasserman DH. The liver. *Curr Biol* 2017; **27**: R1147-R1151 [PMID: 29112863 DOI: 10.1016/j.cub.2017.09.019]
2 **Cheong J**, Bartell N, Peeraphatdit T, Mosli M, Al-Judaibi B. Gastrointestinal and liver manifestations of COVID-19. *Saudi J Gastroenterol* 2020; **26**: 226-232 [PMID: 32367837 DOI: 10.4103/sjg.SJG_147_20]
3 **Wu ZH**, Yang DL. A meta-analysis of the impact of COVID-19 on liver dysfunction. *Eur J Med Res* 2020; **25**: 54 [PMID: 33148326 DOI: 10.1186/s40001-020-00454-x]
4 **Kullar R**, Patel AP, Saab S. Hepatic Injury in Patients With COVID-19. *J Clin Gastroenterol* 2020; **54**: 841-849 [PMID: 32976196 DOI: 10.1097/MCG.0000000000001432]
5 **Kulkarni AV**, Kumar P, Tevethia HV, Premkumar M, Arab JP, Candia R, Talukdar R, Sharma M, Qi X, Rao PN, Reddy DN. Systematic review with meta-analysis: liver manifestations and outcomes in COVID-19. *Aliment Pharmacol Ther* 2020; **52**: 584-599 [PMID: 32638436 DOI: 10.1111/apt.15916]
6 **Ye Z**, Song B. Liver injury in COVID-19: Diagnosis and associated factors. *Liver Int* 2020; **40**: 2040-2041 [PMID: 32359220 DOI: 10.1111/liv.14501]
7 **World Health Organization**. Clinical management of COVID-19: Interim Guidance. 27 May, 2020. [cited 18 May 2021]. Available from: https://www.who.int/publications-detail/clinical-management-of-covid-19
8 **Cai Q**, Huang D, Yu H, Zhu Z, Xia Z, Su Y, Li Z, Zhou G, Gou J, Qu J, Sun Y, Liu Y, He Q, Chen J, Liu L, Xu L. COVID-19: Abnormal liver function tests. *J Hepatol* 2020; **73**: 566-574 [PMID: 32298767 DOI: 10.1016/j.jhep.2020.04.006]
9 **Lv XH**, Yang JL, Deng K. Letter to the Editor: COVID-19-Related Liver Injury: The Interpretation for Aspartate Aminotransferase Needs to Be Cautious. *Hepatology* 2021; **73**: 874 [PMID: 32767788 DOI: 10.1002/hep.31509]
10 **Lei F**, Liu YM, Zhou F, Qin JJ, Zhang P, Zhu L, Zhang XJ, Cai J, Lin L, Ouyang S, Wang X, Yang C, Cheng X, Liu W, Li H, Xie J, Wu B, Luo H, Xiao F, Chen J, Tao L, Cheng G, She ZG, Zhou J, Wang H, Lin J, Luo P, Fu S, Ye P, Xiao B, Mao W, Liu L, Yan Y, Chen G, Huang X, Zhang BH, Yuan Y. Longitudinal Association Between Markers of Liver Injury and Mortality in COVID-19 in China. *Hepatology* 2020; **72**: 389-398 [PMID: 32359177 DOI: 10.1002/hep.31301]
11 **Cichoż-Lach H**, Michalak A. Liver injury in the era of COVID-19. *World J Gastroenterol* 2021; **27**: 377-390 [PMID: 33584070 DOI: 10.3748/wjg.v27.i5.377]
12 **Deidda S**, Tora L, Firinu D, Del Giacco S, Campagna M, Meloni F, Orrù G, Chessa L, Carta MG, Melis A, Spolverato G, Littera R, Perra A, Onali S, Zorcolo L, Restivo A. Gastrointestinal coronavirus disease 2019: epidemiology, clinical features, pathogenesis, prevention, and management. *Expert Rev Gastroenterol Hepatol* 2021; **15**: 41-50 [PMID: 32955375 DOI: 10.1080/17474124.2020.1821653]
13 **Napodano C**, Pocino K, Stefanile A, Marino M, Miele L, Gulli F, Basile V, Pandolfi F, Gasbarrini A, Rapaccini GL, Basile U. COVID-19 and hepatic involvement: The liver as a main actor of the pandemic novel. *Scand J Immunol* 2021; **93**: e12977 [PMID: 32931622 DOI: 10.1111/sji.12977]
14 **Wong YJ**, Tan M, Zheng Q, Li JW, Kumar R, Fock KM, Teo EK, Ang TL. A systematic review and meta-analysis of the COVID-19 associated liver injury. *Ann Hepatol* 2020; **19**: 627-634 [PMID: 32882393 DOI: 10.1016/j.aohep.2020.08.064]
15 **Ghoda A**, Ghoda M. Liver Injury in COVID-19 Infection: A Systematic Review. *Cureus* 2020; **12**: e9487 [PMID: 32879813 DOI: 10.7759/cureus.9487]
16 **Li X**, Xu S, Yu M, Wang K, Tao Y, Zhou Y, Shi J, Zhou M, Wu B, Yang Z, Zhang C, Yue J, Zhang Z, Renz H, Liu X, Xie J, Xie M, Zhao J. Risk factors for severity and mortality in adult COVID-19 inpatients in Wuhan. *J Allergy Clin Immunol* 2020; **146**: 110-118 [PMID: 32294485 DOI: 10.1016/j.jaci.2020.04.006.]
17 **Zhu J**, Ji P, Pang J, Zhong Z, Li H, He C, Zhang J, Zhao C. Clinical characteristics of 3062 COVID-19 patients: A meta-analysis. *J Med Virol* 2020; **92**: 1902-1914 [PMID: 32293716 DOI: 10.1002/jmv.25884]
18 **Alqahtani SA**, Schattenberg JM. Liver injury in COVID-19: The current evidence. *United European Gastroenterol J* 2020; **8**: 509-519 [PMID: 32450787 DOI: 10.1177/2050640620924157]
19 **Chen N**, Zhou M, Dong X, Qu J, Gong F, Han Y, Qiu Y, Wang J, Liu Y, Wei Y, Xia J, Yu T, Zhang X, Zhang L. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. *Lancet* 2020; **395**: 507-513 [PMID: 32007143 DOI: 10.1016/S0140-6736(20)30211-7]
20 **Kukla M**, Skonieczna-Żydecka K, Kotfis K, Maciejewska D, Łoniewski I, Lara LF, Pazgan-Simon M, Stachowska E, Kaczmarczyk M, Koulaouzidis A, Marlicz W. COVID-19, MERS and SARS with

Concomitant Liver Injury-Systematic Review of the Existing Literature. *J Clin Med* 2020; **9** [PMID: 32403255 DOI: 10.3390/jcm9051420]

21 **Richardson S**, Hirsch JS, Narasimhan M, Crawford JM, McGinn T, Davidson KW; the Northwell COVID-19 Research Consortium, Barnaby DP, Becker LB, Chelico JD, Cohen SL, Cookingham J, Coppa K, Diefenbach MA, Dominello AJ, Duer-Hefele J, Falzon L, Gitlin J, Hajizadeh N, Harvin TG, Hirschwerk DA, Kim EJ, Kozel ZM, Marrast LM, Mogavero JN, Osorio GA, Qiu M, Zanos TP. Presenting Characteristics, Comorbidities, and Outcomes Among 5700 Patients Hospitalized With COVID-19 in the New York City Area. *JAMA* 2020; **323**: 2052-2059 [PMID: 32320003 DOI: 10.1001/jama.2020.6775]

22 **Kovalic AJ**, Huang G, Thuluvath PJ, Satapathy SK. Elevated Liver Biochemistries in Hospitalized Chinese Patients With Severe COVID-19: Systematic Review and Meta-analysis. *Hepatology* 2021; **73**: 1521-1530 [PMID: 32692464 DOI: 10.1002/hep.31472]

23 **Youssef M**, H Hussein M, Attia AS, M Elshazli R, Omar M, Zora G, S Farhoud A, Elnahla A, Shihabi A, Toraih EA, S Fawzy M, Kandil E. COVID-19 and liver dysfunction: A systematic review and meta-analysis of retrospective studies. *J Med Virol* 2020; **92**: 1825-1833 [PMID: 32445489 DOI: 10.1002/jmv.26055]

24 **Díaz LA**, Idalsoaga F, Cannistra M, Candia R, Cabrera D, Barrera F, Soza A, Graham R, Riquelme A, Arrese M, Leise MD, Arab JP. High prevalence of hepatic steatosis and vascular thrombosis in COVID-19: A systematic review and meta-analysis of autopsy data. *World J Gastroenterol* 2020; **26**: 7693-7706 [PMID: 33505145 DOI: 10.3748/wjg.v26.i48.7693]

25 **Amin M**. COVID-19 and the liver: overview. *Eur J Gastroenterol Hepatol* 2021; **33**: 309-311 [PMID: 32558697 DOI: 10.1097/MEG.0000000000001808]

26 **Hoffmann M**, Kleine-Weber H, Schroeder S, Krüger N, Herrler T, Erichsen S, Schiergens TS, Herrler G, Wu NH, Nitsche A, Müller MA, Drosten C, Pöhlmann S. SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor. *Cell* 2020; **181**: 271-280.e8 [PMID: 32142651 DOI: 10.1016/j.cell.2020.02.052]

27 **Yan R**, Zhang Y, Li Y, Xia L, Guo Y, Zhou Q. Structural basis for the recognition of SARS-CoV-2 by full-length human ACE2. *Science* 2020; **367**: 1444-1448 [PMID: 32132184 DOI: 10.1126/science.abb2762]

28 **Jothimani D**, Venugopal R, Abedin MF, Kaliamoorthy I, Rela M. COVID-19 and the liver. *J Hepatol* 2020; **73**: 1231-1240 [PMID: 32553666 DOI: 10.1016/j.jhep.2020.06.006]

29 **Remmelink M**, De Mendonça R, D'Haene N, De Clercq S, Verocq C, Lebrun L, Lavis P, Racu ML, Trépant AL, Maris C, Rorive S, Goffard JC, De Witte O, Peluso L, Vincent JL, Decaestecker C, Taccone FS, Salmon I. Unspecific post-mortem findings despite multiorgan viral spread in COVID-19 patients. *Crit Care* 2020; **24**: 495 [PMID: 32787909 DOI: 10.1186/s13054-020-03218-5]

30 **Nardo AD**, Schneeweiss-Gleixner M, Bakail M, Dixon ED, Lax SF, Trauner M. Pathophysiological mechanisms of liver injury in COVID-19. Liver Int. 2021; 41 :20-32 [PMID: 33190346 DOI: 10.1111/liv.14730.]

31 **Shang J**, Wan Y, Luo C, Ye G, Geng Q, Auerbach A, Li F. Cell entry mechanisms of SARS-CoV-2. *Proc Natl Acad Sci U S A* 2020; **117**: 11727-11734 [PMID: 32376634 DOI: 10.1073/pnas.2003138117]

32 **Belouzard S**, Millet JK, Licitra BN, Whittaker GR. Mechanisms of coronavirus cell entry mediated by the viral spike protein. *Viruses* 2012; **4**: 1011-1033 [PMID: 22816037 DOI: 10.3390/v4061011]

33 **Heald-Sargent T**, Gallagher T. Ready, set, fuse! *Viruses* 2012; **4**: 557-580 [PMID: 22590686 DOI: 10.3390/v4040557]

34 **Metawea MI**, Yousif WI, Moheb I. COVID 19 and liver: An A-Z literature review. *Dig Liver Dis* 2021; **53**: 146-152 [PMID: 32988758 DOI: 10.1016/j.dld.2020.09.010]

35 **Ye Q**, Wang B, Mao J. The pathogenesis and treatment of the `Cytokine Storm' in COVID-19. *J Infect* 2020; **80**: 607-613 [PMID: 32283152 DOI: 10.1016/j.jinf.2020.03.037]

36 **Nile SH**, Nile A, Qiu J, Li L, Jia X, Kai G. COVID-19: Pathogenesis, cytokine storm and therapeutic potential of interferons. *Cytokine Growth Factor Rev* 2020; **53**: 66-70 [PMID: 32418715 DOI: 10.1016/j.cytogfr.2020.05.002]

37 **Chousterman BG**, Swirski FK, Weber GF. Cytokine storm and sepsis disease pathogenesis. *Semin Immunopathol* 2017; **39**: 517-528 [PMID: 28555385 DOI: 10.1007/s00281-017-0639-8]

38 **Qin C**, Zhou L, Hu Z, Zhang S, Yang S, Tao Y, Xie C, Ma K, Shang K, Wang W, Tian DS. Dysregulation of Immune Response in Patients With Coronavirus 2019 (COVID-19) in Wuhan, China. *Clin Infect Dis* 2020; **71**: 762-768 [PMID: 32161940 DOI: 10.1093/cid/ciaa248]

39 **Soy M**, Keser G, Atagündüz P, Tabak F, Atagündüz I, Kayhan S. Cytokine storm in COVID-19: pathogenesis and overview of anti-inflammatory agents used in treatment. *Clin Rheumatol* 2020; **39**: 2085-2094 [PMID: 32474885 DOI: 10.1007/s10067-020-05190-5]

40 **Li H**, Liu L, Zhang D, Xu J, Dai H, Tang N, Su X, Cao B. SARS-CoV-2 and viral sepsis: observations and hypotheses. *Lancet* 2020; **395**: 1517-1520 [PMID: 32311318 DOI: 10.1016/S0140-6736(20)30920-X]

41 **Huang C**, Wang Y, Li X, Ren L, Zhao J, Hu Y, Zhang L, Fan G, Xu J, Gu X, Cheng Z, Yu T, Xia J, Wei Y, Wu W, Xie X, Yin W, Li H, Liu M, Xiao Y, Gao H, Guo L, Xie J, Wang G, Jiang R, Gao Z, Jin Q, Wang J, Cao B. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet* 2020; **395**: 497-506 [PMID: 31986264 DOI: 10.1016/S0140-6736(20)30183-5]

42 **Liu J**, Li S, Liu J, Liang B, Wang X, Wang H, Li W, Tong Q, Yi J, Zhao L, Xiong L, Guo C, Tian J, Luo J, Yao J, Pang R, Shen H, Peng C, Liu T, Zhang Q, Wu J, Xu L, Lu S, Wang B, Weng Z, Han C,

Zhu H, Zhou R, Zhou H, Chen X, Ye P, Zhu B, Wang L, Zhou W, He S, He Y, Jie S, Wei P, Zhang J, Lu Y, Wang W, Zhang L, Li L, Zhou F, Wang J, Dittmer U, Lu M, Hu Y, Yang D, Zheng X. Longitudinal characteristics of lymphocyte responses and cytokine profiles in the peripheral blood of SARS-CoV-2 infected patients. *EBioMedicine* 2020; **55**: 102763 [PMID: 32361250 DOI: 10.1016/j.ebiom.2020.102763]

43 **Satış H**, Özger HS, Aysert Yıldız P, Hızel K, Gulbahar Ö, Erbaş G, Aygencel G, Guzel Tunccan O, Öztürk MA, Dizbay M, Tufan A. Prognostic value of interleukin-18 and its association with other inflammatory markers and disease severity in COVID-19. *Cytokine* 2021; **137**: 155302 [PMID: 33002740 DOI: 10.1016/j.cyto.2020.155302]

44 **Klok FA**, Kruip MJHA, van der Meer NJM, Arbous MS, Gommers DAMPJ, Kant KM, Kaptein FHJ, van Paassen J, Stals MAM, Huisman MV, Endeman H. Incidence of thrombotic complications in critically ill ICU patients with COVID-19. *Thromb Res* 2020; **191**: 145-147 [PMID: 32291094 DOI: 10.1016/j.thromres.2020.04.013]

45 **Sonzogni A**, Previtali G, Seghezzi M, Alessio MG, Nebuloni M. Liver and COVID 19 infection: a very preliminary lesson learnt from histological post-mortem findings in 48 patients. 2020 Preprint. Available from: Preprints:2020040438 [DOI: 10.20944/preprints202004.0438.v1]

46 **Lai CC**, Wang CY, Hsueh PR. Co-infections among patients with COVID-19: The need for combination therapy with non-anti-SARS-CoV-2 agents? *J Microbiol Immunol Infect* 2020; **53**: 505-512 [PMID: 32482366 DOI: 10.1016/j.jmii.2020.05.013]

47 **Wu J**, Song S, Cao HC, Li LJ. Liver diseases in COVID-19: Etiology, treatment and prognosis. *World J Gastroenterol* 2020; **26**: 2286-2293 [PMID: 32476793 DOI: 10.3748/wjg.v26.i19.2286]

48 **Fan Z**, Chen L, Li J, Cheng X, Yang J, Tian C, Zhang Y, Huang S, Liu Z, Cheng J. Clinical Features of COVID-19-Related Liver Functional Abnormality. *Clin Gastroenterol Hepatol* 2020; **18**: 1561-1566 [PMID: 32283325 DOI: 10.1016/j.cgh.2020.04.002]

49 **Guner R**, Hasanoglu I, Kayaaslan B, Aypak A, Akinci E, Bodur H, Eser F, Kaya Kalem A, Kucuksahin O, Ates I, Bastug A, Tezer Tekce Y, Bilgic Z, Gursoy FM, Akca HN, Izdes S, Erdem D, Asfuroglu E, Hezer H, Kilic H, Cıvak M, Aydogan S, Buzgan T. Comparing ICU admission rates of mild/moderate COVID-19 patients treated with hydroxychloroquine, favipiravir, and hydroxychloroquine plus favipiravir. *J Infect Public Health* 2021; **14**: 365-370 [PMID: 33647553 DOI: 10.1016/j.jiph.2020.12.017]

50 **RECOVERY Collaborative Group**. , Horby P, Mafham M, Linsell L, Bell JL, Staplin N, Emberson JR, Wiselka M, Ustianowski A, Elmahi E, Prudon B, Whitehouse T, Felton T, Williams J, Faccenda J, Underwood J, Baillie JK, Chappell LC, Faust SN, Jaki T, Jeffery K, Lim WS, Montgomery A, Rowan K, Tarning J, Watson JA, White NJ, Juszczak E, Haynes R, Landray MJ. Effect of Hydroxychloroquine in Hospitalized Patients with Covid-19. *N Engl J Med* 2020; **383**: 2030-2040 [PMID: 33031652 DOI: 10.1056/NEJMoa2022926]

51 **Rainsford KD**, Parke AL, Clifford-Rashotte M, Kean WF. Therapy and pharmacological properties of hydroxychloroquine and chloroquine in treatment of systemic lupus erythematosus, rheumatoid arthritis and related diseases. *Inflammopharmacology* 2015; **23**: 231-269 [PMID: 26246395 DOI: 10.1007/s10787-015-0239-y]

52 **Falcão MB**, Pamplona de Góes Cavalcanti L, Filgueiras Filho NM, Antunes de Brito CA. Case Report: Hepatotoxicity Associated with the Use of Hydroxychloroquine in a Patient with COVID-19. *Am J Trop Med Hyg* 2020; **102**: 1214-1216 [PMID: 32314698 DOI: 10.4269/ajtmh.20-0276]

53 **Vasquez-Bonilla WO**, Orozco R, Argueta V, Sierra M, Zambrano LI, Muñoz-Lara F, López-Molina DS, Arteaga-Livias K, Grimes Z, Bryce C, Paniz-Mondolfi A, Rodríguez-Morales AJ. A review of the main histopathological findings in coronavirus disease 2019. *Hum Pathol* 2020; **105**: 74-83 [PMID: 32750378 DOI: 10.1016/j.humpath.2020.07.023]

54 **Oyelade T**, Alqahtani J, Canciani G. Prognosis of COVID-19 in Patients with Liver and Kidney Diseases: An Early Systematic Review and Meta-Analysis. *Trop Med Infect Dis* 2020; **5** [PMID: 32429038 DOI: 10.3390/tropicalmed5020080]

55 **Kim D**, Adeniji N, Latt N, Kumar S, Bloom PP, Aby ES, Perumalswami P, Roytman M, Li M, Vogel AS, Catana AM, Wegermann K, Carr RM, Aloman C, Chen VL, Rabiee A, Sadowski B, Nguyen V, Dunn W, Chavin KD, Zhou K, Lizaola-Mayo B, Moghe A, Debes J, Lee TH, Branch AD, Viveiros K, Chan W, Chascsa DM, Kwo P, Dhanasekaran R. Predictors of Outcomes of COVID-19 in Patients With Chronic Liver Disease: US Multi-center Study. *Clin Gastroenterol Hepatol* 2021; **19**: 1469-1479.e19 [PMID: 32950749 DOI: 10.1016/j.cgh.2020.09.027]

56 **Marjot T**, Moon AM, Cook JA, Abd-Elsalam S, Aloman C, Armstrong MJ, Pose E, Brenner EJ, Cargill T, Catana MA, Dhanasekaran R, Eshraghian A, García-Juárez I, Gill US, Jones PD, Kennedy J, Marshall A, Matthews C, Mells G, Mercer C, Perumalswami PV, Avitabile E, Qi X, Su F, Ufere NN, Wong YJ, Zheng MH, Barnes E, Barritt AS 4th, Webb GJ. Outcomes following SARS-CoV-2 infection in patients with chronic liver disease: An international registry study. *J Hepatol* 2021; **74**: 567-577 [PMID: 33035628 DOI: 10.1016/j.jhep.2020.09.024]

57 **Chen L**, Huang S, Yang J, Cheng X, Shang Z, Lu H, Cheng J. Clinical characteristics in patients with SARS-CoV-2/HBV co-infection. *J Viral Hepat* 2020; **27**: 1504-1507 [PMID: 32668494 DOI: 10.1111/jvh.13362]

58 **Lippi G**, de Oliveira MHS, Henry BM. Chronic liver disease is not associated with severity or mortality in Coronavirus disease 2019 (COVID-19): a pooled analysis. *Eur J Gastroenterol Hepatol* 2021; **33**: 114-115 [PMID: 32282549 DOI: 10.1097/MEG.0000000000001742]

59 **Sarin SK**, Choudhury A, Lau GK, Zheng MH, Ji D, Abd-Elsalam S, Hwang J, Qi X, Cua IH, Suh JI,

Park JG, Putcharoen O, Kaewdech A, Piratvisuth T, Treeprasertsuk S, Park S, Wejnaruemarn S, Payawal DA, Baatarkhuu O, Ahn SH, Yeo CD, Alonzo UR, Chinbayar T, Loho IM, Yokosuka O, Jafri W, Tan S, Soo LI, Tanwandee T, Gani R, Anand L, Esmail ES, Khalaf M, Alam S, Lin CY, Chuang WL, Soin AS, Garg HK, Kalista K, Batsukh B, Purnomo HD, Dara VP, Rathi P, Al Mahtab M, Shukla A, Sharma MK, Omata M; APASL COVID Task Force, APASL COVID Liver Injury Spectrum Study (APCOLIS Study-NCT 04345640). Pre-existing liver disease is associated with poor outcome in patients with SARS CoV2 infection; The APCOLIS Study (APASL COVID-19 Liver Injury Spectrum Study). *Hepatol Int* 2020; **14**: 690-700 [PMID: 32623632 DOI: 10.1007/s12072-020-10072-8]

60 **Cornberg M**, Buti M, Eberhardt CS, Grossi PA, Shouval D. EASL position paper on the use of COVID-19 vaccines in patients with chronic liver diseases, hepatobiliary cancer and liver transplant recipients. *J Hepatol* 2021; **74**: 944-951 [PMID: 33563499 DOI: 10.1016/j.jhep.2021.01.032]

61 **Zhang C**, Shi L, Wang FS. Liver injury in COVID-19: management and challenges. *Lancet Gastroenterol Hepatol* 2020; **5**: 428-430 [PMID: 32145190 DOI: 10.1016/S2468-1253(20)30057-1]

62 **Bhimraj A**, Morgan RL, Shumaker AH, Lavergne V, Baden L, Cheng VC, Edwards KM, Gandhi R, Muller WJ, O'Horo JC, Shoham S, Murad MH, Mustafa RA, Sultan S, Falck-Ytter Y. Infectious Diseases Society of America Guidelines on the Treatment and Management of Patients with COVID-19. Infectious Diseases Society of America 2021; Version 4.2.0. [Accessed 15 May 2021]. Available from: https://www.idsociety.org/practice-guideline/covid-19-guideline-treatment-and-management/

63 **WHO Rapid Evidence Appraisal for COVID-19 Therapies (REACT) Working Group**; Sterne JAC, Murthy S, Diaz JV, Slutsky AS, Villar J, Angus DC, Annane D, Azevedo LCP, Berwanger O, Cavalcanti AB, Dequin PF, Du B, Emberson J, Fisher D, Giraudeau B, Gordon AC, Granholm A, Green C, Haynes R, Heming N, Higgins JPT, Horby P, Jüni P, Landray MJ, Le Gouge A, Leclerc M, Lim WS, Machado FR, McArthur C, Meziani F, Møller MH, Perner A, Petersen MW, Savovic J, Tomazini B, Veiga VC, Webb S, Marshall JC. Association Between Administration of Systemic Corticosteroids and Mortality Among Critically Ill Patients With COVID-19: A Meta-analysis. *JAMA* 2020; **324**: 1330-1341 [PMID: 32876694 DOI: 10.1001/jama.2020.17023]

64 **Wu C**, Hou D, Du C, Cai Y, Zheng J, Xu J, Chen X, Chen C, Hu X, Zhang Y, Song J, Wang L, Chao YC, Feng Y, Xiong W, Chen D, Zhong M, Hu J, Jiang J, Bai C, Zhou X, Song Y, Gong F. Corticosteroid therapy for coronavirus disease 2019-related acute respiratory distress syndrome: a cohort study with propensity score analysis. *Crit Care* 2020; **24**: 643 [PMID: 33172477 DOI: 10.1186/s13054-020-03340-4]

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MINIREVIEWS

Newer variants of progressive familial intrahepatic cholestasis

Vignesh Vinayagamoorthy, Anshu Srivastava, Moinak Sen Sarma

ORCID number: Vignesh Vinayagamoorthy 0000-0003-4860-683X; Anshu Srivastava 0000-0003-0902-4140; Moinak Sen Sarma 0000-0003-2015-4069.

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Vignesh Vinayagamoorthy, Anshu Srivastava, Moinak Sen Sarma, Department of Pediatric Gastroenterology, Sanjay Gandhi Postgraduate Institute of Medical Sciences, Lucknow 226014, Uttar Pradesh, India

Corresponding author: Anshu Srivastava, MD, Professor, Department of Pediatric Gastroenterology, Sanjay Gandhi Postgraduate Institute of Medical Sciences, Raebareli Road, Lucknow 226014, Uttar Pradesh, India. avanianshu@yahoo.com

Abstract

Progressive familial intrahepatic cholestasis (PFIC) is a heterogeneous group of disorders characterized by defects in bile secretion and presentation with intrahepatic cholestasis in infancy or childhood. The most common types include PFIC 1 (deficiency of FIC1 protein, ATP8B1 gene mutation), PFIC 2 (bile salt export pump deficiency, ABCB11 gene mutation), and PFIC 3 (multidrug resistance protein-3 deficiency, ABCB4 gene mutation). Mutational analysis of subjects with normal gamma-glutamyl transferase cholestasis of unknown etiology has led to the identification of newer variants of PFIC, known as PFIC 4, 5, and MYO5B related (sometimes known as PFIC 6). PFIC 4 is caused by the loss of function of tight junction protein 2 (TJP2) and PFIC 5 is due to NR1H4 mutation causing Farnesoid X receptor deficiency. MYO5B gene mutation causes microvillous inclusion disease (MVID) and is also associated with isolated cholestasis. Children with TJP2 related cholestasis (PFIC-4) have a variable spectrum of presentation. Some have a self-limiting disease, while others have progressive liver disease with an increased risk of hepatocellular carcinoma. Hence, frequent surveillance for hepatocellular carcinoma is recommended from infancy. PFIC-5 patients usually have rapidly progressive liver disease with early onset coagulopathy, high alpha-fetoprotein and ultimately require a liver transplant. Subjects with MYO5 B-related disease can present with isolated cholestasis or cholestasis with intractable diarrhea (MVID). These children are at risk of worsening cholestasis post intestinal transplant (IT) for MVID, hence combined intestinal and liver transplant or IT with biliary diversion is preferred. Immunohistochemistry can differentiate most of the variants of PFIC but confirmation requires genetic analysis.

Key Words: Progressive familial intrahepatic cholestasis; Tight junction protein; Hepatocellular carcinoma; Biliary diversion; Microvillous inclusion disease

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Core Tip: Progressive familial intrahepatic cholestasis (PFIC) manifests with a varying spectrum of clinical features, with some variants progressing rapidly into end stage liver disease. Recently, newer variants of PFIC have been described including PFIC 4 due to tight junction protein 2 (TJP2) mutation, PFIC 5 due to NR1H4 mutation and MYO5B related cholestasis also sometimes known as PFIC 6. TJP2 related PFIC also has a risk of hepatocellular carcinoma. This article describes the pathogenesis and clinical features of the newer variants of PFIC.

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INTRODUCTION

Progressive familial intrahepatic cholestasis (PFIC) is a heterogeneous group of intrahepatic cholestatic disorders caused by a defect in bile transport and secretion. It manifests in infancy or childhood and can progress to end-stage liver disease[1-3]. Genetically confirmed PFIC accounts for 12%-13% of cholestatic disorders in infants and children[4]. Disease variants are classified based on the specific bile transporter defects and all of them have an autosomal recessive inheritance. The three most prominent varieties are familial intrahepatic cholestasis-1, 2 and 3, which are caused by mutations in ATP8B1 gene encoding FIC1, ABCB11 gene encoding bile salt export pump, and ABCB4 gene encoding multidrug resistance protein-3 respectively (Figure 1). Nearly two-thirds of subjects with normal gamma-glutamyl transpeptidase (GGT) cholestasis (normally associated with PFIC except PFIC 3) do not have any mutations identified in ATP8B1 or ABCB11 genes[3]. Detailed mutational analysis in patients with this phenotype has led to the identification of 3 more conditions, often known as PFIC 4, 5, and 6. PFIC 4 is caused by the loss of function of tight junction protein 2 (TJP2)[5], and PFIC 5 is due to NR1H4 mutation causing farnesoid X receptor (FXR) deficiency[6,7]. MYO5B mutation, known to cause microvillous inclusion disease (MVID), is also reported to cause isolated cholestasis and is sometimes known as PFIC 6 though it is not yet recognized by the Online Mendelian Inheritance in Man [8]. The exact incidence of newer variants of PFIC is not known due to the limited number of studies, which are mostly case reports or small case series. Based on the available literature, this review attempts to sensitize physicians to the disease.

GENETICS AND PATHOGENESIS

PFIC 4

TJP2 gene, located in chromosome 9q21 was first discovered in 1991 by Gumbiner *et al* [9]. It encodes a protein called tight junction protein 2 or zona occludens-2. Though named as tight junction protein, it is not present in the tight junction. Instead, TJP2 is a cytosolic protein, involved in maintaining cell-to-cell adhesion by linking the transmembrane tight junction proteins like claudin with the actin cytoskeleton. There are two types of claudin *i.e.*, claudin-1 (CLDN1) and claudin-2 (CLDN2), both of which are localized to the bile canalicular membrane[10]. In TJP2 mutation, CLDN1 fails to localize to the bile canalicular membrane (Figure 2). This results in reduced integrity of the canalicular membrane and reflux of toxic bile acids through the paracellular spaces into hepatocytes, causing hepatocyte damage and cholestasis[11]. TJP2 has a widespread expression, including the respiratory and central nervous systems. This may explain the systemic features reported in a few cases[11]. The detergent action of the bile potentiates damage in the liver, which explains the predominant hepatic manifestations in this condition.

PFIC 5

PFIC 5 is related to a deficiency of the FXR due to loss of function mutation in the *NR1H4* gene located in chromosome 12q23. NR1H4 related PFIC 5 is a less commonly

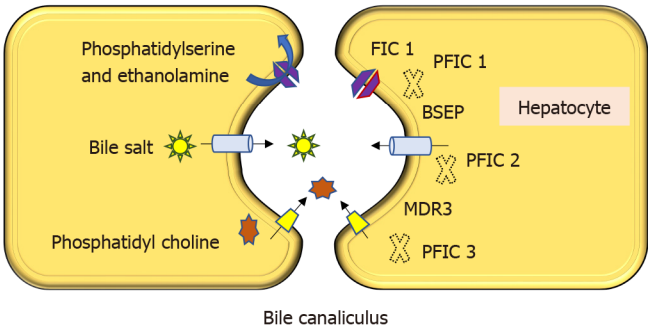

Figure 1 Pathogenesis of progressive familial intrahepatic cholestasis 1, 2 and 3. Familial intrahepatic cholestasis protein 1 is a flippase that helps in movement of phosphatidylserine and phosphatidylethanolamine from the outer to inner leaflet of the plasma membrane of hepatocyte; Bile salt exporter pump exports bile acid from hepatocytes to bile canaliculus; Multidrug resistance protein 3 is a floppase involved in transporting phosphatidylcholine into bile canaliculus. PFIC: Progressive familial intrahepatic cholestasis; FIC1: Familial intrahepatic cholestasis protein 1; BSEP: Bile salt exporter pump; MDR3: Multidrug resistance protein 3.

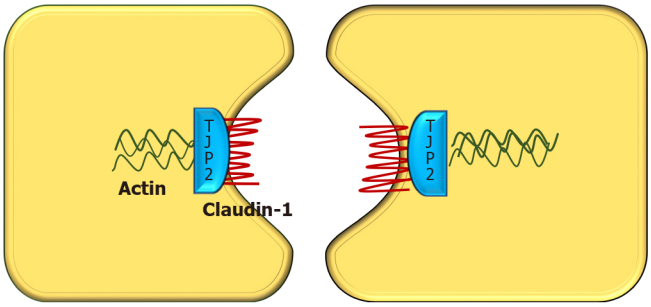

Figure 2 Diagrammatic representation of interaction between various tight junction proteins in hepatocytes. Claudin, tight junction proteins (TJP2), and actin form intercellular cytoskeletal support. Tight junctions prevent mixing of bile and blood. Absence of TJP2 causes a failure of claudin-1 localization at the canalicular membrane, leading to loss of compactness of the tight junctions and leakage of the bile through the paracellular space. TJP2: Tight junction proteins 2.

reported variant, with < 10 cases reported by 2020. FXR, a protein translated from the NR1H4 gene was first described in 1995 by Forman *et al*[12]. It belongs to a nuclear receptor group activated by farnesyl, an intermediate metabolite of the mevalonic acid synthesis pathway. FXR is the master regulator of cholesterol, bile acid, triglyceride and various sterol ring-containing compounds (Vitamin D, carotenoids, retinoids, *etc.*) [13]. In the liver, the FXR acts as a nuclear bile acid-sensing receptor involved in the expression of bile salt export protein (BSEP) and sometimes MDR3[6,14]. Apart from the liver, FXR is also expressed in the small intestine. Whenever bile acid levels are elevated in the ileal enterocytes, FXR is activated to induce the synthesis of fibroblast growth factor 19 (FGF19). FGF 19 is then transported *via* enterohepatic recirculation to the liver, where it binds to the fibroblast growth factor receptor 4/β-Klotho complex, and causes inhibition of bile acid synthesis by repressing CYP7A1. Elevated bile acid inside hepatocytes also activates FXR which induces ABCB11gene transcription, BSEP synthesis, and bile acid export from the liver. Hence, the NR1H4 mutation causes loss of BSEP expression, leading to the accumulation of toxic bile and hepatocellular damage (Figure 3). FXR is also involved in the regulation of coagulation factor synthesis by transactivating fibrinogen and kininogen genes. Thus, the FXR mutation leads to the development of vitamin K independent, early-onset coagulopathy, well before liver failure sets in[6].

Homozygous or compound heterozygous loss of function mutations (c.526C>T and c.419 420insAAA/intragenic 31.7-kb deletion, respectively) have been described[7]. In one woman with intrahepatic cholestasis of pregnancy, *NR1H4* heterozygous variant (c.-1G>T) was found to be associated with cholestasis[15].

PFIC 6

The *MYO5B* gene located in chromosome 18q21.1 encodes an actin-associated molecular motor protein called MYO5B. MYO5B and RAS-related GTP-binding protein 11A (RAB11A) is essential for the epithelial cell polarization in multiple tissues (Figure 4). In hepatocytes, it is important for the localization of ATP-dependent bile canalicular transporters like BSEP to the canalicular membrane, and in the intestine, it

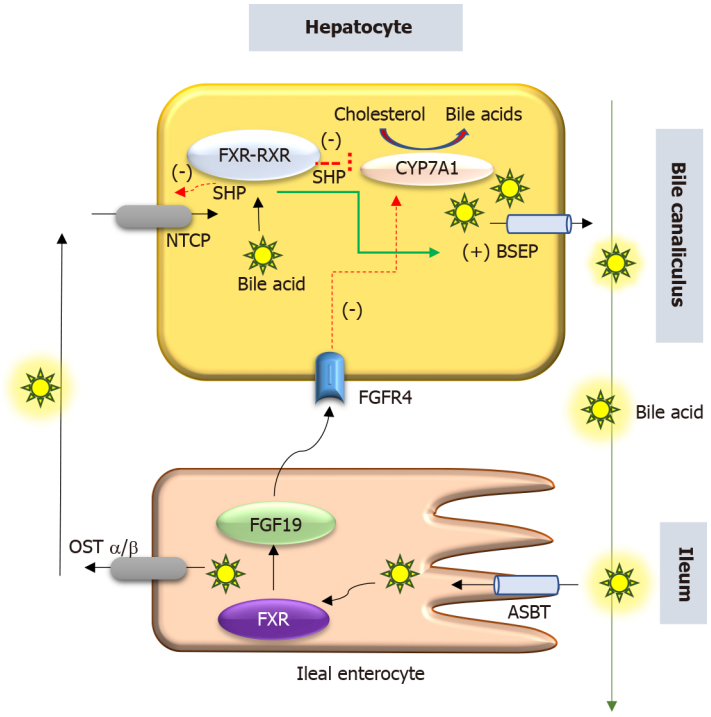

Figure 3 Schematic representation of role of farnesoid X receptor in hepatocyte. Bile acids are transported into the hepatocyte by NTCP. De novo synthesis of bile acids from cholesterol is mediated by CYP7A1. Bile acids and farnesoid X receptor (FXR) interact and enter the nucleus to promote expression of bile salt export protein and short heterodimer partner (SHP). SHP suppresses expression of NTCP and CYP7A1. FXR also induces FGF-19 in ileal enterocytes which inhibits CYP7A1 *via* FGFR4. ASBT: Apical sodium bile transporter, BSEP: Bile salt export pump; FGF-19: Fibroblast growth factor-19; FGFR-4: Fibroblast growth factor receptor-4; FXR: Farnesoid X receptor; NTCP: Na+-taurocholate co-transporting polypeptide; OST α/β: Organic solute transporter; RXR: Retinoid X receptor; SHP: Short heterodimer partner.

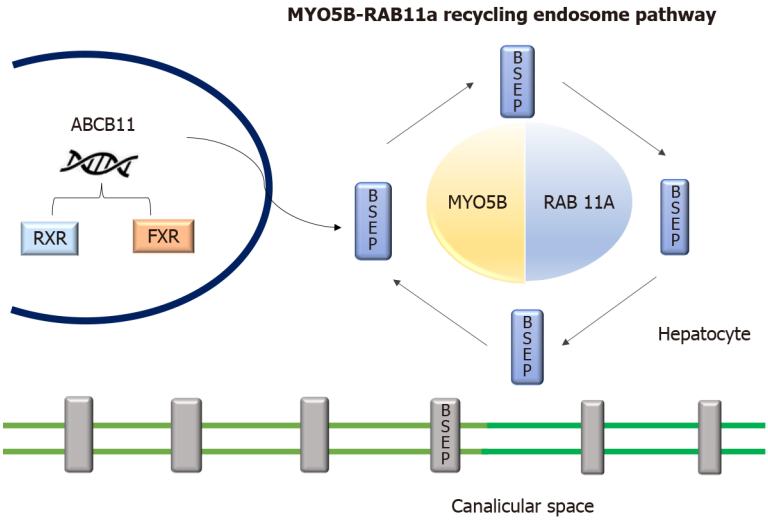

Figure 4 Diagrammatic representation of role of MYO5B and RAS-related GTP-binding protein 11A interaction and endosome recycling pathway and bile salt export pump expression. MYO5B and RAS-related GTP-binding protein 11A (RAB11A) interaction is essential for epithelial cell polarization and BSEP localization to the canalicular membrane. Diminished MYO5B/RAB11A recycling endosome pathway leads to disruption of bile salt export pump localization. ABCB11: ATP Binding Cassette Subfamily B Member 11; BSEP: Bile salt export pump; FXR: Farnesoid X receptor; RAB11A: RAS-related GTP-binding protein 11A; RXR: Retinoid X receptor.

is important for maintaining enterocyte polarity[16]. MYO5B mutations disrupt the MYO5B/RAB11A recycling endosome pathway leading to defective targeting of BSEP [17]. MYO5B gene mutations can result in cholestatic liver disease with or without associated MVID, which presents as intractable diarrhea in infancy[8,18]. Staining of BSEP and MDR3 by immunohistochemistry in these patients is sub-canalicular in the location instead of the regular localization in the canalicular membrane[8].

There is a suggestion that the type of MYO5B mutation affects the clinical presentation[18,19]. Less severe mutations have a loss of canalicular transporter function in hepatocytes without any loss of enterocytes functionality. These patients present with isolated cholestasis. In severe variants of mutations, there is a dysfunction of both bile canalicular transporter and enterocyte polarization. However, a severe loss of enterocyte function leads to a reduced bile acid absorption in the intestine and in turn decreased bile acid load to the hepatocyte, potentially preventing cholestatic manifestations[18]. Patients with MVID more often have biallelic severe mutations in MYO5B. Biallelic mutations in the MYO5B-RAB11A interaction domain are more in MVID than those with isolated cholestasis[20]. Thus, isolated cholestasis appears to reflect relatively mild MYO5B functional deficiency, whereas severe mutations in MYO5B primarily cause MVID[20].

CLINICAL PRESENTATION

Intrahepatic cholestasis is the hallmark of how these 3 genetic conditions present. Most often, patients present with variable combinations of pruritus, jaundice, pale stools, and failure to thrive. The published literature on each of these three entities (TJP2, FXR, and MYO5B) is limited and has been summarized in Tables 1-3 respectively.

PFIC 4

A varying spectrum of clinical presentation, ranging from mild anicteric illness, recurrent jaundice to severe progressive liver disease has been described[5,11]. Incomplete penetrant, homozygous, missense mutations affecting both isoforms of TJP2 have been shown to cause familial hypercholanemia in the Amish population which manifests as a mild anicteric disease with pruritus and steatorrhea. In this condition, the binding of TJP2 to claudins is impaired[21]. Milder mutations of TJP2 are also known to be associated with intrahepatic cholestasis of pregnancy[15].

In the 12 cases reported by Sambrotta *et al*[5], 9 (75%) required liver transplantation (LT) while 2 had portal hypertension. In contrast, none of the 7 cases reported by Zhang *et al*[22] required LT, and cholestasis responded to medical therapy in a majority. Zhang *et al*[22] also showed that truncating or canonical splice-site biallelic TJP2 mutations caused a more severe presentation due to a complete loss of protein expression. In their study, 3 children with severe mutations had growth failure. While the other 3 cases with missense variants had normal growth and sustained response in pruritus with ursodeoxycholic acid (UDCA) and cholestyramine.

All homozygous mutations are predicted to abolish protein translation and a complete loss of function[5]. Mutations involving missense and frame deletion lead to less severe clinical disease due to residual TJP2 protein expression[22]. This suggests the presence of a genotype-phenotype correlation based on the amount of remnant functional TJP2 activity.

There is a higher risk of developing hepatocellular carcinoma (HCC) in these cases, similar to that seen in PFIC 2 patients. Subjects can either present with a space-occupying lesion (SOL) in the liver or are detected to have HCC after LT on histology of the explanted liver[23,24]. This predisposition to HCC highlights the importance of close follow-up and regular monitoring.

PFIC 5

FXR is the master player of bile acid regulation and plays an important role in reducing bile acid-induced hepatotoxicity. Rapidly progressive liver disease and early onset vitamin K independent coagulopathy are the main features of this condition. The details of the 8 published cases are given in Table 2. A majority of patients presented early in the first 3 mo of life and progressed rapidly to liver failure. Patients have markedly increased alpha-fetoprotein and deranged international normalized ratio. Without a liver transplant, 5/8 died in infancy itself. Three cases survived post-liver transplant, of which 2 were found to have liver function abnormality with graft steatosis in the follow-up[6]. This post-transplant hepatic damage may be attributed to the altered enterohepatic circulation and FXR signalling in these cases. The absence of FXR in the intestine leads to low FGF 19 levels and this allows for continued and increased synthesis of bile acids by the liver[25]. Intrahepatic cholestasis of pregnancy has been reported and attributed to the downregulation of BSEP in this condition[26].

Table 1 Clinical characteristics and outcome in patients with *TJP2* mutation

Ref.	*n*	Age at onset of symptoms	Symptoms	Other symptoms	Treatment	Liver transplant	Outcome
Sambrotta *et al*[5]	12	1 wk-3 mo	NC-12/12	Chronic respiratory disease-1, recurrent unexplained hematoma-1	UDCA, PEBD-2	9/12 cases at the age of 1.5-10 yr	Post-transplant-9 (doing well, no disease recurrence); Stable liver disease with PHT-2; Mortality-1 at 13 mo age
Zhang *et al*[22]	7 (M = 6, F = 1)	3 d-2 mo	NC-6/7, pruritus at 7 mo-1/7	Gallstones 2/7	Response to UDCA, cholestyramine	None	Resolved cholestasis (n = 6) over 7-26 mo; Persisting icterus-1
Ge *et al*[46]	1 (F)	6mo	Jaundice, pruritus, FTT	-	Responded to medical treatment	None	Resolved cholestasis
Mirza *et al*[47]	1 (M)	4 yr	Jaundice, pruritus	-	Medical treatment	None	Cirrhosis, PHT with variceal bleed at 15 yr
Wei *et al*[24]	Index case (M) with multiple affected family members[1]	19 yr	Cirrhosis, PHT with variceal bleed, HCC at 22 yr	-	Medical treatment including EVL	23 yr	Well in post-transplant period

[1]Variable severity of liver disease: Cholestatic liver disease requiring transplant, cholestatic liver disease and intrahepatic cholestasis of pregnancy in other affected members.

EVL: Endoscopic variceal ligation; F: Female; FTT: Failure to thrive; HCC: Hepatocellular carcinoma; M: Male; NC: Neonatal cholestasis; PEBD: Partial external biliary diversion; PHT: Portal hypertension; UDCA: Ursodeoxycholic acid.

PFIC associated with MYO5B defects

Patients with MYO5B mutations can present with isolated cholestasis, isolated MVID, or both MVID and cholestasis. Typically, the child presents with jaundice, pruritus, and hepatomegaly. In patients with MVID and cholestasis, the onset of cholestasis may be pre or post-small bowel transplant. The exact explanation as to why some MVID cases develop cholestasis while others do not is unclear but it may be related to the severity of mutation (vide supra). The summary of the clinical presentation of 29 cases with MYO5B mutation, as reported in 4 papers, is shown in Table 3. Even in siblings with the same mutation and presentation with cholestasis, the disease severity may vary[20]. This suggests the possible role of modifier genes or environmental factors. Among Han Chinese children, defects in MYO5B accounted for approximately 20% of cases of idiopathic low-normal GGT intrahepatic cholestasis[20].

INVESTIGATIONS AND THE APPROACH TO DIAGNOSIS

The main steps for making a diagnosis of PFIC and determining the specific type in any given child with cholestasis are as follows: Step 1: Detailed history and physical examination including family history, consanguinity, extraintestinal symptoms, growth, nutritional deficiencies, and features of advanced liver disease; Step 2:

Table 2 Clinical characteristics and outcome in patients with *NR1H4* mutation

Ref.		Sex	Age at onset of symptoms	Age at initial evaluation	Symptoms	Lab parameters			Histology/IHC	Age at LTx	Outcome
						GGT	INR (at onset)	AFP ng/mL			
Gomez-Ospina *et al* [6], 2016	All cases had homozygous mutations										
	[1]Patient 1	F	2 wk	20 mo	J, FTT	53	2	716	Cirrhosis	22 mo	10 yr[4]
	[1]Patient 2	M	2 wk	7 wk	J, FTT	45	2	146000	Fibrosis	4.4 mo	15 mo[4]
	[2]Patient 3	F	6 wk	6 wk	J	59	1.4	13900	Fibrosis	ND	Died 8 mo
	[2]Patient 4	M	Birth	Birth	J, ascites, pleural effusion, ICB		-	-	Fibrosis	ND	Died at 4 wk
Himes *et al*[7], 2020	Patient 5 and 7 had homozygous mutations										
	Patient 5	M	16 mo	17 mo	J, ascites	81	1.9	9610	Cirrhosis	20 mo	Alive at 8 yr of age, no graft steatosis
	[3]Patient 6	M	3 wk	1 mo	J, FTT, hydrothorax	-	-	-	-	ND	Died at 8 mo, liver failure
	[3]Patient 7	F	1 wk	4 mo	J, FTT, hydrothorax	-	-	> 100000	-	ND	Died at 7 mo, liver failure
Chen *et al*[27], 2019	Patient had compound heterozygote mutation										
	Patient 8		N/A	3 mo	J, splenomegaly		3.0	> 80000	-	ND	Died at 5 mo

[1]Family 1.
[2]Family 2.
[3]Family 3.
[4]Post transplant both cases have hepatic steatosis and liver function test abnormalities.
AFP: Alpha fetoprotein; BSEP: Bile salt export pump; F: Female; FTT: Failure to thrive; FXR: Farnesoid X receptor; GGT: Gamma-glutamyltransferase; ICB: Intracranial bleed; IHC: Immunohistochemistry; INR: International normalized ratio; J: Jaundice; LTx: Liver transplantation; MDR3: Multidrug resistance protein 3; M: Male; N/A: Not applicable; ND: Not done.

Complete liver function test with GGT. Low-normal GGT is seen in ATP8B1, ABCB11, TJP2, NR1H4, and MYO5B disease. Early-onset of vitamin K unresponsive coagulopathy is a feature of NR1H4 disease; Step 3: Radiologic imaging. Ultrasonography (USG) of the abdomen is useful to exclude structural causes of neonatal cholestasis, like biliary atresia or choledochal cyst. The presence of biliary radicle dilatation may suggest sclerosing cholangitis, which needs to be confirmed by MRCP. USG is also useful to document features of advanced liver disease like ascites, splenomegaly, dilated portal vein, and collaterals. Gall stones have been reported in TJP2 disease, as also in PFIC 2 and 3. The presence of hepatic SOL raises suspicion of HCC and needs evaluation by triple-phase CT and alpha-fetoprotein. Early HCC is a feature of TJP2 disease; Step 4: Liver histology including immunohistochemistry and next-generation sequencing (NGS). Liver biopsy shows canalicular cholestasis in all three

Table 3 *MYO5B* mutation clinical characteristics and outcome

Ref.		Age at onset of symptom	Age at initial evaluation	Symptoms	Treatment	Lab parameters			Outcome
						GGT (IU/L)	AST (IU/L)	ALT (IU/L)	
Qiu *et al* [20], 2017	*n* = 10, M-8, F-2, 4 had affected siblings	2 d-19 mo	1 mo-10 yr	Jaundice and pruritus; No diarrhea	UDCA, cholestyramine	9-99	24-255	41-432	Recurrent-3, persistent-2, transient cholestasis-2, lost to follow-3, listed for LT -1 (died)
Cockar *et al* [19], 2020	*n* = 6, M-3, F-3	-	6 mo-15 yr	Pruritus with pale stools-6, Jaundice-3; FTT-3; Diarrhea-2, (intractable and settled at 3 yr and 7 yr), gallstone-1	Antipruritic medications-6; PIBD-1; PIBD followed by PEBD-1; ENBD followed by PEBD-1	10-22	-	15-177	1-LT for poor QOL and pruritus; 5-Partial response with mild pruritus while on medications
Gonzales *et al*[8], 2017	*n* = 5, M-4, F-1	-	7-15 mo	Pruritus-5; Jaundice-5; Pale stools-5 hepatomegaly-5; Language delay-1 episodes of severe diarrhea before 3 yr of age-1	UDCA and rifampicin-5; PEBD-1	7-11	31-170	57-207	Followed till 3.5-13.5 yr of age; Fluctuating cholestasis-4; Cholestasis resolved after 1 mo of PEBD, well till 7 yr of age
Girard *et al* [17], 2014	*n* = 8/28 MVID, patients with cholestasis M-5, F-3	3-60 mo		Jaundice, pruritus, hepatomegaly-8; Pre Int Tx-5, post Int Tx-3	Antipruritic medications-8; PIBD followed by PEBD-1; PIBD-1; PEBD-1; Combined liver and Int Tx-1	8-42	51-124	52-121	Follow up till 2.8-14 yr of age, remission-6, partial remission-2; Removal of small bowel graft due to acute rejection in 2 cases improved cholestasis

AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; ENBD: Endonasal biliary drainage; F: Female; FTT: Failure to thrive; GGT: Gamma glutamyl transferase; Int Tx: Intestinal transplant; LT: Liver transplantation; M: Male; MVID: Microvillus inclusion disease; PEBD: Partial external biliary drainage; PIBD: Partial internal biliary drainage; QOL: Quality of life; UDCA: Ursodeoxycholic acid.

types (TJP2, NR1H4, and MYO5B defects) along with a variable degree of fibrosis and giant cell transformation[6]. On electron microscopy, the tight junctions appear elongated and lack the densest part of the zona occludens in PFIC 4[6]. In subjects with MYO5B and liver disease, electron microscopy will show dilatation of the bile canalicular lumen, canalicular thickening, and disappearance of the microvilli apart from cholestasis[17]. Inclusion bodies are not seen in the hepatocytes on transmission electron microscopy, in contrast to the findings in intestines in MVID[17]. The comparative features at histology in these three types are given in Table 4.

A complete panel of immunohistochemistry including BSEP, MDR3, TJP2, FXR, MYO5B, and Claudin1 can help in identifying the subtype of PFIC as shown in Table 4. However, simultaneous NGS for multiple genes (cholestasis panel) is a rapid and affordable way of confirming the molecular diagnosis[27]. A recent study has shown that a molecular genetic diagnosis can be made in a quarter of cases with neonatal cholestasis using NGS[28]. A study with a 66-gene cholestasis panel in 2171 cholestatic children and young adults, had a diagnostic yield of 12% and turnaround time of only 21 d[29]. The simultaneous testing for multiple genes helps in not only confirming the diagnosis but also in excluding other conditions. NGS is becoming the test of choice in the primary evaluation of patients with PFIC phenotype as it is non-invasive in comparison to liver biopsy and immunohistochemistry. For cases in which

Table 4 Comparison of clinical features, laboratory profile and outcome in progressive familial intrahepatic cholestasis 4, 5 and 6

	PFIC 4	PFIC 5	PFIC 6
Gene mutation	TJP2/Zona occludens-2 located in 9q21.11	NR1H4/FXR-located in 12q23.1	MYO5B located in 18q21.1
Clinical features			
Clinical features	Cholestatic jaundice with pruritus	Rapidly progressive neonatal-onset cholestasis with uncorrectable coagulopathy	Cholestasis with pruritus, with/without transient, recurrent or progressive diarrhea (association with MVID)
Extrahepatic features	Neurological and respiratory symptoms	-	-
ICP	Yes	Yes (uncommon)	No
Laboratory parameters			
AST/ALT	Elevated	Moderate elevation	Mild to moderate elevation
GGT	Normal or mild elevation	Normal	Normal
Coagulopathy	Late-onset	Early-onset	Late-onset
Alpha fetoprotein	Normal, elevated in cases with HCC	Elevated	Normal
S. Bile acids	Elevated	Elevated	Elevated
Histopathology			
Canalicular cholestasis	Yes	Yes	Yes
Portal/lobular fibrosis	Yes	Yes	Yes
Giant-cell transformation	Yes	Diffuse	Sparse
Ductular reaction	No	Yes	Yes
Hepatocyte necrosis	Yes	-	-
Cirrhosis	Yes	Yes	Less common
Immunohistochemistry			
BSEP	Present	Absent BSEP staining on bile canaliculus	Abnormally thick, irregular and granular positivity that overflows into subcanalicular area
MDR3	Present	Present	Thickened canalicular staining granular and patchy pattern overflows into subcanalicular area
TJP2	Absent expression in canalicular membrane	Present	Present
Claudin1	Absent or reduced staining on bile canaliculi	Present	Present
FXR	Normal	Absent staining on bile canaliculus	Normal
MYO5B/RAB11	Normal	Normal	Intense, granular staining pattern in hepatocyte cytoplasm, and weak/loss of canalicular expression
Progression	Rapid	Very rapid	Slow
Complications	Hepatocellular carcinoma	Post-transplant graft steatosis similar to PFIC1	Worsening of cholestasis post intestinal transplant
Treatment			
Medical management	UDCA, Rifampicin	Minimal role	UDCA, rifampin, cholestyramine
Biliary diversion	PEBD some role	Not tried	Cholestasis subsides after BD in MVID patients with cholestasis
Liver transplant	Yes	Yes	Yes. Combined liver intestinal transplant in children with MVID and ongoing cholestasis

ALT: Alanine aminotransferase; ASBT: Apical sodium-dependent bile acid transporter; AST: Aspartate aminotransferase; BD: Biliary diversion; BSEP: Bile salt export pump; FXR: Farnesoid X receptor; GGT: Gamma-glutamyl transferase; HCC: Hepatocellular carcinoma; MDR3: Multidrug resistance class 3 glyco-protein; ICP: Intrahepatic cholestasis of pregnancy; MVID: Microvillus inclusion disease; MYO5B: Myosin-5b; NBD: Nasobiliary drainage; PEBD: Partial external biliary drainage; PFIC1Progressive familial intrahepatic cholestasis-1; RAB11: RAS-related GTP-binding protein-11; TJP2: Tight junction protein-2; UDCA: Ursodeoxycholic acid.

the panel yields a negative result and the index of suspicion is high, further testing by the whole exome (WES) or whole-genome (WGS) sequencing may be done. The presence of variables of unknown significance and monoallelic pathogenic/likely pathogenic variants in a significant proportion of cases highlights the complexity of analysis and the need for expertise for proper interpretation. Also, the ongoing discovery of new genes requires expansion of the genetic testing panel from time to time.

DIFFERENTIAL DIAGNOSIS

The main differentials to be considered in a patient with intrahepatic cholestasis with low-normal GGT (< 100 U/L) include bile acid synthetic defect (BASD), arthrogryposis-renal dysfunction-cholestasis (ARC) syndrome, and USP53 related cholestasis, apart from the different types of PFIC (1, 2, 4, 5 and MYO5B associated). Type 3 PFIC (ABCB4) has raised GGT[1,30]. The serum bile acids are raised in PFIC and ARC syndrome, while they are low in the BASD. These entities can be differentiated by their distinct clinical presentation and liver histopathology with immunohistochemistry. However, the confirmation of diagnoses is best done by genetic analysis.

Bile acid synthetic defects

In bile acid synthetic defects (BASD) there is an accumulation of toxic bile acid intermediates in the hepatocytes due to deficiency of various enzymes involved in bile acid synthesis. Patients present with cholestatic jaundice, overt steatorrhea and florid manifestations of fat-soluble vitamin deficiencies like rickets. Pruritus is distinctly uncommon. Sometimes they may also present with neonatal liver failure, and cholestatic liver disease along with neurological manifestations like hypotonia, seizures[31]. BASD is diagnosed with fast-atom bombardment mass spectrometry of urine, which shows the accumulation of the distinct bile acid intermediaries due to a block in the bile acid synthesis pathway. Genetic analysis is confirmatory. Supplementation of cholic acid (CA) and chenodeoxycholic acid (CDCA) along with fat-soluble vitamins is the mainstay of therapy[32].

ARC syndrome

ARC syndrome (MIM 208085) is a rare multisystem disorder with autosomal recessive inheritance. It includes a triad of arthrogryposis, renal tubular acidosis, and neonatal cholestatic jaundice. Some patients may have accompanying features like ichthyosis (approximately 50%), platelet anomalies (approximately 25%), agenesis of the corpus callosum (> 20%), congenital cardiovascular anomalies (approximately 10%), and deafness. The clinical features are very useful to suspect the diagnosis, which is confirmed by a demonstration of mutations in the VPS33B or VIPAR gene. Histopathology shows bile duct paucity, giant cell transformation, bile plugs, and portal fibrosis. Caution is required before proceeding with a renal or liver biopsy due to the increased risk of a life-threatening bleed. Treatment is supportive and includes management of joint contractures, renal tubular acidosis, and cholestasis (UDCA, fat-soluble vitamins)[33].

USP53 related cholestasis

USP53 encodes an enzyme known as ubiquitin carboxyl-terminal hydrolase 53, which belongs to the de-ubiquitinating enzyme family and helps in maintaining cell integrity by interacting with TJP2 in hepatocytes. Whole-exome sequencing in 69 Han Chinese infants, with low GGT cholestasis without any pathological variants in ATP8B1, ABCB11, NR1H4, TJP2, and MYO5B genes, showed the presence of biallelic *USP53* mutations (homozygous or compound heterozygous) in 7 patients[34]. All these children had cholestatic jaundice in infancy and responded to medications (UDCA, cholestyramine). Liver biopsy showed varying levels of lobular disarray and hepatocellular and canalicular cholestasis, rosetting, portal tract fibrosis, ductular prolif-

eration, and giant-cell transformation. Ultrastructural examination in 2 cases revealed abnormality of tight junction complexes and expression of TJP2 and CLDN1 were reduced. Two children also had sensorineural hearing loss. In another report on the novel USP53 mutation, three members from the same family (2 sisters and a cousin) had low-GGT cholestasis, pruritus, elevated transaminases, very high alkaline phosphatase, and sensorineural hearing loss (n = 2). One of them required LT because of intractable pruritus[35].

Alagille syndrome

Alagille syndrome is also known as arteriohepatic dysplasia or syndromic paucity of interlobular bile ducts. This disorder is autosomal dominant with variable phenotypic penetrance. Alagille syndrome is one of the commonest causes of genetic cholestasis [36]. The defining feature is cholestasis with multisystemic involvement. Features include neonatal cholestasis in 95%, extrahepatic biliary hypoplasia, pruritus, xanthoma and associated facial dysmorphism. Structural cardiac defects such as peripheral pulmonary stenosis and septal defects are seen in 88%. Vertebral anomalies, ocular abnormalities most commonly posterior embryotoxon, renal dysplasia, vascular anomalies like Moyamoya disease, carotid and subclavian artery aneurysm are the other systemic features. Genetic analysis reveals *JAG1* mutation in the majority (approximately 90%) and *NOTCH2* mutation in minority[35].

Citrin deficiency

It is caused due to SLC25A13 (Solute Carrier family 25) gene mutation located in chromosome 7q21.3. The disease spectrum includes neonatal intrahepatic cholestasis, failure to thrive and dyslipidemia, and adult-onset type II citrullinemia. Chubby cheeks in infancy are a hallmark finding. These children also have a characteristic history of aversion to carbohydrates and a dietary preference towards a protein and lipid-rich diet[37].

Neonatal ichthyosis-sclerosing cholangitis syndrome

Neonatal Ichthyosis Sclerosing cholangitis is a rare cause of neonatal cholestasis with an autosomal recessive inheritance pattern. It is caused due to a mutation in the CLDN1 gene which encodes the CLDN1 protein located at the tight junction. This condition presents with neonatal cholestasis, cicatricial alopecia, ichthyosis and pruritus. Magnetic resonance cholangiopancreatography will show features of sclerosing cholangitis[38].

Other PFIC subtypes

Amongst the different PFIC subtypes, PFIC 1, 2, 4, 5 and 6 have low-normal GGT cholestasis. The presence of diarrhea is a feature of PFIC 1 and MYO5B disease. While neurological symptoms may be seen in ARC syndrome and sometimes in patients with MVID, a higher risk of HCC is a feature of TJP2 and BSEP deficiency. Table 4 gives the comparison of the clinical features and investigations in TJP2, FXR, and MYO5B defects. A detailed description and comparison of PFIC 1 and 2 are given elsewhere [39].

TREATMENT

Medical management

The main components include counselling of parents in detail, providing adequate nutrition, correcting vitamin deficiencies, controlling pruritus, managing complications like ascites, variceal bleeding *etc.*, growth monitoring, and vaccination[40].

Nutritional therapy: A diet that provides adequate calories (125%-140% of RDA) and protein (2-3 g/kg) with supplementation of medium-chain triglyceride and fat-soluble vitamins is recommended[41]. The doses of vitamin supplementation may need modification based on clinical signs and symptoms of vitamin deficiency and serum level monitoring (if available). Anemia, if present, needs to be corrected. Age-appropriate immunization including vaccination against hepatotropic viruses (hepatitis A and hepatitis B) is essential.

Management of pruritus: Pruritus is one of the most disabling symptoms in these children. Apart from skincare, medications such as UDCA, cholestyramine, rifampicin, naltrexone, and sertraline are used for controlling pruritus. These aspects have been

addressed in detail elsewhere[42]. There are no published reports on the use of FXR agonists like obeticholic acid, or apical sodium–bile acid transporter inhibitors like maralixibat in PFIC 4, 5 and MYO5B related diseases. Long-term follow-up includes growth monitoring, monitoring for nutritional deficiencies, and HCC surveillance, especially in TJP2 related cholestasis.

Biliary diversion

Biliary diversion (BD) takes away bile from the intestine, thereby reducing the reabsorption of bile acids through the enterohepatic circulation[43]. It has an important role in the alleviation of pruritus that is refractory to medical management in PFIC 1 and 2[44]. The role of BD is not well known in the newer variants of PFIC. BD has been tried in MVID patients who developed worsened cholestasis post intestinal transplant and was found to be helpful[17]. In MYO5B mutation, the ongoing cholestatic liver disease worsens after the intestinal transplant, leading to progressive liver fibrosis. Hence combined liver and intestinal transplantation are preferred. But in cases of isolated intestinal transplants, gallbladders should be preserved so that in case the cholestasis worsens, partial external biliary drainage can be attempted. The ileal bypass should be avoided as it removes a part of the transplanted bowel and doesn't result in long-term remission of cholestasis[16].

Liver transplant

LT is to be considered in children with decompensated chronic liver disease, growth failure (not amenable to dietary modification), refractory pruritus, or associated complications like hepato-pulmonary syndrome. In NR1H4 related PFIC, an early transplant may be required due to progressive liver disease with decompensation. Post liver transplant graft steatosis may develop in patients with NR1H4 mutation-associated cholestasis[6].

Genetic counseling

Once a child is confirmed to have PFIC, parents need to be counselled about the nature of the disease and the autosomal recessive pattern of inheritance. A geneticist should be involved in counselling about future pregnancies and testing during pregnancy[45].

CONCLUSION

TJP2, FXR, and MYO5B are recent additions to the three well-known types of PFIC (1, 2, and 3). This review has described the genetics, clinical profile, investigative findings, and treatments of these newer entities. There are gaps in our understanding of these conditions due to the limited literature at present. Advances in bioinformatics and techniques of next-generation gene-sequencing will help us study the genotype-phenotype correlation and synergistic effect of multiple mutations. Despite the recognition of these entities, not all cases with the PFIC phenotype have a confirmed genetic diagnosis, which indicates the presence of other causative genes that are waiting to be discovered.

REFERENCES

1 **Davit-Spraul A**, Gonzales E, Baussan C, Jacquemin E. Progressive familial intrahepatic cholestasis. *Orphanet J Rare Dis* 2009; **4**: 1 [PMID: 19133130 DOI: 10.1186/1750-1172-4-1]

2 **Hori T**, Nguyen JH, Uemoto S. Progressive familial intrahepatic cholestasis. *Hepatobiliary Pancreat Dis Int* 2010; **9**: 570-578 [PMID: 21134824]

3 **Jacquemin E**. Progressive familial intrahepatic cholestasis. *Clin Res Hepatol Gastroenterol* 2012; **36** Suppl 1: S26-S35 [PMID: 23141890 DOI: 10.1016/S2210-7401(12)70018-9]

4 **Baker A**, Kerkar N, Todorova L, Kamath BM, Houwen RHJ. Systematic review of progressive familial intrahepatic cholestasis. *Clin Res Hepatol Gastroenterol* 2019; **43**: 20-36 [PMID: 30236549 DOI: 10.1016/j.clinre.2018.07.010]

5 **Sambrotta M**, Strautnieks S, Papouli E, Rushton P, Clark BE, Parry DA, Logan CV, Newbury LJ, Kamath BM, Ling S, Grammatikopoulos T, Wagner BE, Magee JC, Sokol RJ, Mieli-Vergani G; University of Washington Center for Mendelian Genomics, Smith JD, Johnson CA, McClean P, Simpson MA, Knisely AS, Bull LN, Thompson RJ. Mutations in TJP2 cause progressive cholestatic liver disease. *Nat Genet* 2014; **46**: 326-328 [PMID: 24614073 DOI: 10.1038/ng.2918]

6 **Gomez-Ospina N**, Potter CJ, Xiao R, Manickam K, Kim MS, Kim KH, Shneider BL, Picarsic JL,

Jacobson TA, Zhang J, He W, Liu P, Knisely AS, Finegold MJ, Muzny DM, Boerwinkle E, Lupski JR, Plon SE, Gibbs RA, Eng CM, Yang Y, Washington GC, Porteus MH, Berquist WE, Kambham N, Singh RJ, Xia F, Enns GM, Moore DD. Mutations in the nuclear bile acid receptor FXR cause progressive familial intrahepatic cholestasis. *Nat Commun* 2016; **7**: 10713 [PMID: 26888176 DOI: 10.1038/ncomms10713]

7 **Himes RW**, Mojarrad M, Eslahi A, Finegold MJ, Maroofian R, Moore DD. NR1H4-related Progressive Familial Intrahepatic Cholestasis 5: Further Evidence for Rapidly Progressive Liver Failure. *J Pediatr Gastroenterol Nutr* 2020; **70**: e111-e113 [PMID: 32443034 DOI: 10.1097/MPG.0000000000002670]

8 **Gonzales E**, Taylor SA, Davit-Spraul A, Thébaut A, Thomassin N, Guettier C, Whitington PF, Jacquemin E. MYO5B mutations cause cholestasis with normal serum gamma-glutamyl transferase activity in children without microvillous inclusion disease. *Hepatology* 2017; **65**: 164-173 [PMID: 27532546 DOI: 10.1002/hep.28779]

9 **Gumbiner B**, Lowenkopf T, Apatira D. Identification of a 160-kDa polypeptide that binds to the tight junction protein ZO-1. *Proc Natl Acad Sci U S A* 1991; **88**: 3460-3464 [PMID: 2014265 DOI: 10.1073/pnas.88.8.3460]

10 **Furuse M**, Fujita K, Hiiragi T, Fujimoto K, Tsukita S. Claudin-1 and -2: novel integral membrane proteins localizing at tight junctions with no sequence similarity to occludin. *J Cell Biol* 1998; **141**: 1539-1550 [PMID: 9647647 DOI: 10.1083/jcb.141.7.1539]

11 **Sambrotta M**, Thompson RJ. Mutations in TJP2, encoding zona occludens 2, and liver disease. *Tissue Barriers* 2015; **3**: e1026537 [PMID: 26451340 DOI: 10.1080/21688370.2015.1026537]

12 **Forman BM**, Goode E, Chen J, Oro AE, Bradley DJ, Perlmann T, Noonan DJ, Burka LT, McMorris T, Lamph WW, Evans RM, Weinberger C. Identification of a nuclear receptor that is activated by farnesol metabolites. *Cell* 1995; **81**: 687-693 [PMID: 7774010 DOI: 10.1038/cr.2008.289]

13 **Trauner M**, Claudel T, Fickert P, Moustafa T, Wagner M. Bile acids as regulators of hepatic lipid and glucose metabolism. *Dig Dis* 2010; **28**: 220-224 [PMID: 20460915 DOI: 10.1159/000282091]

14 **Huang L**, Zhao A, Lew JL, Zhang T, Hrywna Y, Thompson JR, de Pedro N, Royo I, Blevins RA, Peláez F, Wright SD, Cui J. Farnesoid X receptor activates transcription of the phospholipid pump MDR3. *J Biol Chem* 2003; **278**: 51085-51090 [PMID: 14527955 DOI: 10.1074/jbc.M308321200]

15 **Dixon PH**, Sambrotta M, Chambers J, Taylor-Harris P, Syngelaki A, Nicolaides K, Knisely AS, Thompson RJ, Williamson C. An expanded role for heterozygous mutations of ABCB4, ABCB11, ATP8B1, ABCC2 and TJP2 in intrahepatic cholestasis of pregnancy. *Sci Rep* 2017; **7**: 11823 [PMID: 28924228 DOI: 10.1038/s41598-017-11626-x]

16 **Lapierre LA**, Kumar R, Hales CM, Navarre J, Bhartur SG, Burnette JO, Provance DW Jr, Mercer JA, Bähler M, Goldenring JR. Myosin vb is associated with plasma membrane recycling systems. *Mol Biol Cell* 2001; **12**: 1843-1857 [PMID: 11408590 DOI: 10.1091/mbc.12.6.1843]

17 **Girard M**, Lacaille F, Verkarre V, Mategot R, Feldmann G, Grodet A, Sauvat F, Irtan S, Davit-Spraul A, Jacquemin E, Ruemmele F, Rainteau D, Goulet O, Colomb V, Chardot C, Henrion-Caude A, Debray D. MYO5B and bile salt export pump contribute to cholestatic liver disorder in microvillous inclusion disease. *Hepatology* 2014; **60**: 301-310 [PMID: 24375397 DOI: 10.1002/hep.26974]

18 **Aldrian D**, Vogel GF, Frey TK, Ayyıldız Civan H, Aksu AÜ, Avitzur Y, Ramos Boluda E, Çakır M, Demir AM, Deppisch C, Duba HC, Düker G, Gerner P, Hertecant J, Hornová J, Kathemann S, Koeglmeier J, Koutroumpa A, Lanzersdorfer R, Lev-Tzion R, Lima R, Mansour S, Meissl M, Melek J, Miqdady M, Montoya JH, Posovszky C, Rachman Y, Siahanidou T, Tabbers M, Uhlig HH, Ünal S, Wirth S, Ruemmele FM, Hess MW, Huber LA, Müller T, Sturm E, Janecke AR. Congenital Diarrhea and Cholestatic Liver Disease: Phenotypic Spectrum Associated with MYO5B Mutations. *J Clin Med* 2021; **10** [PMID: 33525641 DOI: 10.3390/jcm10030481]

19 **Cockar I**, Foskett P, Strautnieks S, Clinch Y, Fustok J, Rahman O, Sutton H, Mtegha M, Fessatou S, Kontaki E, Papaevangelou V, Deheragoda M, Thompson RJ, Grammatikopoulos T. Mutations in Myosin 5B in Children With Early-onset Cholestasis. *J Pediatr Gastroenterol Nutr* 2020; **71**: 184-188 [PMID: 32304554 DOI: 10.1097/MPG.0000000000002740]

20 **Qiu YL**, Gong JY, Feng JY, Wang RX, Han J, Liu T, Lu Y, Li LT, Zhang MH, Sheps JA, Wang NL, Yan YY, Li JQ, Chen L, Borchers CH, Sipos B, Knisely AS, Ling V, Xing QH, Wang JS. Defects in myosin VB are associated with a spectrum of previously undiagnosed low γ-glutamyltransferase cholestasis. *Hepatology* 2017; **65**: 1655-1669 [PMID: 28027573 DOI: 10.1002/hep.29020]

21 **Carlton VE**, Harris BZ, Puffenberger EG, Batta AK, Knisely AS, Robinson DL, Strauss KA, Shneider BL, Lim WA, Salen G, Morton DH, Bull LN. Complex inheritance of familial hypercholanemia with associated mutations in TJP2 and BAAT. *Nat Genet* 2003; **34**: 91-96 [PMID: 12704386 DOI: 10.1038/ng1147]

22 **Zhang J**, Liu LL, Gong JY, Hao CZ, Qiu YL, Lu Y, Feng JY, Li JQ, Li ZD, Wang MX, Xing QH, Knisely AS, Wang JS. TJP2 hepatobiliary disorders: Novel variants and clinical diversity. *Hum Mutat* 2020; **41**: 502-511 [PMID: 31696999 DOI: 10.1002/humu.23947]

23 **Vij M**, Shanmugam NP, Reddy MS, Sankaranarayanan S, Rela M. Paediatric hepatocellular carcinoma in tight junction protein 2 (TJP2) deficiency. *Virchows Arch* 2017; **471**: 679-683 [PMID: 28733884 DOI: 10.1007/s00428-017-2204-1]

24 **Wei CS**, Becher N, Friis JB, Ott P, Vogel I, Grønbæk H. New tight junction protein 2 variant causing progressive familial intrahepatic cholestasis type 4 in adults: A case report. *World J Gastroenterol* 2020; **26**: 550-561 [PMID: 32089630 DOI: 10.3748/wjg.v26.i5.550]

25 **Kliewer SA**, Mangelsdorf DJ. Bile Acids as Hormones: The FXR-FGF15/19 Pathway. *Dig Dis* 2015; **33**: 327-331 [PMID: 26045265 DOI: 10.1159/000371670]

26 **Van Mil SW**, Milona A, Dixon PH, Mullenbach R, Geenes VL, Chambers J, Shevchuk V, Moore GE, Lammert F, Glantz AG, Mattsson LA, Whittaker J, Parker MG, White R, Williamson C. Functional variants of the central bile acid sensor FXR identified in intrahepatic cholestasis of pregnancy. *Gastroenterology* 2007; **133**: 507-516 [PMID: 17681172 DOI: 10.1053/j.gastro.2007.05.015]

27 **Chen HL**, Li HY, Wu JF, Wu SH, Chen HL, Yang YH, Hsu YH, Liou BY, Chang MH, Ni YH. Panel-Based Next-Generation Sequencing for the Diagnosis of Cholestatic Genetic Liver Diseases: Clinical Utility and Challenges. *J Pediatr* 2019; **205**: 153-159.e6 [PMID: 30366773 DOI: 10.1016/j.jpeds.2018.09.028]

28 **Togawa T**, Sugiura T, Ito K, Endo T, Aoyama K, Ohashi K, Negishi Y, Kudo T, Ito R, Kikuchi A, Arai-Ichinoi N, Kure S, Saitoh S. Molecular Genetic Dissection and Neonatal/Infantile Intrahepatic Cholestasis Using Targeted Next-Generation Sequencing. *J Pediatr* 2016; **171**: 171-7.e1 [PMID: 26858187 DOI: 10.1016/j.jpeds.2016.01.006]

29 **Feldman AG**, Sokol RJ. Neonatal cholestasis: emerging molecular diagnostics and potential novel therapeutics. *Nat Rev Gastroenterol Hepatol* 2019; **16**: 346-360 [PMID: 30903105 DOI: 10.1038/s41575-019-0132-z]

30 **de Vree JM**, Jacquemin E, Sturm E, Cresteil D, Bosma PJ, Aten J, Deleuze JF, Desrochers M, Burdelski M, Bernard O, Oude Elferink RP, Hadchouel M. Mutations in the MDR3 gene cause progressive familial intrahepatic cholestasis. *Proc Natl Acad Sci U S A* 1998; **95**: 282-287 [PMID: 9419367 DOI: 10.1073/pnas.95.1.282]

31 **Ravindranath A**, Sen Sarma M, Yachha SK. Bile acid synthetic defects: Simplified approach in a nutshell. *Hepatobiliary Pancreat Dis Int* 2020; **19**: 80-84 [PMID: 31558365 DOI: 10.1016/j.hbpd.2019.09.003]

32 **Gonzales E**, Matarazzo L, Franchi-Abella S, Dabadie A, Cohen J, Habes D, Hillaire S, Guettier C, Taburet AM, Myara A, Jacquemin E. Cholic acid for primary bile acid synthesis defects: a life-saving therapy allowing a favorable outcome in adulthood. *Orphanet J Rare Dis* 2018; **13**: 190 [PMID: 30373615 DOI: 10.1186/s13023-018-0920-5]

33 **Zhou Y**, Zhang J. Arthrogryposis-renal dysfunction-cholestasis (ARC) syndrome: from molecular genetics to clinical features. *Ital J Pediatr* 2014; **40**: 77 [PMID: 25239142 DOI: 10.1186/s13052-014-0077-3]

34 **Zhang J**, Yang Y, Gong JY, Li LT, Li JQ, Zhang MH, Lu Y, Xie XB, Hong YR, Yu Z, Knisely AS, Wang JS. Low-GGT intrahepatic cholestasis associated with biallelic USP53 variants: Clinical, histological and ultrastructural characterization. *Liver Int* 2020; **40**: 1142-1150 [PMID: 32124521 DOI: 10.1111/liv.14422]

35 **Maddirevula S**, Alhebbi H, Alqahtani A, Algoufi T, Alsaif HS, Ibrahim N, Abdulwahab F, Barr M, Alzaidan H, Almehaideb A, AlSasi O, Alhashem A, Hussaini HA, Wali S, Alkuraya FS. Identification of novel loci for pediatric cholestatic liver disease defined by KIF12, PPM1F, USP53, LSR, and WDR83OS pathogenic variants. *Genet Med* 2019; **21**: 1164-1172 [PMID: 30250217 DOI: 10.1038/s41436-018-0288-x]

36 **Mitchell E**, Gilbert M, Loomes KM. Alagille Syndrome. *Clin Liver Dis* 2018; **22**: 625-641 [PMID: 30266153 DOI: 10.1016/j.cld.2018.06.001]

37 **Hayasaka K**. Metabolic basis and treatment of citrin deficiency. *J Inherit Metab Dis* 2021; **44**: 110-117 [PMID: 32740958 DOI: 10.1002/jimd.12294]

38 **Nagtzaam IF**, van Geel M, Driessen A, Steijlen PM, van Steensel MA. Bile duct paucity is part of the neonatal ichthyosis-sclerosing cholangitis phenotype. *Br J Dermatol* 2010; **163**: 205-207 [PMID: 20645982 DOI: 10.1111/j.1365-2133.2010.09794.x]

39 **Srivastava A**. Progressive familial intrahepatic cholestasis. *J Clin Exp Hepatol* 2014; **4**: 25-36 [PMID: 25755532 DOI: 10.1016/j.jceh.2013.10.005]

40 **Jagadisan B**, Srivastava A. Child with Jaundice and Pruritus: How to Evaluate? *Indian J Pediatr* 2016; **83**: 1311-1320 [PMID: 26932879 DOI: 10.1007/s12098-016-2058-6]

41 **Lane E**, Murray KF. Neonatal Cholestasis. *Pediatr Clin North Am* 2017; **64**: 621-639 [PMID: 28502442 DOI: 10.1016/j.pcl.2017.01.006]

42 **Thébaut A**, Debray D, Gonzales E. An update on the physiopathology and therapeutic management of cholestatic pruritus in children. *Clin Res Hepatol Gastroenterol* 2018; **42**: 103-109 [PMID: 29031874 DOI: 10.1016/j.clinre.2017.08.007]

43 **Lemoine C**, Superina R. Surgical diversion of enterohepatic circulation in pediatric cholestasis. *Semin Pediatr Surg* 2020; **29**: 150946 [PMID: 32861450 DOI: 10.1016/j.sempedsurg.2020.150946]

44 **Verkade HJ**, Thompson RJ, Arnell H, Fischler B, Gillberg PG, Mattsson JP, Torfgård K, Lindström E. Systematic Review and Meta-analysis: Partial External Biliary Diversion in Progressive Familial Intrahepatic Cholestasis. *J Pediatr Gastroenterol Nutr* 2020; **71**: 176-183 [PMID: 32433433 DOI: 10.1097/MPG.0000000000002789]

45 **Fonda Allen J**, Stoll K, Bernhardt BA. Pre- and post-test genetic counseling for chromosomal and Mendelian disorders. *Semin Perinatol* 2016; **40**: 44-55 [PMID: 26718445 DOI: 10.1053/j.semperi.2015.11.007]

46 **Ge T**, Zhang X, Xiao Y, Wang Y, Zhang T. Novel compound heterozygote mutations of TJP2 in a Chinese child with progressive cholestatic liver disease. *BMC Med Genet* 2019; **20**: 18 [PMID: 30658709 DOI: 10.1186/s12881-019-0753-7]

47 **Mirza N**, Bharadwaj R, Malhotra S, Sibal A. Progressive familial intrahepatic cholestasis type 4 in an Indian child: presentation, initial course and novel compound heterozygous mutation. *BMJ Case Rep* 2020; **13** [PMID: 32636225 DOI: 10.1136/bcr-2019-234193]

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MINIREVIEWS

Deep learning in hepatocellular carcinoma: Current status and future perspectives

Joseph C Ahn, Touseef Ahmad Qureshi, Amit G Singal, Debiao Li, Ju-Dong Yang

ORCID number: Joseph C Ahn 0000-0001-6994-2870; Touseef Ahmad Qureshi 0000-0002-6683-4556; Amit G Singal 0000-0002-1172-3971; Debiao Li 0000-0001-8560-8231; Ju-Dong Yang 0000-0001-7834-9825.

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Country/Territory of origin: United States

Specialty type: Gastroenterology and hepatology

Joseph C Ahn, Gastroenterology and Hepatology, Mayo Clinic, Rochester, MN 55904, United States

Touseef Ahmad Qureshi, Debiao Li, Biomedical Imaging Research Institute, Cedars-Sinai Medical Center, Los Angeles, CA 90048, United States

Amit G Singal, Internal Medicine, University of Texas Southwestern Medical Center, Dallas, TX 75390, United States

Ju-Dong Yang, Karsh Division of Gastroenterology and Hepatology, Cedars-Sinai Medical Center, Los Angeles, CA 90048, United States

Corresponding author: Ju-Dong Yang, MD, MS, Assistant Professor, Karsh Division of Gastroenterology and Hepatology, Cedars-Sinai Medical Center, 8900 Beverly Blvd, Los Angeles, CA 90048, United States. judong.yang@cshs.org

Abstract

Hepatocellular carcinoma (HCC) is among the leading causes of cancer incidence and death. Despite decades of research and development of new treatment options, the overall outcomes of patients with HCC continue to remain poor. There are areas of unmet need in risk prediction, early diagnosis, accurate prognostication, and individualized treatments for patients with HCC. Recent years have seen an explosive growth in the application of artificial intelligence (AI) technology in medical research, with the field of HCC being no exception. Among the various AI-based machine learning algorithms, deep learning algorithms are considered state-of-the-art techniques for handling and processing complex multimodal data ranging from routine clinical variables to high-resolution medical images. This article will provide a comprehensive review of the recently published studies that have applied deep learning for risk prediction, diagnosis, prognostication, and treatment planning for patients with HCC.

Key Words: Hepatocellular carcinoma; Artificial intelligence; Deep learning

Core Tip: There are emerging roles for deep learning technology in the field of hepato-

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cellular carcinoma (HCC) including HCC risk prediction, as well as diagnosis, prognostication, and treatment planning leveraging readily available data from radiologic and histopathologic medical images. This article will provide a comprehensive review of the recently published studies that have applied deep learning for risk prediction, diagnosis, prognostication, and treatment planning for patients with HCC.

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INTRODUCTION

Hepatocellular carcinoma (HCC) is an aggressive primary liver cancer that develops in the setting of chronic parenchymal liver diseases, and is among the top causes of cancer incidence and mortality worldwide[1,2]. While the burden of HCC has been declining with effective antiviral therapy against hepatitis B virus (HBV) and hepatitis C virus (HCV), HCC incidence related to metabolic syndrome will likely continue to rise due to the dramatic increase in the prevalence of non-alcoholic fatty liver disease (NAFLD) in the general population[3]. Decades of HCC research led to the development of a screening protocol, non-invasive diagnostic modalities based on imaging, and various treatment modalities including surgical, locoregional and systemic therapies[4,5]. However, the overall outcomes of patients with HCC continue to remain poor and there are areas of significant unmet need in risk prediction, early detection, accurate prognostication, and individualized treatments for patients with HCC.

Patients with HCC generate enormous amounts of health data. While promising for researchers, ensuring that such high volumes of data are turned into actionable knowledge can be a significant challenge. Artificial intelligence (AI) is thought to be capable of synthesizing and analyzing multimodal data with superhuman degrees of accuracy or reliability, and recent years have seen a rapid growth in the application of AI to many fields of medicine including hepatology[6]. This "AI revolution" over the past decade has been possible due to the advent of deep learning technology. Deep learning algorithms can process a broad spectrum of medical data from structured numeric data such as vital signs and laboratory values, high dimensional data from multi-omics studies, as well as digitized high-resolution images from various radiologic and histopathologic studies. This review aims to provide an overview as well as highlight examples of the many potential applications of deep learning to improve the care of patients with HCC.

AI, MACHINE LEARNING, AND DEEP LEARNING

AI-based approaches provide a variety of methods for a range of tasks and clinical application including image classification, organ and lesion segmentation, accurate extraction of key imaging features and measurements, tumor detection, stratification of high-risk subjects, prediction of disease and treatment outcome (Figure 1). Advancements in AI in recent years, particularly in the realm of medical image processing and analysis, offer an enormous range of automated tools for extracting precise measurements of biomarkers, revealing complex features, quantifying tissue characteristics and performing radiomics for deep analysis of raw imaging data.

The term "artificial intelligence" encompasses a broad range of technology that enables machines to perform tasks typically thought to require human reasoning and problem-solving skills[7]. "Machine learning" is a branch of AI in which computer algorithms train on sample data to build a mathematical model that makes predictions or decisions without being explicitly programmed to do so[8]. Machine learning algorithms can be broadly divided into supervised and unsupervised learning. Supervised learning algorithms train on sample data with labeled outcome data, and their goal is to learn the relationship between the input data and the outcomes to make

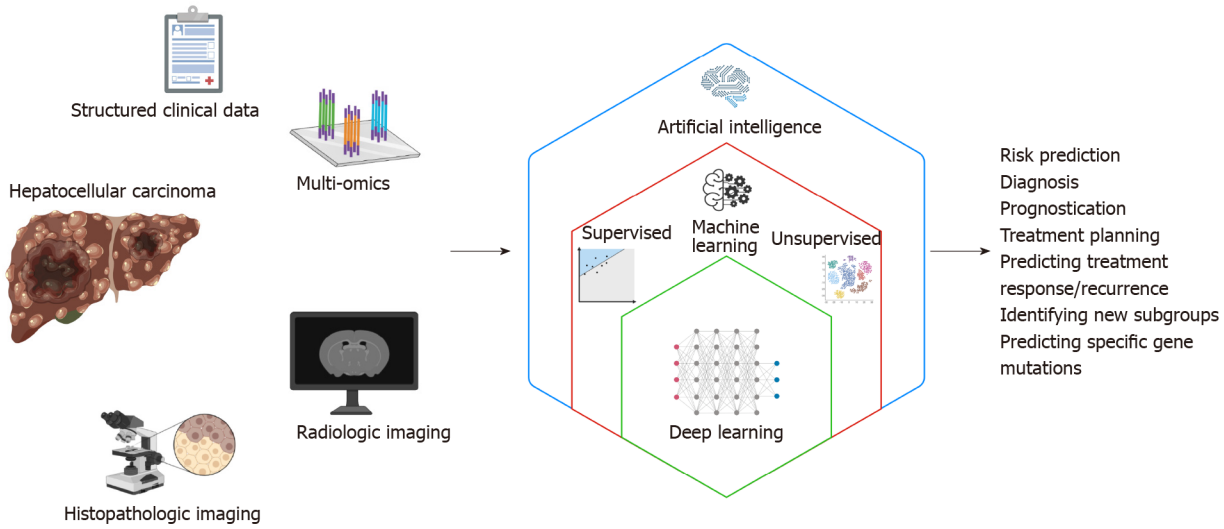

Figure 1 Schematic representation of the relationships between the terms artificial intelligence, machine learning, and deep learning, and how deep learning can utilize multimodal data to improve care for patients with hepatocellular carcinoma.

accurate predictions about the outcome when provided with a new set of input data [9]. Examples of supervised learning algorithms include traditional techniques such as linear regression and logistic regression, as well as more sophisticated techniques including support vector machines, random forest and gradient boosting. On the other hand, unsupervised learning algorithms train on unlabeled sample data and analyze the underlying structure or distribution within the data to discover new clusters or patterns[10]. Examples of unsupervised learning algorithms include K-means and principle component analysis among many others.

Among the various AI-based machine learning algorithms, artificial neural networks (ANNs) consist of layers of interconnected mathematical formulas that enable them to analyze complex non-linear relationships[11]. "Deep learning (DL)" refers to highly complex AI models utilizing multiple layers of ANNs and has recently emerged as a state-of-the-art AI technique for analyzing complex, high-dimensional healthcare data. Some of the commonly used DL techniques include convolutional neural networks (CNNs) and recurrent neural networks (RNNs)[12]. CNNs have connective patterns resembling those of an animal visual cortex and can detect inherent spatial features of high dimensional images. RNNs have connections forming a directed graph along a temporal sequence, and therefore can be highly useful in time series prediction.

It is crucial to recognize that any AI-based machine learning algorithms require external validation in an independent dataset as models could be overfitted and end up overestimating the performance. In this review article, the performance characteristics of the various DL models are from the validation cohorts, and not the original derivation cohorts used to train the algorithms.

HCC CLINICAL DATA

Despite multiple available risk prediction tools for HCC, none have been rigorously validated or endorsed by major liver societies. Currently, HCC surveillance is recommended for patients with cirrhosis and high risk patients with chronic HBV infection [13]. Accurate prediction models utilizing more specific risk factors for HCC development at individual levels would allow health systems to implement targeted screening strategies. Ioannou *et al*[14] trained a RNN to predict HCC development within 3 years using 4 baseline variables and 27 longitudinal variables from 48151 patients with HCV-related cirrhosis in the national Veterans Health Administration. The RNN model significantly outperformed logistic regression and exhibited an area under the curve (AUC) of 0.759 among all samples and an AUC of 0.806 among patients with sustained virologic response. Phan *et al*[15] surveyed 1 million random samples from Taiwan's National Health Insurance Research Database between 2002 to 2010 to predict liver cancer among patients with viral hepatitis. The disease history of

each patient was transformed into a 108 × 998 matrix and applied to a CNN, which predicted liver cancer with an AUC of 0.886 and an accuracy of 0.980. Another study by Nam *et al*[16] constructed a deep neural network to predict 3-year and 5-year incidence of HCC in 424 patients with HBV-related cirrhosis on entecavir therapy. When applied to an external validation cohort of 316 patients, the DL model achieved a Harrell's C-index of 0.782 and significantly outperformed 6 previously reported models based on traditional modeling. The same group also developed another DL model called the AI-based Model of Recurrence after Liver Transplantation (MoRAL-AI) to predict HCC recurrence after liver transplantation using variables such as tumor diameter, age, alpha-fetoprotein (AFP), and prothrombin time[17]. The MoRAL-AI showed significantly better predictive performance compared to conventional models such as the Milan, UCSF, up-to-seven, and Kyoto criteria (C-index = 0.75 *vs* 0.64, 0.62, 0.50, 0.50, respectively; $P < 0.001$).

HCC MULTI-OMICS

Serum AFP has been widely used as a predictive and prognostic biomarker for HCC [18], but AFP has limited sensitivity for detecting early-stage HCC and its levels do not reliably correlate with disease progression[19]. Recent advances in multi-omics related to HCC are expected to address this unmet need for novel biomarkers. Multi-omics refers to an approach to biological analysis which utilizes data sets from multiple "omics", such as the genome, epigenome, transcriptome, proteome, metabolome and microbiome. Multi-omics experiments generate an enormous amount of information, and various machine learning techniques including DL that can help with the computational challenges of processing and analyzing such high dimensional data. Xie *et al*[20] used gene expression profiling of peripheral blood to build an ANN model that classifies HCC patients from a control group. Using a nine-gene expression system, the ANN was able to distinguish HCC patients from controls with an AUC of 0.943, 98% sensitivity, and 85% specificity, although it should be noted that the control group was healthy individuals rather than patients with cirrhosis, which could have overestimated the performance of the model. Choi *et al*[21] proposed a novel network-based DL method to identify prognostic gene signatures *via* G2Vec, a modified Word2Vec model originally used for natural language processing (NLP). When applied to gene expression data for HCC from the Cancer Genome Atlas (TCGA), G2Vec showed superior prediction accuracy for patient outcomes compared to existing gene selection methods and was able to identify two distinct gene modules significantly associated with HCC prognosis. Chaudhary *et al*[22] used RNA se×quencing, miRNA, and methylation data of 360 HCC patients from TCGA to build an autoencoder, which is an unsupervised feed-forward neural network. Using this DL model, they were able to distinguish patients with survival differences and identify specific mutations and pathways as predictors of aggressive tumor behavior.

RADIOLOGY

HCC diagnosis and segmentation

In recent years, there have been remarkable advances in the application of AI for the interpretation of medical imaging, primarily due to the use of DL algorithms using CNN[23]. CNN algorithms trained on ultrasound, computed tomography (CT), or magnetic resonance imaging (MRI) images have shown excellent performances in detection of lesions, classification of lesions, segmentation of organs or anatomic structures, and imaging reconstruction[24].

In 2012, Streba *et al*[25] prospectively studied contrast-enhanced ultrasound images of 112 patients to train an ANN that classified five different types of liver tumors. The ANN showed promising performances with accuracies of 94.5% in the training set and 87.1% in the testing set. In 2017, Hassan *et al*[26] reported using the stacked sparse auto-encoder, an unsupervised DL technique, to segment and classify liver lesions on ultrasound images with a classification accuracy of 97.2%. Additionally, Bharti *et al*[27] built a CNN using echotexture and roughness of liver surface on 754 segmented ultrasound images, which differentiated between normal liver, chronic liver disease, cirrhosis, and HCC with a classification accuracy of 96.6%. Schmauch *et al*[28] also created a CNN which detects and characterizes benign and malignant focal liver lesions on 2-D ultrasound images from 367 patients from various institutions. When

applied to a new dataset of 177 patients, the model achieved a weighted mean AUC of 0.891. Recently, Brehar *et al*[29] conducted a study comparing CNN's performance for HCC detection on ultrasound images against conventional machine learning algorithms including multi-layer perceptron, support vector machines, random forest and AdaBoost. The CNN achieved an AUC of 0.95% with 91.0% accuracy, 94.4% sensitivity, and 88.4% specificity and significantly outperformed the conventional machine learning algorithms. Beyond detecting the actual presence of HCC on ultrasound images, studies have also attempted to predict the risk of future HCC development based on analyzing the ultrasound images of liver parenchyma in patients without HCC. For example, Jin *et al*[30] performed a DL radiomics analysis on 2-D shear wave elastography and corresponding B-mode ultrasound images of 434 chronic HBV patients, which predicted 5-year HCC development with AUC of 0.900 in the test cohort.

In addition to ultrasound images, cross-sectional imaging from CT or MRI studies serve as an extremely abundant and promising source of data for DL. In 2018, Yasaka *et al*[31] used CT image sets of liver masses from 460 patients to train a CNN that can classify liver lesions into five categories of: (1) HCC; (2) Other malignant tumors; (3) Indeterminate masses; (4) Hemangiomas; and (5) Cysts with a median AUC of 0.92. Shi *et al*[32] showed that incorporation of a CNN enabled identification of HCC using a three-phase CT imaging protocol with a diagnostic accuracy similar to that of a four-phase protocol, which would allow patients to receive lower doses of radiation. Segmentation of HCC, liver parenchyma, and other organs on CT scan is very important for determination of tumor extent and treatment planning, but manual contouring of the images is highly time-consuming and subject to inter-observer variability. The 2017 International Conference On Medical Image Computing Computer Assisted Intervention called for a Liver Tumor Segmentation Benchmark (LITS) challenge, encouraging researchers to develop automatic segmentation algorithms to segment liver lesions using 200 CT scans (training: 130; testing: 70) provided by clinical sites around the world. Several teams participating in the challenge have developed DL algorithms with promising performances for HCC segmentation using CT images[33-37]. Beyond the LITS challenge, there are ongoing research efforts to improve segmentation using different architectures of DL networks [38-42].

Hamm *et al*[43] used MRI images from 494 patients to train a CNN which can classify hepatic lesions into six different categories. When applied to random cases in the test set, the CNN outperformed expert radiologists (90% sensitivity and 98% specificity *vs* 82.5% sensitivity and 96.5% specificity) and especially for HCC detection (90% sensitivity *vs* 60%-70% sensitivity). The same group conducted additional studies to make their CNN interpretable by generating highlighted feature maps corresponding to liver lesions[44]. Wu *et al*[45] built a CNN using multiphase MRI images and achieved an AUC of 0.95 for distinguishing Liver Imaging Reporting and Data System (LI-RADS) grade 3 from LI-RADS 4 and 5 lesions for HCC diagnosis. Zhen *et al* [46] also trained a CNN model combining unenhanced MRI images and clinical variables from 1210 patients with liver tumors, which demonstrated diagnostic performances on par with three experienced radiologists using enhanced MRI images.

HCC prognostication, treatment planning, and response to treatment

In addition to serving as accurate and efficient tools for diagnosis of HCC, DL models utilizing radiology data can also be used for prognostication, treatment planning, and assessing tumor response to therapy. Vascular invasion is a key prognostic element in patients with HCC. Recent studies developed CNN models with promising ability to detect microvascular invasion on MRI images of HCC patients undergoing surgical resection[47-49]. An *et al*[50] used an unsupervised CNN-based deformable image registration technique to assess the relationship between ablative margins and local tumor progression in 141 patients with single HCC who underwent microwave ablation, and demonstrated that patients with ablative margins < 5 mm were at significantly higher risk of local tumor progression. Liu *et al*[51] developed a DL radiomics model to predict responses to trans-arterial chemoembolization (TACE) using ultrasound images of 130 HCC patients, which accurately predicted TACE response with an AUC of 0.93. The same group also assessed their ultrasound-based DL radiomics model to predict 2-year progression-free survival among 419 HCC patients and facilitate optimized treatment selection. Peng *et al*[52] trained a residual CNN model to predict response to TACE using CT images from 562 patients with intermediate-stage HCC undergoing TACE, which showed accuracies of 85.1% and 82.8% in two external validation cohorts. Another study developed a DL score for disease-specific survival by using CT images in a cohort of 243 patients with HCC

treated with TACE, with a higher score predicting poor prognosis [hazard ratio (HR): 3.01; 95% cumulative incidence (CI): 2.02-4.50][53]. Finally, Zhang *et al*[54] built a DL-based model predicting overall survival using CT images from 201 patients with unresectable HCC treated with TACE and sorafenib, which achieved superior predictive performance compared to the clinical nomogram (C-index of 0.730 *vs* 0.679, $P = 0.023$).

HCC PATHOLOGY

Automated interpretation of histopathologic images from liver biopsy is another major area of medical imaging in patients with HCC where DL can be utilized. In addition to effectively replicating the human pathologists' jobs of diagnosing and grading HCC, DL models can help identify and analyze additional complex imaging features and patterns which are related to specific mutations and disease prognosis. Lin *et al*[55] used images from multiphoton microscopy of 113 HCC patients to train a CNN with over 90% accuracy for determining HCC differentiation. Kiani *et al*[56] developed a CNN-based "Liver Cancer Assistant" which accurately differentiated hematoxylin and eosin (H&E) images of HCC and cholangiocarcinoma and helped improve the diagnostic performance of nine pathologists. Liao *et al*[57] used TCGA dataset for training a CNN that distinguished HCC from adjacent normal tissues with perfect performance (AUC: 1.00) and predicted the presence of specific somatic mutations with AUCs over 0.70. Wang *et al*[58] trained a CNN for automated segmentation and classification of individual nuclei at single-cell levels on H&E-stained tissue sections of HCC tumors from TCGA, and performed feature extraction to identify 246 quantitative image features. Then, a clustering analysis by an unsupervised learning approach identified three distinct histologic subtypes which were independent of previously established genomic clusters and had different prognosis. Chen *et al*[59] trained a CNN for automatic grading of HCC tumors on histopathological H&E images, which showed 96% accuracy for benign and malignant classification and 89.6% accuracy for the degree of tumor differentiation, and predicted the presence of specific genetic mutations.

Lu *et al*[60] applied three pre-trained CNN models to extract imaging features from HCC histopathology and performed Cox proportional hazards analysis to predict overall survival and disease-free survival, and observed significant correlations between the imaging features and established biological pathways. Saillard *et al*[61] used two DL algorithms based on whole-side digitized histological slides from 194 patients with HCC to predict the survival of patients treated by surgical resection. When tested on an independent validation set from TCGA, both DL models had a higher discriminatory power than a score combining all baseline variables associated with survival. Shi *et al*[62] built an interpretable DL framework using pathologic images from 1445 patients with HCC and developed a "tumor risk score" which showed prognostic performances independent of and superior to clinical staging systems and stratified patients into five groups of different prognosis. A recent study by Yamashita *et al*[63] developed a histopathology-based DL based system which stratified patients with risk scores for postsurgical recurrence of HCC.

FUTURE DIRECTION

There are several key issues to address before DL-based AI models can be universally implemented in real world clinical practice settings. Due to their complexity, DL models are traditionally considered to be "black-box" models, meaning humans cannot understand how the DL models make their predictions. Interpretability of the DL models are crucial for physicians to accept and trust them in everyday clinical practice, and for troubleshooting and improving the models for rare cases. This is being addressed by recent developments in various "explainable AI" techniques but currently there is no clear consensus on the best methodology. Another potential limitation is the generalizability of the individual DL algorithms. Concerns have been raised that AI algorithms developed at highly specialized academic medical centers using their own patients' data may over-represent certain groups of patients and not accurately reflect the real-world population of patients seen at local community hospitals. Finally, AI models, like other prediction models, are often not publicly available, limiting external validation. Independent validation of the proposed model and comparison to old models are as important as deriving new models. Large-scale,

Table 1 Studies applying deep learning for hepatocellular carcinoma

Study	Cohort	Data source	Deep learning	Input	Output	Main findings
Predicting HCC risk using clinical variables						
Ioannou *et al*[14] 2020	48151 HCV cirrhosis (T: 90%, V: 10%)	VHA database	RNN	Clinical variables	Risk of HCC development	RNN predicted HCC development with AUC of 0.759, and AUC of 0.806 among those who achieved SVR
Phan *et al*[15] 2020	6052 HBV and HCV (T: 70%, V: 30%)	Taiwanese NHIRD	CNN	Disease history data	Risk of HCC development	CNN achieved an accuracy of 0.980 and AUC of 0.886 for predicting HCC development among viral hepatitis patients
Nam *et al*[16] 2020	T: 424 HBV cirrhosis; V: 316 HBV cirrhosis	2 Korean centers	ResNet	Clinical variables	Risk of HCC development	DL model achieved an accuracy of 0.763 and AUC of 0.782 in the validation cohort and outperformed previous models
Nam *et al*[17] 2020	T: 349 LT recipients; V: 214 LT recipients	3 Korean LT centers	ResNet	Clinical variables	Recurrent HCC after LT	DL model significantly outperformed conventional models in prediction of post-T HCC recurrence with AUC of 0.75
Multi-omics-based HCC diagnosis and prognostication						
Xie *et al*[20] 2018	T: 133 HCC/54 HV; V: 52 HCC/34 HV	1 center in China	ANN	Gene expression	HCC detection	ANN using nine genes had an AUC of 0.943, 98% sensitivity, and 85% specificity for classifying HCC
Choi *et al*[21] 2018	135 HCC (10-fold CV)	TCGA	G2Vec	Gene expression	HCC prognosis	G2Vec showed significantly higher prediction accuracy for patient outcomes compared to existing gene selection tools
Chaudhary *et al* [22] 2018	T: 360 HCC; V: 220, 221, 166, 40, 27 HCC	TCGA; 5 external datasets	Auto-encoder	RNA-seq, miRNA-seq, methylation	HCC prognosis	DL model distinguished groups with survival differences and identified mutations and pathways predicting aggressive tumor behavior
Radiology-based HCC diagnosis/prediction						
Streba *et al*[25] 2012	112 FLL (10-fold CV)	1 center in Romania	ANN	US images	FLL type	ANN had 87.12% testing accuracy, 93.2% sensitivity, and 89.7% specificity for classifying 5 classes of liver lesions
Hassan *et al*[26] 2017	110 FLL (10-fold CV)	1 center in Egypt	Auto-encoder	US images	FLL type	The proposed system had 97.2% accuracy, 98% sensitivity, and 95.70% specificity for classifying liver lesions
Bharti *et al*[27] 2018	24 normal, 25 CLD, 25 cirrhosis, 20 HCC	1 center in India	CNN	US images	Liver stages	CNN achieved 96.6% classification accuracy for differentiating normal liver, CLD, cirrhosis, and HCC
Schmauch *et al* [28] 2019	T: 367 FLL; V: 177 FLL	Centers in France	ResNet	US images	FLL type	DL model reached mean AUC of 0.935 for focal liver lesion detection and 0.916 for focal liver lesion characterization
Brehar *et al*[29] 2020	T: 200 HCC; V: 68 HCC	1 center in Romania	CNN	US images	HCC detection	CNN achieved AUC of 0.95, accuracy of 0.91, 94.4% sensitivity and 88.4% specificity for HCC detection
Jin *et al*[30] 2021	434 HBV (3:1:1 split)	1 center in China	DL radiomics	US images	Risk of HCC development	DL radiomics model predicted 5-yr HCC development risk with AUC of 0.900 in the test set
Yasaka *et al*[31] 2018	T: 460 liver masses; V: 100 liver masses	1 center in Japan	CNN	CT images	Liver mass type	CNN classified liver lesions into five categories with a median AUC of 0.92
Shi *et al*[32] 2020	449 FLL; (T: 80%, V: 20%)	1 center in China	CNN	CT images	FLL type	CNN applied to three-phase CT protocol images achieved AUC of 0.925 for differentiating HCC

						from other FLLs
Hamm *et al*[43] 2019	T: 434 FLL; V: 60 FLL	1 center in United States	CNN	MRI images	FLL type	CNN achieved 90% sensitivity and 98% specificity for classifying FLLs and AUC of 0.992 for HCC classification
Wang *et al*[44] 2019	T: 434 FLL; V: 60 FLL	1 center in United States	CNN	MRI images	FLL type	Interpretable DL system achieved 76.5% PPV and 82.9% sensitivity for identifying correct radiological features
Wu *et al*[45] 2020	89 liver tumors; (60: 20: 20)	1 center in United States	CNN	MRI images	LI-RADS grading	CNN achieved AUC of 0.95, 90% accuracy, 100% sensitivity and 83.5% PPV for LI-RADS grading of liver tumors
Zhen *et al*[46] 2020	T: 1210 liver tumors; V: 201 liver tumors	1 center in China	CNN	MRI images	Liver tumor type	CNN combined with clinical data showed AUC of 0.985 for classifying HCC with 91.9% agreement with pathology
Radiology-based HCC prognostication, treatment planning, and response to treatment						
Zhang *et al*[47] 2021	T: 158 HCC; V: 79 HCC	1 center in China	CNN	MRI images	MVI in HCC	CNN achieved AUC of 0.72, 55% sensitivity, and 81% specificity for preoperative MVI in HCC patients
Wang *et al*[48] 2020	T: 60 HCC; V: 40 HCC	1 center in China	CNN	MRI images	MVI in HCC	Fusion of deep features from MRI images yielded AUC of 0.79 for MVI prediction in HCC patients
Jiang *et al*[49] 2021	405 HCC; (T: 80%, V: 20%)	1 center in China	CNN	CT images	MVI in HCC	CNN achieved AUC of 0.906 for prediction of MVI. Mean survival was significantly better in the group without MVI
An *et al*[50] 2020	141 single HCC resect MWA	1 center in China	CNN	MRI images	Ablative margin	Deep learning model accurately estimated ablative margins and risk of local tumor progression
Liu *et al*[51] 2020	T: 89 HCC resect TACE; V: 41 HCC rec. TACE	1 center in China	CNN	Ultrasound images	Response to TACE	Deep learning radiomics model predicted tumor response to TACE with AUC of 0.93
Peng *et al*[52] 2020	T: 562 HCC resect TACE; V:227 HCC rec. TACE	3 centers in China	CNN	CT images	Response to TACE	Deep learning model had accuracies of 85.1% and 82.8% for predicting TACE response in 2 validation cohorts
Liu *et al*[53] 2020	243 HCC resect TACE (6:1:3 split)	1 center in China	CNN	CT images	Post-TACE survival	Higher DL score was an independent prognostic factor and predicted overall survival with AUCs of 0.85-0.90
Zhang *et al*[54] 2020	201 HCC resect TACE + sorafenib (T: 120, V: 81)	3 centers in China	CNN	CT images	OS on TACE + sorafenib	Deep learning signature achieved C-index of 0.714 for predicting OS in HCC patients receiving TACE + sorafenib
Histopathology-based HCC diagnosis, subtyping, and outcome predictions						
Lin *et al*[55]2019	113 HCC	1 center in China	CNN	Histopath images	HCC differentiation	CNN achieved an accuracy of 0.941 for determining HCC differentiation on multiphoton microscopy
Kiani *et al*[56] 2020	70 WSI (35 HCC, 35 CC)	TCGA	CNN	Histopath images	HCC *vs* CC	CNN-based "Liver Cancer Assistant" accurately differentiated HCC *vs* cholangiocarcinoma
Liao *et al*[57] 2020	T: 491 HCC; V: 455 HCC	TCGA; 1 center in China	CNN	Histopath images	HCC detection, mutations	CNN distinguished HCC from adjacent tissues with AUC of 1.00 and predicted specific mutations with AUC over 0.70
Wang *et al*[58] 2020	T: 99 HCC; V: 205 HCC	TCGA	CNN	Histopath images	Histological HCC subtype	Unsupervised clustering identified 3 histological subtypes complementing molecular pathways and

						prognostic value
Chen *et al*[59] 2020	T: 402 HCC/89 normal; V: 67 HCC/34 normal	GDC portal; 1 center in China	CNN	Histopath images	HCC grade mutations	CNN achieved 89.6% accuracy for tumor differentiation stage and predicted presence of specific gene mutations
Lu *et al*[60] 2020	421 HCC/105 normal (6-fold CV)	GDC portal	CNN	Histopath images	HCC prognosis	Pre-trained CNN predicted OS using pathology images and identified HCC subgroups with different prognosis
Saillard *et al*[61] 2020	T: 194 HCC; V: 328 HCC	1 French center TCGA	CNN	Histopath images	Survival after HCC resection	CNN models using pathology images predicted survival with C-index 0.75-0.78 and outperformed conventional models
Shi *et al*[62] 2021	T: 1125 HCC; V: 320 HCC	1 center in China; TCGA	CNN	Histopath images	HCC outcomes	Deep learning-based "tumor risk score" was superior to clinical staging and stratified 5 groups of different prognosis
Yamashita *et al* [63] 2021	T: 36 WSI; V: 30 WSI	1 center in United States; TCGA	CNN	Histopath images	Post-surgical recurrence	CNN risk scores outperformed TNM system for predicting recurrence and identified high- and low-risk subgroups

ANN: Artificial neural network; AUC: Area under the curve; CC: Cholangiocarcinoma; CNN: Convolutional neural network; CV: Cross-validation; FLL: Focal liver lesion; GDC: Genomic Data Commons; HBV: Hepatitis B virus; HCC: Hepatocellular carcinoma; HCV: Hepatitis C virus; HV: Healthy volunteers; LT: Liver transplant; MVI: Microvascular invasion; MWA: Microwave ablation; OS: Overall survival; PFS: Progression-free survival; RFA: Radio-frequency ablation; RNN: Recurrent neural network; SR: Surgical resection; STS-net: Spatial transformed similarity network; SVR: Sustained virologic response; T: Training; TCGA: The Cancer Genome Atlas; V: Validation; VHA: Veterans Health Administration; WSI: Whole slide image; CT: Computed tomography; MRI: Magnetic resonance imaging; NHIRD: National Health Insurance Research Database; TNM: Tumor, Nodes, Metastasis; TACE: Trans-arterial chemoembolization; LI-RADS: Liver Imaging Reporting and Data System.

prospective, multi-centered studies involving diverse populations with external validation will be necessary before DL algorithms can be widely accepted.

A currently under-explored, but highly promising and exciting area for the application of DL is the field of autonomous robotics. In a recent editorial, Gumbs *et al* [64] state that while the current form of robotic surgery seems like a form of minimally invasive surgery, the true power of robotic surgery exists in its potential to create autonomous actions. Recently, a DL-based surgical instrument tracking algorithm was able to closely track the instruments during robotic surgery and evaluate the surgeons' performance, demonstrating that DL algorithms can learn the correct steps of robotic surgery[65]. With the help of DL and other AI technologies, it may be possible to imagine a future where fully autonomous robots perform resection of large, complex HCC in ways that no human surgeons can mimic. However, there are significant barriers before the idea of fully autonomous robotic surgery can become a reality, including the current technical limitations of autonomous surgical robotics, as well as the hesitation of patients and providers to fully trust autonomous robots to perform invasive operations. "Explainability" of the DL algorithms will be critical here, as humans would need to be able to understand and correct every single mistake that an autonomous robot makes during surgery. Therefore, for the foreseeable future, DL will most likely remain as a helpful, adjunctive tool to assist human surgeons.

CONCLUSION

This review has provided a comprehensive overview of various ways in which DL algorithms can be employed to assist medical providers and enhance the care of patients with HCC (Table 1). DL algorithms not only can efficiently and accurately replicate the same jobs performed by human physicians, but more importantly can help discover novel biologic pathways and disease subgroups with clinical significance by processing and analyzing complex high-dimensional data in ways impossible for the human brain.

Despite some important limitations to overcome, application of state-of-the-art AI technologies such as DL for the care of patients with HCC is no longer a futuristic idea but is rapidly becoming a reality. Most of the studies covered in this review were published within the past two years, and the number of studies utilizing DL continues

to increase exponentially. We anticipate that DL algorithms will soon take a major role in the diagnosis, prognostication, and treatment of patients with HCC.

REFERENCES

1 **Bray F**, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* 2018; **68**: 394-424 [PMID: 30207593 DOI: 10.3322/caac.21492]

2 **Yang JD**, Hainaut P, Gores GJ, Amadou A, Plymoth A, Roberts LR. A global view of hepatocellular carcinoma: trends, risk, prevention and management. *Nat Rev Gastroenterol Hepatol* 2019; **16**: 589-604 [PMID: 31439937 DOI: 10.1038/s41575-019-0186-y]

3 **Stepanova M**, De Avila L, Afendy M, Younossi I, Pham H, Cable R, Younossi ZM. Direct and Indirect Economic Burden of Chronic Liver Disease in the United States. *Clin Gastroenterol Hepatol* 2017; **15**: 759-766.e5 [PMID: 27464590 DOI: 10.1016/j.cgh.2016.07.020]

4 **Llovet JM**, Kelley RK, Villanueva A, Singal AG, Pikarsky E, Roayaie S, Lencioni R, Koike K, Zucman-Rossi J, Finn RS. Hepatocellular carcinoma. *Nat Rev Dis Primers* 2021; **7**: 6 [PMID: 33479224 DOI: 10.1038/s41572-020-00240-3]

5 **Yang JD**, Heimbach JK. New advances in the diagnosis and management of hepatocellular carcinoma. *BMJ* 2020; **371**: m3544 [PMID: 33106289 DOI: 10.1136/bmj.m3544]

6 **Ahn JC**, Connell A, Simonetto DA, Hughes C, Shah VH. Application of Artificial Intelligence for the Diagnosis and Treatment of Liver Diseases. *Hepatology* 2021; **73**: 2546-2563 [PMID: 33098140 DOI: 10.1002/hep.31603]

7 **Haenlein M**, Kaplan A. A Brief History of Artificial Intelligence: On the Past, Present, and Future of Artificial Intelligence. *Calif Manage Rev* 2019 [DOI: 10.1177/0008125619864925]

8 **Koza JR**, Bennett FH, Andre D, Keane MA. Automated Design of Both the Topology and Sizing of Analog Electrical Circuits Using Genetic Programming. In: Gero JS, Sudweeks F. Artificial Intelligence in Design '96. Dordrecht: Springer Netherlands, 1996: 151-170

9 **Cunningham P**, Cord M, Delany SJ. Supervised Learning. In: Cord M, Cunningham P. Machine Learning Techniques for Multimedia: Case Studies on Organization and Retrieval. Berlin, Heidelberg: Springer Berlin Heidelberg, 2008: 21-49

10 **Ghahramani Z**, Unsupervised Learning. In: Bousquet O, von Luxburg U, Rätsch G. Advanced Lectures on Machine Learning: ML Summer Schools 2003, Canberra, Australia, February 2 - 14, 2003, Tübingen, Germany, August 4 - 16, 2003, Revised Lectures. Berlin, Heidelberg: Springer Berlin Heidelberg, 2004: 72-112

11 **Esteva A**, Robicquet A, Ramsundar B, Kuleshov V, DePristo M, Chou K, Cui C, Corrado G, Thrun S, Dean J. A guide to deep learning in healthcare. *Nat Med* 2019; **25**: 24-29 [PMID: 30617335 DOI: 10.1038/s41591-018-0316-z]

12 **Puttagunta M**, Ravi S. Medical image analysis based on deep learning approach. *Multimed Tools Appl* 2021; 1-34 [PMID: 33841033 DOI: 10.1007/s11042-021-10707-4]

13 **Marrero JA**, Kulik LM, Sirlin CB, Zhu AX, Finn RS, Abecassis MM, Roberts LR, Heimbach JK. Diagnosis, Staging, and Management of Hepatocellular Carcinoma: 2018 Practice Guidance by the American Association for the Study of Liver Diseases. *Hepatology* 2018; **68**: 723-750 [PMID: 29624699 DOI: 10.1002/hep.29913]

14 **Ioannou GN**, Tang W, Beste LA, Tincopa MA, Su GL, Van T, Tapper EB, Singal AG, Zhu J, Waljee AK. Assessment of a Deep Learning Model to Predict Hepatocellular Carcinoma in Patients With Hepatitis C Cirrhosis. *JAMA Netw Open* 2020; **3**: e2015626 [PMID: 32870314 DOI: 10.1001/jamanetworkopen.2020.15626]

15 **Phan DV**, Chan CL, Li AA, Chien TY, Nguyen VC. Liver cancer prediction in a viral hepatitis cohort: A deep learning approach. *Int J Cancer* 2020; **147**: 2871-2878 [PMID: 32761609 DOI: 10.1002/ijc.33245]

16 **Nam JY**, Sinn DH, Bae J, Jang ES, Kim JW, Jeong SH. Deep learning model for prediction of hepatocellular carcinoma in patients with HBV-related cirrhosis on antiviral therapy. *JHEP Rep* 2020; **2**: 100175 [PMID: 33117971 DOI: 10.1016/j.jhepr.2020.100175]

17 **Nam JY**, Lee JH, Bae J, Chang Y, Cho Y, Sinn DH, Kim BH, Kim SH, Yi NJ, Lee KW, Kim JM, Park JW, Kim YJ, Yoon JH, Joh JW, Suh KS. Novel Model to Predict HCC Recurrence after Liver Transplantation Obtained Using Deep Learning: A Multicenter Study. *Cancers (Basel)* 2020; **12** [PMID: 33003306 DOI: 10.3390/cancers12102791]

18 **Behne T**, Copur MS. Biomarkers for hepatocellular carcinoma. *Int J Hepatol* 2012; **2012**: 859076 [PMID: 22655201 DOI: 10.1155/2012/859076]

19 **Tzartzeva K**, Obi J, Rich NE, Parikh ND, Marrero JA, Yopp A, Waljee AK, Singal AG. Surveillance Imaging and Alpha Fetoprotein for Early Detection of Hepatocellular Carcinoma in Patients With Cirrhosis: A Meta-analysis. *Gastroenterology* 2018; **154**: 1706-1718.e1 [PMID: 29425931 DOI: 10.1053/j.gastro.2018.01.064]

20 **Xie H**, Xue YQ, Liu P, Zhang PJ, Tian ST, Yang Z, Guo Z, Wang HM. Multi-parameter gene expression profiling of peripheral blood for early detection of hepatocellular carcinoma. *World J Gastroenterol* 2018; **24**: 371-378 [PMID: 29391759 DOI: 10.3748/wjg.v24.i3.371]

21 **Choi J**, Oh I, Seo S, Ahn J. G2Vec: Distributed gene representations for identification of cancer

prognostic genes. *Sci Rep* 2018; **8**: 13729 [PMID: 30213980 DOI: 10.1038/s41598-018-32180-0]
22 **Chaudhary K**, Poirion OB, Lu L, Garmire LX. Deep Learning-Based Multi-Omics Integration Robustly Predicts Survival in Liver Cancer. *Clin Cancer Res* 2018; **24**: 1248-1259 [PMID: 28982688 DOI: 10.1158/1078-0432.CCR-17-0853]
23 **Chartrand G**, Cheng PM, Vorontsov E, Drozdzal M, Turcotte S, Pal CJ, Kadoury S, Tang A. Deep Learning: A Primer for Radiologists. *Radiographics* 2017; **37**: 2113-2131 [PMID: 29131760 DOI: 10.1148/rg.2017170077]
24 **Azer SA**. Deep learning with convolutional neural networks for identification of liver masses and hepatocellular carcinoma: A systematic review. *World J Gastrointest Oncol* 2019; **11**: 1218-1230 [PMID: 31908726 DOI: 10.4251/wjgo.v11.i12.1218]
25 **Streba CT**, Ionescu M, Gheonea DI, Sandulescu L, Ciurea T, Saftoiu A, Vere CC, Rogoveanu I. Contrast-enhanced ultrasonography parameters in neural network diagnosis of liver tumors. *World J Gastroenterol* 2012; **18**: 4427-4434 [PMID: 22969209 DOI: 10.3748/wjg.v18.i32.4427]
26 **Hassan TM**, Elmogy M, Sallam E-S. Diagnosis of Focal Liver Diseases Based on Deep Learning Technique for Ultrasound Images. *Arab J Sci Eng* 2017; **42**: 3127-3140 [DOI: 10.1007/s13369-016-2387-9]
27 **Bharti P**, Mittal D, Ananthasivan R. Preliminary Study of Chronic Liver Classification on Ultrasound Images Using an Ensemble Model. *Ultrason Imaging* 2018; **40**: 357-379 [PMID: 30015593 DOI: 10.1177/0161734618787447]
28 **Schmauch B**, Herent P, Jehanno P, Dehaene O, Saillard C, Aubé C, Luciani A, Lassau N, Jégou S. Diagnosis of focal liver lesions from ultrasound using deep learning. *Diagn Interv Imaging* 2019; **100**: 227-233 [PMID: 30926443 DOI: 10.1016/j.diii.2019.02.009]
29 **Brehar R**, Mitrea DA, Vancea F, Marita T, Nedevschi S, Lupsor-Platon M, Rotaru M, Badea RI. Comparison of Deep-Learning and Conventional Machine-Learning Methods for the Automatic Recognition of the Hepatocellular Carcinoma Areas from Ultrasound Images. *Sensors (Basel)* 2020; **20** [PMID: 32485986 DOI: 10.3390/s20113085]
30 **Jin J**, Yao Z, Zhang T, Zeng J, Wu L, Wu M, Wang J, Wang Y, Yu J, Zheng R. Deep learning radiomics model accurately predicts hepatocellular carcinoma occurrence in chronic hepatitis B patients: a five-year follow-up. *Am J Cancer Res* 2021; **11**: 576-589 [PMID: 33575088]
31 **Yasaka K**, Akai H, Abe O, Kiryu S. Deep Learning with Convolutional Neural Network for Differentiation of Liver Masses at Dynamic Contrast-enhanced CT: A Preliminary Study. *Radiology* 2018; **286**: 887-896 [PMID: 29059036 DOI: 10.1148/radiol.2017170706]
32 **Shi W**, Kuang S, Cao S, Hu B, Xie S, Chen S, Chen Y, Gao D, Zhu Y, Zhang H, Liu H, Ye M, Sirlin CB, Wang J. Deep learning assisted differentiation of hepatocellular carcinoma from focal liver lesions: choice of four-phase and three-phase CT imaging protocol. *Abdom Radiol (NY)* 2020; **45**: 2688-2697 [PMID: 32232524 DOI: 10.1007/s00261-020-02485-8]
33 **Alirr OI**. Deep learning and level set approach for liver and tumor segmentation from CT scans. *J Appl Clin Med Phys* 2020; **21**: 200-209 [PMID: 33113290 DOI: 10.1002/acm2.13003]
34 **Chlebus G**, Schenk A, Moltz JH, van Ginneken B, Hahn HK, Meine H. Automatic liver tumor segmentation in CT with fully convolutional neural networks and object-based postprocessing. *Sci Rep* 2018; **8**: 15497 [PMID: 30341319 DOI: 10.1038/s41598-018-33860-7]
35 **Deng Z**, Guo Q, Zhu Z. Dynamic Regulation of Level Set Parameters Using 3D Convolutional Neural Network for Liver Tumor Segmentation. *J Healthc Eng* 2019; **2019**: 4321645 [PMID: 30918620 DOI: 10.1155/2019/4321645]
36 **Wardhana G**, Naghibi H, Sirmacek B, Abayazid M. Toward reliable automatic liver and tumor segmentation using convolutional neural network based on 2.5D models. *Int J Comput Assist Radiol Surg* 2021; **16**: 41-51 [PMID: 33219906 DOI: 10.1007/s11548-020-02292-y]
37 **Li X**, Chen H, Qi X, Dou Q, Fu CW, Heng PA. H-DenseUNet: Hybrid Densely Connected UNet for Liver and Tumor Segmentation From CT Volumes. *IEEE Trans Med Imaging* 2018; **37**: 2663-2674 [PMID: 29994201 DOI: 10.1109/TMI.2018.2845918]
38 **Ahn SH**, Yeo AU, Kim KH, Kim C, Goh Y, Cho S, Lee SB, Lim YK, Kim H, Shin D, Kim T, Kim TH, Youn SH, Oh ES, Jeong JH. Comparative clinical evaluation of atlas and deep-learning-based auto-segmentation of organ structures in liver cancer. *Radiat Oncol* 2019; **14**: 213 [PMID: 31775825 DOI: 10.1186/s13014-019-1392-z]
39 **Almotairi S**, Kareem G, Aouf M, Almutairi B, Salem MA. Liver Tumor Segmentation in CT Scans Using Modified SegNet. *Sensors (Basel)* 2020; **20** [PMID: 32164153 DOI: 10.3390/s20051516]
40 **Ayalew YA**, Fante KA, Mohammed MA. Modified U-Net for liver cancer segmentation from computed tomography images with a new class balancing method. *BMC Biomed Eng* 2021; **3**: 4 [PMID: 33641679 DOI: 10.1186/s42490-021-00050-y]
41 **Budak Ü**, Guo Y, Tanyildizi E, Şengür A. Cascaded deep convolutional encoder-decoder neural networks for efficient liver tumor segmentation. *Med Hypotheses* 2020; **134**: 109431 [PMID: 31669758 DOI: 10.1016/j.mehy.2019.109431]
42 **Chen Y**, Wang K, Liao X, Qian Y, Wang Q, Yuan Z, Heng PA. Channel-Unet: A Spatial Channel-Wise Convolutional Neural Network for Liver and Tumors Segmentation. *Front Genet* 2019; **10**: 1110 [PMID: 31827487 DOI: 10.3389/fgene.2019.01110]
43 **Hamm CA**, Wang CJ, Savic LJ, Ferrante M, Schobert I, Schlachter T, Lin M, Duncan JS, Weinreb JC, Chapiro J, Letzen B. Deep learning for liver tumor diagnosis part I: development of a convolutional neural network classifier for multi-phasic MRI. *Eur Radiol* 2019; **29**: 3338-3347 [PMID: 31016442 DOI: 10.1007/s00330-019-06205-9]

44 **Wang CJ**, Hamm CA, Savic LJ, Ferrante M, Schobert I, Schlachter T, Lin M, Weinreb JC, Duncan JS, Chapiro J, Letzen B. Deep learning for liver tumor diagnosis part II: convolutional neural network interpretation using radiologic imaging features. *Eur Radiol* 2019; **29**: 3348-3357 [PMID: 31093705 DOI: 10.1007/s00330-019-06214-8]

45 **Wu Y**, White GM, Cornelius T, Gowdar I, Ansari MH, Supanich MP, Deng J. Deep learning LI-RADS grading system based on contrast enhanced multiphase MRI for differentiation between LR-3 and LR-4/LR-5 liver tumors. *Ann Transl Med* 2020; **8**: 701 [PMID: 32617321 DOI: 10.21037/atm.2019.12.151]

46 **Zhen SH**, Cheng M, Tao YB, Wang YF, Juengpanich S, Jiang ZY, Jiang YK, Yan YY, Lu W, Lue JM, Qian JH, Wu ZY, Sun JH, Lin H, Cai XJ. Deep Learning for Accurate Diagnosis of Liver Tumor Based on Magnetic Resonance Imaging and Clinical Data. *Front Oncol* 2020; **10**: 680 [PMID: 32547939 DOI: 10.3389/fonc.2020.00680]

47 **Zhang Y**, Lv X, Qiu J, Zhang B, Zhang L, Fang J, Li M, Chen L, Wang F, Liu S, Zhang S. Deep Learning With 3D Convolutional Neural Network for Noninvasive Prediction of Microvascular Invasion in Hepatocellular Carcinoma. *J Magn Reson Imaging* 2021; **54**: 134-143 [PMID: 33559293 DOI: 10.1002/jmri.27538]

48 **Wang G**, Jian W, Cen X, Zhang L, Guo H, Liu Z, Liang C, Zhou W. Prediction of Microvascular Invasion of Hepatocellular Carcinoma Based on Preoperative Diffusion-Weighted MR Using Deep Learning. *Acad Radiol* 2020 [PMID: 33303346 DOI: 10.1016/j.acra.2020.11.014]

49 **Jiang YQ**, Cao SE, Cao S, Chen JN, Wang GY, Shi WQ, Deng YN, Cheng N, Ma K, Zeng KN, Yan XJ, Yang HZ, Huan WJ, Tang WM, Zheng Y, Shao CK, Wang J, Yang Y, Chen GH. Preoperative identification of microvascular invasion in hepatocellular carcinoma by XGBoost and deep learning. *J Cancer Res Clin Oncol* 2021; **147**: 821-833 [PMID: 32852634 DOI: 10.1007/s00432-020-03366-9]

50 **An C**, Jiang Y, Huang Z, Gu Y, Zhang T, Ma L, Huang J. Assessment of Ablative Margin After Microwave Ablation for Hepatocellular Carcinoma Using Deep Learning-Based Deformable Image Registration. *Front Oncol* 2020; **10**: 573316 [PMID: 33102233 DOI: 10.3389/fonc.2020.573316]

51 **Liu F**, Liu D, Wang K, Xie X, Su L, Kuang M, Huang G, Peng B, Wang Y, Lin M, Tian J. Deep Learning Radiomics Based on Contrast-Enhanced Ultrasound Might Optimize Curative Treatments for Very-Early or Early-Stage Hepatocellular Carcinoma Patients. *Liver Cancer* 2020; **9**: 397-413 [PMID: 32999867 DOI: 10.1159/000505694]

52 **Peng J**, Kang S, Ning Z, Deng H, Shen J, Xu Y, Zhang J, Zhao W, Li X, Gong W, Huang J, Liu L. Residual convolutional neural network for predicting response of transarterial chemoembolization in hepatocellular carcinoma from CT imaging. *Eur Radiol* 2020; **30**: 413-424 [PMID: 31332558 DOI: 10.1007/s00330-019-06318-1]

53 **Liu QP**, Xu X, Zhu FP, Zhang YD, Liu XS. Prediction of prognostic risk factors in hepatocellular carcinoma with transarterial chemoembolization using multi-modal multi-task deep learning. *EClinicalMedicine* 2020; **23**: 100379 [PMID: 32548574 DOI: 10.1016/j.eclinm.2020.100379]

54 **Zhang L**, Xia W, Yan ZP, Sun JH, Zhong BY, Hou ZH, Yang MJ, Zhou GH, Wang WS, Zhao XY, Jian JM, Huang P, Zhang R, Zhang S, Zhang JY, Li Z, Zhu XL, Gao X, Ni CF. Deep Learning Predicts Overall Survival of Patients With Unresectable Hepatocellular Carcinoma Treated by Transarterial Chemoembolization Plus Sorafenib. *Front Oncol* 2020; **10**: 593292 [PMID: 33102242 DOI: 10.3389/fonc.2020.593292]

55 **Lin H**, Wei C, Wang G, Chen H, Lin L, Ni M, Chen J, Zhuo S. Automated classification of hepatocellular carcinoma differentiation using multiphoton microscopy and deep learning. *J Biophotonics* 2019; **12**: e201800435 [PMID: 30868728 DOI: 10.1002/jbio.201800435]

56 **Kiani A**, Uyumazturk B, Rajpurkar P, Wang A, Gao R, Jones E, Yu Y, Langlotz CP, Ball RL, Montine TJ, Martin BA, Berry GJ, Ozawa MG, Hazard FK, Brown RA, Chen SB, Wood M, Allard LS, Ylagan L, Ng AY, Shen J. Impact of a deep learning assistant on the histopathologic classification of liver cancer. *NPJ Digit Med* 2020; **3**: 23 [PMID: 32140566 DOI: 10.1038/s41746-020-0232-8]

57 **Liao H**, Long Y, Han R, Wang W, Xu L, Liao M, Zhang Z, Wu Z, Shang X, Li X, Peng J, Yuan K, Zeng Y. Deep learning-based classification and mutation prediction from histopathological images of hepatocellular carcinoma. *Clin Transl Med* 2020; **10**: e102 [PMID: 32536036 DOI: 10.1002/ctm2.102]

58 **Wang H**, Jiang Y, Li B, Cui Y, Li D, Li R. Single-Cell Spatial Analysis of Tumor and Immune Microenvironment on Whole-Slide Image Reveals Hepatocellular Carcinoma Subtypes. *Cancers (Basel)* 2020; **12** [PMID: 33260561 DOI: 10.3390/cancers12123562]

59 **Chen M**, Zhang B, Topatana W, Cao J, Zhu H, Juengpanich S, Mao Q, Yu H, Cai X. Classification and mutation prediction based on histopathology H&E images in liver cancer using deep learning. *NPJ Precis Oncol* 2020; **4**: 14 [PMID: 32550270 DOI: 10.1038/s41698-020-0120-3]

60 **Lu L**, Daigle BJ Jr. Prognostic analysis of histopathological images using pre-trained convolutional neural networks: application to hepatocellular carcinoma. *PeerJ* 2020; **8**: e8668 [PMID: 32201640 DOI: 10.7717/peerj.8668]

61 **Saillard C**, Schmauch B, Laifa O, Moarii M, Toldo S, Zaslavskiy M, Pronier E, Laurent A, Amaddeo G, Regnault H, Sommacale D, Ziol M, Pawlotsky JM, Mulé S, Luciani A, Wainrib G, Clozel T, Courtiol P, Calderaro J. Predicting Survival After Hepatocellular Carcinoma Resection Using Deep Learning on Histological Slides. *Hepatology* 2020; **72**: 2000-2013 [PMID: 32108950 DOI: 10.1002/hep.31207]

62 **Shi JY**, Wang X, Ding GY, Dong Z, Han J, Guan Z, Ma LJ, Zheng Y, Zhang L, Yu GZ, Wang XY, Ding ZB, Ke AW, Yang H, Wang L, Ai L, Cao Y, Zhou J, Fan J, Liu X, Gao Q. Exploring prognostic

indicators in the pathological images of hepatocellular carcinoma based on deep learning. *Gut* 2021; **70**: 951-961 [PMID: 32998878 DOI: 10.1136/gutjnl-2020-320930]

63 **Yamashita R**, Long J, Saleem A, Rubin DL, Shen J. Deep learning predicts postsurgical recurrence of hepatocellular carcinoma from digital histopathologic images. *Sci Rep* 2021; **11**: 2047 [PMID: 33479370 DOI: 10.1038/s41598-021-81506-y]

64 **Gumbs AA**, Perretta S, d'Allemagne B, Chouillard E. What is Artificial Intelligence Surgery? *Art Int Surg* 2021; **1**: 1-10 [DOI: 10.20517/ais.2021.01]

65 **Lee D**, Yu HW, Kwon H, Kong HJ, Lee KE, Kim HC. Evaluation of Surgical Skills during Robotic Surgery by Deep Learning-Based Multiple Surgical Instrument Tracking in Training and Actual Operations. *J Clin Med* 2020; **9** [PMID: 32585953 DOI: 10.3390/jcm9061964]

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ORIGINAL ARTICLE

Basic Study

Gut dysbiosis and systemic inflammation promote cardiomyocyte abnormalities in an experimental model of steatohepatitis

Larisse Longo, Pabulo Henrique Rampelotto, Eduardo Filippi-Chiela, Valessa Emanoele Gabriel de Souza, Fernando Salvati, Carlos Thadeu Cerski, Themis Reverbel da Silveira, Cláudia P Oliveira, Carolina Uribe-Cruz, Mário Reis Álvares-da-Silva

ORCID number: Larisse Longo 0000-0002-4453-7227; Pabulo Henrique Rampelotto 0000-0002-8992-9697; Eduardo Filippi-Chiela 0000-0001-8192-3779; Valessa Emanoele Gabriel de Souza 0000-0002-7672-6460; Fernando Salvati 0000-0001-9331-3812; Carlos Thadeu Cerski 0000-0003-0673-5916; Themis Reverbel da Silveira 0000-0001-9867-8650; Cláudia P Oliveira 0000-0002-2848-417X; Carolina Uribe-Cruz 0000-0002-0526-3067; Mário Reis Álvares-da-Silva 0000-0002-5001-246X.

Author contributions: Longo L, Rampelotto PH, Filippi-Chiela E and Álvares-da-Silva MR performed the conceptualization, methodology, formal analysis, investigation, data curation, writing of the original draft, writing-review, and editing; de Souza VEG, Salvati F, and Cerski CT performed the conceptualization, methodology, and formal analysis; da Silveira TR, Oliveira CP and Uribe-Cruz C contributed to the conceptualization, data curation writing the original draft, writing-review and editing.

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Larisse Longo, Pabulo Henrique Rampelotto, Valessa Emanoele Gabriel de Souza, Themis Reverbel da Silveira, Carolina Uribe-Cruz, Mário Reis Álvares-da-Silva, Experimental Laboratory of Hepatology and Gastroenterology, Hospital de Clínicas de Porto Alegre, Porto Alegre 90035-903, Rio Grande do Sul, Brazil

Larisse Longo, Eduardo Filippi-Chiela, Carlos Thadeu Cerski, Carolina Uribe-Cruz, Mário Reis Álvares-da-Silva, Graduate Program in Gastroenterology and Hepatology, Universidade Federal do Rio Grande do Sul, Porto Alegre 90035-003, Rio Grande do Sul, Brazil

Pabulo Henrique Rampelotto, Graduate Program in Genetics and Molecular Biology, Universidade Federal do Rio Grande do Sul, Porto Alegre 90035-903, Rio Grande do Sul, Brazil

Eduardo Filippi-Chiela, Center of Biotechnology, Universidade Federal do Rio Grande do Sul, Porto Alegre 91501-970, Rio Grande do Sul, Brazil

Eduardo Filippi-Chiela, Department of Morphological Sciences, Universidade Federal do Rio Grande do SulPorto Alegre 90050-170, Rio Grande do Sul, Brazil

Eduardo Filippi-Chiela, Experimental Research Center, Hospital de Clínicas de Porto Alegre, Porto Alegre 90035-903, Rio Grande do Sul, Brazil

Fernando Salvati, School of Medicine, Instituto Meridional de Educação-IMED, Passo Fundo 99070-220, Rio Grande do Sul, Brazil

Carlos Thadeu Cerski, Unit of Surgical Pathology, Hospital de Clínicas de Porto Alegre, Porto Alegre 90035-903, Rio Grande do Sul, Brazil

Cláudia P Oliveira, Department of Gastroenterology (LIM07), Faculdade de Medicina da Universidade de São Paulo, São Paulo 01246903, Brazil

Mário Reis Álvares-da-Silva, Division of Gastroenterology, Hospital de Clínicas de Porto Alegre, Porto Alegre 90035-903, Rio Grande do Sul, Brazil

Corresponding author: Larisse Longo, PhD, Postdoc, Experimental Laboratory of Hepatology and Gastroenterology, Hospital de Clínicas de Porto Alegre, Rua Ramiro Barcelos, 2350/Sala 12214, 2° Andar, Porto Alegre 90035-903, Rio Grande do Sul, Brazil.

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larisselongo@hotmail.com

Abstract

BACKGROUND

Cardiovascular disease is the main cause of death in metabolic-associated fatty liver disease, and gut microbiota dysbiosis is associated with both of them.

AIM

To assess the relationship between gut dysbiosis and cardiovascular risk (CVR) in an experimental model of steatohepatitis.

METHODS

Adult male Sprague-Dawley rats were randomized to a control group (n = 10) fed a standard diet and an intervention group (n = 10) fed a high-fat choline-deficient diet for 16 wk. Biochemical, molecular, hepatic, and cardiac histopathology. Gut microbiota variables were evaluated.

RESULTS

The intervention group had a significantly higher atherogenic coefficient, Castelli's risk index (CRI)-I and CRI-II, interleukin-1β, tissue inhibitor of metalloproteinase-1 (all P < 0.001), monocyte chemoattractant protein-1 (P = 0.005), and plasminogen activator inhibitor-1 (P = 0.037) than the control group. Gene expression of miR-33a increased (P = 0.001) and miR-126 (P < 0.001) decreased in the intervention group. Steatohepatitis with fibrosis was seen in the intervention group, and heart computerized histological imaging analysis showed a significant decrease in the percentage of cardiomyocytes with a normal morphometric appearance (P = 0.007), reduction in the mean area of cardiomyocytes (P = 0.037), and an increase of atrophic cardiomyocytes (P = 0.007). There were significant correlations between the cardiomyocyte morphometry markers and those of progression and severity of liver disease and CVR. The intervention group had a lower Shannon diversity index and fewer changes in the structural pattern of gut microbiota (both P < 0.001) than controls. Nine microbial families that are involved in lipid metabolism were differentially abundant in intervention group and were significantly correlated with markers of liver injury and CVR.

CONCLUSION

The study found a link between gut dysbiosis and significant cardiomyocyte abnormalities in animals with steatohepatitis.

Key Words: Animal model; Cardiovascular diseases; Gut microbiota; Metabolic-associated fatty liver disease; Predicted lipid metabolism; Risk cardiovascular; Steatohepatitis

Core Tip: Cardiovascular disease is the main cause of death in metabolic-associated fatty liver disease (MAFLD) and gut microbiota dysbiosis is associated with both. Among the risk factors, we report significant correlations between the presence of atherogenic dyslipidemia, systemic inflammation, endothelial dysfunction, liver fibrogenesis, and gut dysbiosis, all of which contributed to the progression of MAFLD and increased cardiovascular risk.

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INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is the most common form of liver disease and a leading cause of morbidity and mortality in both developed and developing countries[1]. The natural course the disease encompasses a pathological spectrum of liver injury ranging from simple steatosis to steatohepatitis and progressive liver fibrosis that can result in cirrhosis and other complications, including liver decompensation and hepatocellular carcinoma (HCC)[1,2]. Recently, a new nomenclature, metabolic-associated fatty liver disease (MAFLD) was suggested because the disease is not only confined to the liver only, but rather represents a major part of a multisystemic disease that includes cardiovascular manifestations[3-6]. Indeed, cardiovascular disease (CVD) is the leading cause of death in patients with MAFLD, accounting approximately 40%–45% of the total deaths[4,7,8].

The association of steatohepatitis with CVD is related to the metabolic risk factors that they have in common, such as obesity, diabetes mellitus, hypertension, and dyslipidemia. However, multiple studies have shown that steatohepatitis is also independently associated with several markers of subclinical atherosclerosis[4,7,8]. Although the putative pathophysiological mechanisms that link steatohepatitis and CVD are still not completely explained, many nontraditional and emerging risk factors, including proinflammatory cytokines and procoagulant factors (*e.g.*, fibrinogen, plasminogen, and vascular adhesion molecules) are associated with the process[7,9]. Recently, the intestinal microbiome and its highly complex and interdependent interaction with host metabolism, immunity, and disease have opened a new horizon of investigation into the link between these clinical conditions[4,9,10]. Gut microbiota, or the bacterial components and metabolites carried to the liver through the portal vein, overstimulate immune cells and may result in more severe liver damage, inflammation, and fibrosis, thus accelerating the development of steatohepatitis and inducing the systemic inflammation and endothelial dysfunction that promotes increased cardiovascular risk (CVR)[4,10]. Despite considerable progress, understanding of the molecular mechanisms governing microbiota-host interactions is far from complete. Experimental studies are needed to further explore the mechanisms whereby gut microbiota contribute to steatohepatitis-associated CVR.

The goal of this study was to assess the relationships of the gut microbiota, steatohepatitis, and CVR, by describing the crosstalk among gut dysbiosis, associated metabolic predictions, systemic inflammation, endothelial dysfunction, paracrine cell signaling, and cardiomyocyte morphology in an experimental nutritional steatohepatitis model that mimics the metabolic changes found in humans.

MATERIALS AND METHODS

Animals and experimental model

Twenty 60-day-old adult male Sprague-Dawley rats weighing 280-350 g were used. The animals were kept in groups inside two polypropylene boxes in a controlled-temperature environment (22 ± 2 °C) and a 12-h light/dark cycle. All experimental procedures were approved by the Ethics Committee for the Use of Animals (No. 17-0021 and No. 17-0531) and were conducted following the international guidelines for animal welfare. Measures were taken to minimize animal pain and discomfort.

After acclimatization to the environment, the animals were randomized to two experimental groups according to their weight, as previously described[11]. The control group (n = 10) received a standard diet (Nuvilab CR-1, Quimtia S.A., Brazil). The intervention group (n = 10) received a high-fat, choline-deficient diet consisting of 31.5% total fat and enriched with 54.0% trans fatty acids (Rhoster Ltda., Brazil) to induce steatohepatitis. Both groups received water and food ad libitum during the study. After 16 wk of treatment, the animals were fasted for 8 h, anesthetized with isoflurane, and euthanized by cardiac exsanguination. Blood samples were collected and centrifuged to obtain the serum, which was kept at −80 °C until the analyses were performed. Pieces of hepatic and cardiac tissue were fixed in 10% formaldehyde for histopathological evaluation. Feces present in the intestine were collected aseptically and kept at −80 °C for analysis of the gut microbiota.

Atherogenic ratios

Serum total cholesterol (TC), low density lipoprotein-cholesterol (LDLC), high-density lipoprotein cholesterol (HDLC) and triglycerides (TG) were assayed with a Labmax 560[11]. Atherogenic ratios were calculated from the lipid profile and used as a tool for

the prediction of CVR. The ratios included Castelli's risk index (CRI)-I = TC/HDLC, CRI-II = LDLC/HDLC and the atherogenic coefficient (AC) = (TCH − DLC)/HDLC [12].

Systemic inflammation and endothelial dysfunction

The serum markers of inflammation and endothelial dysfunction markers included in the analysis were monocyte chemoattractant protein (MCP)-1, tissue inhibitor of metalloproteinase (TIMP)-1 and plasminogen activator inhibitor (PAI)-1, and were determined by multiplex assay with the Luminex platform (Millipore, Germany). The results were expressed as ng/mL. Serum interleukin (IL)-1β was measured with an enzyme-linked immunosorbent assay kit (Thermo Scientific, United States). Absorbance was measured spectrophotometrically at a wavelength of 450 nm with a Zenyth 200rt microplate reader (Biochrom). The results were expressed in pg/mL. All procedures were performed in duplicate following the manufacturer's instructions.

Analysis of circulating microRNAs

Total RNA was extracted from serum using miRNeasy serum/plasma kits (Qiagen, United States). A cel-miR-39 (1.6×10^8 copies) spike-in control (Qiagen, United States) was added to provide an internal reference. cDNA conversion was performed with 10 ng of total RNA using TaqMan microRNA reverse transcription kits (Applied Biosystems, United States). Amplification of miR-33a, miR-126, miR-499, miR-186 and miR-146a, was performed by quantitative real-time PCR using the TaqMan assay (Applied Biosystems, United States) and expression as normalized against cell-miR-39. The sequences and codes of the assessed miRNAs are listed in Supplementary Table 1 (Private sharing link for Figshare data https://figshare.com/s/2d858620da6b13fe2fec). Values were calculated by the $2^{-(\Delta\Delta Ct)}$ method.

Hepatic histopathological analysis

Formalin-fixed liver tissue samples were embedded in paraffin, sectioned, and stained with hematoxylin and eosin (H&E) and picrosirius red. Histopathological lesions of the different evolutionary stages of liver disease were scored as previously described by Liang *et al*[13]. The score is highly reproduceable and applicable to experimental models in rodents. The analysis was performed by an experienced pathologist who was blinded to the experimental groups. Fibrosis was quantified by morphometric analysis after picrosirius red staining. Ten randomly selected fields were observed *per* animal to measure staining intensity using an Olympus BX51 microscope, and QCapture 64-bit (QImaging) at × 200 magnification. The evaluation was performed using ImageJ (version 1.51p, https://imagej.nih.gov/ij/).

Cardiomyocytes morphometric analysis

Cardiomyocyte morphometric analysis (CMA) was performed based on adaptations of the nuclear morphometric analysis developed by Filippi-Chiela *et al*[14]. Cardiomyocyte size and shape were measured using Image Pro Plus 6.0 (IPP6, Media Cybernetics). H&E images from hearts of animals were acquired. Five different fields were photographed in tissue from each animal using QCapture 64-bit software and an Olympus BX51 microscope. At least 50 cross-sectioned cardiomyocytes of each animal were analyzed. The outlines of single cells were marked using the magic wand tool of IPP6, followed by acquisition the cell area, aspect, area/box, radius ratio, and roundness. The last four measurements were used to define the cardiomyocyte irregularity index (CII) of each cell (CII = area + aspect – area/box + roundness). These variables were used to report the size and shape of single cardiomyocytes. In addition to the average size and regularity, the plot of area *vs* CMA also defined the percentage of normal, hypertrophic, and atrophic cells.

DNA extraction, 16S rRNA sequencing and bioinformatics analysis

A detailed description of the methods used for 16S ribosomal RNA gene sequencing and analyses is provided in the Supplementary Information (Private sharing link for Figshare data https://figshare.com/s/2d858620da6b13fe2fec). Briefly, after DNA extraction, the V4 hypervariable region of the 16S rRNA gene was amplified using 515F–806R primer pair and sequencing was performed with Ion Torrent (Thermo Fisher Scientific, United States). A custom pipeline in Mothur was used for 16S rRNA reads processing. Subsequent analysis of the sequence dataset and data visualization were performed in R using the vegan, phyloseq, ggplot2, and MicrobiomeAnalystR packages or QIIME.

Correlations between analyzed markers

For this analysis, we selected the histopathological NAFLD score, quantification of liver collagen, TIMP-1, MCP-1, and IL-1β as markers of severity and progression of steatohepatitis. For the correlation of CVD risk factors and lipid metabolism, we selected miR-33a, miR-126, PAI-1, CRI-I, CRI-II and AC. We selected the percentage of normal cardiomyocytes, percentage average area of cardiomyocytes, and percentages of atrophic cardiomyocyte morphological characteristics. The overall microbiota composition was correlated with the variables.

Statistical analysis

Data symmetry was tested using the Shapiro-Wilk test. Student-*t* and Mann-Whitney U tests were performed. Spearman's correlation coefficient was performed, with moderate ($0.3 < r < 0.6$), strong ($0.6 < r < 0.9$), or very strong ($0.9 < r < 1.0$) correlations. Quantitative variables were expressed as means ± standard deviation or medians with minimum and maximum values. $P \leq 0.05$ was considered statistically significant. Data were analyzed with SPSS 18.0 (IBM Corp., United States).

RESULTS

Atherogenic ratios, inflammation, and endothelial dysfunction to assess CVR

The results obtained for these parameters are shown in Table 1. There were significant increases in AC), CRI-I, and CRI-II (all $P < 0.001$) in the intervention group, indicating that the animals had an increased CVR. There were significant increases in the serum concentrations of IL-1β ($P = 0.001$), MCP-1 ($P = 0.005$), TIMP-1 ($P < 0.001$), and PAI-1 ($P = 0.037$) in the intervention group compared with the control group. Together, the results suggest the study intervention had increased systemic inflammation and endothelial dysfunction.

Level of circulating microRNAs related to CVR

The levels of circulating microRNAs related to CVR are shown in Figure 1. There was a significant increase in the gene expression of miR-33a ($P = 0.001$) in the intervention group compared with the control group, the opposite was reported for miR-126 ($P < 0.001$). There were no between-group differences in the expression of miR-499 ($P = 0.171$), miR-186 ($P = 0.151$), and miR-146a ($P = 0.151$).

Liver histopathological analysis

No abnormalities were seen in the livers of the control group animals, whereas animals in the intervention group had predominantly microvesicular steatosis along with macrovesicular steatosis of moderate intensity, inflammatory activity, and a mild degree of fibrosis. In the histopathological staging of lesions, seven animals in the intervention group had steatohepatitis and three had simple steatosis. Picrosirius red staining of collagen was more intense ($P < 0.001$) in animals in the intervention group than in the control group (4.10, range: 3.02-6.04 *vs* 1.35, range: 1.21-1.55) relative luminescence units, indicating a significant increase in the deposition of connective tissue fibers in the liver.

Morphometric and histopathological evaluation of cardiomyocytes

Myocardial steatosis was not observed in either the control of intervention group. The evaluation of cardiomyocyte morphometry (*i.e.* size and shape) demonstrated the percentages of normal size, large, or small cells and their shape regularity (Figure 2A). There was a significant decrease in the percentage of cardiomyocytes with a normal morphometric appearance ($P = 0.007$) in the intervention group compared with the control group (Figure 2B). Among the most clinically relevant morphometric changes, there was a significant reduction in the mean area of cardiomyocytes ($P = 0.037$, Figure 2C) and a significant increase in the percentage of atrophic cardiomyocytes in the intervention group ($P = 0.007$, Figure 2D) in relation to the control group. Finally, we separated the animals in the intervention group into two subgroups by the median percentages of normal cardiomyocytes (Figure 2E) and atrophic cardiomyocytes (Figure 2F) and the average area (Figure 2G) and then compared the data. Animals with a percentage of normal cardiomyocytes higher than the median had higher liver tissue levels of TIMP-1, IL-1β, IL-6 and myeloid differentiation primary response (Myd)-88, and lower levels of IL-1β/IL-10 (Figure 2E). Animals with a percentage of atrophic cardiomyocytes above the median had lower liver tissue levels of IL-1β

Table 1 Atherogenic ratios, inflammation and endothelial dysfunction markers in a nutritional model of steatohepatitis

Variable	Control (*n* = 10)	Intervention (*n* = 10)	*P* value
AC	0.6 (0.2–0.9)	2.5 (1.5–3.4)	< 0.001[a]
CRI-I	1.6 (± 0.4)	3.5 (± 1.1)	< 0.001[a]
CRI-II	0.3 (± 0.1)	0.8 (± 0.2)	< 0.001[a]
IL-1β (pg/mL)	367.7 (± 31.2)	465.9 (± 52.7)	0.001[a]
MCP-1 (ng/mL)	2.7 (± 0.6)	3.8 (± 0.9)	0.005[a]
TIMP-1 (ng/mL)	7.1 (± 1.4)	12.4 (± 2.3)	< 0.001[a]
PAI-1 (ng/mL)	0.11 (± 0.05)	0.17 (± 0.06)	0.037[a]

Data are means ± standard deviation or medians (25^{th}-75^{th} percentiles).

[a]$P \leq 0.05$ was considered statistically significant.

AC: Atherogenic coefficient; CRI: Castelli's risk index; IL: Interleukin; MCP: Monocyte chemoattractant protein; PAI: Plasminogen activator inhibitor; TIMP: Tissue inhibitor of metalloproteinase.

(Figure 2F). Animals with an average cardiomyocytes area greater than the median had lower liver tissue levels of tumor necrosis factor-α/IL-10 (Figure 2G).

Gut microbiota diversity and composition

The Shannon diversity index was significantly lower ($P < 0.001$) in intervention than in the control group (Figure 3A). In addition, analysis of similarities (ANOSIM) revealed that the structural pattern of the gut microbiota in intervention group was clearly distinct from that of the control group ($P < 0.001$) by principal coordinates analysis (PCoA) using the Bray-Curtis distance metric (Figure 3B). In terms of composition (*i.e.* taxonomic identification), 1266 bacterial taxa (operational taxonomic units) that belonged to 112 genera, 41 families, and eight phyla were identified. *Firmicutes* (53.1%) and *Bacteroidetes* (43.1%) were the most abundant phyla in all samples. The most abundant families were *Muribaculaceae* (21.7%), *Lachnospiraceae* (20.8%), *Ruminococcaceae* (18.5%), and *Bacteroidaceae* (15.4%, Figure 3C). The four families represented 76.4% of all observed taxa. Differential abundance analysis identified nine families that were associated with the intervention group and one family associated with control group (Linear discriminant analysis score > 2.0; Figure 3D).*Bacteroidaceae*, *Ruminococcaceae*, *Peptostreptococcaceae*, *Peptococcaceae*, *Erysipelotricaceae*, *Clostridiaceae*, *B- urkholderiaceae*, *Streptococcaeae*, and *Tannerellaceae* were differentially abundant in the intervention group. *Lachnospiraceae* was differentially abundant in control group. The distribution of the 41 families and their features are shown in Figure 3E. Most of the taxa prevalent in control group were less prevalent or absent in intervention group. The reverse was also observed.

Lipid metabolism prediction

PCoA using the Bray-Curtis distance metric indicated that the clustering of the predicted lipid metabolic pathways in the study groups was clearly distinct (ANOSIM, $P < 0.001$) As shown in Figure 4A, two samples, R01 and R11, were considered outliers and were not included in further statistical analysis (*e.g.*, LefSe analysis). The distribution of the predicted lipid metabolic pathways is shown in Figure 4B. In total, 12 metabolic pathways were identified in which the between-group difference in the relative frequency was significant ($P < 0.001$, linear discriminant analysis score > 2.0; Figure 4C). The results showed that metabolic pathways involved in sphingolipid metabolism, fatty acid biosynthesis, fatty acid metabolism, steroid hormone biosynthesis, and arachidonic acid metabolism were significantly increased in intervention group, and glycerophospholipid metabolism, glycerolipid metabolism, synthesis and degradation of ketone bodies, biosynthesis of unsaturated fatty acids, alpha-linolenic acid metabolism, linoleic acid metabolism, and ether lipid metabolism were significantly increased in control group.

Correlations between steatohepatitis, CVR, and gut microbiota

The correlations between markers of liver disease progression and severity, CVR factors, cardiomyocyte morphometry and microbiota composition are shown in Table 2. Additional correlations can be found in Supplementary Table 2 (Private

Table 2 Correlation of steatohepatitis, cardiovascular risk, and microbiota composition

Variable[1]		Severity and progression of liver injury				CVR factors and metabolism of lipids						Cardiomyocyte morphometry			Microbiota composition
		Quantification of collagen (picrosirius)	TIMP-1	MCP-1	IL-1β	miR-33a	miR-126	PAI-1	CRI-I	CRI-II	AC	% Normal CAR	Average area of CAR	% Atrophic CAR	
Severity and progression of liver injury	**NAFLD score**	0.879[2]	0.791[2]	0.673[2]	0.347	0.639[2]	-0.777[2]	0.444[3]	0.809[2]	0.820[2]	0.809[2]	-0.519[3]	-0.630[2]	0.721[2]	0.694[2]
	Quantification of collagen (picrosirius)		0.611[2]	0.456[3]	0.752[2]	0.571[3]	-0.683[2]	0.415	0.819[2]	0.821[2]	0.819[2]	-0.205	-0.312	0.238	0.378[2]
	TIMP-1			0.803[2]	0.726[2]	0.728[2]	-0.812[2]	0.535[3]	0.691[2]	0.747[2]	0.691[2]	-0.694[2]	-0.405	0.607[2]	0.539[2]
	MCP-1				0.567[3]	0.492[3]	-0.623[2]	0.336	0.549[3]	0.561[3]	0.549[3]	-0.490[3]	-0.390	0.498[3]	0.232[3]
	IL-1β					0.809[2]	-0.688[3]	0.544[3]	0.645[3]	0.688[2]	0.645[3]	-0.437[3]	-0.393	0.382	0.293[3]
CVR factors and metabolism of lipids	**miR-33a**						-0.655[2]	0.363	0.529[3]	0.603[3]	0.529[3]	-0.704[2]	0.038	0.232	0.160[3]
	miR-126							-0.634[2]	-0.712[2]	-0.730[2]	-0.712[2]	0.459[3]	0.320	-0.364	0.368[2]
	PAI-1								0.487[3]	0.671[2]	0.487[3]	-0.317	0.389	-0.289	0.103
	CRI-I									0.863[2]	1.000[2]	-0.234	-0.459[3]	0.386	0.469[2]
	CRI-II										0.863[2]	-0.399	-0.492[3]	0.551[3]	0.584[2]
	AC											-0.236	-0.457[3]	0.389	0.477[2]
Cardiomyocyte morphometry	**% Normal cardiomyocytes**												0.105	-0.058	
	% Average area of cardiomyocytes													-0.818[2]	
	% Atrophic cardiomyocytes														

[1]Variables were evaluated by Spearman's *r* correlation coefficient: moderate ($0.3 < r < 0.6$), strong ($0.6 < r < 0.9$) or very strong ($0.9 < r < 1.0$).

[2]Correlation significant at the 0.01 level.

[3]Correlation significant at the 0.05 level.

AC: Atherogenic coefficient; CAR: Cardiomyocytes; CRI: Castelli's risk index; CVR: Cardiovascular risk; IL: Interleukin; MCP: Monocyte chemoattractant protein; NAFLD: Nonalcoholic fatty liver disease; PAI: Plasminogen activator inhibitor; TIMP: Tissue inhibitor of metalloproteinase.

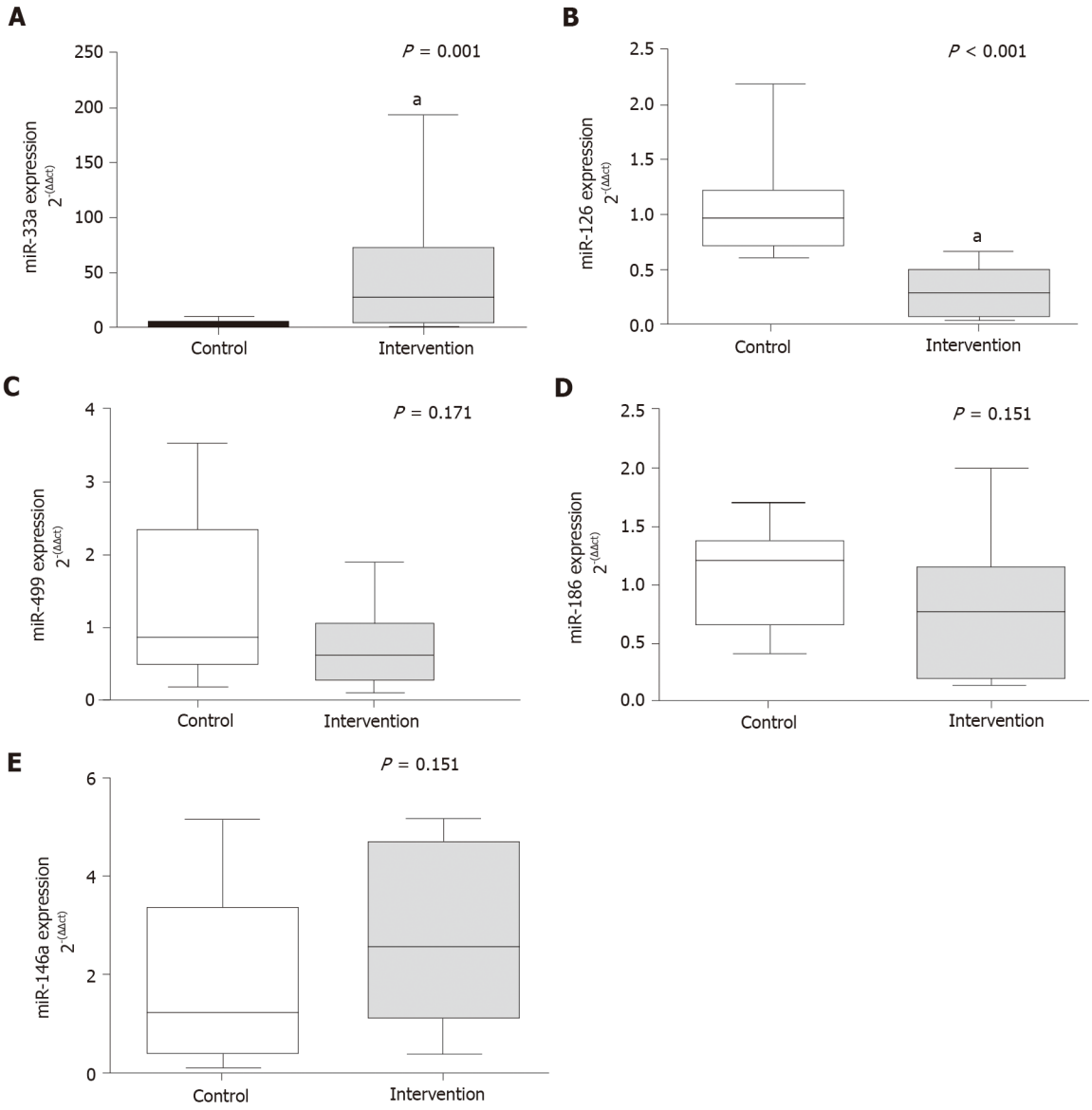


Figure 1 Gene expression of circulating microRNAs. A: miR-33a ($P = 0.001$); B: miR-126 ($P < 0.001$); C: miR-499 ($P = 0.171$); D: miR-186 ($P = 0.151$); E: miR-146a ($P = 0.151$). $^{a}P < 0.05$, Significant effect of the high-fat and choline-deficient diet. Data are medians (25th-75th percentile), Mann-Whitney U test.

sharing link for Figshare data https://figshare.com/s/2d858620da6b13fe2fec). There was a positive correlation between the markers of steatohepatitis severity and progression with CVR factors, such as miR-33a, PAI-1, and atherogenic ratios. Negative correlations were observed for miR-126. Regarding cardiomyocyte morphometry, there were negative correlations between the average area and the percentage of normal cardiomyocytes with the NAFLD score. There was a positive correlation of histopathological NAFLD score with the percentage of atrophic cardiomyocytes, a negative correlation between the percentage of normal cardiomyocytes with MCP-1 and TIMP-1 and a positive correlation of those markers with the percentage of atrophic cardiomyocytes. Furthermore, the average area of cardiomyocytes correlated negatively with atherogenic ratios, CRI-I, CRI-II and AC. miR-33a correlated negatively and miR-126 and positively with the percentage of normal cardiomyocytes.

The composition of the microbiota was positively correlated with markers of liver injury and CVR. The correlation of each family of microorganisms with markers of liver disease progression and severity and CVR factors are shown in Table 3. Significant moderate and strong correlations were observed between nearly all families of bacteria and the hepatic histopathology score, collagen fiber deposition in hepatic tissue, TIMP-1, microRNAs, and atherogenic ratios. Families of interest in the underlying disease including *Bacteroidaceae*, *Clostridiaceae*, *Firmicutes* and *Lactobacillaceae* were correlated with the evaluated markers. No correlation was observed

Table 3 Correlation of gut microbiota at family level, steatohepatitis, and cardiovascular risk factors

Variable[1] (Family)	Severity and progression of liver injury				CVR factors and metabolism of lipids					
	NAFLD score	Quantification of collagen (picrosirius)	TIMP-1	MCP-1	miR-33a	miR-126	PAI-1	CRI-I	CRI-II	AC
Actinomycetaceae					0.584[2]					
Aerococcaceae										
Anaeroplasmataceae		-0.553[2]						-0.614[2]		-0.614[2]
Atopobiaceae	0.627[2]	0.610[2]						0.592[2]	0.663[2]	0.592[2]
Bacillales_unclassified						0.549[2]		-0.548[2]	-0.533[2]	-0.548[2]
Bacteroidaceae	0.836[2]	0.746[2]	0.784[2]		0.689[2]	-0.754[2]		0.662[2]	0.732[2]	0.662[2]
Bacteroidales_unclassified		-0.560[2]							-0.589[2]	-0.492[2]
Burkholderiaceae									0.564[2]	
Clostridiaceae	0.807[2]	0.723[2]	0.645[2]		0.593[2]	-0.669[2]		0.676[2]	0.638[2]	0.676[2]
Clostridiales_unclassified	-0.628[2]	-0.529[2]	-0.535[2]		-0.576[2]				-0.586[2]	-0.525[2]
Clostridiales_vadinBB60	-0.602[2]	-0.671[2]	-0.527[2]		-0.558[2]	0.524[2]		-0.626[2]	-0.502[2]	-0.626[2]
Corynebacteriaceae	-0.669[2]	-0.545[2]	-0.680[2]		-0.782[2]	0.611[2]		-0.571[2]	-0.622[2]	-0.571[2]
Desulfovibrionaceae	-0.806[2]	-0.603[2]	-0.872[2]	-0.776[2]	-0.631[2]	0.755[2]		-0.729[2]	-0.746[2]	-0.729[2]
Eggerthellaceae									0.490[2]	
Firmicutes_unclassified	-0.797[2]	-0.637[2]	-0.687[2]		-0.655[2]	0.594[2]		-0.629[2]	-0.699[2]	-0.629[2]
Gastranaerophilales	-0.822[2]	-0.656[2]	-0.644[2]		-0.643[2]	0.657[2]		-0.698[2]	-0.586[2]	-0.698[2]
Lachnospiraceae	-0.850[2]	-0.653[2]	-0.789[2]	-0.788[2]	-0.613[2]	0.766[2]		-0.643[2]	-0.629[2]	-0.643[2]
Lactobacillaceae	-0.616[2]	-0.633[2]				0.795[2]			-0.529[2]	
Lactobacillales_unclassified										
Micrococcaceae	0.669[2]		0.534[2]			-0.528[2]			0.493[2]	
Mollicutes_RF39_fa	-0.650[2]	-0.618[2]	-0.590[2]		-0.609[2]	0.713[2]		-0.857[2]	-0.768[2]	-0.857[2]
Moraxellaceae	-0.669[2]	-0.536[2]	-0.557[2]		-0.543[2]			-0.599[2]	-0.473[2]	-0.599[2]
Muribaculaceae	-0.816[2]	-0.794[2]			-0.576[2]	0.693[2]	-0.684[2]	-0.827[2]	-0.846[2]	-0.827[2]
Pasteurellaceae										
Prevotellaceae		-0.705[2]				0.603[2]			-0.522[2]	-0.486[2]

Rikenellaceae				-0.679[2]				
Saccharimonadaceae	-0.737[2]	-0.559[2]	-0.619[2]	-0.674[2]	0.656[2]	-0.776[2]	-0.759[2]	-0.776[2]
Staphylococcaceae	-0.734[2]	-0.647[2]	-0.808[2]	-0.838[2]	0.716[2]	-0.616[2]	-0.679[2]	-0.616[2]
Streptococcaceae	0.790[2]	0.726[2]	0.637[2]	0.595[2]	-0.622[2]		0.724[2]	0.515[2]

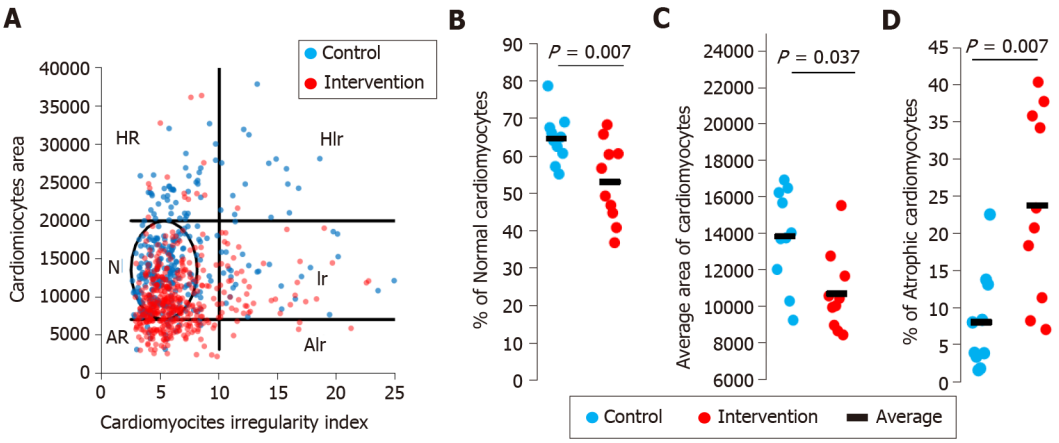
[1]Variables were evaluated by Spearman's *r* correlation coefficient, moderate ($0.3 < r < 0.6$) or strong ($0.6 < r < 0.9$).
[2]Correlation significant at the 0.05 level.
AC: Atherogenic coefficient; CRI: Castelli's risk index; CVR: Cardiovascular risk; MCP: Monocyte chemoattractant protein; NAFLD: Nonalcoholic fatty liver disease; PAI: Plasminogen activator inhibitor; TIMP: Tissue inhibitor of metalloproteinase.

between families of gut microbiota and measurements of cardiomyocyte morphometry.

DISCUSSION

Steatohepatitis and CVD are both associated with metabolic risk factors, including glucose abnormalities, dyslipidemia, chronic inflammation, endothelial dysfunction, and gut dysbiosis. The relationship is recognized in the clinical setting, but the links among steatohepatitis, CVD, and gut dysbiosis needs to be better understood. This study provided evidence of the role of MAFLD as an adjuvant risk factor for the development of CVD. We found that dysbiotic bacteria and their metabolites were translocated to the liver through the ruptured intestinal barrier, causing impaired hepatic triglyceride metabolism, inflammatory responses, and fibrogenesis, which are necessary for the development and progression of MAFLD[11]. We also found significant correlations between the activation of pathophysiological pathways that link MAFLD and increased risk of developing cardiovascular events, such as atherogenic dyslipidemia, systemic inflammation, endothelial dysfunction, gut dysbiosis, and changes in cardiomyocyte morphometry. In this study, the significant associations between steatohepatitis and CVR, justify the screening of MAFLD and its associated risk factors in high-risk patients, in order to intervene effectively, with a focus on new approaches aimed at directing the composition of the intestinal microbiota as a potential therapeutic target.

In a recent publication, we reported that the experimental nutritional model developed in this study is capable of causing marked deposition of body and liver fat, changes in biochemical parameters, activation of microRNAs, receptors, mediators, and inflammatory cytokines, an increase in intestinal permeability, and hepatic histopathological changes, similar to steatohepatitis in humans[11]. This robust experimental model of steatohepatitis of metabolic origin allows evaluating pathophysiological mechanisms related to the development of CVD in MAFLD. We



E

% Normal cardiomyocytes	TIMP-1	IL-1β	Myd88	IL-6 (liver)	IL-1β/IL-10 (liver)
< Median	13.5	3.9	7.3	52914	0.52
> Median	11.2	1.1	2.9	45436	0.64
T Test	0.05	0.04	0.04	0.02	0.01

F

% Atrophic cardiomyocytes	IL-1β (liver)
< Median	2560
> Median	3309
T Test	0.003

G

Average area	TNF-α/IL-10 (liver)
< Median	2.3
> Median	3.4
T Test	0.028

Figure 2 Cardiomyocytes morphometric analysis. The area and cross-sectional shape of cardiomyocytes were determined from images of hematoxylin and eosin-stained tissue. A: Dot plot of cardiomyocyte area *vs* cardiomyocyte irregularity index in control (blue) and intervention (red) groups. Each dot represents a population of cardiomyocytes with different morphometry. N–normal area and shape, Ir–normal area and irregular shape, HR–hypertrophic and regular cardiomyocytes, HIr–hypertrophic and irregular cardiomyocytes, AR–atrophic and regular cardiomyocytes, AIr–atrophic and irregular cardiomyocyte; B: Average area of cardiomyocytes; C: Percentage of normal cardiomyocytes; D: Percentage of atrophic cardiomyocytes; E-G: We segregated the animals in the intervention group into two subgroups and the data were compared. IL: Interleukin; TNF: Tumor necrosis factor.

demonstrated that abnormalities of lipid metabolism and atherogenic ratios were related to greater propensity to develop CVD associated with steatohepatitis. The results are consistent with other experimental and clinical studies[7,15-18]. In addition, we report a significant increase of systemic markers of inflammation and endothelial dysfunction in animals with steatohepatitis. The worsening of the inflammatory state in MAFLD is associated with worse cardiometabolic outcomes. PAI-1 is a marker of endothelial dysfunction, being released in response to low-grade inflammation, free fatty acids, and atherogenic lipoproteins[19,20]. A previous study reporting that an increase in PAI-1 was correlated with the histological severity of MAFLD and alterations in the lipid profile, promoting a more atherogenic phenotype[21]. PAI-1 also plays a vital role in liver fibrosis, promoting increased deposition of extracellular matrix in liver tissue, in which TIMP-1 performs a similar function[22]. In that sense, liver fibrosis can lead to severe hepatic dysfunction and even life-threatening conditions such as liver cirrhosis and HCC. The mechanism of liver fibrosis is multifaceted and, in this study, animals with steatohepatitis had an increase in TIMP-1 concentration and deposition of collagen fibers in liver tissue, markers that significantly correlated with increased CVR.

Assessment of microRNAs has been used for the early detection and monitoring of the progression of MAFLD, and to assess clinical and subclinical CVD. miR-33a inhibits genes involved in high-density lipoprotein synthesis and the reverse transport of cholesterol[23,24]. In this study, animals with steatohepatitis had a significant increase in miR-33a expression that was positively correlated with atherogenic ratios and markers of severity and progression of liver injury. miR-126 expression, which is high in endothelial cells and regulates the migration of inflammatory cells, formation of capillary networks, and cell survival[25], was decreased in animals with steatohepatitis. In fact, there was an inverse correlation between miR-126 expression and


A
Shannon index
4.0
3.5
3.0
B
Control
Intervention
Axis.2 [12.6%]
0.8
0.4
0
-0.4
-0.8
-0.50
-0.25
0.00
0.25
0.50
Axis.1 [45.7%]
C
Control
Intervention
Relative abundance
1.00
0.75
0.50
0.25
0.00
R01 R02 R03 R04 R05 R06 R07 R08 R09 R10
R11 R12 R13 R14 R15 R16 R17 R18 R19 R20
Family
Bacteroidaceae
Muribaculaceae
Lachnospiraceae
Ruminococcaceae
Peptococcaceae
Others
Peptostreptococcaceae
Rikenellaceae
Erysipelotrichaceae
Clostridiaceae_1
D
Control
Bacteroidaceae
Ruminococcaceae
Peptostreptococcaceae
Peptococcaceae
Erysipelotrichaceae
Clostridiaceae_1
Burkholderiaceae
Streptococcaceae
Tannerellaceae
Christensenellaceae
Desulfovibrionaceae
Gastranaerophilales_fa
Lachnospiraceae
Intervention
-5
-4
-3
-2
-1
0
1
2
3
4
5
LDA score

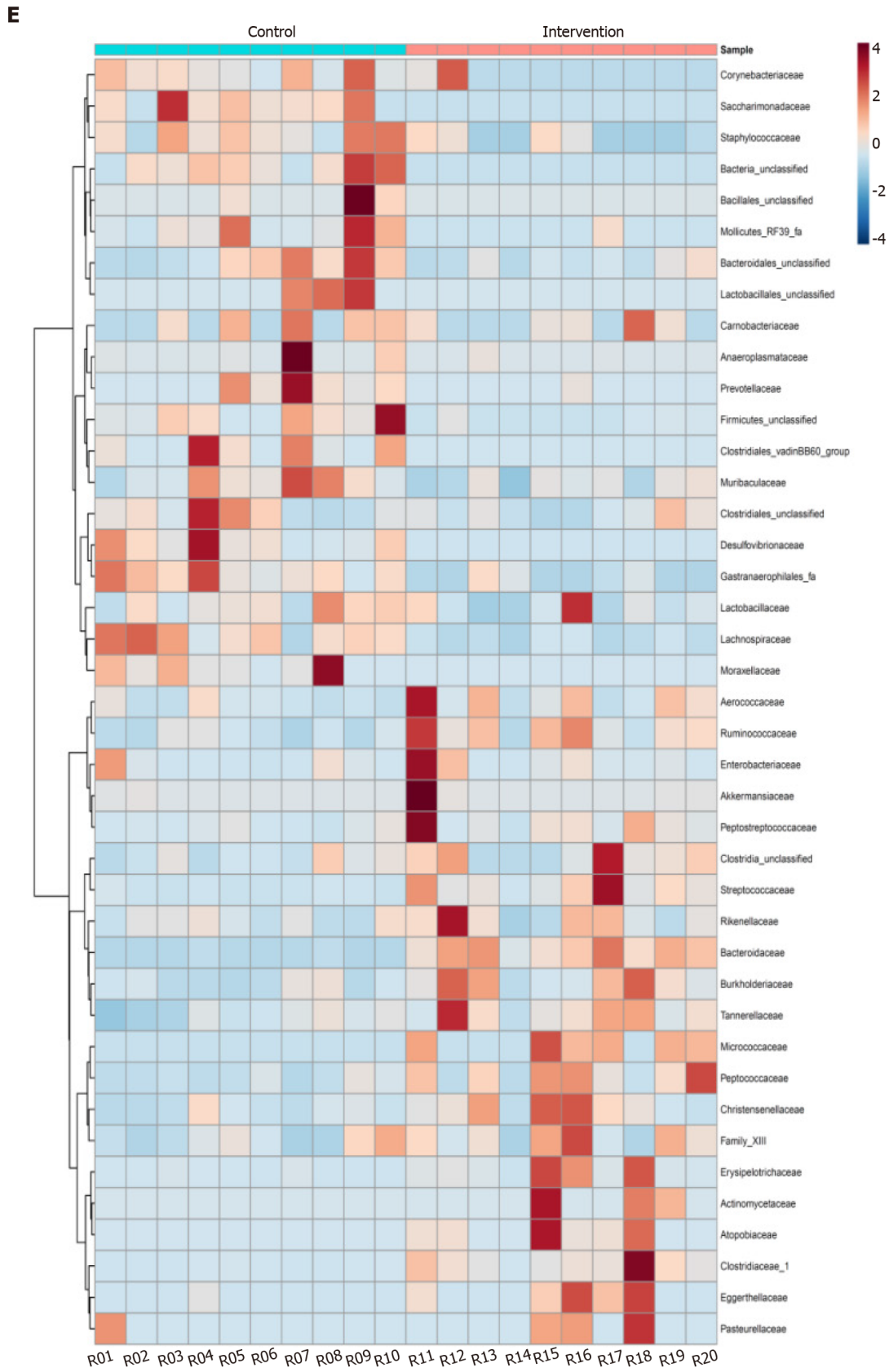


Figure 3 Gut microbiota changes in intervention and control groups. A: Shannon diversity index; B: Principal coordinate analysis based on Bray-Curtis distance metric; C: Relative abundance of gut microbiota at the family level; D: Differential abundance by linear discriminant analysis; E: Heatmap distribution of the 41 families among the samples. LDA: Linear discriminant analysis.

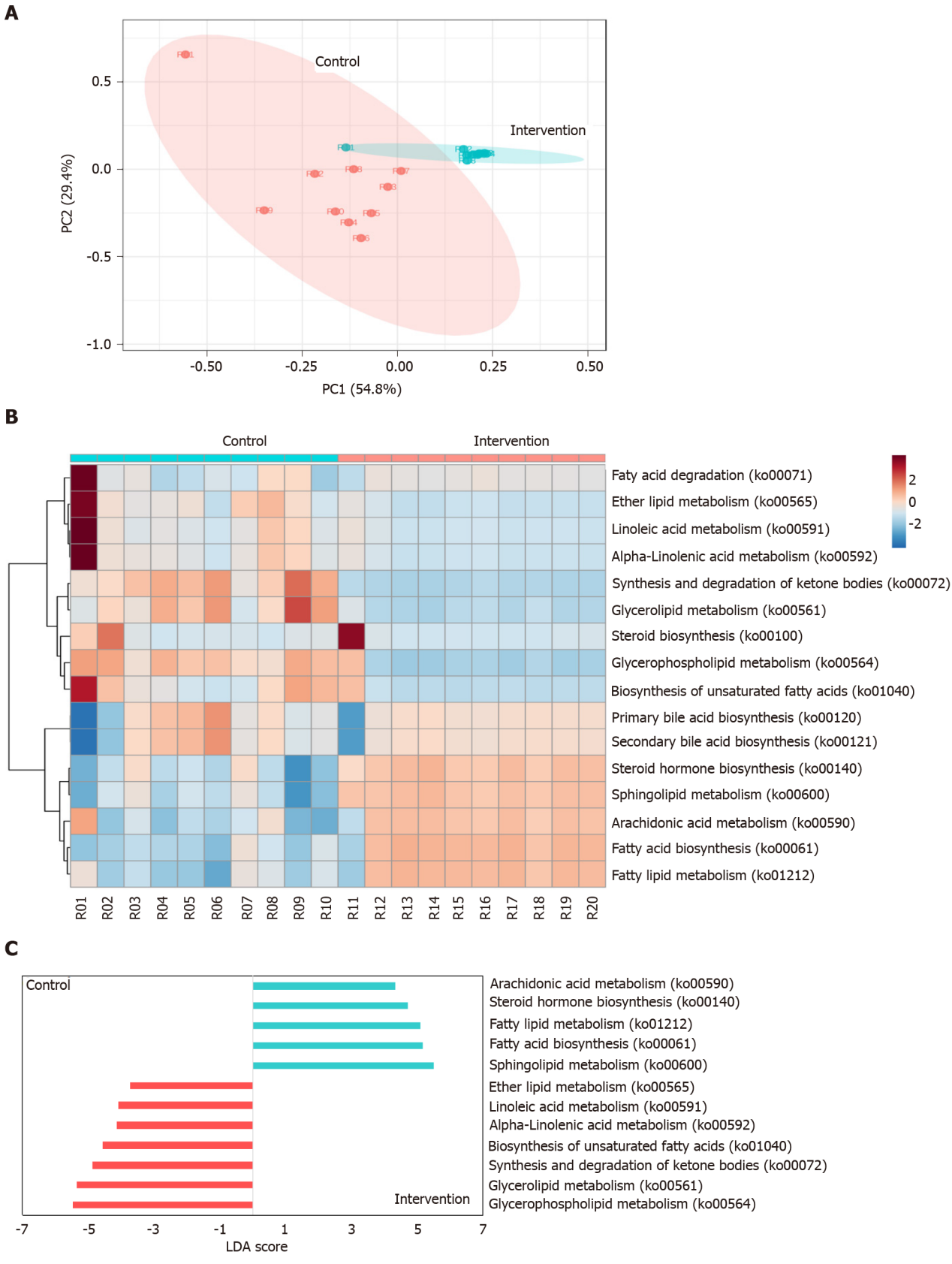


Figure 4 Sixteen predicted functional Kyoto Encyclopedia of Genes and Genomes lipid metabolism pathways in intervention and control group. A: Principal coordinate analysis; B: Heatmap distribution; C: Linear discriminant analysis (LDA) of the 16 differentially abundant KEGG lipid metabolism pathways.

atherogenic ratios, endothelial dysfunction, inflammation, fibrogenesis, and severity of liver injury. As established in the literature, microRNAs act in the epigenetic regulation of intricate processes[24,25]. In this study, we clearly demonstrated that the

expression of miR-33a and miR-126 was involved in the regulation of cholesterol, lipid metabolism, and endothelial dysfunction, and contributed to the development of metabolic disorders and CVD related to steatohepatitis.

The morphometric evaluation of cardiomyocytes was an interesting and innovative analysis in this study, and it found that animals with steatohepatitis had a significant decreases in the percentage of cardiomyocytes with a normal appearance and the mean area of cardiomyocytes relative to the control group. In addition, animals with steatohepatitis had a significant increase in the percentage of atrophic cardiomyocytes. To the best of our knowledge, morphometric analysis of cardiomyocytes in MAFLD has not been previously reported, which makes it difficult to discuss the data obtained. Several cellular processes can be inferred through morphometric analysis, and the method can be used in the diagnosis and prognosis of some clinical conditions[14,26,27]. In this study, we reported that the percentage of normal cardiomyocytes was negatively correlated with the histological severity of liver damage, fibrogenesis, and inflammation. Furthermore, the percentage of atrophic cardiomyocytes correlated positively with the liver injury markers. Clinical manifestations of MAFLD, such as steatosis and inflammation, are additional risk factors for the development of CVD[3,9]. However, the exact mechanisms for this complex relationship are unclear[3,9]. It is likely that several highly interrelated factors contribute to the increase of CVR in steatohepatitis and changes in the morphometry of cardiomyocytes. However, more studies are needed to evaluate the morphometry of cardiomyocytes in more advanced stages of MAFLD.

The "multiple parallel hits" hypothesis highlights the importance of the gut microbiota and seems to provide a more accurate explanation of the pathogenesis of steatohepatitis and its contribution to the increase in CVR[3,10]. The liver is closely related to the intestine both anatomically and functionally, and recent evidence demonstrates that the type and quantity of intestinal microorganisms determine important characteristics related to the pathogenesis and progression of these clinical conditions[28-30]. Our data corroborate with experimental and clinical studies reporting that the development and progression of MAFLD is associated with a significant decrease in the diversity and structure of the bacterial communities of the gut microbiota[29,31,32]. In this study, we report an increase in the abundance of family *Bacteroidaceae* and a decrease in the abundance of *Prevotellaceae* in animals with steatohepatitis. It is known that the diet directly influences the composition of the gut microbiota. Western diets abundant in fat, animal protein, and sugar have been associated with steatohepatitis and increased risk of CVD. That diet favors the abundance of family *Bacteroidaceae*; while diets high in fiber, starch, and plant polysaccharides promote the abundance of family *Prevotellaceae*[30,33,34]. In this study, we report an increase in the abundance of family *Bacteroidaceae* and a decrease in the abundance of *Prevotellaceae* in animals with steatohepatitis, which is consistent with another study[30]. Regarding the increase in the relative abundance of family *Ruminococcacea* observed in the animals of the intervention group, a previous report that demonstrated the *Ruminococcus increased* in more severe disease, especially if advanced hepatic fibrosis was diagnosed. The decrease in its abundance has also been reported in lean steatohepatitis patients[30,35]. There are reports that associate the abundance of *Ruminococcaceae* with the development of CVD[36,37]. However, we found no correlations between the presence of *Ruminococcaceae* and the CVR markers that were assessed in this study. Genus *Ruminococcus* is quite heterogeneous, including both beneficial and deleterious bacteria, making data discussion difficult. Family *Ruminococcaceae* is associated with aerobic fermentation that leads to the production of short chain fatty acids and alcohol, and this can have detrimental effects on intestinal permeability and hepatic inflammation[30,35].

Some of the metabolites produced by gut flora are already biologically active, whereas others are further metabolized by the host, generating secondary mediators that influence the microbiota-host interaction. In this study, we predicted the lipid metabolic pathways that were expressed as a result of the gut dysbiosis observed in steatohepatitis. Animals with steatohepatitis had a significant increase in sphingolipid metabolism. The sphingolipids are membrane lipids that participate in cell division, differentiation, gene expression, and apoptosis. The study data corroborate emerging evidence that support the role of sphingolipids in hepatocellular death, which contributes to the progression of MAFLD[38]. Additionally, there are reports that dysregulation of circulating sphingolipids was independently associated with CVD and subclinical atherosclerosis[39,40]. In this study, arachidonic acid metabolism was significantly increased in animals with steatohepatitis. In addition, a significant decrease in linoleic acid metabolism was reported in this experimental group. Arachidonic acid is synthesized from polyunsaturated fatty acids, and can be derived

from linoleic acid, which is an essential fatty acid[41]. The products resulting from arachidonic acid metabolism are linked to the inflammation and vasodilation of MAFLD and CVD, mainly by the action of the enzyme cyclooxygenase[41,42]. Therefore, as reported in this study, an increase in arachidonic acid metabolism in steatohepatitis and CVD is expected. We report an increase in glycerophospholipid metabolism in animals in the control group. As described by Schnabl and Brenner[43], a high-fat diet causes the gut microbiota to convert choline in the diet to methylamines, consequently reducing the plasma levels of phosphatidylcholine, which is a glycerophospholipid. Phosphatidylcholine is an important constituent of the cell membrane of very low density lipoproteins. Without its presence triglycerides cannot attach to the lipoprotein and start to accumulate in the liver tissue, causing MAFLD [43]. In parallel, there were increases in plasma trimethylamine, and its hepatic metabolism to trimethylamine-N-oxide has been associated with the appearance of CVD. This compound is considered harmful, as it changes the way cholesterol and steroids are metabolized and inhibits the reverse transport of cholesterol, causing the accumulation of fat on the internal walls of arteries[44,45]. Therefore, in this study, the predicted lipid metabolism in animals with steatohepatitis did not include expression of glycerophospholipid metabolism, probably because of the action of the gut microbiota in the metabolic pathway.

CONCLUSION

In summary, it is known that steatohepatitis and CVD have many risk factors in common. Among those, we report significant correlations between the presence of atherogenic dyslipidemia, systemic inflammation, endothelial dysfunction, liver fibrogenesis, and gut dysbiosis, all of which contribute to the progression of MAFLD and increased CVR. In addition, we infer, through the composition of the gut microbiota, which lipid metabolism pathways are activated in animals with steatohepatitis and their relationship with CVR. Subsequent metabolomic studies may aid in elucidating the influence of gut microbial function with the development of cardiometabolic disorders related to steatohepatitis. The gut microbiota may be a potential therapeutic target for both clinical conditions.

ARTICLE HIGHLIGHTS

Research background

Metabolic-associated fatty liver disease (MAFLD), in addition to being a progressive liver disease, is an independent and significant risk factor for the development of cardiovascular disease, and dysbiosis of the intestinal microbiota is associated with both.

Research motivation

The motivation was to explore the mechanisms whereby gut microbiota contribute to steatohepatitis-associated increased cardiovascular risk.

Research objectives

The objective was to assess the relationship between gut dysbiosis and cardiovascular risk in an experimental model of steatohepatitis.

Research methods

Adult male Sprague-Dawley rats were randomized to a control group given a standard diet or an intervention of a high-fat and choline-deficient diet for 16 wk of ten animals each. Biochemical, molecular, hepatic, and cardiac histopathology and gut microbiota variables were evaluated.

Research results

We reported significant correlations between the presence of atherogenic dyslipidemia, systemic inflammation, endothelial dysfunction, liver fibrogenesis and gut dysbiosis, all of which contributed to the progression of MAFLD and increased CVR.

Research conclusions

This study shows that there is a link between gut dysbiosis and significant cardiomyocyte abnormalities in animals with steatohepatitis.

Research perspectives

Metabolomic studies may aid in elucidating the association of gut microbial function with the development of cardiometabolic disorders related to steatohepatitis. The gut microbiota may be a potential therapeutic target for both clinical conditions.

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REFERENCES

1 **Younossi Z**, Anstee QM, Marietti M, Hardy T, Henry L, Eslam M, George J, Bugianesi E. Global burden of NAFLD and NASH: trends, predictions, risk factors and prevention. *Nat Rev Gastroenterol Hepatol* 2018; **15**: 11-20 [PMID: 28930295 DOI: 10.1038/nrgastro.2017.109]

2 **Estes C**, Razavi H, Loomba R, Younossi Z, Sanyal AJ. Modeling the epidemic of nonalcoholic fatty liver disease demonstrates an exponential increase in burden of disease. *Hepatology* 2018; **67**: 123-133 [PMID: 28802062 DOI: 10.1002/hep.29466]

3 **El Hadi H**, Di Vincenzo A, Vettor R, Rossato M. Cardio-Metabolic Disorders in Non-Alcoholic Fatty Liver Disease. *Int J Mol Sci* 2019; **20** [PMID: 31064058 DOI: 10.3390/ijms20092215]

4 **Anstee QM**, Mantovani A, Tilg H, Targher G. Risk of cardiomyopathy and cardiac arrhythmias in patients with nonalcoholic fatty liver disease. *Nat Rev Gastroenterol Hepatol* 2018; **15**: 425-439 [PMID: 29713021 DOI: 10.1038/s41575-018-0010-0]

5 **Eslam M**, Sanyal AJ, George J; International Consensus Panel. MAFLD: A Consensus-Driven Proposed Nomenclature for Metabolic Associated Fatty Liver Disease. *Gastroenterology* 2020; **158**: 1999-2014.e1 [PMID: 32044314 DOI: 10.1053/j.gastro.2019.11.312]

6 **Eslam M**, Newsome PN, Sarin SK, Anstee QM, Targher G, Romero-Gomez M, Zelber-Sagi S, Wai-Sun Wong V, Dufour JF, Schattenberg JM, Kawaguchi T, Arrese M, Valenti L, Shiha G, Tiribelli C, Yki-Järvinen H, Fan JG, Grønbæk H, Yilmaz Y, Cortez-Pinto H, Oliveira CP, Bedossa P, Adams LA, Zheng MH, Fouad Y, Chan WK, Mendez-Sanchez N, Ahn SH, Castera L, Bugianesi E, Ratziu V, George J. A new definition for metabolic dysfunction-associated fatty liver disease: An international expert consensus statement. *J Hepatol* 2020; **73**: 202-209 [PMID: 32278004 DOI: 10.1016/j.jhep.2020.03.039]

7 **Choudhary NS**, Duseja A. Screening of Cardiovascular Disease in Nonalcoholic Fatty Liver Disease: Whom and How? *J Clin Exp Hepatol* 2019; **9**: 506-514 [PMID: 31516267 DOI: 10.1016/j.jceh.2019.02.005]

8 **Niederseer D**, Wernly S, Bachmayer S, Wernly B, Bakula A, Huber-Schönauer U, Semmler G, Schmied C, Aigner E, Datz C. Diagnosis of Non-Alcoholic Fatty Liver Disease (NAFLD) Is Independently Associated with Cardiovascular Risk in a Large Austrian Screening Cohort. *J Clin Med* 2020; **9** [PMID: 32283679 DOI: 10.3390/jcm9041065]

9 **Francque SM**, van der Graaff D, Kwanten WJ. Non-alcoholic fatty liver disease and cardiovascular risk: Pathophysiological mechanisms and implications. *J Hepatol* 2016; **65**: 425-443 [PMID: 27091791 DOI: 10.1016/j.jhep.2016.04.005]

10 **Ji Y**, Yin Y, Li Z, Zhang W. Gut Microbiota-Derived Components and Metabolites in the Progression of Non-Alcoholic Fatty Liver Disease (NAFLD). *Nutrients* 2019; **11** [PMID: 31349604 DOI: 10.3390/nu11081712]

11 **Longo L**, Tonin Ferrari J, Rampelotto PH, Hirata Dellavia G, Pasqualotto A, P Oliveira C, Thadeu Schmidt Cerski C, Reverbel da Silveira T, Uribe-Cruz C, Álvares-da-Silva MR. Gut Dysbiosis and Increased Intestinal Permeability Drive microRNAs, NLRP-3 Inflammasome and Liver Fibrosis in a Nutritional Model of Non-Alcoholic Steatohepatitis in Adult Male Sprague Dawley Rats. *Clin Exp Gastroenterol* 2020; **13**: 351-368 [PMID: 32982365 DOI: 10.2147/CEG.S262879]

12 **Sujatha R**, Kavitha S. Atherogenic indices in stroke patients: A retrospective study. *Iran J Neurol* 2017; **16**: 78-82 [PMID: 28761629]

13 **Liang W**, Menke AL, Driessen A, Koek GH, Lindeman JH, Stoop R, Havekes LM, Kleemann R, van den Hoek AM. Establishment of a general NAFLD scoring system for rodent models and comparison to human liver pathology. *PLoS One* 2014; **9**: e115922 [PMID: 25535951 DOI: 10.1371/journal.pone.0115922]

14 **Filippi-Chiela EC**, Oliveira MM, Jurkovski B, Callegari-Jacques SM, da Silva VD, Lenz G. Nuclear morphometric analysis (NMA): screening of senescence, apoptosis and nuclear irregularities. *PLoS*

One 2012; **7**: e42522 [PMID: 22905142 DOI: 10.1371/journal.pone.0042522]
15 **Siddiqui MS**, Fuchs M, Idowu MO, Luketic VA, Boyett S, Sargeant C, Stravitz RT, Puri P, Matherly S, Sterling RK, Contos M, Sanyal AJ. Severity of nonalcoholic fatty liver disease and progression to cirrhosis are associated with atherogenic lipoprotein profile. *Clin Gastroenterol Hepatol* 2015; **13**: 1000-8.e3 [PMID: 25311381 DOI: 10.1016/j.cgh.2014.10.008]
16 **Targher G**, Lonardo A, Byrne CD. Nonalcoholic fatty liver disease and chronic vascular complications of diabetes mellitus. *Nat Rev Endocrinol* 2018; **14**: 99-114 [PMID: 29286050 DOI: 10.1038/nrendo.2017.173]
17 **Lee HS**, Nam Y, Chung YH, Kim HR, Park ES, Chung SJ, Kim JH, Sohn UD, Kim HC, Oh KW, Jeong JH. Beneficial effects of phosphatidylcholine on high-fat diet-induced obesity, hyperlipidemia and fatty liver in mice. *Life Sci* 2014; **118**: 7-14 [PMID: 25445436 DOI: 10.1016/j.lfs.2014.09.027]
18 **Taher J**, Baker C, Alvares D, Ijaz L, Hussain M, Adeli K. GLP-2 Dysregulates Hepatic Lipoprotein Metabolism, Inducing Fatty Liver and VLDL Overproduction in Male Hamsters and Mice. *Endocrinology* 2018; **159**: 3340-3350 [PMID: 30052880 DOI: 10.1210/en.2018-00416]
19 **Kodaman N**, Aldrich MC, Sobota R, Asselbergs FW, Brown NJ, Moore JH, Williams SM. Plasminogen Activator Inhibitor-1 and Diagnosis of the Metabolic Syndrome in a West African Population. *J Am Heart Assoc* 2016; **5** [PMID: 27697752 DOI: 10.1161/JAHA.116.003867]
20 **Chang ML**, Lin YS, Pao LH, Huang HC, Chiu CT. Link between plasminogen activator inhibitor-1 and cardiovascular risk in chronic hepatitis C after viral clearance. *Sci Rep* 2017; **7**: 42503 [PMID: 28211910 DOI: 10.1038/srep42503]
21 **Jin R**, Krasinskas A, Le NA, Konomi JV, Holzberg J, Romero R, Vos MB. Association between plasminogen activator inhibitor-1 and severity of liver injury and cardiovascular risk in children with non-alcoholic fatty liver disease. *Pediatr Obes* 2018; **13**: 23-29 [PMID: 27764892 DOI: 10.1111/ijpo.12183]
22 **Barrera F**, George J. Prothrombotic factors and nonalcoholic fatty liver disease: an additional link to cardiovascular risk? *Hepatology* 2014; **59**: 16-18 [PMID: 23787943 DOI: 10.1002/hep.26588]
23 **Horie T**, Nishino T, Baba O, Kuwabara Y, Nakao T, Nishiga M, Usami S, Izuhara M, Sowa N, Yahagi N, Shimano H, Matsumura S, Inoue K, Marusawa H, Nakamura T, Hasegawa K, Kume N, Yokode M, Kita T, Kimura T, Ono K. MicroRNA-33 regulates sterol regulatory element-binding protein 1 expression in mice. *Nat Commun* 2013; **4**: 2883 [PMID: 24300912 DOI: 10.1038/ncomms3883]
24 **Koyama S**, Horie T, Nishino T, Baba O, Sowa N, Miyasaka Y, Kuwabara Y, Nakao T, Nishiga M, Nishi H, Nakashima Y, Nakazeki F, Ide Y, Kimura M, Tsuji S, Ruiz Rodriguez R, Xu S, Yamasaki T, Otani C, Watanabe T, Nakamura T, Hasegawa K, Kimura T, Ono K. Identification of Differential Roles of MicroRNA-33a and -33b During Atherosclerosis Progression With Genetically Modified Mice. *J Am Heart Assoc* 2019; **8**: e012609 [PMID: 31242815 DOI: 10.1161/JAHA.119.012609]
25 **Alique M**, Bodega G, Giannarelli C, Carracedo J, Ramírez R. MicroRNA-126 regulates Hypoxia-Inducible Factor-1α which inhibited migration, proliferation, and angiogenesis in replicative endothelial senescence. *Sci Rep* 2019; **9**: 7381 [PMID: 31089163 DOI: 10.1038/s41598-019-43689-3]
26 **Beck AH**, Sangoi AR, Leung S, Marinelli RJ, Nielsen TO, van de Vijver MJ, West RB, van de Rijn M, Koller D. Systematic analysis of breast cancer morphology uncovers stromal features associated with survival. *Sci Transl Med* 2011; **3**: 108ra113 [PMID: 22072638 DOI: 10.1126/scitranslmed.3002564]
27 **Bugrova ML**, Abrosimov DA, Ermolin IL. Ultrastructural Morphological Characterization of Right Atrial and Left Ventricular Rat Cardiomyocytes during Postreperfusion Period. *Bull Exp Biol Med* 2017; **163**: 805-808 [PMID: 29063323 DOI: 10.1007/s10517-017-3908-6]
28 **Lim S**, Taskinen MR, Borén J. Crosstalk between nonalcoholic fatty liver disease and cardiometabolic syndrome. *Obes Rev* 2019; **20**: 599-611 [PMID: 30589487 DOI: 10.1111/obr.12820]
29 **Ezzaidi N**, Zhang X, Coker OO, Yu J. New insights and therapeutic implication of gut microbiota in non-alcoholic fatty liver disease and its associated liver cancer. *Cancer Lett* 2019; **459**: 186-191 [PMID: 31185249 DOI: 10.1016/j.canlet.2019.114425]
30 **Boursier J**, Mueller O, Barret M, Machado M, Fizanne L, Araujo-Perez F, Guy CD, Seed PC, Rawls JF, David LA, Hunault G, Oberti F, Calès P, Diehl AM. The severity of nonalcoholic fatty liver disease is associated with gut dysbiosis and shift in the metabolic function of the gut microbiota. *Hepatology* 2016; **63**: 764-775 [PMID: 26600078 DOI: 10.1002/hep.28356]
31 **Gómez-Zorita S**, Aguirre L, Milton-Laskibar I, Fernández-Quintela A, Trepiana J, Kajarabille N, Mosqueda-Solís A, González M, Portillo MP. Relationship between Changes in Microbiota and Liver Steatosis Induced by High-Fat Feeding-A Review of Rodent Models. *Nutrients* 2019; **11** [PMID: 31505802 DOI: 10.3390/nu11092156]
32 **Demir M**, Lang S, Martin A, Farowski F, Wisplinghoff H, Vehreschild MJGT, Krawczyk M, Nowag A, Scholz CJ, Kretzschmar A, Roderburg C, Lammert F, Goeser T, Kasper P, Steffen HM. Phenotyping non-alcoholic fatty liver disease by the gut microbiota: Ready for prime time? *J Gastroenterol Hepatol* 2020; **35**: 1969-1977 [PMID: 32267559 DOI: 10.1111/jgh.15071]
33 **De Filippo C**, Cavalieri D, Di Paola M, Ramazzotti M, Poullet JB, Massart S, Collini S, Pieraccini G, Lionetti P. Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa. *Proc Natl Acad Sci U S A* 2010; **107**: 14691-14696 [PMID: 20679230 DOI: 10.1073/pnas.1005963107]
34 **Yatsunenko T**, Rey FE, Manary MJ, Trehan I, Dominguez-Bello MG, Contreras M, Magris M, Hidalgo G, Baldassano RN, Anokhin AP, Heath AC, Warner B, Reeder J, Kuczynski J, Caporaso JG,

Lozupone CA, Lauber C, Clemente JC, Knights D, Knight R, Gordon JI. Human gut microbiome viewed across age and geography. *Nature* 2012; **486**: 222-227 [PMID: 22699611 DOI: 10.1038/nature11053]

35 **Duarte SMB**, Stefano JT, Miele L, Ponziani FR, Souza-Basqueira M, Okada LSRR, de Barros Costa FG, Toda K, Mazo DFC, Sabino EC, Carrilho FJ, Gasbarrini A, Oliveira CP. Gut microbiome composition in lean patients with NASH is associated with liver damage independent of caloric intake: A prospective pilot study. *Nutr Metab Cardiovasc Dis* 2018; **28**: 369-384 [PMID: 29482963 DOI: 10.1016/j.numecd.2017.10.014]

36 **Kurilshikov A**, van den Munckhof ICL, Chen L, Bonder MJ, Schraa K, Rutten JHW, Riksen NP, de Graaf J, Oosting M, Sanna S, Joosten LAB, van der Graaf M, Brand T, Koonen DPY, van Faassen M; LifeLines DEEP Cohort Study, BBMRI Metabolomics Consortium, Slagboom PE, Xavier RJ, Kuipers F, Hofker MH, Wijmenga C, Netea MG, Zhernakova A, Fu J. Gut Microbial Associations to Plasma Metabolites Linked to Cardiovascular Phenotypes and Risk. *Circ Res* 2019; **124**: 1808-1820 [PMID: 30971183 DOI: 10.1161/CIRCRESAHA.118.314642]

37 **Toya T**, Corban MT, Marrietta E, Horwath IE, Lerman LO, Murray JA, Lerman A. Coronary artery disease is associated with an altered gut microbiome composition. *PLoS One* 2020; **15**: e0227147 [PMID: 31995569 DOI: 10.1371/journal.pone.0227147]

38 **Yue F**, Xia K, Wei L, Xing L, Wu S, Shi Y, Lam SM, Shui G, Xiang X, Russell R, Zhang D. Effects of constant light exposure on sphingolipidomics and progression of NASH in high-fat-fed rats. *J Gastroenterol Hepatol* 2020; **35**: 1978-1989 [PMID: 32027419 DOI: 10.1111/jgh.15005]

39 **Jiang XC**, Paultre F, Pearson TA, Reed RG, Francis CK, Lin M, Berglund L, Tall AR. Plasma sphingomyelin level as a risk factor for coronary artery disease. *Arterioscler Thromb Vasc Biol* 2000; **20**: 2614-2618 [PMID: 11116061 DOI: 10.1161/01.atv.20.12.2614]

40 **Nelson JC**, Jiang XC, Tabas I, Tall A, Shea S. Plasma sphingomyelin and subclinical atherosclerosis: findings from the multi-ethnic study of atherosclerosis. *Am J Epidemiol* 2006; **163**: 903-912 [PMID: 16611667 DOI: 10.1093/aje/kwj140]

41 **Farrell GC**, Haczeyni F, Chitturi S. Pathogenesis of NASH: How Metabolic Complications of Overnutrition Favour Lipotoxicity and Pro-Inflammatory Fatty Liver Disease. *Adv Exp Med Biol* 2018; **1061**: 19-44 [PMID: 29956204 DOI: 10.1007/978-981-10-8684-7_3]

42 **Futosi K**, Fodor S, Mócsai A. Reprint of Neutrophil cell surface receptors and their intracellular signal transduction pathways. *Int Immunopharmacol* 2013; **17**: 1185-1197 [PMID: 24263067 DOI: 10.1016/j.intimp.2013.11.010]

43 **Schnabl B**, Brenner DA. Interactions between the intestinal microbiome and liver diseases. *Gastroenterology* 2014; **146**: 1513-1524 [PMID: 24440671 DOI: 10.1053/j.gastro.2014.01.020]

44 **Zhao ZH**, Xin FZ, Zhou D, Xue YQ, Liu XL, Yang RX, Pan Q, Fan JG. Trimethylamine N-oxide attenuates high-fat high-cholesterol diet-induced steatohepatitis by reducing hepatic cholesterol overload in rats. *World J Gastroenterol* 2019; **25**: 2450-2462 [PMID: 31171889 DOI: 10.3748/wjg.v25.i20.2450]

45 **Dumas ME**, Kinross J, Nicholson JK. Metabolic phenotyping and systems biology approaches to understanding metabolic syndrome and fatty liver disease. *Gastroenterology* 2014; **146**: 46-62 [PMID: 24211299 DOI: 10.1053/j.gastro.2013.11.001]

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ORIGINAL ARTICLE

Case Control Study

Leukocyte cell-derived chemotaxin-2 and fibroblast growth factor 21 in alcohol-induced liver cirrhosis

Jarosław Jerzy Sak, Andrzej Prystupa, Paweł Kiciński, Dorota Luchowska-Kocot, Ewa Kurys-Denis, Hanna Bis-Wencel

ORCID number: Jarosław Jerzy Sak 0000-0002-8763-0683; Andrzej Prystupa 0000-0003-4628-8911; Paweł Kiciński 0000-0002-0142-4812; Dorota Luchowska-Kocot 0000-0002-1005-6580; Ewa Kurys-Denis 0000-0002-2664-7588; Hanna Bis-Wencel 0000-0003-1425-3378.

Author contributions: Sak JJ and Prystupa A were involved in the conception of the study, data collection and analysis, drafting and revision of the manuscript; Kiciński P and Luchowska-Kocot D were involved in the data collection and analysis; Kurys-Denis E was involved in the drafting and revision of the manuscript; Bis-Wencel H contributed to the data collection and revision of the manuscript.

Institutional review board statement: The study protocol was approved by the Bioethics Committee at Medical University of Lublin, Poland.

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Jarosław Jerzy Sak, Chair and Department of Humanities and Social Medicine, Medical University of Lublin, Lublin 20-093, Poland

Andrzej Prystupa, Department of Internal Medicine, Medical University of Lublin, Lublin 20-081, Poland

Paweł Kiciński, Department of Experimental Hematooncology, Medical University of Lublin, Lublin 20-080, Poland

Dorota Luchowska-Kocot, Department of Medical Chemistry, Medical University of Lublin, Lublin 20-093, Poland

Ewa Kurys-Denis, The Second Department of Radiology, Medical University of Lublin, Lublin 20-081, Poland

Hanna Bis-Wencel, Department of Microbiology and Reproductive Biology, University of Life Sciences in Lublin, Lublin 20-950, Poland

Corresponding author: Jarosław Jerzy Sak, MD, PhD, Academic Research, Additional Professor, Director, Chair and Department of Humanities and Social Medicine, Medical University of Lublin, ul. Chodźki 7 (Collegium Academicum), Lublin 20-093, Poland. jaroslaw.sak@umlub.pl

Abstract

BACKGROUND

The importance of early diagnosis of alcoholic liver disease underscores the need to seek better and especially non-invasive diagnostic procedures. Leukocyte cell-derived chemotaxin-2 (LECT2) has been widely studied to determine its usefulness in monitoring the course of non-alcoholic fatty liver disease but not for alcoholic liver cirrhosis (ALC).

AIM

To determine the concentration of LECT2 in the blood serum of patients in relation to progressive stages of ALC, its relation to fibroblast growth factor 1 (FGF-1) and FGF-21, and to examine the possible wider use of LECT2 in diagnosing ALC.

Data sharing statement: Technical appendix, statistical code, and dataset available from the corresponding author at jaroslaw.sak@umlub.pl. Participants gave informed consent for data sharing.

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METHODS
A retrospective case-control study was conducted with 69 ALC cases and 17 controls with no ALC. Subjects were recruited from the region of Lublin (eastern Poland). Liver cirrhosis was diagnosed based on clinical features, history of heavy alcohol consumption, laboratory tests, and abdominal ultrasonography. The degree of ALC was evaluated according to Pugh-Child criteria (the Pugh-Child score). Blood was drawn and, after centrifugation, serum was collected for analysis. LECT2, FGF-1, and FGF-21 were determined using enzyme-linked immunosorbent assay kits.

RESULTS
The LECT2 Levels in the control group were 18.99 ± 5.36 ng/mL. In the study groups, they declined with the progression of cirrhosis to 11.06 ± 6.47 ng/mL in one group and to 8.06 ± 5.74 ng/mL in the other ($P < 0.0001$). Multiple comparison tests confirmed the statistically significant differences in LECT2 Levels between the control group and both test groups ($P = 0.006$ and $P < 0.0001$). FGF-21 Levels were 44.27 ± 64.19 pg/mL in the first test group, 45.4 ± 51.69 pg/mL in the second ($P = 0.008$), and 13.52 ± 7.51 pg/mL in the control group. The difference between the control group and the second test group was statistically significant ($P = 0.007$).

CONCLUSION
We suggest that LECT2 may be a non-invasive diagnostic factor for alcohol-induced liver cirrhosis. The usefulness of LECT2 for non-invasive monitoring of alcohol-induced liver cirrhosis was indirectly confirmed by the multiple regression model developed on the basis of our statistical analysis.

Key Words: Leukocyte cell-derived chemotaxin-2; Fibroblast growth factor 21; Fibroblast growth factor 1; Alcoholic liver cirrhosis; Pugh-Child score

Core Tip: Leukocyte cell-derived chemotaxin-2 (LECT2) was first described in 1996 as a novel chemotactic factor for neutrophils. It has been widely studied to determine its usefulness for monitoring the course of non-alcoholic fatty liver disease but not for alcoholic liver cirrhosis (ALC). We suggest that LECT2 may be used for the non-invasive diagnosis of ALC.

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INTRODUCTION

Alcoholic liver disease (ALD) occurs in three stages: fatty liver, alcoholic hepatitis, and liver cirrhosis. In the present study, the role of leukocyte cell-derived chemotaxin-2 (LECT2) in the development of alcohol-induced liver cirrhosis was investigated.

In recent decades, there have been significant developments in research on the biochemical possibilities for the early diagnosis and monitoring of non-alcoholic fatty liver disease (NAFLD)[1]. Hepatokines were found to be extremely useful for NAFLD monitoring[2]. Moreover, relationships between the stages of NAFLD and fetuin-A[3,4], selenoprotein-P[5,6], and fibroblast growth factor 21 (FGF-21)[7] have been demonstrated. Fibroblast growth factor mimicking has been developed as a novel therapeutic option[8]. The analogues of hepatokines, such as a pegylated FGF-21 analogue[9], have been used in NAFLD therapies. However, finding similar diagnostic options for ALD remains valid[10]. ALD is among the most prevalent diseases in Western countries. It has recently been recognized as an increasingly serious epidemi-

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ological and therapeutic problem in developing countries[11,12].

Therefore, finding new possibilities for the early diagnosis of ALD, especially novel and precise non-invasive diagnostic procedures, is a real challenge for modern hepatological practice.

LECT2 has been widely studied to determine its usefulness in monitoring the course of NAFLD. According to the available study findings, serum LECT2 concentrations increase with the advancement of NAFLD[13,14]. LECT2 was first described by Yamagoe *et al*[15] in 1996 as a novel chemotactic factor for neutrophils. Subsequent studies identified its expression in human hepatocytes and classified it as a hepatokine [16-18]. Clinical observations have demonstrated that LECT2-associated amyloidosis is a frequent cause of hepatic amyloidosis in the United States[19]. Studies in animal models have reported that LECT2 overexpression increases fibrosis, promotes sinusoid capillarization, and inhibits portal angiogenesis. LECT2 is a functional ligand of Tie1. Xu *et al*[20] suggested that serum LECT2 Levels may be a potential biomarker for the diagnosis or screening of liver fibrosis, and LECT2/Tie1 signaling may be used for the development of new drugs.

It seems that LECT2 could be of great importance in the diagnosis of fatty liver. In a cross-sectional study, Okumura *et al*[13] showed statistically significant higher levels of LECT2 in fatty liver and obesity. However, the possibility of diagnosing and monitoring the course of alcohol-induced liver cirrhosis using LECT2 has not yet been assessed.

The aim of our study was to determine the concentration of LECT2 in the blood serum of patients at progressive stages of alcoholic liver cirrhosis to determine the relation to FGF-1 and FGF-21, and to discuss the possible wider use of LECT2 in the diagnosis of ALC.

MATERIALS AND METHODS

The study protocol was approved by the Bioethics Committee. All patients gave their written informed consent prior to participating in the study.

Patients

The study was conducted at the Department of Internal Medicine, Medical University of Lublin, Poland, and included 69 patients from the region of Lublin (eastern Poland) with alcoholic cirrhosis. Liver cirrhosis was diagnosed based on clinical features, history of heavy alcohol consumption, laboratory tests, and abdominal ultrasonography. Heavy alcohol consumption was defined according to the guidelines of the National Institute on Alcohol Abuse and Alcoholism (NIAAA) as consuming more than four drinks on any day or more than 14 drinks per week for men and three drinks on any day or more than seven drinks per week for women[21]. Patients with alcoholic hepatitis, hepatocellular carcinoma, or viral and autoimmune diseases were excluded from the study. Other exclusion criteria were type 2 diabetes, obesity, acute infections (*e.g.*, pneumonia, spontaneous bacterial peritonitis), acute and chronic heart failure (> NYHA I—*i.e.* slight or marked limitation of physical activity, ordinary physical activity results in fatigue, palpitation, dyspnea), acute and chronic respiratory disorders resulting in respiratory insufficiency, acute kidney injury, and chronic kidney disease (> stage G2—*i.e.* an estimated glomerular filtration rate < 60 mL/min). Both clinical assessments and laboratory tests were used to exclude underlying liver diseases in the control group. The degree of liver cirrhosis was evaluated according to Pugh-Child criteria (the Pugh-Child score), and on that basis, patients were assigned to one of three groups: Pugh-Child (P-Ch) A (n = 21) with stage A, P-Ch B (n = 23) with stage B, and P-Ch C (n = 28) with stage C liver cirrhosis (Table 1). The control group consisted of 17 healthy individuals without liver disease who did not abuse alcohol. Detailed demographic, clinical, and biochemical characteristics of the patients are presented in Tables 1 and 2.

Biochemical measurements

Blood was drawn, and after centrifugation, serum was collected for analysis. Human LECT2, FGF-1, and FGF-21 were determined using enzyme-linked immunosorbent assay (ELISA) kits. All absorbance readings were conducted using an Epoch Microplate Spectrophotometer (BioTek Instrumentals, Inc., Winooski, VT, United States). LECT2 concentrations were determined using a BioVendor Human LECT2 ELISA kit (BioVendor, Laboratorni medicina a.s., Brno, Czech Republic). FGF-1 and FGF-21 concentrations were quantified using sandwich enzyme immunoassay kits

Table 1 Patients' demographics and clinical characteristics

	Control group (*n* = 17)	Liver cirrhosis		*P* value
		Pugh-Child A + B (*n* = 37)	Pugh-Child C (*n* = 32)	
Age (yr)	43.7 ± 14.6	55.7 ± 12.1	55.9 ± 10.2	0.021
Percentage of males (%)	64.3%	73%	72.7%	0.52
Body weight (kg)	67.6 ± 8.9	73 ± 11.4	75.5 ± 12.8	0.17
Height (cm)	173 ± 5.9	174 ± 8	173 ± 7.6	0.64
Duration of alcohol abuse (yr)	-	15.7 ± 8.2	18.7 ± 8.3	0.98
Oesophageal varices (%)	-	32.4%	81.8%	< 0.0001
Encephalopathy (%)	-	32.4%	83.9%	< 0.0001
Ascites (%)	-	40.5%	90.9%	< 0.0001
Total bilirubin (mg/dL)	0.6 ± 0.3	4.6 ± 6.9	10.5 ± 9.2	< 0.0001
INR	-	1.36 ± 0.35	1.95 ± 0.56	< 0.0001
Albumin (g/dL)	-	3.1±0.8	2.4±0.4	0.0002
Total protein (g/dL)	6.3 ± 0.3	6.4 ± 1	5.9 ± 0.9	0.16
Alanine aminotransferase (U/L)	17.9 ± 6	65.3 ± 139.9	50.6 ± 87.3	0.018
Aspartate aminotransferase (U/l)	18.3 ± 7	128.1 ± 173.5	120 ± 164.7	< 0.0001
Platelets (G/L)	231.4 ± 29.8	173 ± 105.4	127.8 ± 72.3	0.0004
Mean corpuscular volume (fL)	84.8 ± 3.5	91.2 ± 9.1	95.5 ± 9	0.0002
Urea (mg/dL)	-	27.5 ± 16.1	58.2 ± 43.7	0.065
Sodium (mmol/l)	140 ± 3.3	133.8 ± 5	131.9 ± 6.7	< 0.0001
Potassium (mmol/L)	4.4 ± 0.4	3.8 ± 0.7	3.9 ± 0.8	0.019
C-reactive protein (mg/L)	2.5 ± 2.3	19.8 ± 21	32.7 ± 27.8	< 0.0001
Angiotensinogen (ng/mL)	1006.91 ± 610.49	1117.04 ± 873.69	1468.7 ± 817.33	0.22

INR: International normalized ratio.

Table 2 Levels of selected biochemical parameters according to the stage of liver cirrhosis

	Control group	Liver cirrhosis		*P* value
		Pugh-Child A + B	Pugh-Child C	
LECT2 (ng/mL)	18.99 ± 5.36	11.06 ± 6.47	8.06 ± 5.74	< 0.0001
FGF-1 (pg/mL)	37.94 ± 40.4	144.77 ± 14.42	164.52 ± 169.46	0.01
FGF-21 (pg/mL)	13.52 ± 7.51	44.27 ± 64.19	45.4 ± 51.69	0.008

LECT2: Leukocyte cell-derived chemotaxin-2; FGF-1: Fibroblast growth factor 1; FGF-21: Fibroblast growth factor 21.

produced by Cloud-Clone Corp. (Katy, TX, United States). Serum samples had been suitably diluted (20-fold dilution for LECT2) or used without dilution (FGF-1 and FGF 21) prior to testing, in accordance with the manufacturers' recommendations. Testing was carried out in accordance with the typical standard applicable for enzyme-linked immunoassays: samples, standards, and blanks were applied to a plate pre-coated with a factor-specific antibody. Subsequently, horseradish peroxidase conjugated avidin was added to each well, and the plate was incubated for one hour at room temperature (LECT2) or at 37°C (FGF-1 and FGF-21). Next, TMB substrate was added; the wells containing biotin-conjugated antibody and enzyme-conjugated avidin exhibited a change in color. The enzyme-substrate reaction was terminated by adding acidic solution, and the absorbance of the complex formed was measured at a

wavelength of 450 nm. The concentrations of the study parameters were determined using a standard curve. Results were multiplied by the dilution factor, when necessary.

Statistical analysis

Statistica 13.3 (TIBCO Software, Inc.) was used for data analysis. Continuous variables were expressed as mean ± SD. Before calculations, variables were checked for normality using the Shapiro-Wilk test. To compare the results between more than two groups, one-way ANOVA and the Kruskal-Wallis test were used, depending on distribution. Correlations among variables were tested using Pearson's and Spearman's correlation tests, depending on distribution. Qualitative variables were shown as indicators of structure (percentage). For intergroup comparisons, the χ^2 test was used. For all tests, $P < 0.05$ was considered statistically significant.

RESULTS

The study group consisted of 69 patients (50 men), including 37 with P-Ch A or P-Ch B cirrhosis and 32 with P-Ch C. The control group included 17 gender-matched individuals ($P = 0.52$). The age of patients in the control group was lower than that of patients with cirrhosis ($P = 0.021$). The duration of alcohol abuse in the study group was, on average, 15.7 ± 8.2 years in the P-Ch A + B subgroup and 18.7 ± 8.3 years in the P-Ch C subgroup.

As expected, patients with liver cirrhosis were characterized by significantly lower albumin levels and higher total bilirubin (TB), alanine aminotransferase, aspartate aminotransferase (AST), international normalized ratio, and C-reactive protein levels (Table 1).

Angiotensinogen levels increased with the progression of cirrhosis, reaching the highest in the P-Ch C group of 1468.7 ± 817.33 ng/mL. However, the differences observed were not statistically significant ($P = 0.22$).

The LECT2 Levels in the control group were 18.99 ± 5.36 ng/mL. With the progression of cirrhosis in the P-Ch A + B group, this value dropped to 11.06 ± 6.47 ng/mL and to 8.06 ± 5.74 ng/mL in the P-Ch C group ($P < 0.0001$) (Table 2). Multiple comparisons confirmed the statistically significant differences in LECT2 Levels between the control group and the P-Ch A + B ($P = 0.006$) and between the control group and P-Ch C ($P < 0.0001$) (Figure 1).

Otherwise, the lowest FGF-1 Level was found in the control group—37.94 ± 40.4 pg/mL—and was higher in patients with cirrhosis, increasing to 144.77 ± 1 in the P-Ch A + B group and to 164.52 ± 169.46 pg/mL in the P-Ch C group ($P < 0.01$). The difference between the control group and P-Ch C was statistically significant ($P = 0.002$) (Table 2).

A similar trend was observed for FGF-21. Its concentration in the control group was 13.52 ± 7.51 pg/mL, 44.27 ± 64.19 pg/mL in the P-Ch A + B group, and 45.4 ± 51.69 pg/mL in the P-Ch C group ($P = 0.008$). The difference between the control group and the P-Ch C group was statistically significant ($P = 0.007$) (Table 2).

The strongest correlations were observed between LECT2 and TB ($r = –0.59$; $P < 0.0001$) and angiotensinogen ($r = –0.51$; $P < 0.0001$) (Table 3).

In the multiple regression model, angiotensinogen, AST, TB, and age were observed to be independent LECT2-related variables (Table 4). This model was statistically significant ($P < 0.0001$) and explained less than two-thirds of variability (adjusted $R^2 = 0.59$).

DISCUSSION

ALD is a serious health consequence of excessive alcohol consumption. The spectrum of clinical-histologic ALD changes includes fatty liver, alcoholic hepatitis, and cirrhosis [22]. It is estimated that over 90% of all heavy drinkers have fatty liver; about 25% of them have alcoholic hepatitis, and 15% have cirrhosis. According to a meta-analysis conducted by Askgaard *et al*[23], the probability of alcoholic liver cirrhosis reaches 16% after 8–12 years of alcoholization; 45% of patients with cirrhosis had been consuming more than 110 g of alcohol daily. The above results correspond to our observations based on a relatively small sample. Alcohol-induced liver cirrhosis accounts for half of all cirrhosis cases in the United States. In recent years, the importance of finding new non-invasive methods to diagnose more severe forms of

Table 3 Correlations between leukocyte cell-derived chemotaxin-2 and other clinical and laboratory parameters (only those statistically significant were included)

Pair of variables		Correlation coefficient	
		R	*P* value
LECT2	Age	-0.29	0.048
	Total bilirubin	-0.59	< 0.0001
	Platelets	0.34	0.02
	Alanine transaminase	-0.43	0.003
	C-reactive protein	-0.4	0.008
	Angiotensinogen	-0.51	< 0.0001
	FGF-1	-0.38	0.004
	FGF-21	-0.39	0.004

LECT2: Leukocyte cell-derived chemotaxin-2; FGF-1: Fibroblast growth factor 1; FGF-21: Fibroblast growth factor 21.

Table 4 Independent factors associated with leukocyte cell-derived chemotaxin-2 concentration (multiple regression)

Effect	B*	SE with B*	B	SE with B	*P* value
Constant			30.64	3.68	< 0.0001
Angiotensinogen	-0.423	0.114	-0.004	0.001	0.001
Alanine aminotransferase	-0.341	0.115	-0.02	0.005	0.005
Total bilirubin	-0.279	0.108	-0.25	0.099	0.014
Age	-0.275	0.109	-0.16	0.064	0.016

B*: Standardized coefficient (Beta). Model: $R = 0.79$; $R^2 = 0.64$, adjusted $R^2 = 0.59$; $P < 0.0001$.

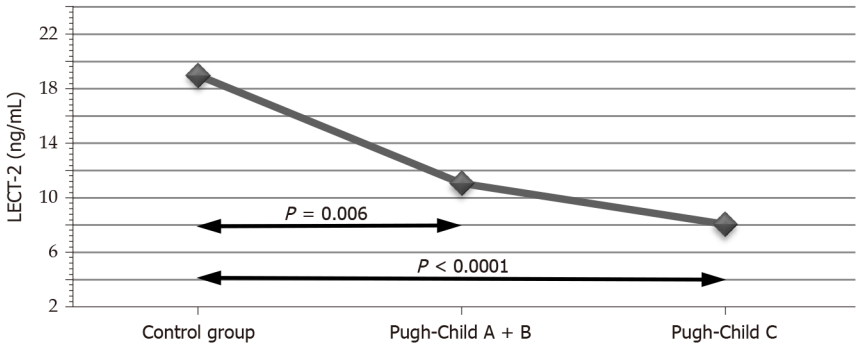

Figure 1 Concentration of leukocyte cell-derived chemotaxin-2 according to the stage of alcoholic liver cirrhosis. LECT-2: Leukocyte cell-derived chemotaxin-2.

ALD and predict prognosis has been strongly emphasized[24,25].

In our study, the serum levels of FGF-1 and FGF-21 in the study groups and control group were determined to obtain biochemical reference points for levels of LECT2. FGF-1 is an angiogenic factor that modifies the migration and proliferation of endothelial cells and regulates the metabolism of lipids and carbohydrates. FGF-1 is involved in response to injury and fibrosis. The highest expression of FGF has been observed in the late stages of hepatic morphogenesis in animal models, as well as during hepatic differentiation in the adult liver. FGF-1 is present in perisinusoidal hepatic stellate cells (HSCs) during liver regeneration. The chronic activation of nonparenchymal HSCs (also called Ito cells and fat-storing cells) is the major contributor to liver fibrogenesis resulting from chronic toxic insult primarily through

its production of extracellular matrix components.

FGF-1 reduces hepatic lipid accumulation independently of insulin and is important in the pathogenesis of NAFLD. Moreover, it has therapeutic potential for the treatment of ischemic disease[26]. Previous studies have demonstrated an inverse relationship between this factor and portal pressure in patients after liver transplantation[27]. In animal model studies, the protective effect of FGF-1 on liver cells was confirmed, as it prevented acute inflammation and apoptosis induced by acetaminophen[28]. The main source of FGF-1 in the human body is liver cells. However, this protein is also expressed in the pancreas, testes, duodenum, and adipose tissue. For this reason, its use as an indicator of liver function is clearly limited, and in recent years this problem has not been studied. Among fibroblast growth factors, FGF-21 has been tested as a marker of liver function[29,30]. According to a Chinese prospective study, this protein is an independent predictor of NAFLD[31]. The possible use of FGF-21 as an NAFLD marker has also been described in an American study conducted in children[32]. However, the above study demonstrated significant relationships between the level of this marker and the prevalence of obesity, with or without insulin resistance. In a study on ALD, Yang *et al*[33] suggested that FGF-21 may indicate a progression from heavy drinking to alcoholic cirrhosis. In their latest study, Willis *et al*[34] indicated that acute high-fat overfeeding augments circulating concentrations of FGF-21, LECT2, and fetuin-A in healthy men. Perhaps a slightly opposite effect than in this subgroup occurs in patients with cirrhosis with regard to correlation of LECT2 and FGF-21. The results of our study showed that LECT2 Levels correlated inversely with FGF-1 and FGF-21 in ALD. However, based on our results, it is not possible to state whether this is specific to ALD. Previous studies have shown that LECT2 could be of great importance in the diagnosis of NAFLD[13,14]. We suggest the need for further, more extensive, including prospective, studies.

Our study is the first attempt to assess the usefulness of LECT2 in the non-invasive diagnosis of alcohol-induced liver cirrhosis. Therefore, the points of reference are scarce. However, considering the above-mentioned studies on the marker function of FGF-21, it is worth noting that our results are compatible with those reported by Yang *et al*[33] In our study, the concentration of FGF-21 in the control group, that is, patients without cirrhosis, was significantly lower compared to both subgroups of the study group. However, the differences in FGF-21 concentrations between the two subgroups (P-Ch A + B and P-Ch C) were not statistically significant. FGF-21 may play an important role in supporting non-invasive diagnostics of alcohol-induced liver cirrhosis and in monitoring the course of NAFLD. We did not find it useful in non-invasive monitoring of alcohol-induced liver cirrhosis, contrary to the level of serum taurine/glycine-conjugated bile acids as a non-invasive marker to predict the severity of alcohol-induced liver cirrhosis, as tested by Yang *et al*[33]. Our results suggest that LECT2 might be used as a diagnostic and monitoring marker to determine the severity of alcohol-induced liver cirrhosis. Its highest statistically significant concentration was observed in the control group. In the study groups, as cirrhosis progressed, the plasma levels of LECT2 dropped. The lowest values of LECT2 were observed in P-Ch C stage patients, that is, in the most advanced stage of the disease.

LECT2 Levels correlated inversely with TB, AST, and angiotensinogen (AGT). Although strong correlations were identified between LECT2 and cirrhosis progression, and between AGT and LECT2, we did not observe an analogous relationship between AGT and cirrhosis progression. We suggest that this may be caused by low sample size and decreased power. The liver's renin-angiotensin system plays an important role in the development of liver cirrhosis. The levels of total bilirubin, AST, and AGT increase as alcohol-induced liver cirrhosis progresses. Higher serum concentration of AGT indicates unfavorable histological remodeling of the liver parenchyma closely related to liver dysfunction. Previous studies on animal models have indicated that AGT plays an important role in NAFLD[35-37]. AGT is an important precursor of hepatic fibrogenesis, which has been confirmed in animal studies[38]. According to the reported data, AGT inhibition could be an effective anti-liver fibrosis strategy.

CONCLUSION

Our research suggests that LECT2 may be used for the non-invasive diagnosis of alcohol-induced liver cirrhosis. The usefulness of LECT2 for non-invasive monitoring of alcohol-induced liver cirrhosis was indirectly confirmed by the multiple regression model developed on the basis of our statistical analysis.

ARTICLE HIGHLIGHTS

Research background

Leukocyte cell-derived chemotaxin-2 (LECT2) has been widely studied to determine its usefulness for monitoring the course of non-alcoholic fatty liver disease but not for alcoholic liver cirrhosis (ALC).

Research motivation

The aim of our study was to assess and discuss LECT2's possible wider use in the diagnosis of ALC.

Research objectives

The purpose of this study was to determine the concentration of LECT2 in the blood serum of patients in accordance with progressive stages of ALC and its relation to fibroblast growth factor 1 (FGF-1) and FGF-21.

Research methods

A study was conducted with an ALC group and a control group with no ALC. The extent of ALC was evaluated according to Pugh-Child criteria (the Pugh-Child score). LECT2, FGF-1, and FGF-21 were determined using enzyme-linked immunosorbent assay kits.

Research results

Our study showed strong correlations between LECT2 and cirrhosis progression. LECT2 levels correlated inversely with FGF-1 and FGF-21.

Research conclusions

LECT2 may be used for the non-invasive diagnosis of alcohol-induced liver cirrhosis.

Research perspectives

Further prospective studies should be conducted to explore whether the inverse correlation of LECT2 and FGF-21 is specific to ALD.

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REFERENCES

1 **Benedict M**, Zhang X. Non-alcoholic fatty liver disease: An expanded review. *World J Hepatol* 2017; **9**: 715-732 [PMID: 28652891 DOI: 10.4254/wjh.v9.i16.715]
2 **Ke Y**, Xu C, Lin J, Li Y. Role of Hepatokines in Non-alcoholic Fatty Liver Disease. *J Transl Int Med* 2019; **7**: 143-148 [PMID: 32010600 DOI: 10.2478/jtim-2019-0029]
3 **Sato M**, Kamada Y, Takeda Y, Kida S, Ohara Y, Fujii H, Akita M, Mizutani K, Yoshida Y, Yamada M, Hougaku H, Takehara T, Miyoshi E. Fetuin-A negatively correlates with liver and vascular fibrosis in nonalcoholic fatty liver disease subjects. *Liver Int* 2015; **35**: 925-935 [PMID: 25627311 DOI: 10.1111/liv.12478]
4 **Celebi G**, Genc H, Gurel H, Sertoglu E, Kara M, Tapan S, Acikel C, Karslioglu Y, Ercin CN, Dogru T. The relationship of circulating fetuin-a with liver histology and biomarkers of systemic inflammation in nondiabetic subjects with nonalcoholic fatty liver disease. *Saudi J Gastroenterol* 2015; **21**: 139-145 [PMID: 26021772 DOI: 10.4103/1319-3767.157556]
5 **Choi HY**, Hwang SY, Lee CH, Hong HC, Yang SJ, Yoo HJ, Seo JA, Kim SG, Kim NH, Baik SH, Choi DS, Choi KM. Increased selenoprotein p levels in subjects with visceral obesity and nonalcoholic Fatty liver disease. *Diabetes Metab J* 2013; **37**: 63-71 [PMID: 23439771 DOI: 10.4093/dmj.2013.37.1.63]
6 **Polyzos SA**, Kountouras J, Mavrouli M, Katsinelos P, Doulberis M, Gavana E, Duntas L. Selenoprotein P in Patients with Nonalcoholic Fatty Liver Disease. *Exp Clin Endocrinol Diabetes* 2019; **127**: 598-602 [PMID: 30625508 DOI: 10.1055/a-0811-9136]
7 **Praktiknjo M**, Djayadi N, Mohr R, Schierwagen R, Bischoff J, Dold L, Pohlmann A, Schwarze-Zander C, Wasmuth JC, Boesecke C, Rockstroh JK, Trebicka J. Fibroblast growth factor 21 is

independently associated with severe hepatic steatosis in non-obese HIV-infected patients. *Liver Int* 2019; **39**: 1514-1520 [PMID: 30916873 DOI: 10.1111/liv.14107]

8 **Ocker M**. Fibroblast growth factor signaling in non-alcoholic fatty liver disease and non-alcoholic steatohepatitis: Paving the way to hepatocellular carcinoma. *World J Gastroenterol* 2020; **26**: 279-290 [PMID: 31988589 DOI: 10.3748/wjg.v26.i3.279]

9 **Sanyal A**, Charles ED, Neuschwander-Tetri BA, Loomba R, Harrison SA, Abdelmalek MF, Lawitz EJ, Halegoua-DeMarzio D, Kundu S, Noviello S, Luo Y, Christian R. Pegbelfermin (BMS-986036), a PEGylated fibroblast growth factor 21 analogue, in patients with non-alcoholic steatohepatitis: a randomised, double-blind, placebo-controlled, phase 2a trial. *Lancet* 2019; **392**: 2705-2717 [PMID: 30554783 DOI: 10.1016/S0140-6736(18)31785-9]

10 **Mueller S**, Seitz HK, Rausch V. Non-invasive diagnosis of alcoholic liver disease. *World J Gastroenterol* 2014; **20**: 14626-14641 [PMID: 25356026 DOI: 10.3748/wjg.v20.i40.14626]

11 **Addolorato G**, Mirijello A, Barrio P, Gual A. Treatment of alcohol use disorders in patients with alcoholic liver disease. *J Hepatol* 2016; **65**: 618-630 [PMID: 27155530 DOI: 10.1016/j.jhep.2016.04.029]

12 **Arab JP**, Roblero JP, Altamirano J, Bessone F, Chaves Araujo R, Higuera-De la Tijera F, Restrepo JC, Torre A, Urzua A, Simonetto DA, Abraldes JG, Méndez-Sánchez N, Contreras F, Lucey MR, Shah VH, Cortez-Pinto H, Bataller R. Alcohol-related liver disease: Clinical practice guidelines by the Latin American Association for the Study of the Liver (ALEH). *Ann Hepatol* 2019; **18**: 518-535 [PMID: 31053546 DOI: 10.1016/j.aohep.2019.04.005]

13 **Okumura A**, Unoki-Kubota H, Matsushita Y, Shiga T, Moriyoshi Y, Yamagoe S, Kaburagi Y. Increased serum leukocyte cell-derived chemotaxin 2 (LECT2) levels in obesity and fatty liver. *Biosci Trends* 2013; **7**: 276-283 [PMID: 24390366]

14 **Yoo HJ**, Hwang SY, Choi JH, Lee HJ, Chung HS, Seo JA, Kim SG, Kim NH, Baik SH, Choi DS, Choi KM. Association of leukocyte cell-derived chemotaxin 2 (LECT2) with NAFLD, metabolic syndrome, and atherosclerosis. *PLoS One* 2017; **12**: e0174717 [PMID: 28376109 DOI: 10.1371/journal.pone.0174717]

15 **Yamagoe S**, Yamakawa Y, Matsuo Y, Minowada J, Mizuno S, Suzuki K. Purification and primary amino acid sequence of a novel neutrophil chemotactic factor LECT2. *Immunol Lett* 1996; **52**: 9-13 [PMID: 8877413 DOI: 10.1016/0165-2478(96)02572-2]

16 **Lebensztejn DM**, Flisiak-Jackiewicz M, Białokoz-Kalinowska I, Bobrus-Chociej A, Kowalska I. Hepatokines and non-alcoholic fatty liver disease. *Acta Biochim Pol* 2016; **63**: 459-467 [PMID: 27262842 DOI: 10.18388/abp.2016_1252]

17 **Yamagoe S**, Mizuno S, Suzuki K. Molecular cloning of human and bovine LECT2 having a neutrophil chemotactic activity and its specific expression in the liver. *Biochim Biophys Acta* 1998; **1396**: 105-113 [PMID: 9524238 DOI: 10.1016/s0167-4781(97)00181-4]

18 **Lan F**, Misu H, Chikamoto K, Takayama H, Kikuchi A, Mohri K, Takata N, Hayashi H, Matsuzawa-Nagata N, Takeshita Y, Noda H, Matsumoto Y, Ota T, Nagano T, Nakagen M, Miyamoto K, Takatsuki K, Seo T, Iwayama K, Tokuyama K, Matsugo S, Tang H, Saito Y, Yamagoe S, Kaneko S, Takamura T. LECT2 functions as a hepatokine that links obesity to skeletal muscle insulin resistance. *Diabetes* 2014; **63**: 1649-1664 [PMID: 24478397 DOI: 10.2337/db13-0728]

19 **Mereuta OM**, Theis JD, Vrana JA, Law ME, Grogg KL, Dasari S, Chandan VS, Wu TT, Jimenez-Zepeda VH, Fonseca R, Dispenzieri A, Kurtin PJ, Dogan A. Leukocyte cell-derived chemotaxin 2 (LECT2)-associated amyloidosis is a frequent cause of hepatic amyloidosis in the United States. *Blood* 2014; **123**: 1479-1482 [PMID: 24415538 DOI: 10.1182/blood-2013-07-517938]

20 **Xu M**, Xu HH, Lin Y, Sun X, Wang LJ, Fang ZP, Su XH, Liang XJ, Hu Y, Liu ZM, Cheng Y, Wei Y, Li J, Li L, Liu HJ, Cheng Z, Tang N, Peng C, Li T, Liu T, Qiao L, Wu D, Ding YQ, Zhou WJ. LECT2, a Ligand for Tie1, Plays a Crucial Role in Liver Fibrogenesis. *Cell* 2019; **178**: 1478-1492. e20 [PMID: 31474362 DOI: 10.1016/j.cell.2019.07.021]

21 National Institute on Alcohol Abuse and Alcoholism (NIAAA). Drinking Levels Defined. [cited 4 Jan 2021]. Available from: https://www.niaaa.nih.gov/alcohol-health/overview-alcohol-consumption/moderate-binge-drinking

22 **Singal AK**, Bataller R, Ahn J, Kamath PS, Shah VH. ACG Clinical Guideline: Alcoholic Liver Disease. *Am J Gastroenterol* 2018; **113**: 175-194 [PMID: 29336434 DOI: 10.1038/ajg.2017.469]

23 **Askgaard G**, Kjær MS, Tolstrup JS. Opportunities to Prevent Alcoholic Liver Cirrhosis in High-Risk Populations: A Systematic Review With Meta-Analysis. *Am J Gastroenterol* 2019; **114**: 221-232 [PMID: 30353053 DOI: 10.1038/s41395-018-0282-6]

24 **Moreno C**, Mueller S, Szabo G. Non-invasive diagnosis and biomarkers in alcohol-related liver disease. *J Hepatol* 2019; **70**: 273-283 [PMID: 30658728 DOI: 10.1016/j.jhep.2018.11.025]

25 **Lucey MR**. Alcohol-Associated Cirrhosis. *Clin Liver Dis* 2019; **23**: 115-126 [PMID: 30454826 DOI: 10.1016/j.cld.2018.09.013]

26 **Xia X**, Babcock JP, Blaber SI, Harper KM, Blaber M. Pharmacokinetic properties of 2nd-generation fibroblast growth factor-1 mutants for therapeutic application. *PLoS One* 2012; **7**: e48210 [PMID: 23133616 DOI: 10.1371/journal.pone.0048210]

27 **Serrano CA**, Ling SC, Verdaguer S, León M, Jarufe N, Guerra JF, Pattillo JC, Benítez C, Villagrán A, Torres J, Concha M, Villarroel L, Dellepiane P, Domínguez P, Martínez J, Gana JC. Portal Angiogenesis in Chronic Liver Disease Patients Correlates with Portal Pressure and Collateral Formation. *Dig Dis* 2019; **37**: 498-508 [PMID: 31067534 DOI: 10.1159/000500115]

28 **Wang X**, Zhang X, Wang F, Pang L, Xu Z, Li X, Wu J, Song Y, Xiao J, Lin H, Liu Y. FGF1 protects

against APAP-induced hepatotoxicity *via* suppression of oxidative and endoplasmic reticulum stress. *Clin Res Hepatol Gastroenterol* 2019; **43**: 707-714 [PMID: 31029643 DOI: 10.1016/j.clinre.2019.03.006]

29 **Yang M**, Xu D, Liu Y, Guo X, Li W, Guo C, Zhang H, Gao Y, Mao Y, Zhao J. Combined Serum Biomarkers in Non-Invasive Diagnosis of Non-Alcoholic Steatohepatitis. *PLoS One* 2015; **10**: e0131664 [PMID: 26121037 DOI: 10.1371/journal.pone.0131664]

30 **Gong Z**, Tas E, Yakar S, Muzumdar R. Hepatic lipid metabolism and non-alcoholic fatty liver disease in aging. *Mol Cell Endocrinol* 2017; **455**: 115-130 [PMID: 28017785 DOI: 10.1016/j.mce.2016.12.022]

31 **Li H**, Dong K, Fang Q, Hou X, Zhou M, Bao Y, Xiang K, Xu A, Jia W. High serum level of fibroblast growth factor 21 is an independent predictor of non-alcoholic fatty liver disease: a 3-year prospective study in China. *J Hepatol* 2013; **58**: 557-563 [PMID: 23142063 DOI: 10.1016/j.jhep.2012.10.029]

32 **Sodhi K**, Bracero L, Feyh A, Nichols A, Srikanthan K, Latif T, Preston D, Shapiro JI, Elitsur Y. Role of Serum Biomarkers in Early Detection of Non-Alcoholic Steatohepatitis and Fibrosis in West Virginian Children. *J Clin Cell Immunol* 2016; **7** [PMID: 27182456 DOI: 10.4172/2155-9899.1000393]

33 **Yang Z**, Kusumanchi P, Ross RA, Heathers L, Chandler K, Oshodi A, Thoudam T, Li F, Wang L, Liangpunsakul S. Serum Metabolomic Profiling Identifies Key Metabolic Signatures Associated With Pathogenesis of Alcoholic Liver Disease in Humans. *Hepatol Commun* 2019; **3**: 542-557 [PMID: 30976744 DOI: 10.1002/hep4.1322]

34 **Willis SA**, Sargeant JA, Yates T, Takamura T, Takayama H, Gupta V, Brittain E, Crawford J, Parry SA, Thackray AE, Varela-Mato V, Stensel DJ, Woods RM, Hulston CJ, Aithal GP, King JA. Acute Hyperenergetic, High-Fat Feeding Increases Circulating FGF21, LECT2, and Fetuin-A in Healthy Men. *J Nutr* 2020; **150**: 1076-1085 [PMID: 31919514 DOI: 10.1093/jn/nxz333]

35 **Takahashi N**, Li F, Hua K, Deng J, Wang CH, Bowers RR, Bartness TJ, Kim HS, Harp JB. Increased energy expenditure, dietary fat wasting, and resistance to diet-induced obesity in mice lacking renin. *Cell Metab* 2007; **6**: 506-512 [PMID: 18054319 DOI: 10.1016/j.cmet.2007.10.011]

36 **Frantz ED**, Penna-de-Carvalho A, Batista Tde M, Aguila MB, Mandarim-de-Lacerda CA. Comparative effects of the renin-angiotensin system blockers on nonalcoholic fatty liver disease and insulin resistance in C57BL/6 mice. *Metab Syndr Relat Disord* 2014; **12**: 191-201 [PMID: 24517411 DOI: 10.1089/met.2013.0129]

37 **Tao XR**, Rong JB, Lu HS, Daugherty A, Shi P, Ke CL, Zhang ZC, Xu YC, Wang JA. Angiotensinogen in hepatocytes contributes to Western diet-induced liver steatosis. *J Lipid Res* 2019; **60**: 1983-1995 [PMID: 31604805 DOI: 10.1194/jlr.M093252]

38 **Lu P**, Liu H, Yin H, Yang L. Expression of angiotensinogen during hepatic fibrogenesis and its effect on hepatic stellate cells. *Med Sci Monit* 2011; **17**: BR248-BR256 [PMID: 21873937 DOI: 10.12659/msm.881928]

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ORIGINAL ARTICLE

Retrospective Study

Biliary complications in recipients of living donor liver transplantation: A single-centre study

Reginia Nabil Guirguis, Ehab Hasan Nashaat, Azza Emam Yassin, Wesam Ahmed Ibrahim, Shereen A Saleh, Mohamed Bahaa, Mahmoud El-Meteini, Mohamed Fathy, Hany Mansour Dabbous, Iman Fawzy Montasser, Manar Salah, Ghada Abdelrahman Mohamed

ORCID number: Reginia Nabil Guirguis 0000-0003-3442-3629; Ehab Hasan Nashaat 0000-0002-7686-6463; Azza Emam Yassin 0000-0002-5764-6078; Wesam Ahmed Ibrahim 0000-0003-1813-5248; Shereen A Saleh 0000-0002-0984-1725; Mohamed Bahaa 0000-0002-8605-4397; Mahmoud El-Meteini 0000-0002-1839-3549; Mohamed Fathy 0000-0001-8000-5722; Hany Mansour Dabbous 0000-0001-5648-7733; Iman Fawzy Montasser 0000-0002-1351-978X; Manar Salah 0000-0001-9909-4016; Ghada Abdelrahman Mohamed 0000-0003-0320-1011.

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Reginia Nabil Guirguis, Ehab Hasan Nashaat, Azza Emam Yassin, Wesam Ahmed Ibrahim, Shereen A Saleh, Ghada Abdelrahman Mohamed, Gastroenterology and Hepatology Unit, Department of Internal Medicine, Faculty of Medicine, Ain Shams University, Cairo 11591, Egypt

Mohamed Bahaa, Mahmoud El-Meteini, Mohamed Fathy, Department of General Surgery, Faculty of Medicine, Ain Shams University, Cairo 11591, Egypt

Hany Mansour Dabbous, Iman Fawzy Montasser, Manar Salah, Department of Tropical Medicine, Faculty of Medicine, Ain Shams University, Cairo 11591, Egypt

Corresponding author: Ghada Abdelrahman Mohamed, MD, Lecturer, Gastroenterology and Hepatology Unit, Department of Internal Medicine, Faculty of Medicine, Ain Shams University, El-Khalifa El-Maamon Street, Abbassia, Cairo 11591, Egypt.
ghadaabdelrahman@med.asu.edu.eg

Abstract

BACKGROUND

Biliary complications (BCs) after liver transplantation (LT) remain a considerable cause of morbidity, mortality, increased cost, and graft loss.

AIM

To investigate the impact of BCs on chronic graft rejection, graft failure and mortality.

METHODS

From 2011 to 2016, 215 adult recipients underwent right-lobe living-donor liver transplantation (RT-LDLT) at our centre. We excluded 46 recipients who met the exclusion criteria, and 169 recipients were included in the final analysis. Donors' and recipients' demographic data, clinical data, operative details and postoperative course information were collected. We also reviewed the management and outcomes of BCs. Recipients were followed for at least 12 mo post-LT until December 2017 or graft or patient loss.

RESULTS

The overall incidence rate of BCs including biliary leakage, biliary infection and biliary stricture was 57.4%. Twenty-seven (16%) patients experienced chronic

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graft rejection. Graft failure developed in 20 (11.8%) patients. A total of 28 (16.6%) deaths occurred during follow-up. BCs were a risk factor for the occurrence of chronic graft rejection and failure; however, mortality was determined by recurrent hepatitis C virus infection.

CONCLUSION
Biliary complications after RT-LDLT represent an independent risk factor for chronic graft rejection and graft failure; nonetheless, effective management of these complications can improve patient and graft survival.

Key Words: Biliary complications; Living donor liver transplantation; Retrospective analysis; Bile leak; Biliary stricture; Risk factors; Mortality; Graft rejection

Core Tip: We included 169 right lobe living-donor liver transplantation recipients in this retrospective study. The overall incidence rate of biliary complications including biliary leakage, biliary infection and biliary stricture was 57.4%. Twenty-seven (16%) patients experienced chronic graft rejection. Graft failure developed in 20 (11.8%) patients. A total of 28 (16.6%) deaths occurred during follow-up. Biliary complications were an independent risk factor for the occurrence of chronic graft rejection and failure; however, mortality was determined by unresolved recurrent hepatitis C virus infection. In conclusion, biliary complications represent an independent risk factor for chronic graft rejection and graft failure; nonetheless, effective management of these complications can improve patient and graft survival.

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INTRODUCTION

Liver transplantation (LT) is a life-saving therapeutic modality for patients with end-stage hepatic disease[1]. Despite considerable progress in LT surgical performance and peri-operative management, post-LT biliary complications (BCs) remain a considerable cause of morbidity, mortality, increased cost, and graft loss[2,3].

Living-donor liver transplantation (LDLT) is a well-established substitute to deceased-donor LT (DDLT)[4,5]. LDLT has potential advantages over DDLT, such as lower cost, superior graft vitality, shorter cold ischemia time, and lower prevalence of steroid-resistant graft rejection[6]. However, it has been reported that LDLT is related to higher post-LT morbidity, hospitalization rates and duration of stay. This is mainly referred to the higher incidence rate of BCs in LDLT ranging from 10% to 67% compared to DDLT[7-9], which could be attributed to the technically challenging biliary reconstruction during LDLT[9]. Technical skilfulness is mandatory to reduce the incidence of BCs[10], and the most critical key step is to maintain the blood supply to the biliary ducts in donor surgery[11].

Post-LT BCs include biliary strictures (BSs), biliary leaks (BLs), and biliary infection. There are two types of BLs post-LDLT: Anastomotic and cut surface BLs[12,13]. BLs occur commonly at the T-tube insertion site and less frequently at the anastomosis site [14]. Most BLs occur within the first post-transplant month and are mostly related to inadequate surgical skills or biliary duct ischemia[15].

BSs are the most common BC, accounting for 40% of BCs following LT. Like BLs, BSs are more prevalent post-LDLT when compared to DDLT, mostly due to the more technically challenging biliary anastomosis in LDLT due to the small-sized ducts requiring multiple biliary anastomoses[7,16]. BSs typically present after one month post-LT; in addition, they can be anastomotic or non-anastomotic[12]. Anastomotic

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strictures account for approximately 80% of post-LT BSs and commonly occur in LDLT and at the anastomotic site[7,17]. Non-anastomotic strictures account for approximately 10%-25% of post-LT BSs[18]. BSs are mainly linked to surgical skills, patients with small-sized ducts, donor-recipient bile duct size mismatch, longer operative time, total ischemia time, local ischemia, chronic rejection, older donor age, donor and recipient gender matching and initial disease recurrence like primary sclerosing cholangitis (PSC)[2,3,19,20].

Duct-to-duct anastomosis (DDA) has developed into the preferred biliary reconstruction method due to its benefits of a shorter total operative time, less incidence of post-operative infections, more physiological enteric functions and the enablement of access to the biliary tree in case of complications. Roux-en-Y hepaticojejunostomy (RYHJ) is performed in the case of re-transplantation or short or diseased bile ducts[21]. However, diversity in the results regarding the superiority of both of the two biliary reconstruction and suturing techniques is still present[3,8,15,22].

Similarly, the use of biliary drainage remains controversial[10]. The post-LT stent represents a method for biliary tract decompression, as well as the facilitation of postoperative cholangiography[22]. However, this technique is predisposed to BL at the entry site and thus has become less commonly used[14]. Also, temporary internal biliary stents may be applied to cross the anastomosis site[19]; however, it has been reported that the incidence of BCs may increase with this technique[23].

There is considerable overlap in the diagnostic and therapeutic modalities in patients with post-LT BCs. Frequently used diagnostic modalities include abdominal ultrasonography, computed tomography scan, magnetic retrograde cholangiopancreatography (MRCP), magnetic resonance imaging, percutaneous transhepatic cholangiography (PTC) and endoscopic retrograde cholangiopancreatography (ERCP). Currently, the preferred imaging method for the biliary tract is MRCP; it provides a guide for further interventional approaches[14].

In the case of isolated deranged liver functions post-LT, it is crucial to make an accurate diagnosis of other parenchymal hepatic diseases such as acute or chronic rejection, drug-induced hepatotoxicity, recurrence of primary cholestatic disease or viral hepatitis to further apply the appropriate management plan. Liver biopsy is a conclusive diagnostic procedure for these patients[4,7].

The management of BCs depends on a multidisciplinary approach including endoscopic, percutaneous and surgical interventions. Currently, ERCP is the preferable first-line therapeutic modality, especially in cases of DDA[4,17]. The success rate of this technique is variable, ranging from 51% to 100%[24]. If ERCP fails, PTC can be tried; also, it is the preferred therapeutic modality in cases of RYHJ. Surgical intervention is a last option for BCs management[2,20]. However, the optimal strategy for managing post-LT BCs remains undefined.

Based on the published literature, BC causes significant morbidity following LDLT. If not managed properly, it leads to cholestasis, progressive bridging fibrosis, secondary biliary cirrhosis and eventually graft failure. Hence, we aimed to investigate its impact on chronic graft rejection, graft failure and mortality.

MATERIALS AND METHODS

Study design

This retrospective cohort study was conducted at Ain Shams Centre for Organ Transplantation, Ain Shams Specialized Hospital, Cairo, Egypt, from January 2011 to December 2016. This study was performed according to the ethical guidelines of the Declaration of Helsinki and was approved by the ethical review board of the Faculty of Medicine, Ain Shams University (No. FMASU MD 187/2016), which waived the requisite of informed consent owing to the retrospective nature of the study.

During the study period, 215 adult recipients underwent right lobe-LDLT (RL-LDLT) at our centre. We excluded 46 patients who met the exclusion criteria, and 169 recipients were enrolled in the final analysis. We included cirrhotic patients who met the transplantation criteria of our institution [a Child-Pugh score of ≥ 7 and model for end-stage liver disease (MELD) score of ≥ 15]. Patients with hepatocellular carcinoma (HCC) were enrolled if they met the Milan criteria, defined as a single lesion ≤ 5 cm or up to three lesions of ≤ 3 cm each with the absence of vascular invasion and extrahepatic metastases[25]. We excluded patients with cholestatic hepatic diseases [primary biliary cirrhosis (PBC) or PSC] and early postoperative mortality and patients lost on follow-up (Figure 1).

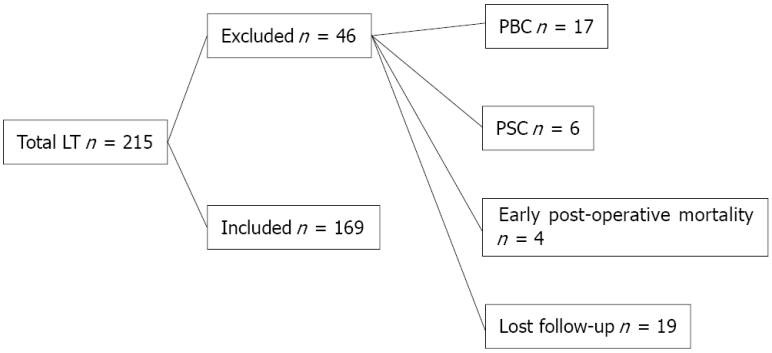

Figure 1 Flow chart of study cohort. LT: Liver transplantation; PBC: Primary biliary cholangitis; PSC: Primary sclerosing cholangitis.

Donors' and recipients' demographic data, clinical data, operative details and postoperative course information were collected. We also reviewed the management and outcomes of BCs. Recipients were followed for at least 12 mo post-LT until December 2017 or graft or patient loss.

Study definitions

The following BCs and their management were recorded from data files:

BL: Clinically suspected due to the existence of bile in the surgical drains or the presence of an intra-abdominal biloma and confirmed by imaging studies.

Biliary infection: Clinically suspected due to fever, abdominal pain, rigours, biochemical cultures and elevated inflammatory markers, including levels of C-reactive protein.

BS: Clinically suspected due to jaundice, pruritus, and elevated levels of serum bilirubin and/or alkaline phosphatase and confirmed by imaging studies as a narrowing at any site of the biliary tree whether at an anastomotic or non-anastomotic site with proximal dilatation.

Diagnosis of other clinical outcomes

Graft failure: Confirmed by histological evidence as graft cirrhosis, the need for re-transplantation because of graft failure and/or allograft-associated mortality.

Chronic ductopenic graft rejection: Proven by liver biopsy.

Recurrent hepatitis C virus (HCV) infection: Proven by high viral load, elevated transaminases and liver biopsy.

Institutional surgical technique for right lobe living donor liver transplantation

A right-lobe graft was used without the middle hepatic vein by the piggyback technique. Biliary anastomosis was done by DDA with an end-to-end interrupted style using absorbable polydioxanone (PDS-II; Ethicon) 6-0 sutures[26]. A ductoplasty was conducted if one duct was approximately twice the size of the other. A routine external biliary stent was inserted for three months post-operation. Three drains were placed postoperatively: In the right subphrenic space, the right Morrison's pouch and at the cut surface of the graft. Internal biliary stents were used selectively if indicated. Arterial reconstruction was described previously[27]. The ratio of graft weight to recipient body weight was used to assess the relation of the graft size for recipients [27]. The accepted ratio was 1.2 ± 0.2%. All recipients had the same ABO blood group as the donors.

Statistical methods

Data were analysed using IBM© SPSS© Statistics version 23 (IBM© Corp., Armonk, NY) and MedCalc© version 18.2.1 (MedCalc© Software bv, Ostend, Belgium). Non-parametric numerical variables were presented as medians and interquartile ranges, whereas between-group differences were analysed using the Mann-Whitney test and, in the case of paired data, the Wilcoxon signed-rank test. Parametric numerical data were shown as mean ± standard deviation, and between-group differences were analysed using a *t*-test and, in the case of paired data, a paired *t*-test. Nominal

variables were shown as number and percentage, and differences were analysed using Pearson's chi-squared test or Fisher's exact test. Ordinal data were analysed using the chi-squared test for trend. Multivariable binary logistic regression analysis was used to define the independent risk factors. Univariable time-to-event analysis was done using the Kaplan-Meier method. Cox proportional hazard regression analysis was used for multivariable time-to-event analysis. Two-sided *P* values of < 0.05 were considered statistically significant.

RESULTS

This study included 169 adult RL-LDLT recipients. At the time of operation, the mean age of the recipient was 50 ± 8 years, and 150 (88.8%) were male. The indications for LT were HCC [60 (35.5%)] and liver cirrhosis because of HCV [148 (87.6%)], hepatitis B virus (HBV) [5 (3%)], HCV and HBV coinfection [4 (2.4%)], and other aetiologies including vascular, autoimmune, and cryptogenic cirrhosis [12 (7.1%); Tables 1 and 2].

Prior to LT, 33 (19.52%) patients were HCV RNA negative, and 136 (80.46%) were HCV RNA positive. Thirty-one (18.3%) patients received antiviral treatment prior to LT. Forty-one (24.3%) patients experienced recurrent HCV infection, which was resolved in 37 (90.2%) patients (Table 1). Before the direct-acting antivirals (DAA) era, a Peg-interferon alfa-2a/Ribavirin (Peg-IFN/RBV) regimen was used for eligible patients, whereas after the availability of DAA therapy, sofosbuvir/daclatasvir ± RBV, sofosbuvir/simeprevir and ledipasvir/sofosbuvir regimens were used.

The majority of grafts had one or two ducts [both *n* = 78 (46.2%)], and the majority of patients needed one anastomosis [109 (64.5%)]. One to two stents were used in the majority of grafts [71 (42%) and 79 (46.7%), respectively; Table 1].

Fourteen (8.3%) patients experienced arterial complications; 12 patients had hepatic artery thrombosis (HAT), and two patients had hepatic artery stenosis (HAS; Table 1). In case HAT was detected not beyond two weeks post-LT, re-exploration was done, and after implementing inflow from the hepatic artery as well as backflow from the graft artery by embolectomy, re-anastomosis was conducted. In case of late presented HAT, interventional radiology and anticoagulation were done. In the case of HAS, a stent was inserted.

Development and management of BCs

Among the 169 RT-LDLT recipients included in this study, minor BLs occurred in 55 patients (32.5%) and stopped spontaneously without further management. Only in nine (16.4%) patients were pigtail insertion and further interventional management needed. Ninety-seven (57.4%) patients suffered from biliary infection; it mostly occurred early [91 (93.81%)], and 13 (7.7%) patients had three or more episodes (Table 1).

Sixty (35.5%) patients developed BS, most of which were anastomotic [59 (98.33%)], presented late [45 (75%)] and in one to two episodes [43 (25.4%)]. Most patients [45/60 (75%)] were HCV PCR positive during the occurrence of BS. Twenty-seven (45%) patients were not eligible for HCV antiviral treatment, while 14 (23.3%), 13 (21.7%) and 6 (10%) patients were treated before, during, and after the occurrence of BS, respectively (Table 1). Risk factors for BS were BL, biliary infection (especially if early or frequent), chronic graft rejection and longer graft arterialization time (Tables 3, 4 and Figure 2). In the multivariate analysis, graft arterialisation time > 130 min and biliary infection were the two determinants of BS (Table 5).

With respect to the management of BCs, ERCP with stenting ± dilatation was done for 60 (35.5%) patients, with 18 (10.7%) patients needing ≥ 3 ERCP sessions. PTC was attempted only in 8 (4.7%) patients, with one patient needing another session. These methods only failed in one patient who needed surgical reconstruction of BSs (Table 1).

Chronic graft rejection

Twenty-seven (16%) patients experienced chronic graft rejection. It was determined by biliary infection (especially if early or frequent), BS (especially if early or frequent), the need of ERCP (especially if multiple sessions), the number of stents used for BS treatment, hospital admission (especially if frequent) and recurrent HCV infection (Tables 1, 6 and Figure 3). The impact of these parameters on graft rejection was further demonstrated by multivariate analysis and Kaplan-Meier analysis (Table 7, Figure 4, and Supplementary material).

Table 1 Descriptive categorical data for the whole study population

Variable		*n* (%)
Etiology of cirrhosis	HCV	148 (87.6)
	HBV	5 (3)
	Combined HCV & HBV	4 (2.4)
	Others	12 (7.1)
Hepatocellular carcinoma	-	109 (64.5)
	+	60 (35.5)
Donors' gender	Male	141 (83.4)
	Female	28 (16.6)
Recipients' gender	Male	150 (88.8)
	Female	19 (11.2)
HCV PCR viremia prior to transplantation	Negative	33 (19.52)
	Below 200 000 IU	59 (34.91)
	200000 to 2 million	69 (40.82)
	More than 2 million	8 (4.73)
Antiviral treatment for HCV prior to transplantation	-	138 (81.7)
	+	31 (18.3)
Arterial complications	-	155 (91.7)
	+	14 (8.3)
Number of anastomosis	1 Anastomosis	109 (64.5)
	2 Anastomosis	57 (33.7)
	3 Anastomosis	3 (1.8)
Number of ducts	1 Duct	78 (46.2)
	2 Ducts	78 (46.2)
	3 Ducts	12 (7.1)
	4 Ducts	1 (0.6)
Number of stents introduced at surgery	Nil	7 (4.1)
	1 Stent	71 (42)
	2 Stents	79 (46.7)
	3 Stents	11 (6.5)
	4 Stents	1 (0.6)
Immunosuppressant	Tacrolimus	118 (69.8)
	Cyclosporine	51 (30.2)
Biliary leakage	-	114 (67.5)
	+	55 (32.5)
Need of pigtail catheter for biloma	-	46 (83.6)
	+	9 (16.4)
Biliary infection	-	72 (42.6)
	+	97 (57.4)
Frequency of biliary infection	1-2 Episodes	84 (49.7)
	≥ 3 Episodes	13 (7.7)
Biliary stricture	-	109 (64.5)

	+	60 (35.5)
Frequency of biliary stricture	1-2 Episodes	43 (25.4)
	≥ 3 Episodes	17 (10.1)
Need for ERCP	-	109 (64.5)
	+	60 (35.5)
Frequency of ERCP	1-2 ERCP	42 (24.9)
	≥ 3 ERCP	18 (10.7)
Need for PTC	-	161 (95.3)
	+	8 (4.7)
Frequency of PTC	1 PTC	7 (4.1)
	2 PTC	1 (0.6)
Surgical intervention for stricture	-	168 (99.4)
	+	1 (0.6)
HCV PCR during occurrence of stricture	Negative	15 (25)
	Below 200 000 IU	15 (25)
	200000 to 2 million	19 (31.7)
	More than 2 million	11 (18.3)
HCV antiviral treatment in relation to stricture diagnosis	No treatment	27 (45)
	Before stricture	14 (23.3)
	During occurrence of stricture	13 (21.7)
	After stricture	6 (10)
Admission related to BC	-	95 (56.2)
	+	74 (43.8)
Mortality	-	141 (83.4)
	+	28 (16.6)
Cause of mortality (total number: 28)	Biliary sepsis	5 (17.9)
	Graft rejection	4 (14.3)
	Recurrent HCV	3 (10.7)
	Other causes	16 (57.1)
Chronic rejection	-	142 (84)
	+	27 (16)
Recurrent HCV infection	-	128 (75.7)
	+	41 (24.3)
Resolution of recurrent HCV	-	4 (9.8)
	+	37 (90.2)
Graft failure	-	149 (88.2)
	+	20 (11.8)
Causes of graft failure (total number: 20)	Biliary sepsis	5 (25)
	Graft rejection	6 (30)
	Recurrent HCV	3 (15)
	Other causes	6 (30)
Early biliary infection (total = 97)	-	6 (6.18)
	+	91 (93.81)

Early biliary stricture (total = 60)	-	45 (75)
	+	15 (25)

Data presented in number (*n*) and percentage (%). HCV: Hepatitis C virus; HBV: Hepatitis B virus; PCR: Polymerase chain reaction; ERCP: Endoscopic retrograde cholangiopancreatography; PTC: Percutaneous transhepatic cholangiography; BC: Biliary complication.

Table 2 Descriptive numerical data for the whole study population

Variable	Data
MELD score	16 ± 4
Child score	10 ± 2
Donors' age (yr)	27 ± 6
Donors' BMI (kg/m^2)	24 ± 3
Recipient's age (yr)	50 ± 8
Recipient's BMI (kg/m^2)	28 ± 4
Total bilirubin (mg/dL)	2.6 (1.9-3.8)
Direct bilirubin (mg/dL)	1.3 (0.7-2.1)
Alkaline phosphatase (IU/L)	104 ± 48
Gamma-glutamyl transferase (IU/L)	36 (19-61)
Platelets (10^9/L)	79 ± 35
Cold ischemia time (min)	49 ± 24
Warm ischemia time (min)	48 ± 20
Graft arterialization time (min)	141 ± 51
Time to biliary infection (d)	16 (11-30)
Time to biliary stricture (d)	150 (120-218)
Time to mortality (d)	285 (55-808)
Time to chronic graft rejection (d)	490 (230-920)
Time to recurrent HCV (d)	391 (180-714)
Time to graft failure (d)	556 (135-1267)

Data are presented as mean ± SD or median and range. MELD: Model for end-stage liver disease; BMI: Body mass index; HCV: Hepatitis C virus.

Graft failure

Graft failure developed in 20 (11.8%) patients; the causes were chronic graft rejection [6 (30%)], biliary infection [5 (25%)], recurrent HCV infection [3 (15%)], and other causes [6 (30%); Table 1]. BL, the need for pigtail catheter insertion, biliary infection (especially if frequent), recurrent HCV infection and non-response to HCV therapy were the risk factors of graft failure (Tables 8, 9 and Figure 5). Kaplan-Meier survival analysis further proved the impact of major BL and biliary infection on graft survival (Figure 6).

Mortality

A total of 28 (16.6%) deaths occurred during follow-up. The aetiologies of mortality were biliary infection [5 (17.9%)], chronic graft rejection [4 (14.3%)], recurrent HCV infection [3 (10.7%)], and other causes [16 (57.1%); Table 1]. Unresolved recurrent HCV infection was the only risk factor for mortality (Table 10 and Figure 7). This was further proved by Kaplan-Meier survival analysis (Figure 8).

Table 3 Risk factors for biliary strictures: Categorical factors

Variable		Biliary strictures		OR	CI		*P* value[1]
		No stricture (*n* = 109)	Stricture (*n* = 60)		95% LCL	95% UCL	
		n, Row %	*n*, Row %				
Etiology of cirrhosis	HCV	95 (64.2)	53 (35.8)				0.142[2]
	Isolated HBV	5 (100)	0 (0)				
	Combined HCV & HBV	1 (25)	3 (75)				
	Causes other than viral hepatitis	8 (66.7)	4 (33.3)				
Donors' gender	Male	90 (63.8)	51 (36.2)	0.8	0.4	2.0	0.684
	Female	19 (67.9)	9 (32.1)				
Recipients' gender	Male	96 (64)	54 (36)	0.8	0.3	2.3	0.704
	Female	13 (68.4)	6 (31.6)				
HCV PCR viremia prior to transplantation	Negative	20 (60.6)	13 (39.4)				0.768[3]
	Below 200000 IU	41 (69.5)	18 (30.5)				
	200000 to 2 million	44 (63.8)	25 (36.2)				
	More than 2 million	4 (50)	4 (50)				
Antiviral treatment prior to transplantation	-	92 (66.7)	46 (33.3)	1.6	0.7	3.6	0.214
	+	17 (54.8)	14 (45.2)				
Hepatocellular carcinoma	-	71 (65.1)	38 (34.9)	1.1	0.6	2.1	0.815
	+	38 (63.3)	22 (36.7)				
Arterial complications	-	102 (65.8)	53 (34.2)	1.9	0.6	5.8	0.255[2]
	+	7 (50)	7 (50)				
Number of anastomoses	One	70 (64.2)	39 (35.8)				0.910[3]
	Two	37 (64.9)	20 (35.1)				
	Three	2 (66.7)	1 (33.3)				
Number of ducts	1 Duct	50 (64.1)	28 (35.9)				0.857[3]
	2 Ducts	52 (66.7)	26 (33.3)				
	3 Ducts	6 (50)	6 (50)				
	4 Ducts	1 (100)	0 (0)				
Number of stents	Nil	5 (71.4)	2 (28.6)				0.578[3]
	1 Stent	43 (60.6)	28 (39.4)				
	2 Stents	53 (67.1)	26 (32.9)				
	3 Stents	7 (63.6)	4 (36.4)				
	4 Stents	1 (100)	0 (0)				
Immunosuppressant	Tacrolimus	81 (68.6)	37 (31.4)	1.8	0.9	3.5	0.087
	Cyclosporine	28 (54.9)	23 (45.1)				
Biliary leakage	-	80 (70.2)	34 (29.8)	2.1	1.1	4.1	**0.026**
	+	29 (52.7)	26 (47.3)				
Biliary infection	-	62 (86.1)	10 (13.9)	6.6	3.0	14.4	**< 0.001**
	+	47 (48.5)	50 (51.5)				
Frequency of biliary infection	Nil	62 (86.1)	10 (13.9)				**< 0.0013**

	1-2 Episodes	45 (53.6)	39 (46.4)				
	≥ 3 Episodes	2 (15.4)	11 (84.6)				
Early biliary infection	-	64 (82.1)	14 (17.9)	4.7	2.3	9.5	**< 0.001**
	+	45 (49.5)	46 (50.5)				
Chronic graft rejection	-	99 (69.7)	43 (30.3)	3.9	1.7	9.2	**0.001**
	+	10 (37)	17 (63)				
Recurrent HCV	-	87 (68)	41 (32)	1.8	0.9	3.8	0.096
	+	22 (53.7)	19 (46.3)				

[1]Pearson chi-squared test unless otherwise indicated.
[2]Fisher's exact test.
[3]Chi-squared test for trend.
Data are presented as number (*n*) and percentage (%). OR: Odds ratio; LCL: Lower confidence limit; UCL: Lower confidence limit. HCV: Hepatitis C virus; HBV: Hepatitis B virus; PCR: Polymerase chain reaction.

Table 4 Risk factors for biliary stricture: Numerical factors

Variable	No biliary stricture (*n* = 109)	Biliary stricture (*n* = 60)	*P* value[1]
MELD score	15 (13-18)	15 (13-19)	0.588
CHILD score	10 (9-11)	9 (8-11)	0.198
Donors' age (yr)	27 (23-30)	25 (24-30)	0.727
Donors' BMI (kg/m^2)	25 (23-26)	24 (22-26)	0.155
Recipient's age (yr)	51 (46-56)	52 (48-55)	0.961
Recipient's BMI (kg/m^2)	27 (25-30)	27 (26-30)	0.219
Total bilirubin (mg/dL)	2.6 (1.9-3.7)	2.5 (1.9-4.1)	0.911
Direct bilirubin (mg/dL)	1.3 (0.8-2.1)	1.3 (0.7-1.9)	0.405
Alkaline phosphatase (IU/L)	99 (70-118)	84 (68-143)	0.982
GGT (IU/L)	36 (19-63)	34 (22-60)	0.992
Platelets (10^9/L)	70 (51-104)	68 (51-102)	0.830
Cold ischemia time (min)	45 (30-60)	45 (30-60)	0.929
Warm ischemia time (min)	45 (35-60)	45 (35-60)	0.860
Graft arterialization time (min)	120 (90-150)	155 (120-205)	**< 0.001**

[1]Mann-Whitney U test.
Data are presented as median and interquartile range (IQR). MELD: Model for end-stage liver disease; BMI: Body mass index.

Table 5 Multivariable binary logistic regression model for prediction of biliary stricture

Variable	*P* value	Odds ratio	95%CI
Graft arterializations time > 130 min	**0.001**	3.705	1.669-8.224
Biliary leakage	0.649	1.208	0.536-2.726
> 1 Episode of biliary infection	**< 0.0001**	9.892	4.086-23.952
Chronic graft rejection	0.173	2.088	0.725-6.014

CI: Confidence interval.

DISCUSSION

LT is considered the only curative therapeutic option for patients with end-stage hepatic disease. Several complications, especially BC, still endanger its short and long-term outcomes[21,28,29]. Many studies have focused on BC to improve care for transplanted recipients; however, data on long-term outcomes remains scarce[28].

The BC incidence rate is extremely diverse between centres. The overall incidence of BC, including BL, biliary infection and BS, in our study was 57.4%. This rate is comparable to previous reports[17,30-33]; however, it is higher than other published data[8,15,21,34,35]. This difference can be attributed to the heterogeneous structure between the different studies regarding the type of graft, surgical techniques and the inconsistent inclusion of biliary infection and bile stones as a part of BC.

In addition to surgical techniques, several risk factors for BC have been defined in the published literature[3,7,14,21,36], such as older recipients and donors, female recipients and recipients of female donors, ABO mismatch, a prolonged anhepatic phase and prolonged ischemia times. However, the current study and other investigators[15,22,34] were unable to establish any of these conditions as risk factors for BC. This may be attributed to the inclusion of only ABO-matched living grafts, the younger age of our donors and recipients and the male predominance in our cohort.

Additionally, cholestatic liver diseases and the use of RYHJ technique were independent risk factors for BS in previous reports[15,37]. However, this is not the case in our study because DDA was used in all the grafts; besides, we excluded patients with PBC and PSC from the final analysis to avoid the bias of primary disease recurrence as a confounding factor during analysis of BC.

In accordance with published data[15,17], no association between BC and MELD score was observed. This result differs from studies recognizing a higher MELD score as a risk factor for BC[3,28,34]. This can be explained by the lower MELD scores in our patients. Also, these conflicting results may reflect the well-established limits of the MELD score in predicting post-LT outcomes[38].

The ideal material and style of sutures in biliary reconstruction has been argued since the early development of LT. Kaldas *et al*[17] reported that the use of non-absorbable sutures for biliary reconstruction was an independent risk factor for BC. However, this was not the case in the present study due to the different suture material.

In accordance with previous results[22], we observed that the occurrence of BS was not related to the number of bile ducts or stent insertion. In contrast, Miyagi *et al*[8] and Ogiso *et al*[34] identified the number of bile ducts as a risk factor for BC. Furthermore, Senter-Zapata *et al*[15] reported that internal biliary stents and T-tube insertion were risk factors for BC post-LT. However, in our centre, we prefer external drainage for easy accessibility of biliary ducts for postoperative cholangiography to manage any strictures[22]; on the contrary, other centres do not prefer this due to the higher incidence of postoperative BL and biliary infections[14].

BCs are mostly identified in the first three to 12 mo post-LT[8,17]. Similarly, in consistence with other reports[7,15,17,30,31,33], we detected BL early in 55/169 (32.5%) patients, and BS in 60/169 (35.5%) patients. The majority of BSs were anastomotic and presented late.

In a similar management plan as other centres[22,24,29,30,34], minor BLs were treated conservatively; nonetheless, major BL required percutaneous drainage and/or stenting. ERCP was the treatment of choice for all patients. PTC was the treatment option if ERCP failed, and surgical intervention was performed as a last option.

In consistence with our results, other investigators[7,8,21,39] observed that BL and cholangitis were risk factors for the development of BS. This can be explained by the inflammatory process with the resultant progression of fibrosis and stricture formation [40].

In agreement with Rammohan *et al*[39], we identified longer arterialization time as a risk factor for BS. This finding is predictable because biliary tract vascularization is supplied exclusively by the hepatic artery[41-43], and a longer arterialization time of the graft may cause biliary ischemia and subsequently BS[28].

In contrast to the present and Ogiso *et al*[34] studies, other investigators[15,17,28,29,41] reported that hepatic artery complications were linked to the incidence of BC. This conflicting result can be attributed to the low incidence of arterial complications in our cohort as well as the early effective intervention for such complications.

It was previously reported that graft rejection and BC are interrelated conditions[15,35]; however, there are limited data concerning the impact of BC on chronic graft rejection. The incidence rate of chronic ductopenic rejection in our study was 27 (16%) patients; 23 (85.18%),17 (63%) and 13 (48.1%) of them had biliary infection, BS and BL,

Table 6 Relation between biliary complications and chronic graft rejection

Variable		No Chronic graft rejection (*n* = 142), *n* (%)	Chronic graft rejection (*n* = 27), *n* (%)	OR	CI		*P* value[1]
					95% LCL	95% UCL	
Biliary leakage	-	100 (87.7)	14 (12.3)	2.2	1.0	5.1	0.059
	+	42 (76.4)	13 (23.6)				
Insertion of pigtail catheter for biliary leakage	-	135 (84.4)	25 (15.6)	1.5	0.3	7.9	0.637[2]
	+	7 (77.8)	2 (22.2)				
Biliary infection	-	68 (94.4)	4 (5.6)	5.3	1.7	16.1	**0.001**
	+	74 (76.3)	23 (23.7)				
Frequency of biliary infection	Nil	68 (94.4)	4 (5.6)				**< 0.0013**
	1-2 Episodes	68 (81)	16 (19)				
	≥ 3 Episodes	6 (46.2)	7 (53.8)				
Early biliary infection	-	73 (93.6)	5 (6.4)	4.7	1.7	13.0	**0.002**
	+	69 (75.8)	22 (24.2)				
Biliary stricture	-	99 (90.8)	10 (9.2)	3.9	1.7	9.2	**0.001**
	+	43 (71.7)	17 (28.3)				
Frequency of biliary strictures	Nil	99 (90.8)	10 (9.2)				**0.0013**
	1-2 Episodes	32 (74.4)	11 (25.6)				
	≥ 3 Episodes	11 (64.7)	6 (35.3)				
Early biliary stricture	-	134 (87)	20 (13)	5.9	1.9	17.9	**0.0032**
	+	8 (53.3)	7 (46.7)				
Need for ERCP	-	99 (90.8)	10 (9.2)	3.9	1.7	9.2	**0.001**
	+	43 (71.7)	17 (28.3)				
Frequency of ERCP	Nil	99 (90.8)	10 (9.2)				**0.0013**
	1-2 ERCP	31 (73.8)	11 (26.2)				
	≥ 3 ERCP	12 (66.7)	6 (33.3)				
Number of stents introduced for stricture	Nil	102 (91.1)	10 (8.9)				**0.0023**
	1-2 stents	25 (73.5)	9 (26.5)				
	≥ 3 stents	15 (65.2)	8 (34.8)				
Need for PTC	-	136 (84.5)	25 (15.5)	1.8	0.3	9.5	0.615[2]
	+	6 (75)	2 (25)				
Frequency of PTC	Nil	136 (84.5)	25 (15.5)				0.190[3]
	1 PTC	6 (85.7)	1 (14.3)				
	2 PTC	0 (0)	1 (100)				
Surgical intervention for stricture	-	141 (83.9)	27 (16.1)	0.8	0.8	0.9	1.000[2]
	+	1 (100)	0 (0)				
HCV PCR at occurrence of stricture	Negative	10 (66.7)	5 (33.3)				0.660[3]
	Below 200000 IU	12 (80)	3 (20)				
	200000 to 2 million	15 (78.9)	4 (21.1)				
	More than 2 million	6 (54.5)	5 (45.5)				
Antiviral treatment in relation to stricture	Not given	21 (77.8)	6 (22.2)				0.536[2]
	Before stricture	9 (64.3)	5 (35.7)				

	After stricture	4 (66.7)	2 (33.3)				
	During occurrence of stricture	9 (69.2)	4 (30.8)				
Admission related to BC	-	85 (89.5)	10 (10.5)	2.5	1.1	5.9	**0.028**
	+	57 (77)	17 (23)				
Frequency of admissions related to biliary complications	Nil	85 (89.5)	10 (10.5)				**0.0023**
	1-2	35 (87.5)	5 (12.5)				
	≥ 3	22 (64.7)	12 (35.3)				
Recurrent HCV	-	116 (90.6)	12 (9.4)	5.6	2.3	13.3	**< 0.001**
	+	26 (63.4)	15 (36.6)				
Resolution of recurrent HCV	-	1 (25)	3 (75)	0.2	0.0	1.7	0.130[2]
	+	25 (67.6)	12 (32.4)				

[1]Pearson chi-squared test unless otherwise indicated.
[2]Fisher's exact test.
[3]Chi-squared test for trend.
Data are presented as number (*n*) and percentage (%). OR: Odds ratio; LCL: Lower confidence limit; UCL: Lower confidence limit; ERCP: Endoscopic retrograde cholangiopancreatography; PTC: Percutaneous transhepatic cholangiography; PCR: Polymerase chain reaction; HCV: Hepatitis C virus; BC: Biliary complication.

Table 7 Multivariable binary logistic regression model for prediction of chronic graft rejection

Variable	*P* value	Odds ratio	95%CI
Biliary infection	**0.001**	4.301	1.97-8.224
Early biliary infection	0.061	1.105	0.89-1.20
Frequency of biliary infection	**0.025**	1.208	0.536-2.726
Biliary stricture	**< 0.0001**	3.882	4.056-9.952
Need for ERCP	**0.02**	2.91	1.85-7.97
Frequency of ERCP	0.074	1.098	0.99-1.114
Number of stents	0.62	1.22	0.57-2.42
Admission related to BCs	0.082	1.102	0.99-1.40
Frequency of admission	0.51	1.73	0.56-7.5
Recurrent HCV	**0.032**	3.11	1.97-8.07

CI: Confidence interval; ERCP: Endoscopic retrograde cholangiopancreatography; HCV: Hepatitis C virus; BC: Biliary complication.

respectively. Additionally, chronic graft rejection was a risk factor for BS. Similar findings were reported by other investigators[44]. This is consistent with the histopathological findings of chronic ductopenic rejection where ductal inflammation and proliferation are seen in early stages and biliary duct fibrosis with progressive ductopenia is seen in late stages, which is manifested as intrahepatic BS by MRCP[45].

Biliary infection was a risk factor for chronic graft rejection and graft failure, which is explained by interrupted immunosuppressive therapy during times of sepsis[46,47].

In agreement with previous results[15,17,34,48], we found that the main reasons for graft failure were chronic ductopenic rejection, biliary infection, BL, and recurrent HCV infection, while Egeli *et al*[49] reported that HCC recurrence was the main cause of graft failure. This is justified by the inclusion of many patients beyond Milan criteria in their study.

In contrast to Mathur *et al*[50] and in consistence with other investigators[8,17,34,41], there was no association between BS and graft failure. This proves that early detection and efficient management of BS can prevent graft loss.

Table 8 Relation between biliary complications and graft failure

Variable		No graft failure (*n* = 149), *n* (%)	Graft failure (*n* = 20), *n* (%)	OR	CI		*P* value[1]
					95% LCL	95% UCL	
Biliary leakage	-	105 (92.1)	9 (7.9)	2.9	1.1	7.5	**0.022**
	+	44 (80)	11 (20)				
Insertion of pigtail catheter	-	144 (90)	16 (10)	7.2	1.8	29.6	**0.012**[2]
	+	5 (55.6)	4 (44.4)				
Biliary infection	-	68 (94.4)	4 (5.6)	3.4	1.1	10.5	**0.029**
	+	81 (83.5)	16 (16.5)				
Frequency of biliary infection	Nil	68 (94.4)	4 (5.6)				**0.021**[3]
	1-2 Episodes	71 (84.5)	13 (15.5)				
	≥ 3 Episodes	10 (76.9)	3 (23.1)				
Early biliary infection	-	73 (93.6)	5 (6.4)	2.9	1.0	8.3	**0.043**
	+	76 (83.5)	15 (16.5)				
Biliary stricture	-	98 (89.9)	11 (10.1)	1.6	0.6	4.0	0.345
	+	51 (85)	9 (15)				
Frequency of biliary stricture	Nil	98 (89.9)	11 (10.1)				0.168[3]
	1-2 Episodes	38 (88.4)	5 (11.6)				
	≥ 3 Episodes	13 (76.5)	4 (23.5)				
Early biliary stricture	-	137 (89)	17 (11)	2.0	0.5	7.9	0.392[2]
	+	12 (80)	3 (20.0)				
Need for ERCP	-	98 (89.9)	11 (10.1)	1.6	0.6	4.0	0.345
	+	51 (85)	9 (15.0)				
Frequency of ERCP	Nil	98 (89.9)	11 (10.1)				0.188[3]
	1-2 ERCP	37 (88.1)	5 (11.9)				
	≥ 3 ERCP	14 (77.8)	4 (22.2)				
Number of stents introduced for stricture	Nil	101 (90.2)	11 (9.8)				0.136[3]
	1-2 Stents	30 (88.2)	4 (11.8)				
	≥ 3 Stents	18 (78.3)	5 (21.7)				
Need for PTC	-	142 (88.2)	19 (11.8)	1.1	0.1	9.2	1.000[2]
	+	7 (87.5)	1 (12.5)				
Frequency of PTC	Nil	142 (88.2)	19 (11.8)				0.374[3]
	1 PTC	7 (100)	0 (0)				
	2 PTC	0 (0)	1 (100)				
Surgical intervention for stricture	-	148 (88.1)	20 (11.9)	0.9	0.8	0.9	1.000[2]
	+	1 (100)	0 (0)				
HCV PCR at occurrence of stricture	Negative	13 (86.7)	2 (13.3)				0.292[3]
	Below 200000 IU	13 (86.7)	2 (13.3)				
	200000 to 2 million	18 (94.7)	1 (5.3)				
	More than 2 million	7 (63.6)	4 (36.4)				
Antiviral treatment in relation to stricture	Not given	24 (88.9)	3 (11.1)				0.836[2]
	Before stricture	11 (78.6)	3 (21.4)				
	After stricture	5 (83.3)	1 (16.7)				

	During occurrence of stricture	11 (84.6)	2 (15.4)				
Admission related to BC	-	85 (89.5)	10 (10.5)	1.3	0.5	3.4	0.551
	+	64 (86.5)	10 (13.5)				
Frequency of admissions related to BC	Nil	85 (89.5)	10 (10.5)				0.119
	1-2 ERCP	38 (95)	2 (5)				
	≥3 ERCP	26 (76.5)	8 (23.5)				
Recurrent HCV infection	-	118 (92.2)	10 (7.8)	3.8	1.5	10.0	**0.010**[2]
	+	31 (75.6)	10 (24.4)				
Resolution of recurrent HCV	-	0 (0)	4 (100)	6.2	3.0	12.8	**0.002**[2]
	+	31 (83.8)	6 (16.2)				

[1]Pearson chi-squared test unless otherwise indicated.
[2]Fisher's exact test.
[3]Chi-squared test for trend.
Data are presented as number (*n*) and percentage (%). OR: Odds ratio; LCL: Lower confidence limit; UCL: Lower confidence limit; ERCP: Endoscopic retrograde cholangiopancreatography; PTC: Percutaneous transhepatic cholangiography; PCR: Polymerase chain reaction; HCV: Hepatitis C virus; BC: Biliary complication.

Table 9 Multivariable binary logistic regression model for prediction of graft failure

Variable	*P* value	Odds ratio	95%CI
Biliary leakage	**0.021**	1.82	1.34-5.57
Insertion of pigtail catheter	**0.010**	3.76	1.45-11.83
Biliary infection	**0.032**	3.11	1.03-9.06
Early biliary infection	0.05	1.34	0.65-2.86
Frequency of biliary infection	**0.001**	2.52	1.28-4.91
Nonresponse to HCV anti-viral therapy	**0.001**	3.6	1.8-9.34
Recurrent HCV	**0.001**	3.56	1.86-10.71

CI: Confidence interval; HCV: Hepatitis C virus.

In the current study, recurrent HCV infection was a risk factor for chronic graft rejection, graft failure and mortality. This is predictable due to the aggressive course of HCV recurrence in LT recipients through direct cytotoxic effects on the graft, resulting in graft failure[48,49,51-53]. It is noteworthy that DAA were not FDA approved during the first three years of the study duration; thus, many patients were ineligible for the Peg-IFN/RBV regimen at that time.

Similar to Takagi *et al*[54] study, the overall mortality rate for recipients was 28 (16.56%). Unresolved HCV recurrence was the only significant risk factor for mortality, while BC had no impact on recipients' survival in the present study. This is similar to previous results[17,21,39,41,49]. In contrast, other investigators[15,33] observed a worse survival rate in recipients with BC. This indicates that early detection and effective management of BC can improve recipients' survival[2,17].

This study has the strength of being large volume with a long duration of follow-up, as well as the exclusion of LDLT recipients because of cholestatic hepatic diseases; however, it is limited by being a single-centre retrospective study. Multi-centre large-scale studies are required to comprehensively investigate the risk factors for the occurrence and impacts of BC.

CONCLUSION

In conclusion, biliary complications after RT-LDLT represent an independent risk

Table 10 Relation between biliary complications and mortality

Variable		Survivors (*n* = 141), *n* (%)	Non-survivors (*n* = 28), *n* (%)	OR	CI		*P* value[1]
					95% LCL	95% UCL	
Biliary leakage	-	97 (85.1)	17 (14.9)	1.4	0.6	3.3	0.405
	+	44 (80)	11 (20)				
Biliary infection	-	60 (83.3)	12 (16.7)	1.0	0.4	2.2	1.000[2]
	+	81 (83.5)	16 (16.5)				
Frequency of biliary infection	Nil	60 (83.3)	12 (16.7)				0.940[3]
	1-2 Episodes	70 (83.3)	14 (16.7)				
	≥ 3 Episodes	11 (84.6)	2 (15.4)				
Early biliary infection	-	64 (82.1)	14 (17.9)	0.8	0.4	1.9	0.655[2]
	+	77 (84.6)	14 (15.4)				
Biliary stricture	-	89 (81.7)	20 (18.3)	0.7	0.3	1.7	0.401[2]
	+	52 (86.7)	8 (13.3)				
Frequency of biliary strictures	Nil	89 (81.7)	20 (18.3)				0.396[3]
	1-2 Episodes	37 (86)	6 (14)				
	≥ 3 Episodes	15 (88.2)	2 (11.8)				
Early biliary stricture	-	128 (83.1)	26 (16.9)	0.8	0.2	3.6	1.000[2]
	+	13 (86.7)	2 (13.3)				
Need for ERCP	-	89 (81.7)	20 (18.3)	0.7	0.3	1.7	0.401[2]
	+	52 (86.7)	8 (13.3)				
Frequency of ERCP	Nil	89 (81.7)	20 (18.3)				0.375[3]
	1-2 ERCP	36 (85.7)	6 (14.3)				
	≥ 3 ERCP	16 (88.9)	2 (11.1)				
Number of stents introduced for stricture	Nil	92 (82.1)	20 (17.9)				0.520[3]
	1-2 Stents	29 (85.3)	5 (14.7)				
	≥ 3 Stents	20 (87)	3 (13)				
Need for PTC	-	134 (83.2)	27 (16.8)	0.7	0.1	6.0	1.000[2]
	+	7 (87.5)	1 (12.5)				
Frequency of PTC	Nil	134 (83.2)	27 (16.8)				0.674[3]
	1 PTC	7 (100)	0 (0)				
	2 PTC	0 (0)	1 (100)				
Surgical intervention for stricture	-	140 (83.3)	28 (16.7)				1.000[2]
	+	1 (100)	0 (0)				
HCV PCR at occurrence of stricture	Negative	12 (80)	3 (20)				0.849[3]
	Below 200 000 IU	14 (93.3)	1 (6.7)				
	200000 to 2 million	18 (94.7)	1 (5.3)				
	More than 2 million	8 (72.7)	3 (27.3)				
Antiviral treatment in relation to stricture	Not given	23 (85.2)	4 (14.8)				1.000[2]
	Before stricture	12 (85.7)	2 (14.3)				
	After stricture	5 (83.3)	1 (16.7)				
	During occurrence of stricture	12 (92.3)	1 (7.7)				

Admission related to BC	-	75 (78.9)	20 (21.1)	0.5	0.2	1.1	0.076[2]
	+	66 (89.2)	8 (10.8)				
Recurrent HCV	-	108 (84.4)	20 (15.6)	1.3	0.5	3.2	0.560[2]
	+	33 (80.5)	8 (19.5)				
Resolution of recurrent HCV (n = 41)	-	0 (0)	4 (9.7)	9.3	3.7	23.3	**0.001**[2]
	+	33 (80.4)	4 (9.7)				

[1]Pearson chi-squared test unless otherwise indicated.
[2]Fisher's exact test.
[3]Chi-squared test for trend.
Data are presented as number (n) and percentage (%). OR: Odds ratio; LCL: Lower confidence limit; UCL: Lower confidence limit; ERCP: Endoscopic retrograde cholangiopancreatography; PTC: Percutaneous transhepatic cholangiography; PCR: Polymerase chain reaction; HCV: Hepatitis C virus; BC: Biliary complication.

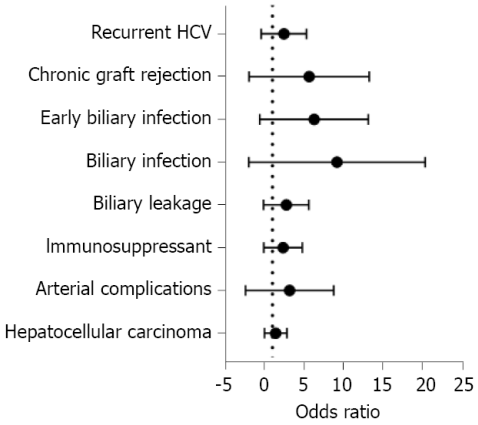

Figure 2 Forest plot for risk factors for biliary strictures. HCV: Hepatitis C virus.

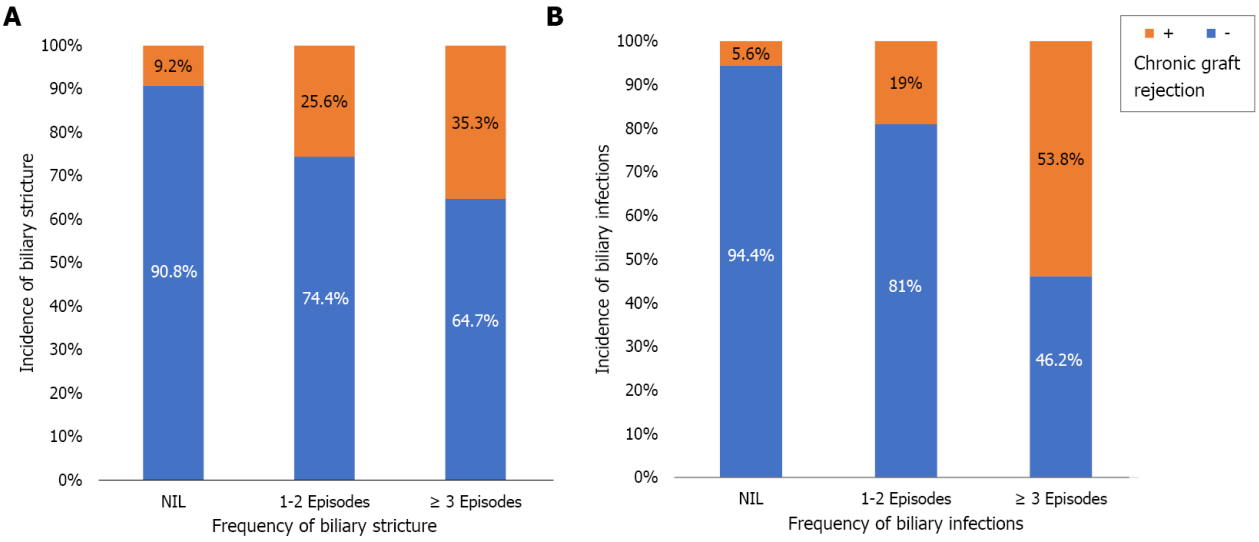

Figure 3 Incidence of chronic graft rejection according to the occurrence of biliary strictures (A) and biliary infections (B).

factor for chronic graft rejection and graft failure; nonetheless, effective management of these complications can improve patient and graft survival.

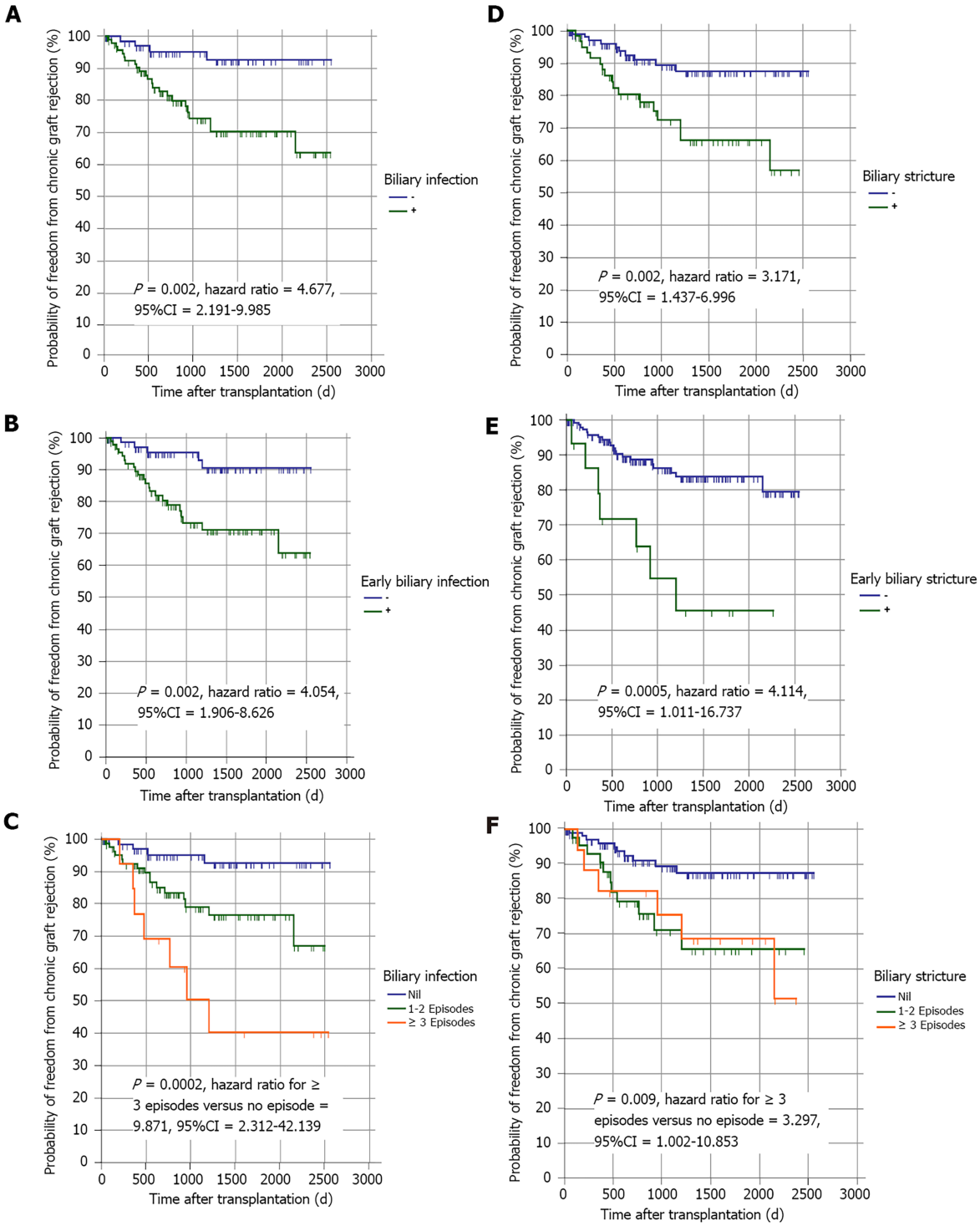

Figure 4 Kaplan-Meier curves. A-C: The curves showing the probability of chronic graft rejection in patients regarding the occurrence (A), timing (B), and frequency (C) of biliary infection; D-F: The curves showing the probability of chronic graft rejection in patients regarding the occurrence (D), timing (E), and frequency (F) of biliary strictures.

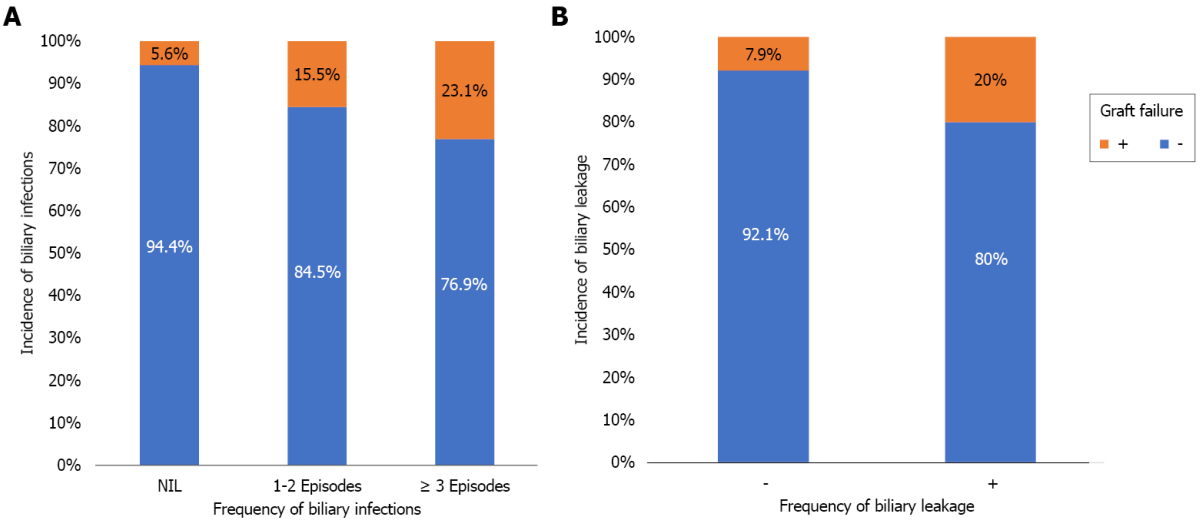

Figure 5 Incidence of graft failure according to the occurrence of biliary infections (A) and biliary leakage (B).

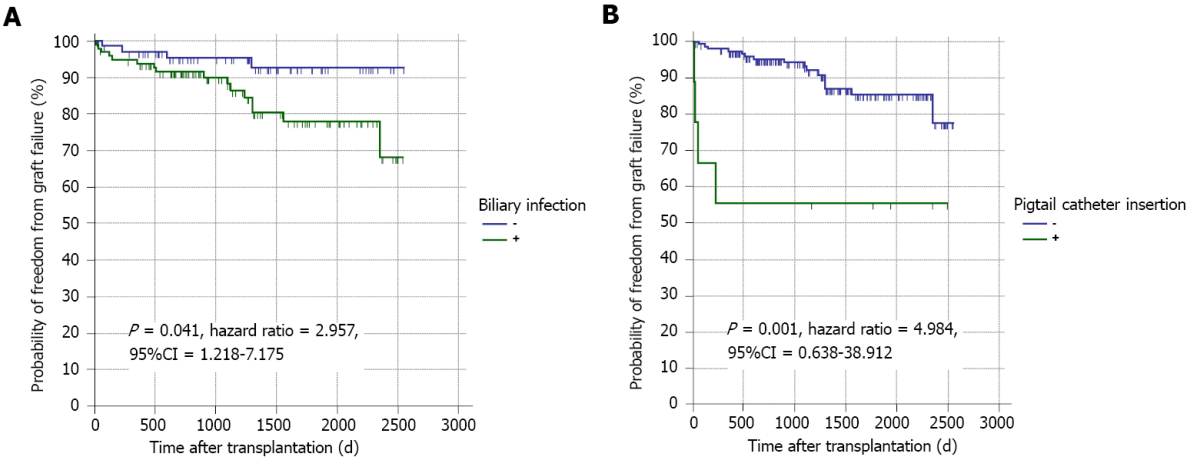

Figure 6 Kaplan-Meier curves. The curves showing the probability of graft failure in patients regarding the occurrence of biliary infection (A) and large bile leaks as indicated by pigtail insertion (B).

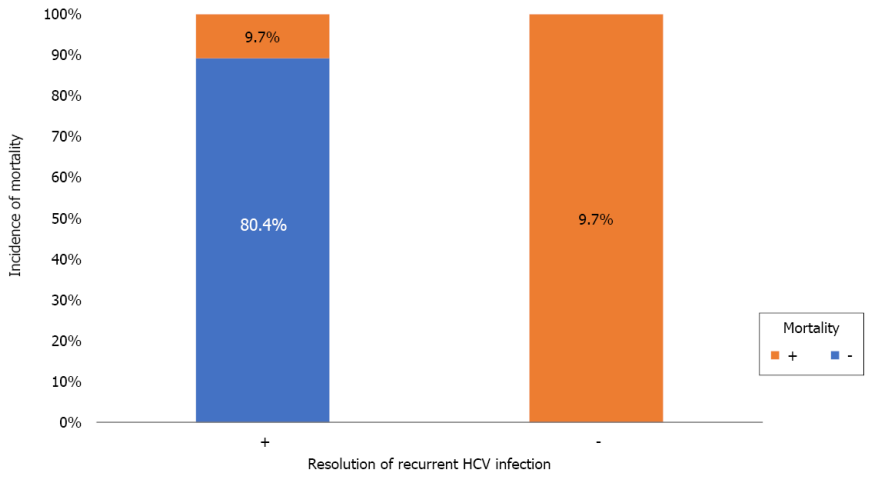

Figure 7 Mortality rate in patients with or without resolution of recurrent hepatitis C virus in patient with biliary stricture. HCV: Hepatitis C virus.

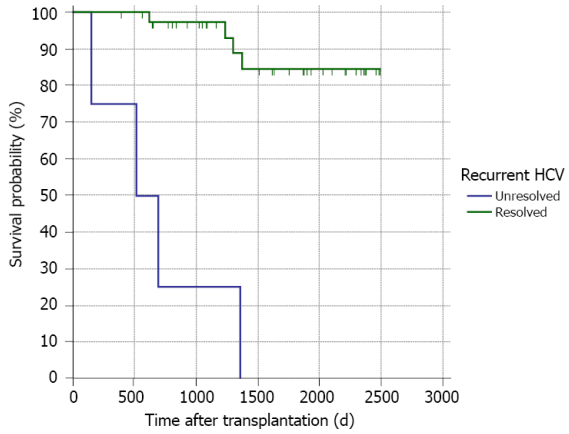

Figure 8 Kaplan-Meier curves showing the survival probability of patients with resolved or unresolved hepatitis C virus. HCV: Hepatitis C virus.

ARTICLE HIGHLIGHTS

Research background

Despite considerable progress in liver transplantation (LT) surgical performance and peri-operative management, post-LT biliary complications (BCs) remain a considerable cause of morbidity, mortality, increased cost, and graft loss.

Research motivation

Many studies have focused on biliary complications to improve care for transplanted recipients; however, data on long-term outcomes remain scarce.

Research objectives

We aimed to investigate the impact of BCs after right lobe-LDLT (RL-LDLT) on chronic graft rejection, graft failure and mortality.

Research methods

From 2011 to 2016, 215 adult recipients underwent RL-LDLT at our centre. We excluded 46 recipients who met the exclusion criteria, and 169 recipients were included in the final analysis. Donors' and recipients' demographic data, clinical data, operative details and postoperative course information were collected. We also reviewed the management and outcomes of BCs. Recipients were followed for at least 12 mo post-LT until December 2017 or graft or patient loss.

Research results

The overall incidence rate of BCs including biliary leakage, biliary infection and biliary stricture was 57.4%. Twenty-seven (16%) patients experienced chronic graft rejection. Graft failure developed in 20 (11.8%) patients. A total of 28 (16.6%) deaths occurred during follow-up. BCs were a risk factor for the occurrence of chronic graft rejection and failure; however, mortality was determined by recurrent hepatitis C virus infection.

Research conclusions

Biliary complications after RT-LDLT represent an independent risk factor for chronic graft rejection and graft failure; nonetheless, effective management of these complications can improve patient and graft survival.

Research perspectives

Multi-centre large-scale studies are required to comprehensively investigate the risk factors for the occurrence and impacts of BC.

REFERENCES

1 **Jadlowiec CC**, Taner T. Liver transplantation: Current status and challenges. *World J Gastroenterol* 2016; **22**: 4438-4445 [PMID: 27182155 DOI: 10.3748/wjg.v22.i18.4438]
2 **Chang JH**, Lee I, Choi MG, Han SW. Current diagnosis and treatment of benign biliary strictures after living donor liver transplantation. *World J Gastroenterol* 2016; **22**: 1593-1606 [PMID: 26819525 DOI: 10.3748/wjg.v22.i4.1593]
3 **Nemes B**, Gámán G, Doros A. Biliary complications after liver transplantation. *Expert Rev Gastroenterol Hepatol* 2015; **9**: 447-466 [PMID: 25331256 DOI: 10.1586/17474124.2015.967761]
4 **Tsujino T**, Isayama H, Kogure H, Sato T, Nakai Y, Koike K. Endoscopic management of biliary strictures after living donor liver transplantation. *Clin J Gastroenterol* 2017; **10**: 297-311 [PMID: 28600688 DOI: 10.1007/s12328-017-0754-z]
5 **Rela M**, Reddy MS. Living donor liver transplant (LDLT) is the way forward in Asia. *Hepatol Int* 2017; **11**: 148-151 [PMID: 28097531 DOI: 10.1007/s12072-016-9780-z]
6 **Doyle MB**, Maynard E, Lin Y, Vachharajani N, Shenoy S, Anderson C, Earl M, Lowell JA, Chapman WC. Outcomes with split liver transplantation are equivalent to those with whole organ transplantation. *J Am Coll Surg* 2013; **217**: 102-12; discussion 113 [PMID: 23639200 DOI: 10.1016/j.jamcollsurg.2013.03.003]
7 **Rao HB**, Prakash A, Sudhindran S, Venu RP. Biliary strictures complicating living donor liver transplantation: Problems, novel insights and solutions. *World J Gastroenterol* 2018; **24**: 2061-2072 [PMID: 29785075 DOI: 10.3748/wjg.v24.i19.2061]
8 **Miyagi S**, Kakizaki Y, Shimizu K, Miyazawa K, Nakanishi W, Hara Y, Tokodai K, Nakanishi C, Kamei T, Ohuchi N, Satomi S. Arterial and biliary complications after living donor liver transplantation: a single-center retrospective study and literature review. *Surg Today* 2018; **48**: 131-139 [PMID: 28439714 DOI: 10.1007/s00595-017-1515-9]
9 **Baker TB**, Zimmerman MA, Goodrich NP, Samstein B, Pomfret EA, Pomposelli JJ, Gillespie BW, Berg CL, Emond JC, Merion RM. Biliary reconstructive techniques and associated anatomic variants in adult living donor liver transplantations: The adult-to-adult living donor liver transplantation cohort study experience. *Liver Transpl* 2017; **23**: 1519-1530 [PMID: 28926171 DOI: 10.1002/lt.24872]
10 **Lee SG**. A complete treatment of adult living donor liver transplantation: a review of surgical technique and current challenges to expand indication of patients. *Am J Transplant* 2015; **15**: 17-38 [PMID: 25358749 DOI: 10.1111/ajt.12907]
11 **Chok KS**, Lo CM. Prevention and management of biliary anastomotic stricture in right-lobe living-donor liver transplantation. *J Gastroenterol Hepatol* 2014; **29**: 1756-1763 [PMID: 24909190 DOI: 10.1111/jgh.12648]
12 **Samstein B**, Smith AR, Freise CE, Zimmerman MA, Baker T, Olthoff KM, Fisher RA, Merion RM. Complications and Their Resolution in Recipients of Deceased and Living Donor Liver Transplants: Findings From the A2ALL Cohort Study. *Am J Transplant* 2016; **16**: 594-602 [PMID: 26461803 DOI: 10.1111/ajt.13479]
13 **Simoes P**, Kesar V, Ahmad J. Spectrum of biliary complications following live donor liver transplantation. *World J Hepatol* 2015; **7**: 1856-1865 [PMID: 26207167 DOI: 10.4254/wjh.v7.i14.1856]
14 **Girometti R**, Pancot M, Como G, Zuiani C. Imaging of liver transplantation. *Eur J Radiol* 2017; **93**: 295-307 [PMID: 28545872 DOI: 10.1016/j.ejrad.2017.05.014]
15 **Senter-Zapata M**, Khan AS, Subramanian T, Vachharajani N, Dageforde LA, Wellen JR, Shenoy S, Majella Doyle MB, Chapman WC. Patient and Graft Survival: Biliary Complications after Liver Transplantation. *J Am Coll Surg* 2018; **226**: 484-494 [PMID: 29360615 DOI: 10.1016/j.jamcollsurg.2017.12.039]
16 **Oh HC**. Percutaneous Transhepatic Cholangioscopy in Bilioenteric Anastomosis Stricture. *Clin Endosc* 2016; **49**: 530-532 [PMID: 27642850 DOI: 10.5946/ce.2016.125]
17 **Kaldas FM**, Korayem IM, Russell TA, Agopian VG, Aziz A, DiNorcia J, Farmer DG, Yersiz H, Hiatt JR, Busuttil RW. Assessment of Anastomotic Biliary Complications in Adult Patients Undergoing High-Acuity Liver Transplant. *JAMA Surg* 2019; **154**: 431-439 [PMID: 30758485 DOI: 10.1001/jamasurg.2018.5527]
18 **Lee DW**, Jo HH, Abdullah J, Kahaleh M. Endoscopic Management of Anastomotic Strictures after Liver Transplantation. *Clin Endosc* 2016; **49**: 457-461 [PMID: 27744664 DOI: 10.5946/ce.2016.130]
19 **Koo PT**, Medici V, Tabibian JH. Anastomotic Biliary Stricture Development after Liver Transplantation in the Setting of Retained Prophylactic Intraductal Pediatric Feeding Tube: Case and Review. *Case Reports Hepatol* 2018; **2018**: 4707389 [PMID: 30363933 DOI: 10.1155/2018/4707389]
20 **Crismale JF**, Ahmad J. Endoscopic Management of Biliary Issues in the Liver Transplant Patient. *Gastrointest Endosc Clin N Am* 2019; **29**: 237-256 [PMID: 30846151 DOI: 10.1016/j.giec.2018.11.003]
21 **Hong SY**, Hu XG, Lee HY, Won JH, Kim JW, Shen XY, Wang HJ, Kim BW. Longterm Analysis of Biliary Complications After Duct-to-Duct Biliary Reconstruction in Living Donor Liver Transplantations. *Liver Transpl* 2018; **24**: 1050-1061 [PMID: 29633539 DOI: 10.1002/lt.25074]
22 **Azzam AZ**, Tanaka K. Biliary complications after living donor liver transplantation: A retrospective analysis of the Kyoto experience 1999-2004. *Indian J Gastroenterol* 2017; **36**: 296-304 [PMID: 28744748 DOI: 10.1007/s12664-017-0771-3]
23 **Santosh Kumar KY**, Mathew JS, Balakrishnan D, Bharathan VK, Thankamony Amma BSP,

Gopalakrishnan U, Narayana Menon R, Dhar P, Vayoth SO, Sudhindran S. Intraductal Transanastomotic Stenting in Duct-to-Duct Biliary Reconstruction after Living-Donor Liver Transplantation: A Randomized Trial. *J Am Coll Surg* 2017; **225**: 747-754 [PMID: 28916322 DOI: 10.1016/j.jamcollsurg.2017.08.024]

24 **Elwir S**, Thompson J, Amateau SK, Trikudanathan G, Attam R, Hassan M, Kandaswamy R, Pruett T, Lake J, Chinnakotla S, Freeman ML, Arain MA. Endoscopic Management of Biliary Leaks and Strictures After Living Donor Liver Transplantation: Optimizing Techniques for Successful Management. *Dig Dis Sci* 2017; **62**: 244-252 [PMID: 27866300 DOI: 10.1007/s10620-016-4367-z]

25 **Mazzaferro V**, Regalia E, Doci R, Andreola S, Pulvirenti A, Bozzetti F, Montalto F, Ammatuna M, Morabito A, Gennari L. Liver transplantation for the treatment of small hepatocellular carcinomas in patients with cirrhosis. *N Engl J Med* 1996; **334**: 693-699 [PMID: 8594428 DOI: 10.1056/NEJM199603143341104]

26 **Ishiko T**, Egawa H, Kasahara M, Nakamura T, Oike F, Kaihara S, Kiuchi T, Uemoto S, Inomata Y, Tanaka K. Duct-to-duct biliary reconstruction in living donor liver transplantation utilizing right lobe graft. *Ann Surg* 2002; **236**: 235-240 [PMID: 12170029 DOI: 10.1097/00000658-200208000-00012]

27 **Tanaka K**, Uemoto S, Tokunaga Y, Fujita S, Sano K, Nishizawa T, Sawada H, Shirahase I, Kim HJ, Yamaoka Y. Surgical techniques and innovations in living related liver transplantation. *Ann Surg* 1993; **217**: 82-91 [PMID: 8424706 DOI: 10.1097/00000658-199301000-00014]

28 **Kaltenborn A**, Gutcke A, Gwiasda J, Klempnauer J, Schrem H. Biliary complications following liver transplantation: Single-center experience over three decades and recent risk factors. *World J Hepatol* 2017; **9**: 147-154 [PMID: 28217251 DOI: 10.4254/wjh.v9.i3.147]

29 **Coelho JCU**, Leite LO, Molena A, Freitas ACT, Matias JEF. BILIARY COMPLICATIONS AFTER LIVER TRANSPLANTATION. *Arq Bras Cir Dig* 2017; **30**: 127-131 [PMID: 29257849 DOI: 10.1590/0102-6720201700020011]

30 **Rao HB**, Koshy AK, Sudhindran S, Prabhu NK, Venu RP. Paradigm shift in the management of bile duct strictures complicating living donor liver transplantation. *Indian J Gastroenterol* 2019; **38**: 488-497 [PMID: 32065353 DOI: 10.1007/s12664-019-01000-2]

31 **Iesari S**, Inostroza Núñez ME, Rico Juri JM, Ciccarelli O, Bonaccorsi-Riani E, Coubeau L, Laterre PF, Goffette P, De Reyck C, Lengelé B, Gianello P, Lerut J. Adult-to-adult living-donor liver transplantation: The experience of the Université catholique de Louvain. *Hepatobiliary Pancreat Dis Int* 2019; **18**: 132-142 [PMID: 30850341 DOI: 10.1016/j.hbpd.2019.02.007]

32 **Boraschi P**, Donati F, Pacciardi F, Ghinolfi D, Falaschi F. Biliary complications after liver transplantation: Assessment with MR cholangiopancreatography and MR imaging at 3T device. *Eur J Radiol* 2018; **106**: 46-55 [PMID: 30150050 DOI: 10.1016/j.ejrad.2018.07.009]

33 **Hafeez Bhatti AB**, Dar FS, Qureshi AI, Khan NY, Zia HH, Khan EUD, Khan NA, Salih M, Shah NH. Failure to rescue in living donor liver transplantation: Patterns and predictors. *Int J Surg* 2017; **44**: 281-286 [PMID: 28694002 DOI: 10.1016/j.ijsu.2017.07.026]

34 **Ogiso S**, Kamei H, Onishi Y, Kurata N, Jobara K, Kawashima H, Ogura Y. Decreased long-term graft survival in persistent biliary complications after right-lobe living-donor liver transplantation. *Clin Transplant* 2020; **34**: e13771 [PMID: 31846118 DOI: 10.1111/ctr.13771]

35 **Dogan N**, Hüsing-Kabar A, Schmidt HH, Cicinnati VR, Beckebaum S, Kabar I. Acute allograft rejection in liver transplant recipients: Incidence, risk factors, treatment success, and impact on graft failure. *J Int Med Res* 2018; **46**: 3979-3990 [PMID: 29996675 DOI: 10.1177/0300060518785543]

36 **Memeo R**, Piardi T, Sangiuolo F, Sommacale D, Pessaux P. Management of biliary complications after liver transplantation. *World J Hepatol* 2015; **7**: 2890-2895 [PMID: 26689137 DOI: 10.4254/wjh.v7.i29.2890]

37 **Pena Polanco NA**, Levy C, Martin EF. Cholestatic Liver Diseases After Liver Transplant. *Clin Liver Dis* 2017; **21**: 403-420 [PMID: 28364821 DOI: 10.1016/j.cld.2016.12.011]

38 **Rana A**, Hardy MA, Halazun KJ, Woodland DC, Ratner LE, Samstein B, Guarrera JV, Brown RS Jr, Emond JC. Survival outcomes following liver transplantation (SOFT) score: a novel method to predict patient survival following liver transplantation. *Am J Transplant* 2008; **8**: 2537-2546 [PMID: 18945283 DOI: 10.1111/j.1600-6143.2008.02400.x]

39 **Rammohan A**, Govil S, Vargese J, Kota V, Reddy MS, Rela M. Changing pattern of biliary complications in an evolving liver transplant unit. *Liver Transpl* 2017; **23**: 478-486 [PMID: 28152569 DOI: 10.1002/lt.24736]

40 **Daniel K**, Said A. Early Biliary complications after liver transplantation. *Clin Liver Dis (Hoboken)* 2017; **10**: 63-67 [PMID: 30992762 DOI: 10.1002/cld.654]

41 **Jeong S**, Wang X, Wan P, Sha M, Zhang J, Xia L, Tong Y, Luo Y, Xia Q. Risk factors and survival outcomes of biliary complications after adult-to-adult living donor liver transplantation. *United European Gastroenterol J* 2017; **5**: 997-1006 [PMID: 29163966 DOI: 10.1177/2050640616688994]

42 **Kim PT**, Fernandez H, Gupta A, Saracino G, Ramsay M, McKenna GJ, Testa G, Anthony T, Onaca N, Ruiz RM, Klintmalm GB. Low Measured Hepatic Artery Flow Increases Rate of Biliary Strictures in Deceased Donor Liver Transplantation: An Age-Dependent Phenomenon. *Transplantation* 2017; **101**: 332-340 [PMID: 27941438 DOI: 10.1097/TP.0000000000001564]

43 **Nacif LS**, Ducatti L, Andraus W, D'Albuquerque LC. Hepatic Artery Thrombosis after Orthotopic Liver Transplantation. *Adv Res Gastroentero Hepatol* 2015; **1**: 555560 [DOI: 10.19080/ARGH.2015.01.555560]

44 **Mocchegiani F**, Vincenzi P, Lanari J, Montalti R, Nicolini D, Svegliati Baroni G, Risaliti A, Vivarelli M. Immunological risk factors in biliary strictures after liver transplantation. *Ann Transplant* 2015;

20: 218-224 [PMID: 25892243 DOI: 10.12659/AOT.892393]

45 **Koukoulis GK**, Shen J, Karademir S, Jensen D, Williams J. Cholangiocytic apoptosis in chronic ductopenic rejection. *Hum Pathol* 2001; **32**: 823-827 [PMID: 11521226 DOI: 10.1053/hupa.2001.26465]

46 **Tannuri AC**, Lima F, Mello ES, Tanigawa RY, Tannuri U. Prognostic factors for the evolution and reversibility of chronic rejection in pediatric liver transplantation. *Clinics (Sao Paulo)* 2016; **71**: 216-220 [PMID: 27166772 DOI: 10.6061/clinics/2016(04)07]

47 **Horster S**, Bäuerlein FJ, Mandel P, Raziorrouh B, Hopf C, Stemmler HJ, Guba M, Angele M, Stangl M, Rentsch M, Frey L, Kaspar M, Kaczmarek I, Eberle J, Nickel T, Gruener N, Zachoval R, Diepolder H. Influence of hepatitis C virus infection and high virus serum load on biliary complications in liver transplantation. *Transpl Infect Dis* 2013; **15**: 306-313 [PMID: 23489913 DOI: 10.1111/tid.12069]

48 **Huesing-Kabar A**, Dohna CZ, Heinzow H, Cicinnati VR, Beckebaum S, Schmidt M, Gerth HU, Pohlen M, Wilms C, Palmes D, Schmidt HH, Kabar I. Risk factors for allograft failure in liver transplant recipients. *Z Gastroenterol* 2018; **56**: 745-751 [PMID: 29341040 DOI: 10.1055/s-0043-125225]

49 **Egeli T**, Unek T, Ağalar C, Derici S, Ozbilgin M, Akarasu M, Bacakoglu A, Ellidokuz H, Astarcıoglu I. Analysis of Causes and Risk Factors for Late Mortality After Liver Transplant: How Can We Obtain Better Long-Term Survival? *Exp Clin Transplant* 2020; **18**: 182-187 [PMID: 29863452 DOI: 10.6002/ect.2017.0346]

50 **Mathur AK**, Nadig SN, Kingman S, Lee D, Kinkade K, Sonnenday CJ, Welling TH. Internal biliary stenting during orthotopic liver transplantation: anastomotic complications, post-transplant biliary interventions, and survival. *Clin Transplant* 2015; **29**: 327-335 [PMID: 25604635 DOI: 10.1111/ctr.12518]

51 **Berge E**, Otón E, Reina Z, Díaz L, Márquez A, Cejas L, Acosta S, Pérez F. Predictors of Poor Prognosis in Recurrent Hepatitis C After Liver Transplantation. *Transplant Proc* 2016; **48**: 2997-2999 [PMID: 27932129 DOI: 10.1016/j.transproceed.2016.07.043]

52 **Shiba H**, Hashimoto K, Kelly D, Fujiki M, Quintini C, Aucejo F, Uso TD, Yerian L, Yanaga K, Matsushima M, Eghtesad B, Fung J, Miller C. Risk stratification of allograft failure secondary to hepatitis C recurrence after liver transplantation. *Hepatol Res* 2016; **46**: 1099-1106 [PMID: 26833562 DOI: 10.1111/hepr.12661]

53 **Llovet LP**, Sciarrone S, Rodríguez-Tajes S, Montironi C, Mescoli C, Rugge M, Crespo G, Burra P, Forns X, Diaz A, Londoño MC. Ductular reaction and hepatocyte ballooning identify patients with fibrosing cholestatic hepatitits after liver transplantation. *Gastroenterol Hepatol* 2020; **43**: 14-21 [PMID: 31495536 DOI: 10.1016/j.gastrohep.2019.07.006]

54 **Takagi K**, Domagala P, Polak WG, Ijzermans JNM, Boehnert MU. Right posterior segment graft for living donor liver transplantation: A systematic review. *Transplant Rev (Orlando)* 2020; **34**: 100510 [PMID: 31495539 DOI: 10.1016/j.trre.2019.100510]

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ORIGINAL ARTICLE

Retrospective Study

Liver function tests and metabolic-associated fatty liver disease: Changes in upper normal limits, does it really matter?

Roberta Forlano, Benjamin H Mullish, Ameet Dhar, Robert D Goldin, Mark Thursz, Pinelopi Manousou

ORCID number: Roberta Forlano 0000-0003-4746-7065; Benjamin H Mullish 0000-0001-6300-3100; Ameet Dhar 0000-0003-1349-4620; Robert D Goldin 0000-0001-5184-4519; Mark Thursz 0000-0002-8218-192X; Pinelopi Manousou 0000-0002-5363-1565.

Author contributions: Forlano R performed the research and wrote the paper; Mullish BH, Dhar A, Goldin RD, Thursz MR provided clinical advice and contributed to the draft; Manousou P designed the research and supervised the report.

Institutional review board statement: This study was considered a service evaluation project, using routinely collected patient data, therefore no ethical approval was required under the UK policy framework for health and social care.

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Roberta Forlano, Benjamin H Mullish, Ameet Dhar, Mark Thursz, Pinelopi Manousou, Liver Unit/Department of Metabolism, Digestion and Reproduction, Faculty of Medicine, Imperial College London, London W2 1NY, United Kingdom

Robert D Goldin, Centre for Pathology, Department of Medicine, Imperial College London, London W2 1NY, United Kingdom

Corresponding author: Pinelopi Manousou, MD, PhD, Senior Lecturer, Liver Unit/Department of Metabolism, Digestion and Reproduction, Faculty of Medicine, Imperial College London, Exhibition Road, London W2 1NY, United Kingdom. p.manousou@imperial.ac.uk

Abstract

BACKGROUND

Metabolic-associated fatty liver disease (MAFLD) is the commonest cause of abnormal liver function tests (LFTs). Current upper normal of limit (UNL) of LFTs was derived from a "healthy" population, where undiagnosed MAFLD and viral hepatitis might be suspected.

AIM

To evaluated potential implications of changes in UNL of alanine aminotransferase (ALT) in MAFLD.

METHODS

We retrospectively assessed consecutive first referrals with a diagnosis of MAFLD from 2010 to 2017. The conventional UNL of ALT was 45 IU/L for men and 34 IU/L for women, while a low UNL of ALT was 30 IU/L for men and 19 IU/L for women. The UNL of aspartate aminotransferase (AST) was 40 IU/L.

RESULTS

Total 436 patients were enrolled; of these, 288 underwent liver biopsy. Setting a lower UNL reduced the percentage of those with significant disease despite normal ALT; specifically, patients with advanced fibrosis (F ≥ F3) or definite "metabolic-associated steato-hepatitis (MASH)" (NAS ≥ 5) within normal ALT decreased from 10% to 1% and from 28% to 4% respectively. However, the proportion of those with elevated ALT and no evidence of advanced fibrosis or "definite MASH" increased from 39% to 47% and from 3% to 19%. Overall, LFTs performed poorly in distinguishing "definite MASH" from simple steatosis (receiver operating characteristic areas under the curves 0.59 for ALT and 0.55 for

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AST).

CONCLUSION
Liver function tests might both under- and overestimate MASH-related liver disease. Reducing the UNL might not be beneficial and imply an increase in healthcare burden. Risk stratification in MAFLD should rely on a combination of risk factors, not on LFTs alone.

Key Words: Metabolic-associated fatty liver disease; Liver function tests; Alanine aminotransferase; Fibrosis; Stiffness

Core Tip: In the United Kingdom, the hepatologists receive increasing demand for secondary care services to investigate liver function tests (LFTs), especially with the suspicion of metabolic-associated fatty liver disease (MAFLD). With current upper normal limit (UNL), patients without liver diseases but elevated LFTs is high (27%), as well as those with significant fibrosis or metabolic-associated steato-hepatitis and normal LFTs (10%). Here, we aimed to evaluate the potential implications of changes in UNL of LFTs. Our data show that reducing the UNL would lead to an increase in overall healthcare burden. In MAFLD, the risk-stratification should rely on a combination of risk factors, rather than on LFTs alone.

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INTRODUCTION

Metabolic-associated fatty liver disease (MAFLD) is emerging as the most prevalent chronic liver disease worldwide secondary to the epidemic of obesity and metabolic syndrome. MAFLD also represents the commonest cause of abnormal liver function tests (LFTs) in Western countries[1]. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are enzymes which transfer amino groups to different substrates, with ALT being more liver-specific[2]. Notably, the patient's metabolic status (such as the presence of obesity and/or insulin resistance) may directly influence LFTs values[3,4]. Moreover, current upper normal limits (UNL) were derived in a population with highly-prevalent MAFLD but unrecognised as a disease entity at the time. As such, several studies have questioned whether current UNL of ALT should be revised although giving contrasting results[5,6].

LFTs are often the first-line investigation for any suspected liver disease with or without imaging[2]. However, the role of LFTs in diagnosing metabolic-associated steato-hepatitis (MASH)-related liver disease, such as the presence of advance fibrosis and/or steatohepatitis, is currently limited. In particular, the full spectrum of MAFLD has been reported in patients with normal LFTs[7,8]. Although histology represents the "gold standard" for diagnosing and staging MASH, the costs and invasive nature of the procedure limit its widespread applicability. Therefore, non-invasive markers are an established part of the investigation of MAFLD. In particular, transient elastography has been validated as marker of fibrosis and represents the typical second-line investigation for MAFLD[2,9].

The aim of this study was to evaluate potential implications of lowering the UNL of ALT in patients with a clinical or histological diagnosis of MAFLD.

MATERIALS AND METHODS

Study population

We retrospectively assessed all consecutive referrals with a clinical or histological diagnosis of MAFLD followed-up at the Liver Unit of St. Mary's Hospital, Imperial College Healthcare NHS Trust, from January 2010 to May 2017.

At the time of liver biopsy or Liver Stiffness Measurement, clinical parameters were recorded, including demographic, anthropometric and biochemical data. The use of steatogenic drugs, chronic alcohol consumption, as well as other liver disease were considered as exclusion criteria[9]. Fibrosis-4 index and non-alcoholic fatty liver disease (NAFLD) fibrosis score were calculated based on published formulas[10,11].

The conventional upper normal limit (UNL) of ALT from the Imperial College NHS Trust laboratory was 45 IU/L for men and 34 IU/L for women. The effect of the application of a lower value of ALT was then investigated. This UNL was set at 30 IU/L for men and 19 IU/L for women, in keeping with previous studies aiming to increase the sensitivity in diagnosing active chronic hepatitis C in the general population[5]. Similarly, this lower ALT UNL helped with differentiating active from inactive chronic hepatitis B carriers[12].

The whole study population was then stratified into three subgroups: the group with ALT higher than the conventional UNL (ALT ≥ 45 IU/L for men and ≥ 34 IU/L for women), the group with ALT within the conventional and the low UNL (ALT 31-45 IU/L for men and 20-34 UI/L for women), and the group with ALT lower than the low UNL (ALT ≤ 30 IU/L for men and ≤ 19 IU/L for women). The UNL for AST was set as 40 IU/L, as per laboratory range.

Liver stiffness measurement

Liver stiffness measurement (LSM) was obtained using FibroScan™. Scans were performed after 4 h fasting. LSM was interpreted according to interquartile range/median ratio: "poorly reliable" LSM values were not considered[13]. Advanced fibrosis was defined as LSM ≥ 8.1 kPa[14].

Liver histology

Liver biopsies were performed using a 16-Gauge Trucut needle (Argon, Athens Tx, USA). Specimens were formalin-fixed and paraffin-embedded; thick sections were stained with Hematoxylin and Eosin and Sirius Red. All biopsies were scored using the NASH CRN scoring system. Advanced fibrosis was defined as fibrosis stage ≥ F3. "Definite MASH", "possible MASH" and "non-MASH" were defined as per NAFLD activity score (NAS)[15].

Statistical analysis

The distribution of variables was explored using the Shapiro-Wilk test. Since the variables were normally distributed, continuous variables were expressed as medians and SD, and categorical variables were expressed as relative frequencies. Differences between the groups were tested using one-way ANOVA for categorical and Mann-Whitney or Kruskal Wallis for categorical variables. Correlation was measured using Pearson's Rho coefficient. Receiver operating characteristic (ROC) areas under the curves (AUROC) were used to assess the diagnostic performance of ALT and AST. Statistical analysis was performed using SPSS© (version 24.0; SPSS Inc. Chicago, IL).

Ethics

This study was considered a service evaluation project, using routinely collected patient data, therefore no ethical approval was required under the United Kingdom (UK) policy framework for health and social care.

RESULTS

Alanine aminotransferase and liver stiffness measurement

Four hundred thirty-six patients underwent LSM. Overall, 330 (76%) patients had ALT higher than the conventional UNL, 73 (17%) had ALT within the conventional and the low UNL and 33 (7%) had ALT lower than the low UNL. AST and γ-GT levels only were significantly different between the three groups ($P < 0.0001$ and $P = 0.008$ respectively). There was no difference in terms of use of statin therapy between the groups (Table 1).

Table 1 Anthropometric and clinical characteristics of the whole population, stratified into three groups according to alanine aminotransferase levels

Variable	ALT lower than the low cut-off (*n* = 33)	ALT within the conventional and the low cut-off (*n* = 73)	ALT higher than the conventional cut-off (*n* = 330)	*P* value
Age (yr)	52 ± 13.3	52.1 ± 12.1	52.5 ± 13.1	0.52
BMI (kg/m^2)	29.9 ± 4.2	30 ± 5.5	29.3 ± 4.5	0.23
T-Cholesterol (mmol/L)	4.2 ± 1.4	4.4 ± 1	4.7 ± 2	0.3
HDL (mmol/L)	1 ± 0.3	1.1 ± 0.3	1 ± 0.8	0.81
LDL (mmol/L)	2.4 ± 1.1	2.5 ± 0.9	2.6 ± 1	0.27
Triglycerides (mmol/L)	1.9 ± 1	1.6 ± 0.9	1.7 ± 1.4	0.28
HbA1c (mmol/L)	41 ± 21	42 ± 16	45 ± 15.8	0.75
AST (IU/L)	25 ± 8	31 ± 7.7	51 ± 37	< 0.0001[1]
γGT (IU/L)	32 ± 41	38 ± 62	81 ± 76	0.008[1]
Platelet (10^9 /L)	208 ± 70	225 ± 72	229 ± 72	0.39
Albumin (g/L)	40 ± 6.1	41 ± 3.4	41 ± 3.2	0.62
Ferritin (μg/L)	58 ± 145	104 ± 150	163 ± 120	0.13
Male gender	21 (65)	52 (62)	207 (63)	0.13
Diabetes Mellitus	19 (58)	46 (55)	161 (49)	0.12
Ethnicity				
Caucasian	17 (5)	35 (48)	163 (49)	0.79
Arab	8 (24)	11 (15)	66 (20)	0.31
Hispanic and Latinos	2 (6)	5 (6)	20 (7)	0.99
South Asian	4 (12)	11 (15)	41 (12)	0.95
East Asian	1 (3)	6 (6)	25 (7)	0.26
African/Afrocaribbean	1 (3)	5 (6)	15 (4)	0.73
Hypertension	15 (45)	33 (39)	112 (34)	0.2
Dyslipidemia	13 (39)	37 (44)	141 (43)	0.93
Statin treatment	14 (42)	34 (46)	152 (46)	0.54

[1]Significantly different. Data present as mean ± SD or *n* (%). ALT: Alanine aminotransferase; BMI: Body mass index; HDL: High-density lipoprotein; LDL: Low-density lipoprotein; HbA1c: Hemoglobin A1C; AST: Aspartate aminotransferase.

Using the conventional UNL as reference, 10% of the patients had evidence of advanced fibrosis (LSM ≥ 8.1 kPa) despite normal ALT. When the low UNL for ALT was applied, this percentage reduced to 3%. However, applying the low UNL determined also the increase in the proportion of those with elevated ALT but not showing evidence of advanced fibrosis (LSM ≥ 8.1 kPa) from 42% to 52% (Supplementary Figure 1).

In the whole population, there was no linear association between ALT and age, as Pearson's correlation was not significant (Rho = -0.86, *P* = 0.07). Moreover, the distribution of ALT across age groups was similar when patients were further stratified per gender (Kruskal Wallis).

Alanine aminotransferase and liver histology

A subgroup of 288 patients underwent a liver biopsy. Overall, 220 (78%) patients had ALT higher than the conventional UNL, 50 (17%) had ALT within the conventional and the low UNL and 18 (5%) had ALT lower than the low UNL.

Using the conventional UNL as reference, 10% of patients had advanced fibrosis (F ≥ F3) on histology despite normal ALT. When the low UNL for ALT was applied, this percentage reduced to 1%. However, applying the low UNL determined also the increase in the proportion of those with elevated ALT but not showing advanced

fibrosis from 39% to 47% (Figure 1). Similarly, lowering the UNL of ALT, the percentage of those with "definite MASH" (NAS ≥ 5) despite normal ALT decreased from 28% to 4%, whilst the percentage of patients without "definite MASH" but showing elevated ALT increased from 3% to 19% (Figure 2).

Overall, FIB-4 and NAFLD fibrosis scored performed better than ALT in diagnosing F > F3. Specifically, the AUROC of ALT for diagnosing F ≥ F3 was 0.45 (95%CI: 0.38-0.53, P = 0.05) compared to 0.71 (95%CI: 0.63-0.79, P = 0.0001) for FIB-4 and 0.65 (95%CI: 0.59-0.72, P = 0.0001) for NAFLD fibrosis score. However, ALT, FIB-4 and NAFLD fibrosis score performed similarly in diagnosing "definite MASH". In particular, the AUROC of ALT was 0.55 (95%CI: 0.47-0.62, P = 0.049), compared to 0.47 (95%CI: 0.39-0.54, P = 0.01) for FIB-4 and 0.5 (95%CI: 0.42-0.58, P = 0.05) for NAFLD fibrosis score (Figure 3A and B).

Aspartate aminotransferase and liver stiffness measurement

Overall, 235 (54%) patients had elevated AST and 201 (46%) had normal AST. ALT, γ-GT and ferritin only were significantly different between the groups (P < 0.0001, P < 0.0001 and P = 0.008 respectively). There was no difference in terms of statin therapy (Supplementary Table 1).

Advanced fibrosis (LSM ≥ 8.1 kPa) was diagnosed despite normal AST in 16% of the cases, while the proportion of those with elevated AST but LSM <8.1 kPa was 27%.

In the whole population, there was no linear association between AST and age, as Pearson's correlation was not significant (Rho = 0.01, P = 0.99). Moreover, the distribution of AST across age groups was similar when patients were further stratified per gender (Kruskal Wallis).

Aspartate aminotransferase and liver histology

In the subgroup of patients who underwent a liver biopsy, 155 (54%) patients had elevated AST and 133 (46%) had normal AST. Advanced fibrosis (F ≥ F3) was diagnosed despite normal AST in 21% of the cases, while the proportion of those with elevated AST and no advanced fibrosis (F ≥ F3) was 26%. "Definite MASH" was diagnosed in presence of normal AST in 37% cases.

Overall, FIB-4 and NAFLD fibrosis scored performed better than AST in diagnosing F > F3, while the three performed similarly in diagnosing "definite MASH". Specifically, the AUROC of AST for diagnosing F ≥ F3 was 0.56 (95%CI: 0.49-0.64, P = 0.05) and 0.59 (95%CI: 0.52-0.67, P = 0.049) for diagnosing "definite MASH" (Figure 3A and B).

DISCUSSION

Metabolic-associated Fatty Liver Disease is a major cause of chronic liver disease and the commonest cause of elevated liver enzymes[16,17]. In the UK, referrals for abnormal LFTs are increasing (> 300 referrals/year), and this often represents the first step in diagnosing MAFLD[18].

Several factors may influence ALT, such as age, gender, BMI, insulin resistance and triglycerides[3,4,19]. Overall, ALT is more commonly elevated than AST in chronic liver disease, with the notable exception of alcohol-induced liver injury[20]. Since transaminases are released following hepatocellular injury, AST and ALT are markers of cytolysis and not necessarily associated with inflammation or steatosis[21]. Nevertheless, LFTs are often used as a surrogate markers to assess the anti-inflammatory effect in clinical trials in MAFLD[22].

While the diagnosis and management of MAFLD has been streamlined in secondary and tertiary care centres, there is still a high variability in how the disease is assessed within the community. In particular, general practitioners (GPs) in primary care rely heavily on LFTs measurement, consistent with pragmatic guidelines which have been developed only recently in the UK[2]. It is also evident from a recent survey study that diagnosing MAFLD is perceived as challenging even to experienced GPs, with the overall perception of overlooking the disease especially in high-risk groups[23].

In this retrospective cohort of patients diagnosed with MAFLD, LFTs were frequently normal despite the presence of advanced liver disease. Moreover, transaminases could not distinguish simple steatosis from "definite MASH" (AUROC 0.59 for ALT and 0.55 for AST) at first referral, giving false reassurance in 10%-15% of patients. Conversely, decision-making based on LFTs alone might have implied unnecessary second-line investigations in approximately 27%-42% of cases. Our results confirm that non-invasive markers based on blood tests (*i.e.*, FIB-4 and NAFLD fibrosis score)

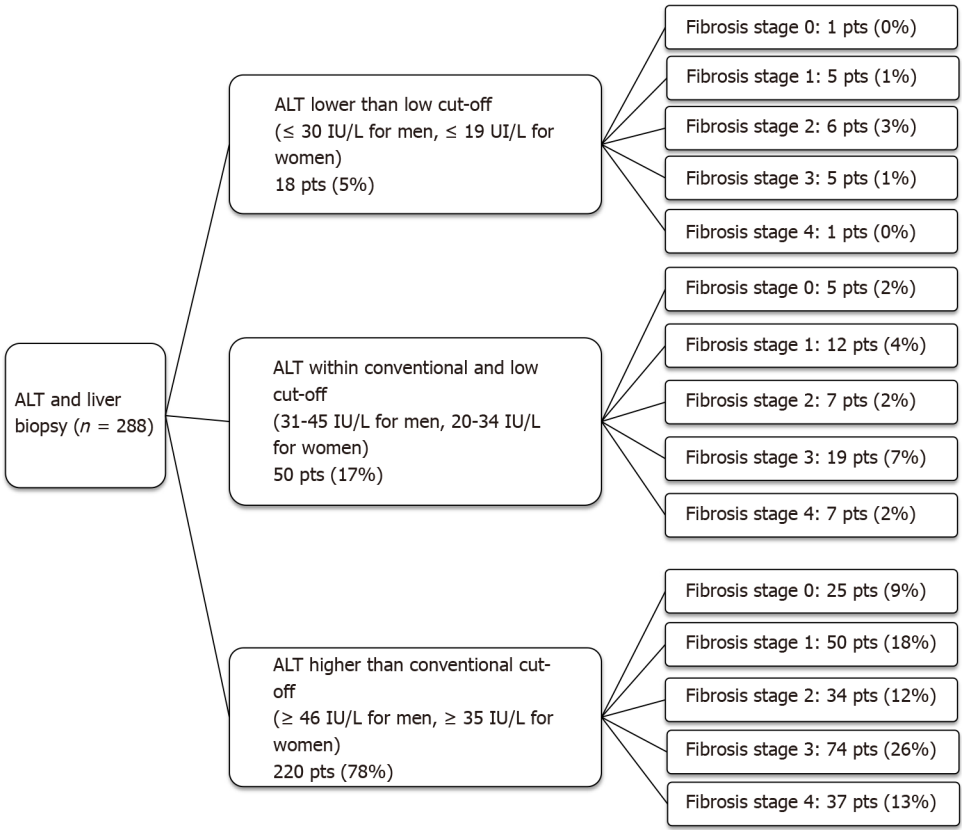

Figure 1 Fibrosis stage in three subgroups of patients stratified for alanine aminotransferase levels. ALT: Alanine aminotransferase; pts: Patients.

perform better than LFTs alone in assessing the severity of liver disease from NAFLD.

The actual normal ALT value is an area of ongoing controversy. Differences in the UNL used between studies are consistent, resulting from laboratory setting and populations tested[24]. Interestingly, the ALT normal range has been derived from "healthy" subjects in the general population[1], where MAFLD and obesity were highly prevalent[24]. Moreover, the UNL was first described in the 1980s, when LFTs were used to rule out 'non-A and non-B hepatitis' positivity amongst blood donors, in a time when anti-HCV antibodies were not available[25]. As such, both undiagnosed cases of MAFLD and chronic viral hepatitis may have contributed to the current definition of the UNL.

In this cohort, when a lower UNL was applied, the proportion of patients with advanced fibrosis or definite MASH on biopsy and normal biochemistry fell substantially, providing a rationale for revising current UNL. However, reducing the ALT normal range might lead to an increase in unnecessary second-line investigations (from 27% to 33% in based on histology this population) for a disease which is already highly prevalent in the general population. As a result, health costs would overwhelm the healthcare system with no clear clinical benefit[5].

CONCLUSION

Liver function tests might both underestimate and overestimate MASH-associated liver disease. Changing the UNL of ALT is not beneficial, as it might increase healthcare burden. Referral/management pathways and risk-stratification strategies are most needed for primary and they should rely on a combination of risk factors and non-invasive markers, not on LFTs alone.

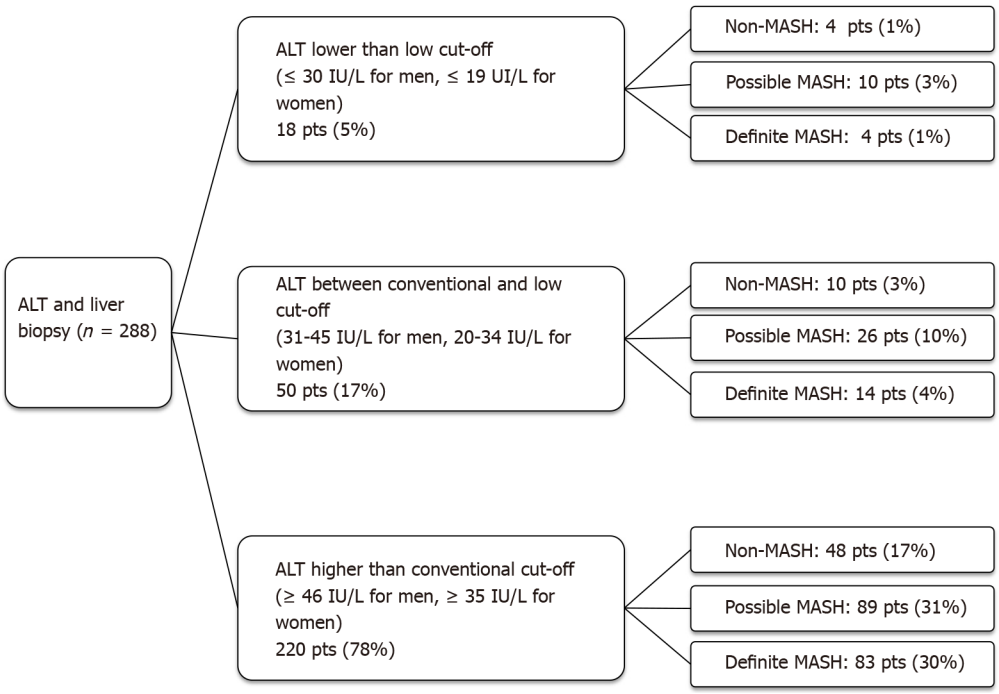

Figure 2 Diagnosis of metabolic-associated steato-hepatitis in three subgroups of patients stratified for alanine aminotransferase levels. ALT: Alanine aminotransferase; pts: Patients; MASH: Metabolic-associated steato-hepatitis.

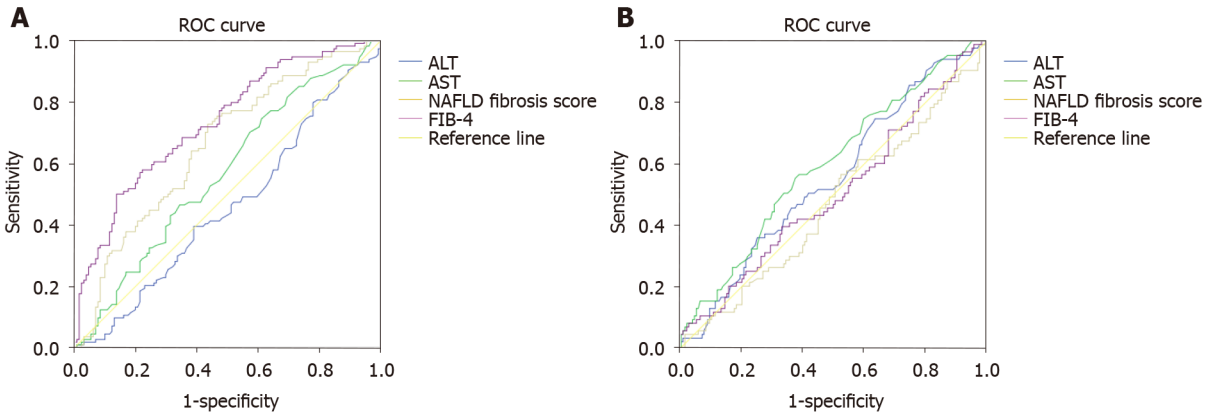

Figure 3 Receiver operating characteristic areas under the curves for liver function tests and non-invasive markers of fibrosis for diagnosis advanced fibrosis (F ≥ F3) and definite metabolic-associated steato-hepatitis (Non-alcoholic fatty liver disease activity score ≥ 5). A: Liver function tests and non-invasive markers of fibrosis for diagnosis advanced fibrosis (F ≥ F3); B: Liver function tests and non-invasive markers of fibrosis for diagnosis definite metabolic-associated steato-hepatitis (Non-alcoholic fatty liver disease activity score ≥ 5). ROC: Receiver operating characteristic; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; NAFLD: Non-alcoholic fatty liver disease; FIB: Fibrosis.

ARTICLE HIGHLIGHTS

Research background

Elevated liver function tests (LFTs) often represent the main reason for referring patients with metabolic-associated fatty liver disease (MAFLD) to secondary and tertiary care.

Research motivation

In MAFLD, liver function tests may both under and over-estimate liver disease. Moreover, difference in upper normal limit (UNL) of LFTs is consistent across the literature.

Research objectives
As such, we investigated the potential use of different UNLs of LFTs in MAFLD.

Research methods
We evaluated the use of a lower UNL of ALT *vs* histology and liver stiffness measurement in a cohort of 436 patients with non-alcoholic fatty liver disease in a tertiary care centre.

Research results
Modifying the upper normal limit of LFTs does not improve the diagnostic performance of the test in MAFLD.

Research conclusions
In MAFLD, the risk-stratification should rely on a combination of risk factors and non-invasive markers, rather than on LFTs alone.

Research perspectives
Future research should focus on identifying biomarkers for diagnosing metabolic-associated steato-hepatitis and advanced fibrosis.

REFERENCES

1 **Liangpunsakul S**, Chalasani N. Unexplained elevations in alanine aminotransferase in individuals with the metabolic syndrome: results from the third National Health and Nutrition Survey (NHANES III). *Am J Med Sci* 2005; **329**: 111-116 [PMID: 15767815 DOI: 10.1097/00000441-200503000-00001]

2 **Newsome PN**, Cramb R, Davison SM, Dillon JF, Foulerton M, Godfrey EM, Hall R, Harrower U, Hudson M, Langford A, Mackie A, Mitchell-Thain R, Sennett K, Sheron NC, Verne J, Walmsley M, Yeoman A. Guidelines on the management of abnormal liver blood tests. *Gut* 2018; **67**: 6-19 [PMID: 29122851 DOI: 10.1136/gutjnl-2017-314924]

3 **Maximos M**, Bril F, Portillo Sanchez P, Lomonaco R, Orsak B, Biernacki D, Suman A, Weber M, Cusi K. The role of liver fat and insulin resistance as determinants of plasma aminotransferase elevation in nonalcoholic fatty liver disease. *Hepatology* 2015; **61**: 153-160 [PMID: 25145475 DOI: 10.1002/hep.27395]

4 **Fracanzani AL**, Valenti L, Bugianesi E, Andreoletti M, Colli A, Vanni E, Bertelli C, Fatta E, Bignamini D, Marchesini G, Fargion S. Risk of severe liver disease in nonalcoholic fatty liver disease with normal aminotransferase levels: a role for insulin resistance and diabetes. *Hepatology* 2008; **48**: 792-798 [PMID: 18752331 DOI: 10.1002/hep.22429]

5 **Prati D**, Taioli E, Zanella A, Della Torre E, Butelli S, Del Vecchio E, Vianello L, Zanuso F, Mozzi F, Milani S, Conte D, Colombo M, Sirchia G. Updated definitions of healthy ranges for serum alanine aminotransferase levels. *Ann Intern Med* 2002; **137**: 1-10 [PMID: 12093239 DOI: 10.7326/0003-4819-137-1-200207020-00006]

6 **Wejstål R**, Hansson G, Lindholm A, Norkrans G. Persistent alanine aminotransferase elevation in healthy Swedish blood donors--mainly caused by obesity. *Vox Sang* 1988; **55**: 152-156 [PMID: 3238949 DOI: 10.1111/j.1423-0410.1988.tb05083.x]

7 **Kunde SS**, Lazenby AJ, Clements RH, Abrams GA. Spectrum of NAFLD and diagnostic implications of the proposed new normal range for serum ALT in obese women. *Hepatology* 2005; **42**: 650-656 [PMID: 16037946 DOI: 10.1002/hep.20818]

8 **Mofrad P**, Contos MJ, Haque M, Sargeant C, Fisher RA, Luketic VA, Sterling RK, Shiffman ML, Stravitz RT, Sanyal AJ. Clinical and histologic spectrum of nonalcoholic fatty liver disease associated with normal ALT values. *Hepatology* 2003; **37**: 1286-1292 [PMID: 12774006 DOI: 10.1053/jhep.2003.50229]

9 **European Association for the Study of the Liver (EASL)**; European Association for the Study of Diabetes (EASD); European Association for the Study of Obesity (EASO). EASL-EASD-EASO Clinical Practice Guidelines for the management of non-alcoholic fatty liver disease. *J Hepatol* 2016; **64**: 1388-1402 [PMID: 27062661 DOI: 10.1016/j.jhep.2015.11.004]

10 **Sterling RK**, Lissen E, Clumeck N, Sola R, Correa MC, Montaner J, S Sulkowski M, Torriani FJ, Dieterich DT, Thomas DL, Messinger D, Nelson M; APRICOT Clinical Investigators. Development of a simple noninvasive index to predict significant fibrosis in patients with HIV/HCV coinfection. *Hepatology* 2006; **43**: 1317-1325 [PMID: 16729309 DOI: 10.1002/hep.21178]

11 **Angulo P**, Hui JM, Marchesini G, Bugianesi E, George J, Farrell GC, Enders F, Saksena S, Burt AD, Bida JP, Lindor K, Sanderson SO, Lenzi M, Adams LA, Kench J, Therneau TM, Day CP. The NAFLD fibrosis score: a noninvasive system that identifies liver fibrosis in patients with NAFLD. *Hepatology* 2007; **45**: 846-854 [PMID: 17393509 DOI: 10.1002/hep.21496]

12 **Assy N**, Beniashvili Z, Djibre A, Nasser G, Grosovski M, Nseir W. Lower baseline ALT cut-off

values and HBV DNA levels better differentiate HBeAg- chronic hepatitis B patients from inactive chronic carriers. *World J Gastroenterol* 2009; **15**: 3025-3031 [PMID: 19554656 DOI: 10.3748/wjg.15.3025]

13 **Boursier J**, Zarski JP, de Ledinghen V, Rousselet MC, Sturm N, Lebail B, Fouchard-Hubert I, Gallois Y, Oberti F, Bertrais S, Calès P; Multicentric Group from ANRS/HC/EP23 FIBROSTAR Studies. Determination of reliability criteria for liver stiffness evaluation by transient elastography. *Hepatology* 2013; **57**: 1182-1191 [PMID: 22899556 DOI: 10.1002/hep.25993]

14 **Cassinotto C**, Boursier J, de Lédinghen V, Lebigot J, Lapuyade B, Cales P, Hiriart JB, Michalak S, Bail BL, Cartier V, Mouries A, Oberti F, Fouchard-Hubert I, Vergniol J, Aubé C. Liver stiffness in nonalcoholic fatty liver disease: A comparison of supersonic shear imaging, FibroScan, and ARFI with liver biopsy. *Hepatology* 2016; **63**: 1817-1827 [PMID: 26659452 DOI: 10.1002/hep.28394]

15 **Kleiner DE**, Brunt EM, Van Natta M, Behling C, Contos MJ, Cummings OW, Ferrell LD, Liu YC, Torbenson MS, Unalp-Arida A, Yeh M, McCullough AJ, Sanyal AJ; Nonalcoholic Steatohepatitis Clinical Research Network. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. *Hepatology* 2005; **41**: 1313-1321 [PMID: 15915461 DOI: 10.1002/hep.20701]

16 **Armstrong MJ**, Houlihan DD, Bentham L, Shaw JC, Cramb R, Olliff S, Gill PS, Neuberger JM, Lilford RJ, Newsome PN. Presence and severity of non-alcoholic fatty liver disease in a large prospective primary care cohort. *J Hepatol* 2012; **56**: 234-240 [PMID: 21703178 DOI: 10.1016/j.jhep.2011.03.020]

17 **Younossi Z**, Anstee QM, Marietti M, Hardy T, Henry L, Eslam M, George J, Bugianesi E. Global burden of NAFLD and NASH: trends, predictions, risk factors and prevention. *Nat Rev Gastroenterol Hepatol* 2018; **15**: 11-20 [PMID: 28930295 DOI: 10.1038/nrgastro.2017.109]

18 **Sheridan DA**, Aithal G, Alazawi W, Allison M, Anstee Q, Cobbold J, Khan S, Fowell A, McPherson S, Newsome PN, Oben J, Tomlinson J, Tsochatzis E. Care standards for non-alcoholic fatty liver disease in the United Kingdom 2016: a cross-sectional survey. *Frontline Gastroenterol* 2017; **8**: 252-259 [PMID: 29067150 DOI: 10.1136/flgastro-2017-100806]

19 **Piton A**, Poynard T, Imbert-Bismut F, Khalil L, Delattre J, Pelissier E, Sansonetti N, Opolon P. Factors associated with serum alanine transaminase activity in healthy subjects: consequences for the definition of normal values, for selection of blood donors, and for patients with chronic hepatitis C. MULTIVIRC Group. *Hepatology* 1998; **27**: 1213-1219 [PMID: 9581673 DOI: 10.1002/hep.510270505]

20 **Kim WR**, Flamm SL, Di Bisceglie AM, Bodenheimer HC; Public Policy Committee of the American Association for the Study of Liver Disease. Serum activity of alanine aminotransferase (ALT) as an indicator of health and disease. *Hepatology* 2008; **47**: 1363-1370 [PMID: 18366115 DOI: 10.1002/hep.22109]

21 **Verma S**, Jensen D, Hart J, Mohanty SR. Predictive value of ALT levels for non-alcoholic steatohepatitis (NASH) and advanced fibrosis in non-alcoholic fatty liver disease (NAFLD). *Liver Int* 2013; **33**: 1398-1405 [PMID: 23763360 DOI: 10.1111/liv.12226]

22 **Ratziu V**. A critical review of endpoints for non-cirrhotic NASH therapeutic trials. *J Hepatol* 2018; **68**: 353-361 [PMID: 29223369 DOI: 10.1016/j.jhep.2017.12.001]

23 **Standing HC**, Jarvis H, Orr J, Exley C, Hudson M, Kaner E, Hanratty B. GPs' experiences and perceptions of early detection of liver disease: a qualitative study in primary care. *Br J Gen Pract* 2018; **68**: e743-e749 [PMID: 30249611 DOI: 10.3399/bjgp18X699377]

24 **Neuschwander-Tetri BA**, Unalp A, Creer MH; Nonalcoholic Steatohepatitis Clinical Research Network. Influence of local reference populations on upper limits of normal for serum alanine aminotransferase levels. *Arch Intern Med* 2008; **168**: 663-666 [PMID: 18362260 DOI: 10.1001/archinternmed.2007.131]

25 **Pratt DS**, Kaplan MM. Evaluation of abnormal liver-enzyme results in asymptomatic patients. *N Engl J Med* 2000; **342**: 1266-1271 [PMID: 10781624 DOI: 10.1056/NEJM200004273421707]

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ORIGINAL ARTICLE

Retrospective Study

Use of oral vancomycin in children with autoimmune liver disease: A single centre experience

Angelo Di Giorgio, Anna Tulone, Emanuele Nicastro, Lorenzo Norsa, Aurelio Sonzogni, Lorenzo D'Antiga

ORCID number: Angelo Di Giorgio 0000-0003-0363-5565; Anna Tulone 0000-0002-7112-2182; Emanuele Nicastro 0000-0002-4518-9375; Lorenzo Norsa 0000-0003-3322-2921; Aurelio Sonzogni 0000-0001-5001-9533; Lorenzo D'Antiga 0000-0001-7150-3148.

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Angelo Di Giorgio, Anna Tulone, Emanuele Nicastro, Lorenzo Norsa, Lorenzo D'Antiga, Pediatric Hepatology, Gastroenterology and Transplantation, Hospital Papa Giovanni XXIII , Bergamo 24127, Italy

Aurelio Sonzogni, Liver Pathology, Hospital Papa Giovanni XXIII, Bergamo 24127, Italy

Corresponding author: Angelo Di Giorgio, MD, PhD, Pediatric Hepatology, Gastroenterology and Transplantation, Hospital Papa Giovanni XXIII , Piazza Oms 1, Bergamo 24127, Italy. adigiorgio@asst-pg23.it

Abstract

BACKGROUND

Previous reports showed some beneficial effect of oral vancomycin treatment (OVT) in children with primary sclerosing cholangitis; conversely, the experience in patients with other autoimmune liver diseases (AILD), including autoimmune hepatitis (AIH) and autoimmune sclerosing cholangitis (ASC), is scant.

AIM

To assess the response to immunosuppressive treatment (IS) and to OVT in children diagnosed with AILD.

METHODS

Retrospective study of children diagnosed with AIH (normal biliary tree at cholangiography) and ASC (abnormal biliary tree at cholangiography) in the last 10 years. All underwent standard immunosuppressive therapy (IS), but non-responders received also OVT. Biochemical remission [normal aspartate aminotransferase (AST)] and immunological remission (normal IgG and negative autoantibodies) rates and Sclerosing Cholangitis Outcomes in Pediatrics (SCOPE) index were assessed and compared during the follow up.

RESULTS

75 children were included [69% female, median age 10.5 years (5.6-13.4 years), AIH = 54, ASC= 21]. Sixty-three patients (84%, AIH = 52, ASC = 11) were treated with standard IS and 61 achieved biochemical remission, whereas 12 not responding to IS [16%, F = 75%, median age 13.5 years, (12.2-15.7), 10 with ASC] required OVT and 8 achieved biochemical remission. Overall OVT increased the biochemical remission rate of the whole group of AILD patients from 81% (61/75) to 92% (69/75). Median values of AST, alanine aminotransferase (ALT) and

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gamma-glutamyl transferase (GGT) decreased significantly after OVT start ($P < 0.05$). Complete normalization of livers enzymes (AST, ALT and GGT) was observed in 6/12 patients (50%). Decrease in SCOPE index score was reported in 5/12 patients (42%). At last follow up (median of 4.4 years, range 0.6-13.8 years) all 75 patients are alive, 6 (8%, 1 with ASC) successfully discontinued medications, 1 (with ASC) required liver transplantation.

CONCLUSION
Children with AIH and ASC respond well to IS treatment. OVT may represent a valuable treatment option to achieve biochemical remission in patients not responding to standard IS. These promising preliminary results suggest that a prospective study is indicated to define the efficacy of OVT in AILD.

Key Words: Autoimmune hepatitis; Autoimmune sclerosing cholangitis; Autoimmune liver disease; Vancomycin; Children; Liver transplantation

Core Tip: Experience with oral vancomycin in children with autoimmune liver disease (AILD) is limited. We enrolled 75 children [median age 10.5 years (5.6-13.4)], 54 with autoimmune hepatitis and 21 with autoimmune sclerosing cholangitis; 63/75 achieved remission by standard immunosuppressive therapy (IS), whereas 12/75 (16%) required oral vancomycin treatment (OVT). In 6/12 patients (50%) the response was complete, whereas it was partial in 2/12 (17%), and absent in 4/12 (33%). Overall OVT increased the remission rate of the whole group of AILD patients from 81% to 92%. OVT may represent a valuable treatment option in children with AILD who do not respond to standard IS.

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INTRODUCTION

Pediatric autoimmune liver disease (AILD) is a progressive inflammatory condition including autoimmune hepatitis (AIH), (diagnosed with the standard criteria) and autoimmune sclerosing cholangitis (ASC), (defined as patients fulfilling the criteria for AIH but with an abnormal biliary tree at cholangiography)[1-3].

Children with AILD respond well to immunosuppressive (IS) treatment, although some patients progress to cirrhosis despite normal liver enzymes; a low proportion (30%-40%) achieve immunological remission (normal IgG and negative autoantibodies), and only a small percentage (10%-20%) can stop medications successfully, maintaining remission off treatment[3,4]. Furthermore, children with ASC have a higher need for liver transplantation (LT) compared to AIH, suggesting that bile duct damage may progress despite IS treatment[1-4].

Empirical use of candidate therapies for AILD has significantly increased in the last decades, in the attempt of finding effective medications to normalize liver enzymes and improve outcomes; oral vancomycin is one of the most common drugs empirically used in patients with SC[5-7]. Oral vancomycin is supposed to have an immunomodulatory effect by inducing an increase of T-regs lymphocytes and TGF-β (both with anti-inflammatory activity) without alterations in Th1 or Th2 cytokine production patterns [6-9]. Cox *et al*[12] reported benefits from oral vancomycin treatment (OVT) in children with primary SC (PSC) and inflammatory bowel disease (IBD). Interestingly, OVT seems to be able to modify the gut microbiota and bile acid metabolism, that may have a protective effect on PSC recurrence after LT[10-12].

Previous studies have offered information on the use of OVT in adults and children with PSC; conversely the experience with OVT in children with AIH or ASC not responding to standard IS is very limited[5-7].

In our center we empirically used oral vancomycin in a small series of children with AIH and ASC not responding to standard IS to gather insights that could guide us to the design of a prospective clinical trial.

In this study, we aimed to review our cohort of pediatric patients with AILD to assess: (1) The response to standard IS treatment; and (2) The efficacy of OVT to achieve biochemical and immunological remission in patients not responding to standard IS.

MATERIALS AND METHODS

Data collection

We reviewed retrospectively the medical records of children diagnosed with AILD (AIH or ASC) at Hospital Papa Giovanni XXIII, Bergamo, Italy, between 2010 and 2021. During this period of time all patients were diagnosed by the standard diagnostic criteria including magnetic resonance cholangiopancreatography (MRCP) performed at diagnosis; OVT was regularly adopted in patients not responding to standard treatment. Biochemical and clinical features, histology, and data on outcomes were collected in all patients and compared between the two groups divided according to the diagnosis (AIH *vs* ASC) and OVT.

Diagnosis of autoimmune liver disease

The diagnosis of AILD was based on elevated transaminases and IgG levels, positive autoantibodies, compatible liver histology, and exclusion of other liver diseases[13]. A lower threshold for autoantibody positivity was applied to children compared to adults, *i.e.*, titre ≥ 1:20 for antinuclear antibodies (ANA) and smooth muscle antibodies (SMA) and ≥ 1:10 for anti-liver kidney microsomal type 1 (anti-LKM-1) were used, as indicated by the International Autoimmune Hepatitis Group (IAIHG) consensus statement on liver autoimmune serology[14] and more recently by the European Society of Paediatric Gastroenterology, Hepatology and Nutrition[3]. Patients without cholangiopathy on MRCP were diagnosed as AIH type 1 (AIH-1, positivity for SMA and/or ANA) or type 2 (AIH-2, positivity for LKM-1 and/or LC1)[1,3]. Patients with cholangiopathy were diagnosed as ASC[1,3]. Patients with histological diagnosis of ASC but normal cholangiogram were classified as small duct ASC[3].

Clinical presentation was classified as: (1) Acute (malaise, nausea/vomiting, abdominal pain, jaundice, dark urine, pale stools); (2) Insidious (fatigue, headache, amenorrhoea, joint pain); and (3) Asymptomatic (incidental finding of abnormal liver function tests during investigation of non-hepatic conditions, including IBD). Protocol and description of autoantibodies detection and histological features suggestive for biliopathy are reported in our previous studies[4].

Treatment protocol

IS treatment consisted of first line use of oral prednisone at a dose of 2 mg/kg/d (up to a maximum of 60 mg/d) for 10-14 d followed by 4-6 wk tapering schedule to reach a total maintenance dose of 5 or 2.5 mg/d (depending on age). Blood tests during induction of remission were checked weekly to monitor the response to treatment and side effects. If the response was not satisfactory, azathioprine was added [starting dose 0.5 mg/kg/d, increased weekly to 1.5 mg/kg/d (maximum dose 2-2.5 mg/kg/d) in the absence of side effects or leukopenia] until normal transaminase levels were achieved. Mycophenolate mofetil (MMF, as second line treatment) and calcineurin inhibitors (cyclosporine or tacrolimus, as third line treatment) were used when standard treatment failed or azathioprine was contraindicated. Patients with ASC were also administered ursodehoxycholic acid (UDCA) at the dose of 15-20 mg/kg/d [3,15].

Patients not responding to standard immunosuppression underwent liver biopsy to assess the degree of inflammation and the stage of biliopathy as per criteria defined in our previous study[16].

OVT was given to patients who did not respond to first/second line treatment and who had on histology features of biliopathy without (or mild) portal-periportal inflammation. OVT was started at the dose of 50 mg/kg/d (divided in 3 doses, maximum dose 1500 mg/d), for 6 mo. In patients who did not respond, OVT was discontinued after 6 mo, whereas it was continued in responders.

Conversely, in children having on histology moderate/severe inflammatory infiltrate, a temporary increase of oral prednisone and conversion from azathioprine to MMF or from MMF to tacrolimus were prescribed[3], and OVT was not commenced.

We considered OVT-related side-effects the following symptoms: Fever, chills, rash, fatigue, gastroenterological symptoms (abdominal pain, persistent diarrhea), nephrotoxicity, neutropenia, ototoxicity, thrombocytopenia, antibiotic-resistant infections and neurological symptoms[5].

Response to treatment

Biochemical remission was defined as normal transaminase levels; immunological remission was normal transaminase and IgG levels with negative/Low titer (ANA/SMA < 1:20) of autoantibodies; histological remission was the absence of inflammation on liver histology. Relapse was defined as transaminase levels ≥ 2-fold the upper limit of normal (ULN)[3].

In patients receiving OVT, the values of aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transferase (GGT), serum IgG and autoantibodies were reported before and after treatment.

Biochemical response to OVT was classified as follows:

Complete response: AST, ALT and GGT returning within normal values (NV);

Partial response: AST, ALT or GGT levels decreasing to < 1.5 × ULN, but not reaching NV;

No response: No significant changes in liver enzymes.

Discontinuation of IS treatment was attempted in patients with normal transaminases and IgG, negative or low positive titer of autoantibodies at least 3 years after starting IS treatment, and no inflammation on follow up histology[3].

SCOPE index

The Sclerosing Cholangitis Outcomes in Pediatrics (SCOPE) index includes 5 parameters which correlate with long-term outcomes in children with SC. The model stratifies patients as low, medium, or high risk based on progression to transplant or death (rates of < 1%, 3%, or 9% annually) and to hepatobiliary complications, including portal hypertension or biliary strictures (rates of 2%, 6%, and 13% annually)[17]. In this study, we assessed whether the SCOPE index score was improved, stable or worsened after OVT.

Statistical analysis

Data are reported as medians and interquartile range unless specified differently. Baseline measures and data on outcome were compared between AIH and ASC to see whether they differed. Paired *t* test/Mann-Whitney *U* test were used for continuous variables and chi-square/Fisher exact test for categorical variables. A *P* value of 0.05 or less was assigned significance. The analysis was performed with IBM-SPSS 13.0 for Windows. The statistical methods of this study were reviewed by one of the authors (EN) who is an expert statistician.

RESULTS

Seventy-five patients were diagnosed with AILD [AIH = 54 (type 1 *n* = 42, type 2 *n* = 12), ASC = 21] during the study period. Median age at diagnosis was 10.5 years (5.6-13.4) without differences between the two groups ($P > 0.05$). Female predominance was 69% (AIH = 72 %, ASC = 62%). The most common type of presentation was acute (35%, 43% in AIH *vs* 14% in ASC, $P = 0.011$), followed by asymptomatic (33%) and insidious (32%), the latter more common in ASC group (57% in ASC *vs* 22% in AIH, $P = 0.005$). IBD was reported in 18 patients [24%, ulcerative colitis (UC) in 12, Crohn's disease (CD) in 2 and IBD-unclassified (IBD-U) in 4 patients], mainly in ASC group (57% *vs* 11% in AIH group, $P < 0.001$). Associated autoimmune disorders were reported in 13/75 patients (17%, AIH = 17% and ASC = 14%) including coeliac disease in 4 (with AIH), autoimmune thyroiditis in 3 (1 with AIH), diabetes mellitus type 1 in 2 (both with AIH), psoriasis in 2 (both with AIH), idiopathic arthritis in 1 (with ASC), nephrotic syndrome in 1 (with ASC).

Baseline features

At diagnosis, all but one patient (F, with ASC, already on treatment for IBD) had raised transaminases; GGT was increased in 63 patients (84%) and normal in 12 (16%, all with AIH). Median values of AST, ALT, GGT, total bilirubin, ALT/AST ratio,

Table 1 Laboratory and histological features at diagnosis of 75 children with autoimmune liver disease

	All patients, *n* = 75	AIH, *n* = 54	ASC, *n* = 21	*P* value
Biochemical profile				
AST U/L (NV ≤ 45)	438 (129-982)	678 (204-1200)	150 (94-333)	< 0.001
GGT U/L (NV ≤ 50)	116 (60-296)	107 (54-196)	360 (68-607)	< 0.001
Total bilirubin (NV ≤ 1 mg/dL)	1.7 (0.6-4.5)	2.7 (0.6-5.3)	1.2 (0.7-2.5)	0.05
ALP (NV ≤ 350 U/L)	296 (204-469)	283 (199-462)	301 (242-494)	0.328
ALP/AST ratio	0.7 (0.3-2.2)	0.4 (0.2-1.6)	2.3 (0.7-3.5)	0.002
Albumin (NV: 30-50 g/dL)	42 (38-44)	42 (37-44)	42 (40-46)	0.082
INR (NV: 0.9-1.2)	1.2 (1.1-1.5)	1.2 (1.1-1.6)	1.1 (1.0-1.2)	< 0.05
Platelet (10^9/L)	252 (180-350)	234 (167-314)	319 (251-393)	< 0.05
Autoimmune profile				
ANA (≥ 1:20): *n* (%)	55 (73)	38 (70)	17 (81)	0.777
SMA (≥ 1:20): *n* (%)	53 (71)	38 (70)	15 (71)	1
Anti-LKM-1 (≥ 1:10): *n* (%)	12 (16)	12 (22)	0 (0)	< 0.05
Anti-LC1: *n* (%)	9 (12)	9 (17)	0 (0)	< 0.05
ANCA: *n* (%)	37 (49)	22 (41)	15 (71)	< 0.05
IgG g/dL (NV: 0.5-1.8 g/dL)	2.0 (1.4-3.2)	2.3 (1.4-3.3)	1.7 (1.5-2.2)	0.325
IgG > ULN: *n* (%)	51 (68)	37 (69)	14 (67)	1
Histology, *n* (%)				
Interface hepatitis	51 (68)	42 (78)	12 (57)	0.09
Fibrosis	61 (81)	42 (78)	19 (90)	0.324
Cirrhosis	17 (23)	15 (28)	2 (10)	0.127
Features of biliopathy[1]	62 (83)	37 (68)	17 (81)	0.764

[1]It includes at least one of the following: inflammatory injury of the bile duct, ductular reaction, periductular fibrosis, biliary metaplasia, granulomatous cholangitis[16]. Values are expressed as median and interquartile ranges.

AIH: Autoimmune hepatitis; ASC: Autoimmune sclerosing cholangitis; AST: Aspartate aminotransferase; GGT: Gamma-glutamyl transferase; ALP: Alkaline phosphatase; INR: International normalized ratio; ANA: Antinuclear antibody; SMA: Smooth muscle antibody; LKM-1: Liver-kidney microsome antibody type 1; LC1: Liver cytosol antibody type 1; SLA: Liver soluble antigen; ANCA: Anti-neutrophil cytoplasmic LT antibodies; ULN: Upper limit of normal; NV: Normal value.

international normalized ratio and platelets were significantly different between AIH and ASC (Table 1). Autoantibodies were positive in all (100%). No patient with ASC was positive for anti-LKM-1 and/or LC-1. Positivity for anti-neutrophil cytoplasmatic antibodies was more common in ASC patients (71% *vs* 41% in AIH, $P < 0.05$). Raised IgG was reported in 68% of patients (51/75) without differences between the two groups ($P > 0.05$). Liver biopsy was performed in all patients with similar prevalence of interface hepatitis, cirrhosis and biliary features in the two groups ($P > 0.05$) (Table 1).

Response to treatment in the whole group

Medications used in our cohort of patients are reported in Table 2. The association between prednisone/azathioprine was more common in AIH patients (52% *vs* 10% in ASC, $P < 0.001$); conversely the association between prednisone/MMF/OVT was commonly used in ASC patients (23% *vs* 2% in AIH, $P = 0.005$) (Table 2).

Sixty-nine patients (92%, AIH = 96% *vs* ASC = 81%, $P = 0.048$) normalized transaminase levels and achieved biochemical remission at a median of 0.1 years (0.1-0.5) after starting standard medical treatment; 74 patients (98%, AIH = 100% and ASC = 95%) reduced AST levels to < 2 × ULN (AST NV 45 IU/L).

Sixty-eight patients (91%) normalized GGT levels at a median of 0.3 years (0.2-0.9) after starting standard medical treatment. Median time to GGT normalization tended to be significantly higher in ASC patients ($P = 0.06$); 71 patients (95%, AIH 98% and

Table 2 Response to medical treatment and outcome of 75 patients with autoimmune liver diseases

Variables	All patients, *n* = 75	AIH, *n* = 54	ASC, *n* = 21	*P* value
Treatment, *n*				
Prednisone alone	26 (35%)	19 (35%)	7 (33%)	1
Prednisone + Azathioprine	30 (40%)	28 (52%)	2 (10%)	< 0.001
Prednisone + MMF	5 (7%)	3 (5%)	2 (10%)	0.615
Prednisone + Vancomycin	4 (5%)	1 (2%)	3 (14%)	0.064
Prednisone + Azathioprine + Vancomycin	2 (3%)	0 (0%)	2 (10%)	0.075
Prednisone + MMF + Vancomycin	6 (8%)	1 (2%)	5 (23%)	0.005
Prednisone + Tacrolimus	1 (1%)	1 (2%)	0	NA
Cyclosporine	1 (1%)	1 (2%)	0	NA
Response to treatment				
Normal AST (NV ≤ 45 U/L): *n*	69 (92%)	52 (96%)	17 (81%)	0.048
Time to normalize AST (yr)	0.1 (0.1-0.5)	0.2 (0.1-0.6)	0.1 (0.1-0.2)	0.19
GGT (< 50 UI/L), *n*	68 (91%)	51 (94%)	17 (81%)	0.811
Time to normalize GGT (yr)	0.3 (0.2-0.6)	0.3 (0.2-0.5)	0.3 (0.2-1.1)	0.062
Immunological remission[1]: *n*	25 (33%)	22 (40%)	3 (14%)	0.032
Time to immunological remission	3.1 (2.2-4.2)	3.8 (2.9-4.3)	3.4 (3.2-3.7)	0.86
Relapse of AILD during treatment, *n*				
At least one relapse	36 (48%)	22 (41%)	14 (67%)	< 0.070
1 relapse alone	26 (35%)	17 (31%)	9 (43%)	0.421
≥ 2 relapses	10 (13%)	5 (9%)	5 (24%)	0.131
Outcome at last follow up				
Median follow up, yr (range)	4.4 (0.6-13.8)	4.1 (1.2-11.7)	4.5 (0.6-13.8)	0.079
Alive	75 (100%)	54 (100%)	21 (100%)	NA
OFF-IS therapy	6 (8%)	5 (9%)	1 (5%)	0.666
Medical treatment	68 (91%)	49 (91%)	19 (90%)	1
Liver transplant	1 (1%)	0 (0%)	1 (5%)	0.28

[1]Normal aspartate aminotransferase, normal IgG, and negative or low titer autoantibodies.

AIH: Autoimmune hepatitis; ASC: Autoimmune sclerosing cholangitis; AST: Aspartate aminotransferase; MMF: Mycophenolate mofetil; GGT: Gamma-glutamyl transferase; IS: Immunosuppressive; NA: Not applicable; ALID: Autoimmune liver disease; NV: Normal value.

ASC = 85%) reduced GGT levels to < 2 × ULN (GGT < 100 U/L).

One patient with ASC (F, age at diagnosis 13.1 years, with CD) did not respond to first and second line treatment and required LT (details below). Immunological remission was achieved in 25 patients (33%, AIH 40% and ASC = 14%) at a median of 3.1 years (2.2-4.2) after starting standard IS treatment.

Thirty-six patients experienced at least 1 episode of relapse (1 episode *n* = 16 patients; ≥ 2 episodes *n* = 10) managed with a temporary increase of prednisolone dose in 10 patients, with the addition of azathioprine in 15, and conversion from azathioprine to MMF in 11. Suboptimal adherence to treatment was detected in 8% (*n* = 3, AIH = 2, ASC = 1) of those who relapsed.

Treatment with OVT in non-responders

Of 75 patients, 12 [16%, F = 75%, median age 13.5 years, (12.2-15.7)] required OVT after a median time from the diagnosis of 2.2 years (0.8-4.3) (Table 3). Ten patients were diagnosed with ASC and 2 with AIH; 10/12 had IBD (83%) (Table 3). Liver biopsy performed before starting OVT showed absent (or mild) inflammatory infiltrate in all, and biliary features including inflammatory injury of the bile duct in 8 (67%) patients,

Table 3 Demographic, biochemical and histological features of 12 patients with autoimmune liver disease treated with oral vancomycin

Patients/ diagnosis	Gender	Age at diagnosis (yr)	Type of presentation	IBD	Splenomegaly[1]	IgG > ULN	Auto-antibodies	Histology				Medications
								Interface hepatitis	Fibrosis	Cirrhosis	Biliopathy[3]	
AIH	F	4.2	Asymptomatic	IC	Not	Yes	SMA 1:40; p-ANCA	No	Yes	No	Yes	Pred/MMF/UDCA/Mesa
AIH	F	10.9	Asymptomatic	UC	Not	Yes	ANA 1:320; SMA 1:160; p-ANCA positive	Yes	Yes	No	Yes	Pred/UDCA/Mesa
ASC	F	16	Insidious	None	Yes	Not	ANA 1:160; p-ANCA positive	Yes	Yes	Yes	No	Pred/MMF/UDCA
ASC	M	4.3	Asymptomatic	CD	Not	Yes	ANA 1:160; SMA 1:160; p-ANCA +++	Yes	Yes	No	Yes	Pred/UDCA/Mesa
ASC	F	8.6	Insidious	UC	Not	Not	SMA 1:40; p-ANCA positive	No	No	No	Yes	Pred/AZA/UDCA/Mesa
ASC	F	12.1	Insidious	UC[2]	Not	Not	SMA 1:40; p-ANCA positive	No	No	No	Yes	Pred/AZA/UDCA
ASC	M	14.1	Insidious	None	Not	Not	SMA 1:40; p-ANCA positive	Yes	Yes	No	Yes	Pred/AZA/UDCA
ASC	M	14.3	Acute	UC	Not	Yes	ANA 1:320; SMA 1:320	Yes	No	No	Yes	Pred/UDCA/Mesa
ASC	F	13.8	Asymptomatic	UC	Yes	Yes	ANA 1:640; p-ANCA positive	No	Yes	Yes	Yes	Pred/MMF/UDCA/Mesa
ASC	F	5.1	Acute	IC	Not	Not	ANA 1:160; p-ANCA positive	No	Yes	No	Yes	Pred/MMF/UDCA/Mesa
ASC	F	13.1	Acute	CD	Yes	Yes	ANA 1:80; p-ANCA positive	No	Yes	No	Yes	Pred/MMF/UDCA/Mesa
ASC	F	3.6	Asymptomatic	UC	Yes	Not	ANA 1:160; SMA 1:80; p-ANCA positive	Yes	Yes	No	Yes	Pred/MMF/UDCA/Mesa

[1]Spleen size detected on liver scan o magnetic resonance cholangiopancreatography.
[2]Patient underwent colectomy at age of 14 years.
[3]It includes at least one of the following: Inflammatory injury of the bile duct, ductular reaction, periductular fibrosis, biliary metaplasia, granulomatous cholangitis[16].
AIH: Autoimmune hepatitis; ASC: Autoimmune sclerosing cholangitis; IBD: Inflammatory bowel disease; UC: Ulcerative colitis, CD: Crohn disease; IC: Indeterminate colitis; ULN: Upper limit of normal; F: Female; M: Male; ANA: Anti-nuclear antibody; SMA: Smooth muscle antibody; ANCA: Anti-neutrophil cytoplasmic antibodies; Pred: Prednisone; UDCA: Ursodeoxycholic acid; MMF: Mycophenolate mofetil; Mesa: Mesalazyne.

ductular reaction in 11 (92%), biliary metaplasia in 7 (58%), and periductular fibrosis in 6 (50%). Need for OVT was significantly higher in ASC group compared to AIH [10/12 (83%) in ASC *vs* 2/54 (4%) in AIH, $P < 0.001$]. Immunological profile, histology and medications are reported in Table 3.

Median values of AST, ALT and GGT significantly decreased during OVT [AST levels from 107 UI/L (83-158) to 38 UI/L (31-65), $P = 0.010$; ALT from 160 UI/L (140-335) to 40 UI/L (37-87), $P = 0.008$; GGT from 279 (150-498) to 63 (32-143), $P = 0.005$] (Figure 1).

AST levels decreased in 10/12 patients (83%, within normal range in 8 patients and < 1.5 × ULN in 2), ALT levels in 9/12 patients (75%, within normal range in 7 patients and < 1.5 × ULN in 2), and GGT levels in 8/12 patients (67%, within normal range in 6 patients and < 1.5 × ULN in 2) (Table 2). Median time to normalization of AST, ALT and GGT levels were 2 mo (1.7-3.2), 5 mo (2.7-6.2), and 5 mo (3.2-6.0) respectively.

A complete response to OVT (normalization of AST, ALT and GGT) was observed in 6/12 patients (50%, cases *n*. 1, 2, 4, 5, 8, 10), a partial response in 2/12 (17%, cases *n*. 3 and 9) (Table 4).

After OVT, the percentage of patients who achieved biochemical remission increased overall from 81% (61/75 patients) to 92% (69/75), [from 93% (50/54) to 96% (52/54) in AIH, and from 52% (11/21) to 81% (17/21) in ASC] (Figure 2). Similarly, the percentage of patients who normalized GGT levels increased after OVT, mainly in ASC patients (from 62% to 81%) (Figure 2). No significant changes were observed in the other biochemical parameters including total bilirubin, serum albumin, and platelet count, nor in the prevalence of high IgG and positive autoantibodies ($P > 0.05$).

Based on SCOPE index score, all 6 patients who showed a complete response to OVT were classified as low risk (cases 1, 2) or medium risk (cases 4, 5, 8, 10); the other 6 patients (cases 3, 6, 7, 9, 11, 12) were classified as high risk. Decrease in SCOPE index score was reported in 5/12 patients (42%), from high to medium risk in 2 patients (cases 7, 9) and from medium to low risk in 3 (cases 4, 5, 8) (Table 4). After a median time of 24 mo (range 1-99), none of 12 patients complained of side effects related to OVT.

Four of 12 patients (33%, cases 6, 7, 11, 12, all with ASC) did not respond to OVT. One patient (*n*. 6) underwent colectomy at the age of 14 years due to a severe form of IBD. She never normalized her liver enzymes. A course of OVT was commenced at the age of 15.2 years, was not successful and was therefore discontinued 6 mo later. At the age of 16 years she was diagnosed also with juvenile arthritis, and was treated with adalimumab. Another patient (*n*. 7) achieved histological remission 3 years after the diagnosis, and IS treatment was gradually discontinued. Six months later he developed a relapse of ASC not responding to prednisone and azathioprine. A follow up liver biopsy showed fibro-obliterative lesions around the bile ducts and OVT was commenced, though without success. One patient (*n*. 11) developed progressive cholestasis and complications of portal hypertension requiring LT at age 17 years. One year later she developed ASC disease recurrence requiring re-transplantation at age 21 years. A second ASC recurrence occurred 10 mo later leading to multiple episodes of cholangitis. A new course of OVT was commenced unsuccessfully. The patient was re-listed for the third LT.

The last patient (*n*. 12) did not respond to first and second line treatment nor to OVT and developed features of portal hypertension (splenomegaly and hypersplenism) and incomplete cirrhosis on histology.

Outcome

At last follow up (median of 4.4 years, range 0.6-13.8 years) all patients are alive. Only 1 patient (F, with ASC) underwent LT at the age of 17 years and re-LT at the age of 21 years, due to recurrence of ASC (details above). Of 74 patients not requiring LT, 68 (92%) at last follow-up were still on medical treatment. In one patient (*n*. 5) who responded to OVT, we tried to reduce the dose of vancomycin from 1500 mg/d (divided in 3 doses) to 1000 mg/d (in 2 doses). However, few weeks later, AST and GGT increased 3 × ULN and normalized again when OVT went back to full dose (1500 mg/times for day).

Based on histological remission, IS withdrawal was attempted in 8 patients [7 females, median age 10.4 years (8.1-15.1), 7 AIH-1, 1 ASC] after a median of 4.0 years (3.9-5.3) from the diagnosis; 2/8 (*n*. 1,2) received OVT at the age of 5.4 and 11.8 years respectively. Two of these 8 patients (F, both with AIH-1) relapsed 1 and 4 mo after stopping treatment and responded successfully to IS treatment re-introduction. The other 6 (8%), including 1 patient with ASC, remained off treatment. One patient (*n*.1), discontinued prednisone and MMF 7.6 years after the diagnosis remaining on OVT alone, and her AST and GGT levels remained normal. Sixteen months later (at age of

Table 4 Biochemical response to oral vancomycin and Sclerosing Cholangitis Outcomes in Pediatrics index score of 12 patients with autoimmune liver disease treated with oral vancomycin

Patients/ diagnosis	Age at OVT (yr)	AST (NV ≤ 45 U/L)				ALT (NV ≤ 45 U/L)				GGT (NV ≤ 50 U/L)				Response to OVT [1]	SCOPE index score[2]		Time on OVT (mo)	OVT side-effect	Overall FU[3] (mo)
		Before OVT	After OVT	TTN	Result	Before OVT	After OVT	TTN	Result	Before OVT	After OVT	TTN	Result		Before OVT	After OVT			
AIH	5.4	212	39	4 mo	NV	147	17	6 mo	NV	73	22	6 mo	NV	Complete	3 low risk	0 low risk	99	None	113
AIH	11.8	251	31	2 mo	NV	359	39	9 mo	NV	26	34	8 mo	NV	Complete	3 low risk	0 low risk	72	None	73
ASC	16.8	98	47	3 mo	< 1.5 NV	140	70	4	< 1.5 NV	39	83	4	< 1.5 NV	Partial	8 high risk	8 high risk	16	None	26
ASC	4.8	86	28	7 d	NV	156	38	14 d	NV	84	44	14 d	NV	Complete	4 medium risk	1 low risk	37	None	39
ASC	13.1	60	14	1 mo	NV	365	38	3 mo	NV	68	27	4 mo	NV	Complete	5 medium risk	2 low risk	31	None	84
ASC	15.2	71	40	14 mo	NV	140	56	14 mo	< 1.5 NV	52	164	12 mo	-	None	6 high risk	6 high risk	6	None	68
ASC	17.4	113	65	1 mo	< 1.5 NV	205	141	1 mo	-	49	226	1 mo	-	None	6 high risk	4 medium risk	3	None	52
ASC	15	407	30	6 mo	NV	856	35	6 mo	NV	61	28	1 mo	NV	Complete	5 medium risk	2 low risk	40	None	49
ASC	17.3	102	37	2 mo	NV	111	36	2 mo	NV	135	82	5 mo	< 1.5 NV	Partial	6 high risk	4 medium risk	18	None	61
ASC	12.5	76	31	2 mo	NV	124	40	7 mo	NV	86; TX	42	6 mo	NV	Complete	5 medium risk	4 medium risk	47	None	135
ASC	13.9	123	155	-	-	165	154	-	-	165	1800	-	-	None	8 high risk	8 high risk	6	None	86; TX
ASC	13.2	141	135	-	-	156	180	-	-	71 mo (range 26-165)	136	-	-	None	7 high risk	7 high risk	4	None	165
Response to OVT					10/12 (83%)				9/12 (75%)				8/12 (67%)				Median: 34 (range 1-99)		71 (range 26-165)

[1]Complete response is defined as "normalization of all three liver enzymes [aspartate aminotransferase (AST), alanine aminotransferase (ALT) and gamma-glutamyl transferase (GGT)]", partial response as "AST, ALT or GGT levels

decreasing to < 1.5 × ULN without reaching normal value", and no response as "no significant changes in liver enzymes".
[2]Sclerosing Cholangitis Outcomes in Pediatrics: Points 0-3: Low risk; Points 4-5: Medium risk; Points 6-11: High risk.
[3]Time from diagnosis to last follow up visit.
AIH: Autoimmune hepatitis; ASC: Autoimmune sclerosing cholangitis; OVT: Oral vancomycin treatment; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; GGT: Gamma-glutamyl transferase; TTN: Time to normalize or to achieve the lowest value; NV: Normal value; FU: Follow up; SCOPE: Sclerosing Cholangitis Outcomes in Pediatrics.

13.8 years) on routine blood tests she had an increase of AST and GGT > 3 × ULN. The patient confessed a low adherence to treatment; once she re-started OVT regularly, AST and GGT returned normal.

DISCUSSION

In pediatrics, there are few published studies focusing on the differences between AIH and ASC. Furthermore, experience on empirical use of oral vancomycin in children with AILD not responding to standard immunosuppression is limited.

In this study, MRCP performed at diagnosis allowed us to differentiate children with AIH from those with ASC, and see whether they differ in terms of characteristics at presentation, response to medical treatment and outcome.

Our results show that characteristics at presentation were different between AIH and ASC, similarly to other studies[4,18]. All patients with ASC were positive for ANA and/or SMA, none for anti-LKM-1 confirming the rare association between LKM-1 positivity and ASC[18-20]. IBD was more common in ASC patients compared those with AIH, UC being more common[4,18-21]. On histology, cirrhosis was reported in 23% of patients, similar to previous studies (from 11% to 68%), suggesting a late diagnosis in a proportion of cases[4,18,19]. Features of biliopathy were equally reported in AIH and ASC confirming that both conditions are not easily distinguishable on histological ground making the cholangiogram the only effective tool to differentiate patients with AIH from those with ASC[16,18].

Pediatric patients with AILD respond well to IS treatment although the efficacy of second and third line treatment remains to be demonstrated, particularly in patients ASC[3].

The first study reporting benefits from OVT in children with ASC and IBD (n = 3 patients) was reported by Cox *et al*[12] in 1998. In that study OVT was administered to 3 patients (1 aged 15 years and 2 aged 14 years) diagnosed with PSC and IBD who showed improvements in gastrointestinal symptoms and liver enzymes after OVT[12].

However, this is the first study that aims to assess consistently the efficacy of OVT in a cohort of children and adolescents with AIH and ASC who did not respond to standard treatment and were treated according to a single protocol.

At our center OVT was given to children with AILD who failed to respond to first/second line IS treatment and had, on histology, features of biliopathy without (or

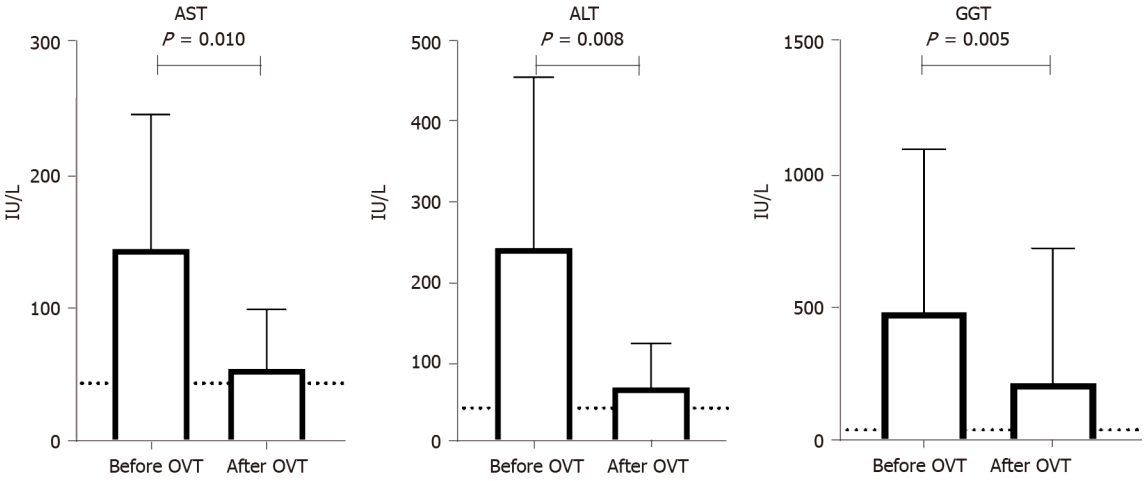

Figure 1 Aspartate aminotransferase, alanine aminotransferase and gamma-glutamyl transferase levels before and after oral vancomycin treatment (*n* = 12 patients). AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; GGT: Gamma-glutamyl transferase; OVT: Oral vancomycin treatment.

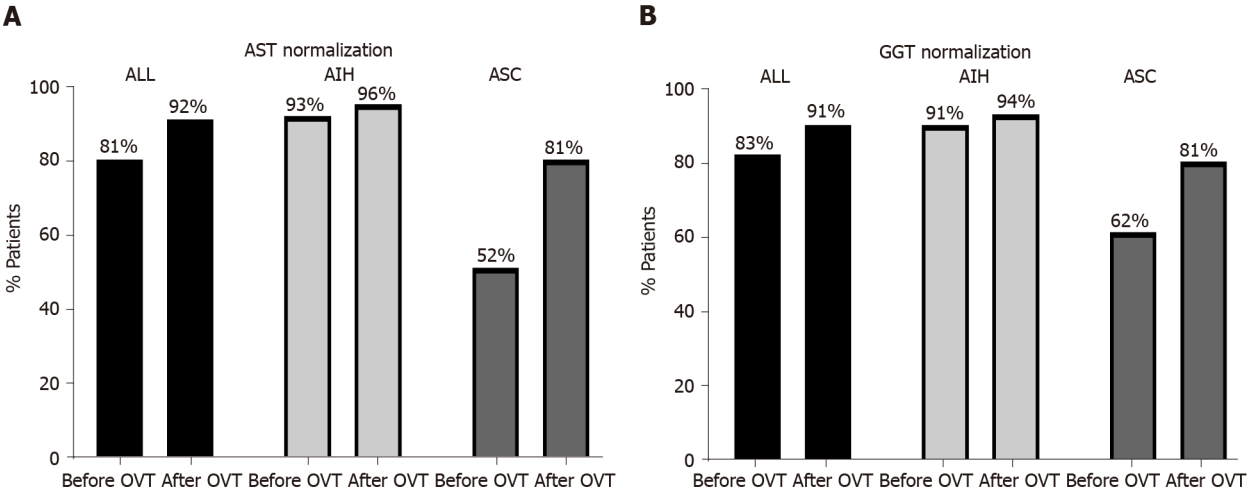

Figure 2 Percentage of patients (*n* = 75) who normalized aspartate aminotransferase and gamma-glutamyl transferase levels before and after oral vancomycin treatment. AST: Aspartate aminotrasferase; GGT: Gamma-glutamyl trasferase; OVT: Oral vancomycin treatment; AIH: Autoimmune hepatitis; ASC: Autoimmune sclerosing cholangitis.

mild) inflammation. To our opinion, in these patients an escalation of IS therapy (third line treatment) was not indicated due to the absence of significant lymphoplasmacytic infiltrate.

In this cohort a high proportion of patients normalized transaminases and GGT levels on standard IS; the majority of patients (40%) required an association between prednisone plus azathioprine, mainly in AIH group. Of interest, 10/12 patients who required OVT had ASC and 2/12 with AIH; on histology all had strong features of biliopathy, with mild or no inflammation.

Similarly to our study, improvements in liver enzymes after OVT were reported in Davies *et al*[7]'s study (*n* = 14 children with PSC and IBD), and in two randomized clinical trials on a total of 64 adult patients with PSC[5,6]. In Abarbanel *et al*[8]'s study the authors showed that all children with PSC and IBD experienced a reduction in GGT and ALT levels and improvement of biliary imaging, biopsies of the liver and intestine, and IBD symptoms while on OVT. In our study, median time to normalize liver enzymes ranged from 2 to 5 mo suggesting that a course of OVT should last at least 6 mo before assessing a biochemical response to treatment. Of note, no improvements were observed in the other biochemical parameters similar to Davies *et al*[7].

In a recent prospective study including pediatric patients (42% with small and 48% with large duct PSC), 49% (22/45), 20% (9/40), and 62.2% (28/45) of children experienced normalization of GGT, ALP, and ALT, respectively. Of note, the biochemical response to OVT was more favorable in the pediatric compared to the adult group. Besides, a significant proportion of patients showed improvements on histologic features and cholangiopathy[22]. Conversely, in a recent retrospective study on a large cohort of children with PSC the authors did not show improvement in outcomes of children treated with OVT or UDCA compared to those with "no treatment"[23], although several limitations were recorded in the study design[24]. The median OVT dose in Deneau *et al*[23]'s study was 21 mg/kg/d, which was substantially lower than the 50 mg/kg/d typically used in our and others' studies[5,6].

In Tabibian *et al*[6]'s work (n = 35 adult patients with PSC) the authors experienced a significant improvement in pruritus only in the high-dose vancomycin group. In our study we observed a temporary increase in AST and GGT levels after OVT dose reduction. In Cox *et al*[12]'s study, 3 children with SC and IBD had a normalization of liver tests while on OVT and return to abnormal values upon OVT discontinuation. These results confirm the efficacy of OVT and the importance of maintaining full doses regularly during the treatment.

The mechanisms by which OVT leads to biochemical improvement are still undefined. Previous studies suggested that OVT may have an immunomodulatory effect on regulatory T cells (Treg)[5,6-8]. Some authors suggest that the response to OVT is likely due to its antimicrobial effects on unknown pathogens or normal flora that cause abnormal immunological reactions following migration to the liver[7]. Several lines of experimental evidence from animal models demonstrate that enteric dysbiosis and/or administration of bacterial antigens can lead to hepatobiliary inflammation with various features of PSC[6]. In this study we found that the prevalence of IBD was similar in patients responding to OVT compared to those not responding, suggesting no role of IBD in the pathogenic mechanism of OVT.

Overall, the need for OVT emerged mainly in ASC group, and the percentage of patients who achieved the biochemical remission increased mainly in ASC group (from 52% to 81%) rather than in AIH (from 93% to 96%) (Figure 2)[4,25].

Of 75 patients, only 33% achieved immunological remission and no significant changes in IgG levels and autoantibody positivity were observed after OVT. This may imply an ongoing disease activity despite normal transaminase levels, possibly explaining the low proportion of children able to stop treatment successfully (8% in this study).

Interestingly, all 6 patients who showed a complete response to OVT were classified as low or medium SCOPE index strata, none as high risk, suggesting that probably the patients achieving a biochemical response to OVT are those with a milder disease activity. Similar results were reported in Deneau *et al*[17]'s study showing that a low SCOPE index at treatment initiation was an independent predictor of response. Moreover, the authors showed that the rate of response to OVT was similar in the group that started it as primary treatment and another that had it as second line[17]. Remarkably, in this study, OVT was associated with prednisone alone in 3 cases (100% responded to treatment) and with a second IS drug in the other 9 (55% responded to treatment, $P > 0.05$).

The decrease in the SCOPE index score (42% in this study) may suggest a potential benefit of OVT on long-term outcomes. Similar results were reported in a triple blinded, randomized, placebo-controlled clinical trial on adult patients with PSC where the analysis showed a significant decrease in the Mayo PSC score in the vancomycin group at the third month comparing to the baseline evaluation[5].

Similarly to previous studies, we did not observe side effects or infectious complications from long-term OVT during the study period[6,7,22]. However, whether the use of this antibiotic may lead to vancomycin-resistant organisms is still an open issue. All 4 patients not responding to OVT (all with ASC) showed a progression of liver disease. One patient developed recurrence of ASC after the LT (twice) and did not respond to OVT confirming the high recurrence rate post-LT[3]. Differently from our experience, OVT has been reported to be effective in the treatment of a pediatric patient with recurrent PSC after LT, suggesting a disease mechanism with some causes external to the liver—potentially from the gut bacteria[26].

Overall, the outcome in our cohort was excellent, with 100% of patients alive at last follow up and 8% off IS treatment. Only 1 patient required LT, although the median follow up of our cohort of patients is relatively short.

CONCLUSION

This is the first study reporting data on the consistent use of OVT in children with AILD not responding to standard treatment. Our results show that AIH and ASC have different characteristics at presentation although both respond well to medical therapy. For children not responding to standard IS, OVT may represent a valuable option to achieve biochemical remission, particularly in ASC patients. This study adds timely insights into the highly engaged discussion about the use of OVT for children with AILD, confirming the need of further structured studies assessing the efficacy and safety of OVT as well as its potential benefits on long-term outcomes.

ARTICLE HIGHLIGHTS

Research background

Pediatric autoimmune liver disease (AILD) includes autoimmune hepatitis (AIH) and autoimmune sclerosing cholangitis (ASC). Children with AILD not responding to standard immunosuppression (IS) may progress to end-stage liver disease and require liver transplantation.

Research motivation

Despite the absence of strong evidences the empirical use of candidate therapies has significantly increased in the last decades. Oral vancomycin has an immunomodulatory effect and it has been used in patients with primary sclerosing cholangitis. In pediatrics, the experience with oral vancomycin treatment (OVT) in patients with AIH or ASC is very limited.

Research objectives

In this study we evaluated: (1) The response to standard IS in a large cohort of pediatric patients with AILD; and (2) The efficacy of OVT to normalize transaminases (biochemical remission) and to achieve immunological remission in patients not responding to standard IS.

Research methods

Retrospective study of children diagnosed with AILD (AIH or ASC) at Hospital Papa Giovanni XXIII, Bergamo, Italy, in the last decade. Response to IS treatment and need for OVT was reported in all patients and compared between the two groups (AIH *vs* ASC).

Research results

Seventy-five patients diagnosed with AILD were included in this study (median age 10.5 years, range 5.6-13.4; F = 69%); 12 patients (16%, 10 with ASC) required OVT. Response to OVT was observed in 75% of patients and the percentage of those who achieved biochemical remission increased overall from 81% to 92%. Decrease in Sclerosing Cholangitis Outcomes in Pediatrics (SCOPE) index was reported in 42% of patients.

Research conclusions

This study shows that OVT may be considered as a valuable treatment option to achieve biochemical remission in children with AILD not responding to standard IS. Decrease in SCOPE index after OVT may suggest improvements in the long-term outcome.

Research perspectives

These promising preliminary results suggest that further prospective studies are needed to better define the efficacy of OVT in AILD.

REFERENCES

1 **Liberal R**, Vergani D, Mieli-Vergani G. Paediatric Autoimmune Liver Disease. *Dig Dis* 2015; **33** Suppl 2: 36-46 [PMID: 26641670 DOI: 10.1159/000440708]

2 **European Association for the Study of the Liver**. EASL Clinical Practice Guidelines: Autoimmune

hepatitis. *J Hepatol* 2015; **63**: 971-1004 [PMID: 26341719 DOI: 10.1016/j.jhep.2015.06.030]
3 **Mieli-Vergani G**, Vergani D, Baumann U, Czubkowski P, Debray D, Dezsofi A, Fischler B, Gupte G, Hierro L, Indolfi G, Jahnel J, Smets F, Verkade HJ, Hadžić N. Diagnosis and Management of Pediatric Autoimmune Liver Disease: ESPGHAN Hepatology Committee Position Statement. *J Pediatr Gastroenterol Nutr* 2018; **66**: 345-360 [PMID: 29356770 DOI: 10.1097/MPG.0000000000001801]
4 **Di Giorgio A**, Hadzic N, Dhawan A, Deheragoda M, Heneghan MA, Vergani D, Mieli-Vergani G, Samyn M. Seamless Management of Juvenile Autoimmune Liver Disease: Long-Term Medical and Social Outcome. *J Pediatr* 2020; **218**: 121-129.e3 [PMID: 31955873 DOI: 10.1016/j.jpeds.2019.11.028]
5 **Rahimpour S**, Nasiri-Toosi M, Khalili H, Ebrahimi-Daryani N, Nouri-Taromlou MK, Azizi Z. A Triple Blinded, Randomized, Placebo-Controlled Clinical Trial to Evaluate the Efficacy and Safety of Oral Vancomycin in Primary Sclerosing Cholangitis: a Pilot Study. *J Gastrointestin Liver Dis* 2016; **25**: 457-464 [PMID: 27981301 DOI: 10.15403/jgld.2014.1121.254.rah]
6 **Tabibian JH**, Weeding E, Jorgensen RA, Petz JL, Keach JC, Talwalkar JA, Lindor KD. Randomised clinical trial: vancomycin or metronidazole in patients with primary sclerosing cholangitis - a pilot study. *Aliment Pharmacol Ther* 2013; **37**: 604-612 [PMID: 23384404 DOI: 10.1111/apt.12232]
7 **Davies YK**, Cox KM, Abdullah BA, Safta A, Terry AB, Cox KL. Long-term treatment of primary sclerosing cholangitis in children with oral vancomycin: an immunomodulating antibiotic. *J Pediatr Gastroenterol Nutr* 2008; **47**: 61-67 [PMID: 18607270 DOI: 10.1097/MPG.0b013e31816fee95]
8 **Abarbanel DN**, Seki SM, Davies Y, Marlen N, Benavides JA, Cox K, Nadeau KC, Cox KL. Immunomodulatory effect of vancomycin on Treg in pediatric inflammatory bowel disease and primary sclerosing cholangitis. *J Clin Immunol* 2013; **33**: 397-406 [PMID: 23054338 DOI: 10.1007/s10875-012-9801-1]
9 **Buness C**, Lindor KD, Miloh T. Oral Vancomycin Therapy in a Child with Primary Sclerosing Cholangitis and Severe Ulcerative Colitis. *Pediatr Gastroenterol Hepatol Nutr* 2016; **19**: 210-213 [PMID: 27738604 DOI: 10.5223/pghn.2016.19.3.210]
10 **Vrieze A**, Out C, Fuentes S, Jonker L, Reuling I, Kootte RS, van Nood E, Holleman F, Knaapen M, Romijn JA, Soeters MR, Blaak EE, Dallinga-Thie GM, Reijnders D, Ackermans MT, Serlie MJ, Knop FK, Holst JJ, van der Ley C, Kema IP, Zoetendal EG, de Vos WM, Hoekstra JB, Stroes ES, Groen AK, Nieuwdorp M. Impact of oral vancomycin on gut microbiota, bile acid metabolism, and insulin sensitivity. *J Hepatol* 2014; **60**: 824-831 [PMID: 24316517 DOI: 10.1016/j.jhep.2013.11.034]
11 **Davies YK**, Tsay CJ, Caccamo DV, Cox KM, Castillo RO, Cox KL. Successful treatment of recurrent primary sclerosing cholangitis after orthotopic liver transplantation with oral vancomycin. *Case Rep Transplant* 2013; **2013**: 314292 [PMID: 23509657 DOI: 10.1155/2013/314292]
12 **Cox KL**, Cox KM. Oral vancomycin: treatment of primary sclerosing cholangitis in children with inflammatory bowel disease. *J Pediatr Gastroenterol Nutr* 1998; **27**: 580-583 [PMID: 9822326 DOI: 10.1097/00005176-199811000-00015]
13 **Sebode M**, Hartl J, Vergani D, Lohse AW; International Autoimmune Hepatitis Group (IAIHG). Autoimmune hepatitis: From current knowledge and clinical practice to future research agenda. *Liver Int* 2018; **38**: 15-22 [PMID: 28432836 DOI: 10.1111/Liv.13458]
14 **Vergani D**, Alvarez F, Bianchi FB, Cançado EL, Mackay IR, Manns MP, Nishioka M, Penner E; International Autoimmune Hepatitis Group. Liver autoimmune serology: a consensus statement from the committee for autoimmune serology of the International Autoimmune Hepatitis Group. *J Hepatol* 2004; **41**: 677-683 [PMID: 15464251 DOI: 10.1016/j.jhep.2004.08.002]
15 **Kerkar N**, Chan A. Autoimmune Hepatitis, Sclerosing Cholangitis, and Autoimmune Sclerosing Cholangitis or Overlap Syndrome. *Clin Liver Dis* 2018; **22**: 689-702 [PMID: 30266157 DOI: 10.1016/j.cld.2018.06.005]
16 **Di Giorgio A**, D'Adda A, Marseglia A, Sonzogni A, Licini L, Nicastro E, D'Antiga L. Biliary features in liver histology of children with autoimmune liver disease. *Hepatol Int* 2019; **13**: 510-518 [PMID: 31069759 DOI: 10.1007/s12072-019-09948-1]
17 **Deneau MR**, Mack C, Perito ER, Ricciuto A, Valentino PL, Amin M, Amir AZ, Aumar M, Auth M, Broderick A, DiGuglielmo M, Draijer LG, Tavares Fagundes ED, El-Matary W, Ferrari F, Furuya KN, Gupta N, Hochberg JT, Homan M, Horslen S, Iorio R, Jensen MK, Jonas MM, Kamath BM, Kerkar N, Kim KM, Kolho KL, Koot BGP, Laborda TJ, Lee CK, Loomes KM, Martinez M, Miethke A, Miloh T, Mogul D, Mohammad S, Mohan P, Moroz S, Ovchinsky N, Palle S, Papadopoulou A, Rao G, Rodrigues Ferreira A, Sathya P, Schwarz KB, Shah U, Shteyer E, Singh R, Smolka V, Soufi N, Tanaka A, Varier R, Vitola B, Woynarowski M, Zerofsky M, Zizzo A, Guthery SL. The Sclerosing Cholangitis Outcomes in Pediatrics (SCOPE) Index: A Prognostic Tool for Children. *Hepatology* 2021; **73**: 1074-1087 [PMID: 32464706 DOI: 10.1002/hep.31393]
18 **Sokollik C**, McLin VA, Vergani D, Terziroli Beretta-Piccoli B, Mieli-Vergani G. Juvenile autoimmune hepatitis: A comprehensive review. *J Autoimmun* 2018; **95**: 69-76 [PMID: 30344030 DOI: 10.1016/j.jaut.2018.10.007]
19 **Terziroli Beretta-Piccoli B**, Mieli-Vergani G, Vergani D. Serology in autoimmune hepatitis: A clinical-practice approach. *Eur J Intern Med* 2018; **48**: 35-43 [PMID: 29056396 DOI: 10.1016/j.ejim.2017.10.006]
20 **Giorgio AD**, Vergani D, Mieli-Vergani G. Cutting edge issues in juvenile sclerosing cholangitis. *Dig Liver Dis* 2021 [PMID: 34289942 DOI: 10.1016/j.dld.2021.06.028]
21 **Smolka V**, Karaskova E, Tkachyk O, Aiglova K, Ehrmann J, Michalkova K, Konecny M,

Volejnikova J. Long-term follow-up of children and adolescents with primary sclerosing cholangitis and autoimmune sclerosing cholangitis. *Hepatobiliary Pancreat Dis Int* 2016; **15**: 412-418 [PMID: 27498582 DOI: 10.1016/s1499-3872(16)60088-7]

22 **Ali AH**, Damman J, Shah SB, Davies Y, Hurwitz M, Stephen M, Lemos LM, Carey EJ, Lindor KD, Buness CW, Alrabadi L, Berquist WE, Cox KL. Open-label prospective therapeutic clinical trials: oral vancomycin in children and adults with primary sclerosing cholangitis. *Scand J Gastroenterol* 2020; **55**: 941-950 [PMID: 32633158 DOI: 10.1080/00365521.2020.1787501]

23 **Deneau MR**, Mack C, Mogul D, Perito ER, Valentino PL, Amir AZ, DiGuglielmo M, Draijer LG, El-Matary W, Furuya KN, Gupta N, Hochberg JT, Horslen S, Jensen MK, Jonas MM, Kerkar N, Koot BGP, Laborda TJ, Lee CK, Loomes KM, Martinez M, Miethke A, Miloh T, Mohammad S, Ovchinsky N, Rao G, Ricciuto A, Sathya P, Schwarz KB, Shah U, Singh R, Vitola B, Zizzo A, Guthery SL. Oral Vancomycin, Ursodeoxycholic Acid, or No Therapy for Pediatric Primary Sclerosing Cholangitis: A Matched Analysis. *Hepatology* 2021; **73**: 1061-1073 [PMID: 32946600 DOI: 10.1002/hep.31560]

24 **Assis DN**, Levy C. Oral Vancomycin or Ursodeoxycholic Acid for Pediatric Primary Sclerosing Cholangitis? *Hepatology* 2021; **73**: 887-889 [PMID: 33403699 DOI: 10.1002/hep.31702]

25 **Rojas CP**, Bodicharla R, Campuzano-Zuluaga G, Hernandez L, Rodriguez MM. Autoimmune hepatitis and primary sclerosing cholangitis in children and adolescents. *Fetal Pediatr Pathol* 2014; **33**: 202-209 [PMID: 24754367 DOI: 10.3109/15513815.2014.898721]

26 **Tabibian JH**, Talwalkar JA, Lindor KD. Role of the microbiota and antibiotics in primary sclerosing cholangitis. *Biomed Res Int* 2013; **2013**: 389537 [PMID: 24232746 DOI: 10.1155/2013/389537]

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ORIGINAL ARTICLE

Retrospective Study

Trends of alcoholic liver cirrhosis readmissions from 2010 to 2018: Rates and healthcare burden associated with readmissions

Asim Kichloo, Zain El-Amir, Dushyant Singh Dahiya, Farah Wani, Jagmeet Singh, Dhanshree Solanki, Ehizogie Edigin, Precious Eseaton, Asad Mehboob, Hafeez Shaka

ORCID number: Asim Kichloo 0000-0003-4788-8572; Zain El-Amir 0000-0001-7649-5634; Dushyant Singh Dahiya 0000-0002-8544-9039; Farah Wani 0000-0002-4683-6845; Jagmeet Singh 0000-0001-7179-1020; Dhanshree Solanki 0000-0001-8655-225X; Ehizogie Edigin 0000-0003-1093-1661; Precious Eseaton 0000-0001-5955-6060; Asad Mehboob 0000-0001-0000-0000; Hafeez Shaka 0000-0002-9456-4581.

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Institutional review board statement: As the National Readmission Database lacks patient and hospital-specific identifiers, this study was exempt from the Institutional Review Board (IRB) approval as per guidelines put forth by our

Asim Kichloo, Zain El-Amir, Dushyant Singh Dahiya, Department of Internal Medicine, Central Michigan University College of Medicine, Saginaw, MI 48602, United States

Asim Kichloo, Farah Wani, Department of Internal Medicine, Samaritan Medical Center, Watertown, NY 13601, United States

Jagmeet Singh, Department of Internal Medicine, Guthrie Robert Packer Hospital, Sayre, PA 18840, United States

Dhanshree Solanki, Department of Internal Medicine, Rutgers University, New Brunswick, NJ 07103, United States

Ehizogie Edigin, Hafeez Shaka, Department of Internal Medicine, John H Stroger Hospital of Cook County, Chicago, IL 60612, United States

Precious Eseaton, Department of Internal Medicine, University of Benin School of Medicine, Edo 300213, Nigeria

Asad Mehboob, Division of Gastroenterology, Department of Internal Medicine, Covenant Healthcare, Saginaw, MI 48602, United States

Corresponding author: Dushyant Singh Dahiya, MD, Doctor, Department of Internal Medicine, Central Michigan University College of Medicine, 1000 Houghton Ave, Saginaw, MI 48602, United States. dush.dahiya@gmail.com

Abstract

BACKGROUND

Alcoholic liver cirrhosis (ALC) is a chronic liver disease with varying disease severity. Readmissions of ALC are associated with poor outcomes.

AIM

To identify and assess trends of readmissions for ALC over an eight-year period.

METHODS

This retrospective interrupted trend study analysed 30-d readmissions of ALC in the United States from 2010 to 2018 using the National Readmissions Database. Hospitalization for ALC was the reason for index admission obtained using the International Classification of Diseases codes (571.2 and K70.3X). Biodemographic

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characteristics and hospitalization trends were highlighted over time. A multivariate regression analysis model was used to calculate the trend for risk-adjusted odds of 30-d all-cause ALC readmissions, ALC specific readmission rate, ALC readmission proportion, inpatient mortality, mean length of stay (LOS) and mean total hospital cost (THC) following adjustments for age, gender, grouped Charlson Comorbidity Index, insurance, mean household income, and hospital characteristics.

RESULTS

There was a trend towards increasing total 30-d readmissions of ALC from 7660 in 2010 to 15085 in 2018 ($P < 0.001$). Patients readmitted for ALC were noted to have an increasing comorbidity burden over time. We noted a rise in the risk-adjusted 30-d all-cause readmission of ALC from 24.9% in 2010 to 29.9% in 2018 ($P < 0.001$). ALC-specific readmission rate increased from 6.3% in 2010 to 8.4% in 2018 ($P < 0.001$) while ALC readmission proportion increased from 31.4% in 2010 to 36.3% in 2018 ($P < 0.001$). Inpatient mortality for 30-d readmissions of ALC declined from 10.5% in 2010 to 8.2% in 2018 ($P = 0.0079$). However, there was a trend towards increasing LOS from 5.6 d in 2010 to 6.3 d in 2018 ($P < 0.001$) and increasing THC from 13790 dollars in 2010 to 17150 dollars in 2018 ($P < 0.001$). The total days of hospital stay attributable to 30-d readmissions of ALC increased by 119.2% while the total attributable hospital costs increased by 149% by the end of 2018.

CONCLUSION

There was an increase in the 30-d readmission rate and comorbidity burden for ALC; however, inpatient mortality declined. Additionally, there was a trend towards increasing LOS and THC for these readmissions.

Key Words: Alcoholic liver cirrhosis; Readmissions; Epidemiology; Trends; Mortality

Core Tip: This retrospective interrupted trend study analysed 30-d readmissions of alcoholic liver cirrhosis (ALC) in the United States from 2010-2018. There was a trend towards increasing 30-d all-cause readmission rate and ALC-specific readmission rate for the study period. However, inpatient mortality was noted to have a declining trend from 10.5% in 2010 to 8.2% in 2018 ($P = 0.0079$). The total days of hospital stay attributable to ALC readmissions increased by 119.2% and total attributable hospital costs increased by 149% during the study period.

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INTRODUCTION

Alcohol use disorders are known to affect millions worldwide, and alcohol consumption is directly associated with liver disease mortality. Alcoholic liver disease varies in severity and prognosis based on several factors, including the pattern of alcohol consumption, duration of alcohol consumption, amount of alcohol consumption, the presence or absence of liver inflammation, nutritional status, genetic predisposition, and diet[1]. Alcoholic liver cirrhosis (ALC) is closely associated not only with the duration of alcohol consumption, but also the amount of undiluted alcohol consumed[1]. Although many patients with significant alcohol consumption develop fatty liver disease, not all patients with alcoholic liver disease progress to liver cirrhosis. It has also been postulated that genetic and environment factors may also

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play a key role in the development of ALC. Liver cirrhosis is reported to have significant mortality, morbidity, and reduced life expectancy. In fact, the median survival of patients with advanced ALC is reported to be around 1-2 years. Additionally, patients with decompensated cirrhosis who abstain from alcohol use have a reported 5-year survival rate of 60%, compared to the 30% survival rate in patients who continue with alcohol consumption[1]. It has previously been reported that a high proportion of patients with liver cirrhosis are readmitted within 30 d or 90 d, underscoring the risk of readmission in these patients[2].

While data on the morbidity and mortality of ALC has been reported in literature, there is paucity of information on the trends of readmissions after an index hospitalization for ALC. The purpose of this study was to identify the trends of readmissions, total hospital charges, and length of stay (LOS) over an eight-year study period while also examining changes in the demographic of ALC readmissions over time. Furthermore, as National Readmission Database (NRD) stores data on inpatient admissions in the form of International Classification of Diseases (ICD) codes, we used the codes 571.2 and K70.3X to include all patients with ALC in our study[3].

MATERIALS AND METHODS

Design and data source

This was a retrospective interrupted trends study involving adult hospitalizations for ALC in in the United States from 2010-2018. Data was extracted from the NRD which is the largest, publicly available, all-payer, inpatient healthcare readmission database in the United States, drawn from the Agency for Healthcare Research and Quality Healthcare Cost and Utilization Project (HCUP) State Inpatient Databases[3]. The NRD is an annual file constructed using one calendar year of discharge data. Discharges included in the NRD were treated at community hospitals (excluding rehabilitation or long-term acute care hospitals) and a majority of these discharges had patient linkage numbers that were verified and not questionable. Discharge weights were calculated using post-stratification for hospital characteristics (census region, urban-rural location, teaching status, bed size, and hospital control) and patient characteristics [sex and five age groups (0, 1-17, 18-44, 45-64, and 65 and older)]. The NRD contains discharge data from 28 geographically dispersed states accounting for 59.7% of the total United States population and 58.7% of all United States hospitalizations. It comprises both patient and hospital-level information. Up to 40 discharge diagnoses and 25 procedures are collected for each patient using the ICD-9 and ICD-10 codes. Diagnose were classified as principal diagnosis which was the reason for hospitalization, and secondary diagnosis which was any other discharge diagnosis. Hospitals were stratified according to ownership control, the number of beds, teaching status, urban/rural location, and geographic region. Furthermore, the NRD allows for weighted analysis to obtain 100% of the United States hospitalizations within a given year[3].

Study population

The study involved hospitalizations from NRD for 2010, 2012, 2014, 2016 and 2018 with ALC as the reason for index admission using ICD codes (571.2 and K70.3X). Individuals less than 18 years of age, December and elective hospitalizations were excluded from the study. Using unique hospitalization identifiers, index hospitalizations were identified and one subsequent hospitalization within 30 d was tagged as a readmission. Furthermore, traumatic admissions were excluded from the readmission data. December admissions were excluded when searching for index admissions as these hospitalizations would lack data for at least 30 d following discharge to determine if there was a readmission according to the study design. The comorbidity burden was assessed using Sundararajan's adaptation of the modified Deyo's Charlson Comorbidity Index[4].

Outcome measures

The biodemographic and hospitalization trends of the studied populations were highlighted over time. The 30-d all-cause ALC readmission rate, the ALC specific readmission rate, ALC readmission proportion, trends in inpatient mortality rate, mean LOS and mean THC were calculated. Total hospital cost was obtained using the HCUP Cost-to-Charge Ratio files and adjusted for inflation using the Medical Expenditure Panel Survey index for hospital care with 2018 as the reference point[5,6].

Statistical analysis

Data analysis was performed using Stata® Version 16 software (StataCorp, Texas, United States). All analyses were conducted using the weighted samples for national estimates in adjunct with HCUP regulations for using the NRD database. A multivariate regression analysis was used to calculate the trends of risk-adjusted odds of 30-d all-cause ALC readmission rate, the ALC specific readmission rate, ALC readmission proportion, trends in inpatient mortality rate, mean LOS and mean THC following adjustment for age, sex, grouped Charlson Comorbidity Index, insurance type, mean household income, and hospital characteristics. All *P* values were 2 sided with 0.05 set as the threshold for statistical significance.

Ethical considerations

The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. As the NRD does not include patient-specific and hospital-specific identifiers, this study was exempt from the Institutional Review Boards as per guidelines put forth by the IRB for research on database studies.

Data availability statement

The NRD is a large publicly available all-payer inpatient care database in the United States, containing data on more than 18 million hospital stays per year[3]. Its large sample size provides sufficient data for analysis across hospital types and the study of readmissions for relatively uncommon disorders and procedures.

RESULTS

Biodemographic and hospital characteristics of ALC readmissions

Details of characteristics of readmissions for ALC within the included years for the study are shown in Table 1. There has been a yearly increase in the total number of 30-d readmissions for ALC from 7660 in 2010 to 15085 in 2018 ($P < 0.001$). Most readmissions were noted for men but there was a decreasing trend in the proportion of male readmissions ($P < 0.001$). Patients readmitted for ALC had an increasing comorbidity burden with time. We also noted a rising trend of readmissions for small bed-sized and metropolitan teaching hospitals.

Trends in ALC readmission outcomes

There was a steady rise in the rate of risk-adjusted 30-d all-cause ALC readmissions from 24.9% in 2010 to 29.9% in 2018 ($P < 0.001$). We also noted increasing risk-adjusted 30-d ALC specific readmission rate from 6.3% in 2010 to 8.4% in 2018 ($P < 0.001$) and increasing ALC readmission proportion from 31.4% in 2010 to 36.3% in 2018 ($P < 0.001$) (Table 1 and Figure 1). In-patient mortality for 30-d readmissions of ALC showed a decreasing trend from 10.5% in 2010 to 8.2% in 2018 ($P = 0.0079$). However, there was a trend towards increasing LOS from 5.6 d in 2010 to 6.3 d in 2018 ($P < 0.001$) and increasing THC from 13790 dollars in 2010 to 17150 dollars in 2018 ($P < 0.001$) (Table 2).

Cost burden of ALC readmissions

The total days of hospital stay attributable to 30-d readmissions of ALC increased by 119.2% from 43244 d in 2010 to 94789 d in 2018, while the total attributable hospital costs increased by 149% from 104 million dollars in 2010 to over 259 million dollars by the end of 2018.

DISCUSSION

Total number of readmissions and demographics of readmissions

There has been a yearly increase in the total number of 30-d readmissions of ALC in the United States. This may be due to rising alcohol use, high-risk drinking habits and DSM-IV alcohol use disorders[7]. Prior research has established a strong positive co-relation between rising alcohol use disorders and alcoholic liver disease such as ALC. In our study, most 30-d ALC readmissions were for males, but a decreasing trend was noted in the proportion of male readmissions. A study examining privately insured

Table 1 Demographic characteristics and hospitalization trends for 30-d readmissions of alcoholic liver cirrhosis in the United States from 2010–2018

Variable	Year				
	2010	2012	2014	2016	2018
Number of readmissions	7660	8211	8753	13278	15085
Mean age (yr)	53.5 ± 0.5	53.6 ± 0.4	53.6 ± 0.4	54.0 ± 0.3	54.2 ± 0.3
Male (%)	72.5	73.1	72.2	68.3	67.4
Charlson comorbidity					
Index (CCI) Score (%)					
0	2.8	2.4	2.2	0.6	0.6
1	15.7	15.1	13.0	1.4	13.2
2	7.5	6.5	6.9	7.3	6.3
≥ 3	74.0	76.0	78.0	78.4	79.8
Insurance type					
Medicare	27.6	28.2	29.3	30.0	30.5
Medicaid	40.5	42.0	42.1	41.6	40.6
Private	21.4	20.0	20.4	22.5	21.8
Uninsured	10.5	9.7	8.3	6.0	7.2
Household income					
Quartile (%)					
1	34.6	36.2	34.0	34.2	33.2
2	23.8	25.6	28.3	27.4	29.2
3	23.4	22.4	22.0	23.5	22.6
4	18.2	15.8	15.6	14.8	15.0
Hospital characteristics					
Hospital bed size (%)					
Small	9.3	9.0	12.4	11.6	14.3
Medium	22.3	22.7	26.4	25.9	25.9
Large	68.4	68.2	61.2	62.5	59.8
Teaching status (%)					
Metropolitan non-teaching	40.4	39.1	28.0	26.1	20.5
Metropolitan teaching	52.6	53.7	66.8	69.5	75.4
Non-metropolitan	7.0	7.3	5.2	4.3	4.1
Hospital Volume (Quintiles)					
Q1	2.4	2.3	2.2	1.9	1.5
Q2	6.6	5.8	6.0	5.2	5.5
Q3	12.6	12.5	12.0	10.6	11.3
Q4	21.8	22.0	20.1	20.1	20.7
Q5	56.6	57.4	59.7	62.2	61.1

individuals with alcoholic cirrhosis noted that 32% of patients with alcoholic cirrhosis were women[8]. Our findings may reflect a rise in alcohol use, alcohol use disorders, and high-risk drinking behaviours in women, which is consistent with findings in current the literature[7].

Table 2 Trends of rates and outcomes for 30-d readmissions of alcoholic liver cirrhosis in the United States from 2010–2018

Outcomes	Year					*P* value[1]
	2010	2012	2014	2016	2018	
All-cause readmission rate (%)	24.9	25.1	25.1	29.8	29.9	< 0.001[1]
ALC specific readmission rate (%)	6.3	6.2	6.2	7.7	8.4	< 0.001[1]
ALC readmission proportion (%)	31.4	30.9	30.7	33.5	36.3	< 0.001[1]
Inpatient mortality (%)	10.5	9.7	9.2	8.3	8.2	0.008[1]
Mean length of stay (d)	5.6	5.6	5.6	6.4	6.3	0.001[1]
Mean total hospital cost (USD)	13790	14206	13612	17602	17150	< 0.001[1]

[1]Statistically significant. ALC: Alcoholic liver cirrhosis. ALC: Alcoholic liver cirrhosis.

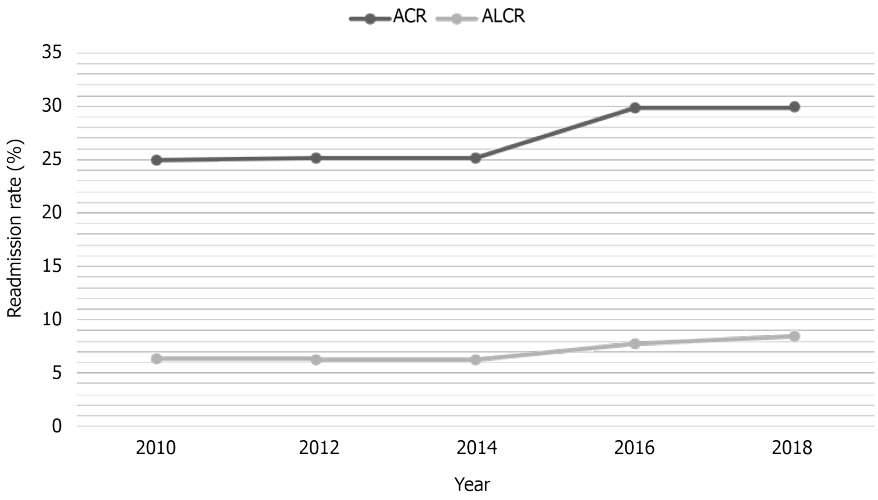

Figure 1 Trends for 30-d readmissions of alcoholic liver cirrhosis (ALC) in the United States from 2010–2018. ACR: All-cause readmissions, ALCR: Alcoholic liver cirrhosis-specific readmissions.

Recent reports have also indicated that women with alcohol use disorder may experience more barriers to treatment than men. Additionally, women are less likely to access treatment for alcohol use disorders than men. The reasons for these differences in treatment across genders are numerous and include low perception for the need of treatment, feelings of shame and guilt, concurrent disorders, economic disparities, insurance disparities, and employment status[9]. The rise of alcohol use disorders and rising consumption of alcohol by women along with differences in treatment between genders may, in part, explain the down trend noted in males over the eight-year study period. Targeted treatments plans or treatment plan elements that aim to address gaps in the treatment for alcohol use disorders may help prevent ALC and help in the management of ALC patients with alcohol use disorders. Research also suggests that treatment outcomes for women are best when given in women-only programs that have women-specific content focus[9]. Thus, creating targeted treatment programs for women may be an effective way of reducing ALC readmissions and promoting abstinence from alcohol use, a key component of ALC treatment strategies[10].

Patients readmitted for ALC had increasing comorbidity burden with time. Comorbidities are known to increase mortality and affect the overall prognosis in patients with liver cirrhosis, but it is important to recognize complications and distinguish them from comorbidities in cirrhotics[11]. Previous reports have indicated that increased alcohol consumption, high-risk consumption behaviours and increased alcohol use disorders in the United States not only constitute a public health crisis, but also increase the risk of numerous comorbidities associated with alcohol use. Alcohol use disorders and increased alcohol consumption are well known risk factors for morbidity and mortality in patients with hypertension, cardiovascular disease, stroke,

cirrhosis, certain cancers, type 2 diabetes mellitus, infections, and injuries. Moreover, alcohol use disorders and high-risk alcohol consumption are both associated with numerous psychiatric disorders[7]. As previous studies have indicated, understanding the impact of comorbidities on cirrhosis can help generate tailored treatment regimens for patients with ALC[11]. The rising comorbidity burden with time may also reflect the need for increased interventions specifically based on comorbid conditions.

Trends for ALC readmission outcomes and cost

There was a steady rise in the risk-adjusted 30-d all-cause ALC readmission rate. We also noted increasing risk-adjusted 30-d ALC specific readmission rate and ALC readmission proportion. A study investigating patients with ALC found that these patients were more likely to be disproportionately sicker at presentation and were readmitted more often than their non-ALC counterparts[8]. Additionally, hospital readmissions have been reported to occur more frequently in patients with cirrhosis. In general, research noted that early readmission reflects poor quality of care, and previous studies have reported a pooled rate of 26% for 30-d readmissions for cirrhosis. These readmissions negative impact inpatient mortality. The rising rate of readmissions in patients with ALC suggests that there may be room for improvement in caring for patients with ALC with the hope of reducing readmissions as has been suggested in previous cirrhosis-related readmissions studies[12]. Previous studies have also found that initial ALC admissions have most often resulted in readmissions secondary to acute complications from cirrhosis and substance abuse, while in patients without ALC, readmissions were most commonly due to acute cirrhosis complications and complications from cancer[2]. The rise in ALC-related readmissions found in our study may reflect increased alcohol use, closely related to the amount of undiluted alcohol consumed and the duration of consumption[1]. This reflects the need for enrolment of patients with ALC into alcohol rehabilitation programs on index admission, extensive patient education, regular outpatient follow-ups and early effective alcohol use disorder treatments in the outpatient setting to prevent development and readmissions in ALC patients.

Inpatient mortality showed a decreasing trend in our study; however, there was a trend towards increasing LOS and THC. The total days of hospital stay attributable to ALC readmissions increased by 119.2%, and total attributable hospital costs increased by 149% from 104 dollars million in 2010 to over 259 million dollars by 2018. Inpatient charges for patients with liver cirrhosis are substantial and have been consistently increasing[13]. Cirrhosis has resulted in considerable resource utilization and expenditure, despite acceptable hospital survival. Critically ill patients with liver cirrhosis have historically been perceived as not only having a poor prognosis, but also substantial costs of care, which is elucidated by our findings[14]. Alcohol liver diseases such as ALC are reportedly account for more than half of the charges associated with liver cirrhosis. This significant cost associated with ALC is driven by the volume of both admissions and readmissions of the same patients. Previous reports have suggested that effective alcohol use disorder interventions can help reduce costs related to inpatient cirrhosis management[13]. Treatments that have been proven to be cost-effective and in some cases cost-saving for ALC include one-on-one physician counselling and medication-assisted therapies[15]. These have been shown to improve outcomes in patients with compensated ALC[15].

Strengths and limitations

This study has several strengths that can be appreciated. The population used for this study is drawn from the NRD, which is believed to be a large, multi-ethnic hospital-based registry in the United States. This study also examines eight-year data and numerous demographic characteristics of ALC hospitalizations, offering a comprehensive, thorough, and contemporary overview of ALC readmissions in the United States. However, as with any study, there are limitations that should be noted. Data from the NRD is subject to all biases associated with retrospective studies. Additionally, the NRD does not contain data on the hospital course and treatment aspects of the disease. Moreover, the NRD reports information on hospitalizations rather than from individual patients. Thus, patients with numerous readmissions would be included more than once in the data set. The database also uses ICD codes to store information and therefore may have coding errors. Finally, the NRD does not include information about the severity of ALC at the time of admission.

Despite these limitations, the large sample size, outcomes of the study, and analysis techniques make for a study that provides a current perspective on a major healthcare burden while aiming to encourage further discourse and future controlled prospective studies on ALC hospitalizations and readmissions.

CONCLUSION

ALC is a chronic liver disease with a known healthcare and economic burden, morbidity, and mortality with the potential to result in readmissions. This retrospective, interrupted trends study examined adult hospitalizations for ALC in in the United States. We found a yearly increase in the total number of 30-d readmissions for ALC and an increasing comorbidity burden with time which may reflect the rise in alcohol use disorders and comorbid conditions in patients with ALC. There was a steady rise in the risk-adjusted 30-d all-cause ALC readmission rate, risk-adjusted 30-d ALC-specific readmission rate and 30-d ALC readmission proportion. This may reflect the need for better management of ALC in an outpatient setting. Medication-assisted therapies and counselling may be highly cost-effective ways to reduce ALC readmissions. Inpatient mortality notably showed a decreasing trend for the study period. However, there was a trend towards increasing LOS and THC. Ultimately, improved management of ALC and associated conditions like alcohol use disorder and high-risk alcohol consumption may help reduce readmissions and resultant healthcare burdens associated with readmissions.

ARTICLE HIGHLIGHTS

Research background

Readmissions of alcoholic liver cirrhosis (ALC) are associated with poor outcomes.

Research motivation

There is paucity of data on the trends of 30-d readmissions of ALC in the United States despite it being a significant healthcare burden.

Research objectives

The primary objective of this study was to identify and assess trends of 30-d readmissions of ALC in the United States over an eight-year period.

Research methods

This retrospective interrupted trend study used the National Readmissions Database to identify all 30-d readmissions of ALC. Multivariate regression analysis was used to calculate the trend for risk-adjusted odds of 30-d all-cause ALC readmissions, ALC specific readmission rate, ALC readmission proportion, mortality, mean length of stay (LOS) and mean total hospital cost (THC).

Research results

There was a trend towards increasing total 30-d readmissions of ALC, risk-adjusted 30-d all-cause ALC readmission, ALC specific readmission rate, and ALC readmission proportion. However, inpatient mortality declined from 10.5% in 2010 to 8.2% in 2018. The total days of hospital stay attributable to 30-d readmissions of ALC increased by 119.2% while the total attributable hospital costs increased by 149% by the end of 2018.

Research conclusions

The total number of 30-d readmissions of ALC increased; however, inpatient mortality declined. There was a trend towards increasing LOS and THC for these readmissions.

Research perspectives

Future studies are needed to investigate the treatment aspects of ALC in an inpatient setting. Additionally, the impact of early enrollment of patients into alcohol rehabilitation programs, patient education, regular outpatient follow-up and early effective alcohol use disorder treatments in the outpatient setting to prevent readmissions of ALC in is yet to be determined.

REFERENCES

1 **Bruha R**, Dvorak K, Petrtyl J. Alcoholic liver disease. *World J Hepatol* 2012; **4**: 81-90 [PMID: 22489260 DOI: 10.4254/wjh.v4.i3.81]

2 **Tapper EB**, Halbert B, Mellinger J. Rates of and Reasons for Hospital Readmissions in Patients

With Cirrhosis: A Multistate Population-based Cohort Study. *Clin Gastroenterol Hepatol* 2016; **14**: 1181-1188.e2 [PMID: 27085758 DOI: 10.1016/j.cgh.2016.04.009]

3 **Agency for Healthcare Research and Quality**. Overview of the Nationwide Readmissions Database (NRD). 2018. [cited 20 May 2021]. Available from: https://www.hcup-us.ahrq.gov/nrdoverview.jsp

4 **Sundararajan V**, Quan H, Halfon P, Fushimi K, Luthi JC, Burnand B, Ghali WA; International Methodology Consortium for Coded Health Information (IMECCHI). Cross-national comparative performance of three versions of the ICD-10 Charlson index. *Med Care* 2007; **45**: 1210-1215 [PMID: 18007172 DOI: 10.1097/mLr.0b013e3181484347]

5 **Agency for Healthcare Research and Quality**. Cost-to-Charge Ratio Files. [cited 20 May 2021]. Available from: https://www.hcup-us.ahrq.gov/db/ccr/costtocharge.jsp

6 **Dunn A**, Grosse SD, Zuvekas SH. Adjusting Health Expenditures for Inflation: A Review of Measures for Health Services Research in the United States. *Health Serv Res* 2018; **53**: 175-196 [PMID: 27873305 DOI: 10.1111/1475-6773.12612]

7 **Grant BF**, Chou SP, Saha TD, Pickering RP, Kerridge BT, Ruan WJ, Huang B, Jung J, Zhang H, Fan A, Hasin DS. Prevalence of 12-Month Alcohol Use, High-Risk Drinking, and DSM-IV Alcohol Use Disorder in the United States, 2001-2002 to 2012-2013: Results From the National Epidemiologic Survey on Alcohol and Related Conditions. *JAMA Psychiatry* 2017; **74**: 911-923 [PMID: 28793133 DOI: 10.1001/jamapsychiatry.2017.2161]

8 **Mellinger JL**, Shedden K, Winder GS, Tapper E, Adams M, Fontana RJ, Volk ML, Blow FC, Lok ASF. The high burden of alcoholic cirrhosis in privately insured persons in the United States. *Hepatology* 2018; **68**: 872-882 [PMID: 29579356 DOI: 10.1002/hep.29887]

9 **McCrady BS**, Epstein EE, Fokas KF. Treatment Interventions for Women With Alcohol Use Disorder. *Alcohol Res* 2020; **40**: 08 [PMID: 32742894 DOI: 10.35946/arcr.v40.2.08]

10 **Walsh K**, Alexander G. Alcoholic liver disease. *Postgrad Med J* 2000; **76**: 280-286 [PMID: 10775280 DOI: 10.1136/pmj.76.895.280]

11 **Jepsen P**. Comorbidity in cirrhosis. *World J Gastroenterol* 2014; **20**: 7223-7230 [PMID: 24966593 DOI: 10.3748/wjg.v20.i23.7223]

12 **Orman ES**, Ghabril M, Emmett TW, Chalasani N. Hospital Readmissions in Patients with Cirrhosis: A Systematic Review. *J Hosp Med* 2018 [PMID: 29694458 DOI: 10.12788/jhm.2967]

13 **Barritt AS 4th**, Jiang Y, Schmidt M, Hayashi PH, Bataller R. Charges for Alcoholic Cirrhosis Exceed All Other Etiologies of Cirrhosis Combined: A National and State Inpatient Survey Analysis. *Dig Dis Sci* 2019; **64**: 1460-1469 [PMID: 30673984 DOI: 10.1007/s10620-019-5471-7]

14 **Shawcross DL**, Austin MJ, Abeles RD, McPhail MJW, Yeoman AD, Taylor NJ, Portal AJ, Jamil K, Auzinger G, Sizer E, Bernal W, Wendon JA. The impact of organ dysfunction in cirrhosis: survival at a cost? *J Hepatol* 2012; **56**: 1054-1062 [PMID: 22245890 DOI: 10.1016/j.jhep.2011.12.014]

15 **Avanceña ALV**, Miller N, Uttal SE, Hutton DW, Mellinger JL. Cost-effectiveness of alcohol use treatments in patients with alcohol-related cirrhosis. *J Hepatol* 2021; **74**: 1286-1294 [PMID: 33326815 DOI: 10.1016/j.jhep.2020.12.004]

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ORIGINAL ARTICLE

Observational Study

New stem cell autophagy surrogate diagnostic biomarkers in early-stage hepatocellular carcinoma in Egypt: A pilot study

Tarek Yosef, Wesam Ahmed Ibrahim, Marwa Matboli, Amina Ahmed Swilam, Sarah El-Nakeep

ORCID number: Tarek Yosef 0000-0002-0003-7548; Wesam Ahmed Ibrahim 0000-0003-1813-5248; Marwa Matboli 0000-0002-6852-3954; Amina Ahmed Swilam 0000-0002-1464-1269; Sarah El-Nakeep 0000-0003-2830-5052.

Author contributions: Yosef T supervised the conduction of the study; Ibrahim WA and El-Nakeep S followed the clinical collection of data and the availability of patients; El-Nakeep S and Matboli M formulated the research question and its applicability and wrote the final draft; Matboli M conducted the laboratory analysis; Swilam AA collected the data from the patients; all authors revised and accepted the final submitted manuscript.

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Informed consent statement: Informed consent was obtained from each participant. Both the

Tarek Yosef, Wesam Ahmed Ibrahim, Sarah El-Nakeep, Gastroenterology and Hepatology Unit, Department of Internal Medicine, Faculty of Medicine, Ain Shams University, Cairo 11591, Egypt

Marwa Matboli, Department of Biochemistry, Faculty of Medicine, Ain Shams University, Cairo 11591, Egypt

Amina Ahmed Swilam, Department of Internal Medicine, Health Affair Directorate, Ministry of Health and Population, Cairo 11591, Egypt

Corresponding author: Sarah El-Nakeep, MD, Associate Professor, Gastroenterology and Hepatology Unit, Department of Internal Medicine, Faculty of Medicine, Ain Shams University, Ramsees Street, Cairo 11591, Egypt. sarahnakeep@gmail.com

Abstract

BACKGROUND

Stem cell autophagy disruption is responsible for the development of hepatocellular carcinoma (HCC). Many non-coding RNAs are linked to the activation and inhibition of certain genes. The *SQSTM1* gene controls stem cell autophagy as shown in previous studies. The upregulation of *SQSTM1* is associated with the inhibition of autophagy in cancerous stem cells in patients with HCC.

AIM

To determine whether serum microRNA, hsa-miR-519d, is linked to *SQSTM1* gene and whether they could be used as diagnostic biomarkers for early-stage HCC.

METHODS

In silico analysis was performed to determine the most correlated genes of autophagy with microRNAs. *SQSTM1* and hsa-miR-519d were validated through this pilot clinical study. This study included 50 Egyptian participants, who were classified into three subgroups: Group 1 included 34 patients with early-stage HCC, Group 2 included 11 patients with chronic liver disease, and Group 3 (control) included 5 healthy subjects. All patients were subjected to full laboratory investigations, including viral markers and alpha-fetoprotein (AFP), abdominal ultrasound, and clinical assessment with the Child–Pugh score calculation. Besides, the patients with HCC underwent triphasic computed tomography with contrast to diagnose and determine the tumor site, size, and number. Quantitative

patients and controls were randomly selected.

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real-time polymerase chain reaction was used to assess hsa-miR-519d-3p and *SQSTM1* in the serum of all the study participants.

RESULTS

Hsa-miR-519d-3p was significantly upregulated in patients with HCC compared with those with chronic liver disease and healthy subjects with an area under the curve (AUC) of 0.939, with cutoff value 8.34, sensitivity of 91.2%, and specificity of 81.8%. *SQSTM1* was upregulated with an AUC of 0.995, with cutoff value 7.89, sensitivity of 97.1%, and specificity of 100%. AFP significantly increased in patients with HCC with an AUC of 0.794, with cutoff value 7.30 ng/mL, sensitivity of 76.5%, and specificity of 72.7%.

CONCLUSION

This study is the first to show a direct relation between *SQSTM1* and hsa-miR-519d-3p; they are both upregulated in HCC. Thus, they could be used as surrogate diagnostic markers for stem cell autophagy disturbance in early-stage HCC.

Key Words: Autophagy; Hepatocellular carcinoma; miRNA; miR-519d; Stem cell; *SQSTM1*

Core Tip: Hepatocellular carcinoma (HCC) is the most common primary liver cancer. HCC is associated with poor prognosis due to difficult discovery at an early stage. The molecular pathophysiology behind HCC is not yet fully understood. Autophagy is one of the important affected pathways in HCC pathogenesis. In this study we used *in silico* analysis to determine a new molecular pathway and find the underlying background controlling genetic and epigenetic pathways. We found that autophagy-controlling gene *SQSTM1* is related to hsa-miR-519d-3p. Also, we found that their use as early detecting biomarkers for HCC diagnosis are more efficient than the currently used alpha-fetoprotein.

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INTRODUCTION

The scientists who discovered the mechanism of autophagy were awarded a Nobel Prize, and autophagy subsequently became a topic of great scientific interest for researchers. Autophagy is defined as cellular "self-eating," where lysosomal degradation of cellular elements occurs[1-3]. This process has three types: Chaperone-mediated autophagy, microautophagy, and macroautophagy. Autophagy is considered a "dynamic cellular recycling"[4] and provides cancerous cell preservation through the production of amino acids from degraded proteins[5]. The activation of autophagy increases resistance to cisplatin and sorafenib in patients with hepatocellular carcinoma (HCC); this could be reversed upon deactivation[6].

The discovery of "epigenetic–genetic" links is an important area of research. Studies on the regulation of targeted genes by microRNAs (miRNAs) must answer two questions: The mechanism of regulation and the effect of dysfunction on specific cancerous molecular pathways[7].

MiR-519d dysregulation is not only linked to the initiation and progression of many cancers as breast[8], skin[9], and gastrointestinal cancers[10,11] but also associated with obesity[12].

SQSTM1, also known as p62 protein, is a multifunctional protein responsible for various stress-induced cellular functions, including apoptosis and autophagy; its coding gene is the *SQSTM1* gene located on chromosome 5[13]. The impairment of

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autophagy causes the accumulation of p62 proteins in the hepatoma cells of mice[14]. Meanwhile, its upregulation significantly contributes to the resistance of hepatoma cells to sorafenib[15]. *SQSTM1* was initially believed to only control several cellular metabolic pathways, including the mechanistic target of rapamycin, nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), and mitogen-activated protein kinase signaling pathways, but later was also linked to the control of selective autophagy[16].

Here, in this study, we used *in silico* analysis to search for a new link among epigenetic–genetic biomarkers to identify and detect their relationship with early-stage HCC. We found significant *in silico* data relation between hsa-miR-519d-3p and *SQSTM1* and their link to HCC pathophysiology. We clinically validated the data by examining serum samples to assess their ability to be used in the diagnosis of HCC.

MATERIALS AND METHODS

This was a cross-sectional study conducted on randomly selected 50 Egyptian participants from outpatient clinics and inpatients attending the Gastroenterology and Hepatology Unit of the Internal Medicine Department at Ainshams University Hospitals, Faculty of Medicine, Ain Shams University, Cairo, Egypt.

The participants were divided into three groups

Group 1: Consisted of 34 patients with HCC that were diagnosed according to the American Association for the Study of Liver Diseases practice guidelines and staged according to the Barcelona Clinic Liver Cancer as stages A to D[17].

Group 2: Consisted of 11 patients with chronic liver disease.

Group 3: Consisted of 5 healthy subjects (control), who were enlisted during routine checkups and as volunteers.

Inclusion criteria for the study

Age more than 18 years.

The ability to provide informed consent.

Proven diagnosis of HCC according to the American Association for the Study of Liver Diseases practice guidelines for group 1[17].

Exclusion criteria for the study

Patients actively undergoing chemotherapy or radiation therapy for HCC.

Patients with other malignancies or treated within the last 5 years.

The Internal Medicine Department, Faculty of Medicine, Ain Shams University, approved this study's protocol in 2016 for ethics of conducting the study and in accordance with the ethical standards of the Declaration of Helsinki. Informed consent was obtained from each participant. Both the patients and controls were randomly selected. This study was not funded.

Data of samples

The following parameters were documented for the participants: Full personal history and thorough clinical examination.

Laboratory investigations included the following: (1) Liver function: Serum albumin, prothrombin time and international normalized ratio, and total and direct bilirubin; (2) Liver enzymes: Serum aspartate transaminase, alanine transaminase, alpha-fetoprotein (AFP), hepatitis C virus antibody, and hepatitis B virus surface antigen (HBsAg); and (3) Abdominal ultrasound: Tumor size, the number of nodules, local spread, lymph node metastasis, cirrhosis, and the presence of ascites.

Triphasic spiral contrast-enhanced computed tomography in the HCC group.

Biomarker identification and bioinformatics analysis

Bioinformatics analysis was performed to retrieve biomarkers relevant to HCC based on previous microarray studies. This step included the following.

Biomarker retrieval and verification: In this concern, we used the public databases, including miRDB, miRTargetLink Human, GeneCards, and Human Protein Atlas, to choose a set of miRNAs and its targeted messenger RNA (mRNA) that is related to HCC. According to the data retrieved, we chose the microRNA-519d, hsa-miR-519d-

3p, and the targeted mRNA, *SQSTM1*, as a point to be studied in relation to HCC. *In silico* analysis is shown in detail in Figure 1.

Sample collection: Blood was collected from all participants in a plain test tube. These blood samples were left at room temperature for a minimum of 30 min to allow complete blood clotting.

The clotted blood samples were centrifuged for 20 min.

The serum was carefully separated and transferred to 1.5 mL aliquots and stored at 80 °C until assayed.

An identifier was used to label each serum sample to protect the confidentiality of the participants.

Laboratory work

Extraction of total RNA: An miRNEasy RNA isolation kit (Qiagen, Hilden, Germany) was used to extract total RNA from the serum samples according to the manufacturer's instructions. The RNA concentration and integrity were assessed using an Ultraspec 1000 UV/visible spectrophotometer (Amersham Pharmacia Biotech, Cambridge, United Kingdom). Then, 72 µL diethyl pyrocarbonate–water was added to 3 µL RNA solution (dilution 1:25). The sample was read at 260 nm for RNA detection and 280 nm for protein detection using a spectrophotometer. Next, 40 µg RNA/mL is equivalent to 1 absorbance, so the concentration of RNA in a sample (µg/mL) = sample absorbance at 260 nm × 40/1 × dilution factor (25). The samples were considered to have high RNA quality if the RNA–protein ratio (260:280 ratio) was more than 1.8–2.

Reverse transcription-polymerase chain reaction: The extracted total RNA underwent reverse transcription into cDNA using a miScript II RT Kit (Qiagen) according to the manufacturer's protocol using a Hybaid thermal cycler (Thermo Electron, Waltham, MA, United States).

Quantitative reverse transcription-polymerase chain reaction: RNA levels were examined by quantitative reverse transcription-polymerase chain reaction (qRT-PCR) to ensure sensitive and specific RNA detection and quantification with high amplification efficacy. All PCR primers were obtained from Qiagen. All steps followed the manufacturer's suggested protocol.

Quantitative PCR for the detection of *SQSTM1* mRNA: The relative expression of *SQSTM1* mRNA was assessed using a QuantiTect SYBR Green PCR Kit (Qiagen) on a Rotor-Gene real-time PCR detection system (Qiagen) with specific primers provided by Qiagen. Beta-actin (ACTB) was used as a housekeeping gene.

The QuantiTect SYBR Green PCR Kit provides accurate real-time quantification of cDNA targets in an easy-to-handle format. The kit can be used in real-time two-step RT-PCR of RNA targets following reverse transcription with the fluorescent dye SYBR Green I in the master mix, which enables the analysis of many targets without having to synthesize target-specific labeled probes. It uses the SYBR Green I dye to detect PCR products by binding to double-stranded DNA formed during the PCR. It binds to each new copy of double-stranded DNA generated during each PCR cycle. The result is an increase in fluorescence intensity proportional to the number of double-stranded PCR products produced.

High specificity and sensitivity in PCR are achieved using the hot-start enzyme HotStarTaq DNA Polymerase together with a specialized PCR buffer. In addition, the buffer contains ROX dye, allowing fluorescence normalization on certain cyclers. The kit has been optimized for use with any real-time cycler, including Rotor-Gene® cyclers. A melting point analysis was performed to monitor the homogeneity and specificity of the quantitative PCR (qPCR) products.

qPCR for the detection of hsa-miR-519d-3p: A relative miRNA expression level for hsa-miR-519d-3p was analyzed by mixing the total cDNA with the reagent provided in the miScript SYBR Green PCR Kit (Qiagen) according to the manufacturer's suggested protocol, in addition to the manufacturer-provided miScript universal primer. RNU-6 was used as a housekeeping gene.

For detecting mature miRNA, cDNA prepared in a reverse transcription reaction using miScript HiSpec Buffer or miScript HiFlex Buffer serves as the template for real-time PCR analysis using a miRNA-specific miScript Primer Assay (forward primer) and the miScript SYBR Green PCR Kit, which contains the miScript Universal Primer (reverse primer) and QuantiTect SYBR Green PCR Master Mix.

Figure 1 Bioinformatic search and validation of the newly diagnostic biomarkers. A: miR-519d-3p and *SQSTM1* as a targeted mRNA according to miRDB (http://mirdb.org/cgi-bin/search.cgi?searchType=miRNA&full=mirbase&searchBox=MIMAT0002853); B: A network of 923 genes targeted by hsa-miR-519d-3p, along with focusing on *SQSTM1* in the network (miRTargetLink Human) (https://ccb-web.cs.uni-saarland.de/mirtargetlink/network.php?type=miRNA&qval=hsa-miR-519d-3p); C: The expression of miR-519d in liver tissue and other tissues (https://www.genecards.org/); D: The tissue expression of *SQSTM1* is low in hepatocytes of healthy liver tissue (www.proteinatlas.org); E: The expression of *SQSTM1* in cancers and liver cancer specifically (www.proteinatlas.org).

PCR result analysis: The PCR program for the SYBR Green-based qPCR was as follows: Denaturation at 95 °C for 15 min; 40 cycles of denaturation for 10 s at 94 °C; annealing for 30 s at 55 °C; and extension for 34 s at 70 °C. Each reaction was performed in duplicate. A Rotor-Gene manual was used to calculate the threshold cycle (Ct) value of each sample. Any Ct value greater than 36 was considered negative. We used the melting curve analysis software of Applied Biosystems to analyze our results. The melting curves were analyzed to affirm the specificities of the amplicons for the SYBR Green-based PCR amplification. The ($2^{-\Delta\Delta Ct}$) relative quantification RQ technique was used to measure the expression of the RNA-based biomarker panel.

The housekeeping genes, ACTB and RNU-6, were used as an invariant internal control to normalize the raw data of the samples and compare these results with a reference sample.

Statistical analysis

Statistical analyses of the obtained data were performed using SPSS, version 23 (IBM Corp., Armonk, NY, United States).

To describe the studied sample, quantitative data are presented as minimum, maximum, mean, and standard deviation for parametric data and median and interquartile range (IQR) for nonparametric data. Qualitative data are presented as

count and percentage.

Student's t-test was used to compare quantitative data between two independent groups, and the Mann–Whitney U-test was used for nonparametric data.

One-way analysis of variance was performed to compare quantitative data when more than two groups were to be compared; then, a post-hoc test was used to detect the difference between individual groups for parametric data, and the Kruskal–Wallis test was used for nonparametric data.

The chi-square test and Fisher's exact test were used to compare qualitative data between different groups.

The receiver operating characteristic (ROC) curve was used to measure diagnostic validity and determine the best cutoff value for some variables.

P values less than 0.05 denote statistical significance. In addition, concerning the level of significance: *P* values represent the level of significance, *P* values more than 0.05 are non-significant, *P* values less than 0.05 are significant, and *P* values less than 0.01 are highly significant.

RESULTS

We conducted this study on 50 Egyptian participants divided into three groups: 34 patients in the HCC group, 11 patients in the chronic liver infection group, and 5 healthy participants as the control group.

The age of all participants was more than 18 years with a mean of 58.88 ± 8.08 years, 56.18 ± 16.26 years, and 55.40 ± 22.09 years in the HCC, chronic liver infection, and control groups, respectively, with a non-statistically significant *P* value (0.72). In addition, a non-significant difference was observed between the malignant and non-malignant groups (*i.e.* patients in the chronic liver infection group added to the control group) with a *P* value of 0.53.

Gender differences were observed among the study groups—HCC group: Male = 67.6% and female = 32.4%; chronic liver infection group: Male = 81.8% and female = 18.2%); and healthy group: Male = 60% and female = 40%. The difference between the three study groups was statistically non-significant with a *P* value of 0.63 (Table 1). Liver function and laboratory data are shown in Table 2.

Hepatitis C antibody was prevalent in 88.2% of the patients with HCC, whereas all patients with chronic liver disease were positive, and no subjects in the control group were positive for hepatitis C virus antibody. HBsAg was prevalent in 5.9% of the patients with HCC, whereas none of the subjects in the chronic liver disease and control groups were positive for HBsAg. These data are shown in Table 3.

Our results concerning hsa-miR-519d-3p showed data from the qRT-PCR. These data were reported in delta–delta Ct [DDCT or -(ΔΔCT)] and RQ calculated as follows: $RQ = 2^{-ddCT} = 2^{-\Delta\Delta CT}$ (Table 4 and Figure 2A).

The results of serum miRNA (miR-519d) in the three study groups, reported in RQ, showed that in the HCC group, serum miRNA was 41.94 (IQR: 18.25–139.10); in the chronic liver infection group, serum miRNA was 5.98 (IQR: 3.14–8.28), and in the control group, serum miRNA was 1.17 (IQR: 1.16–1.21), with a highly significant *P* value (< 0.001) (Table 4). These data suggest that hsa-miR-519d-3p is significantly upregulated in the HCC group compared with the chronic liver infection and control groups. The ROC curve to assess the validity of the results of qRT-PCR of hsa-miR-519d in the serum in differentiating the HCC and chronic liver infection groups with the best cutoff value of ≥ 8.34, sensitivity of 91.2%, and specificity of 81.8% is shown in Figure 3A.

The second part of this study focused on the serum level of *SQSTM1* in HCC and whether it can be used as a significant biomarker. The data we obtained from qRT-PCR using the RQ of the serum *SQSTM1* gene in comparing the three study groups from Table 4 and Figure 2B showed that *SQSTM1* was 33.91 (IQR: 14.83–132.51) in the HCC group, 3.68 (IQR: 2.28–5.50) in the chronic liver infection group, and 0.84 (IQR: 0.76–0.99) in the control group with a highly significant *P* value (< 0.001). The ROC curve to assess the validity of the results of qRT-PCR of *SQSTM1* in the serum to differentiate the HCC and chronic liver infection groups with the best cutoff value of ≥ 7.89, sensitivity of 97.1%, and specificity of 100% is shown in Figure 3B.

When we divided the groups into the malignant and non-malignant groups, we found similar results (Figure 4).

The ROC curve to assess the validity of the RQ results of qRT-PCR of hsa-miR-519d in the serum among the malignant and non-malignant groups with the best cutoff value of ≥ 8.34, sensitivity of 91.2% and specificity of 87.5% is shown in Figure 4A**.** The

Table 1 The ages in the different groups of the study (mean ± SD)

Age	HCC (*n* = 34)	Chronic liver infection (*n* = 11)	Control (*n* = 5)	F[1]	*P* value
	58.88 ± 8.08	56.18 ± 16.26	55.40 ± 22.09	0.34	0.72 NS

[1]One-way analysis of variance. HCC: Hepatocellular carcinoma; NS: Non-significant; SD: Standard deviation.

Table 2 Significance of the differences in laboratory data between the three study groups (mean ± SD)

Variable	HCC (*n* = 34)	Chronic liver infection (*n* = 11)	Control (*n* = 5)	F[1]	*P* value
INR	1.37[a] ± 0.20	1.35[a] ± 0.30	1.07[b] ± 0.08	3.93	0.03 S
Serum albumin (g/dL)	2.94 ± 0.42	3.03 ± 0.73	3.40 ± 0.25	1.95	0.15 NS
AST[2] (IU/L)	50.00[a] ± 38.00–102.00	23.00[b] ± 15.00–39.00	15.00[b] ± 14.00–18.00	16.21	< 0.001 HS
ALT[2] (IU/L)	40.50[a] ± 28.00–73.30	22.00 ± 15.00–38.00	10.00[b] ± 8.00–15.00	12.69	0.002 HS
Total bilirubin[2] (mg/dL)	1.60[a] ± 1.10–2.20	1.10 ± 0.50–1.60	0.40[b] ± 0.30–0.50	14.91	0.001 HS
Direct bilirubin[2] (mg/dL)	0.70[a] ± 0.50–1.10	0.30[b] ± 0.10–0.60	0.10[b] ± 0.10–0.20	15.84	< 0.001 HS

[1]One-way analysis of variance (a, b Post-hoc test).
[2]Kruskal–Wallis test (median and interquartile range).
[a]$P < 0.05$.
[b]$P < 0.01$.
ALT: Alanine transaminase; AST: Aspartate transaminase; HCC: Hepatocellular carcinoma; HS: Highly significant; INR: International normalized ratio; NS: Non-significant; SD: Standard deviation.

Table 3 Hepatitis virus B and C infections in the three study groups

Variable		HCC (*n* = 34), (%)	Chronic liver infection (*n* = 11), (%)	Control (*n* = 5), (%)	(X^2)[1]	*P* value
HCVAb	Positive	30 (88.2)	11 (100.0)	0 (0.0)	18.32 FE	< 0.001 HS
	Negative	4 (11.8)	0 (0.0)	5 (100.0)		
HBsAg	Positive	2 (5.9)	0 (0.0)	0 (0.0)	0.78 FE	1.00 NS
	Negative	32 (94.1)	11 (100.0)	5 (100.0)		

[1]The chi-square test (FE: Fisher's exact test). HBsAg: Hepatitis B virus surface antigen; HCC: Hepatocellular carcinoma; HCVAb: Hepatitis C virus antibody; HS: Highly significant; NS: Non-significant.

ROC curve to assess the validity of the RQ results of qRT-PCR of *SQSTM1* in the serum among the malignant and non-malignant groups with the best cutoff value of ≥ 7.89, sensitivity of 97.1%, and specificity of 100% is shown in Figure 4B.

Furthermore, in this study, AFP was 62.60 (IQR: 8.20–600.80) in the HCC group, 3.50 (IQR: 2.50–20.00) in the chronic liver infection group, and 0.70 (IQR: 0.50–1.00) in the control group (Table 5). These results show that AFP is elevated with high statistical significance in patients with HCC as compared to other groups, with a *P* value of < 0.001. The constructed ROC curve to compare AFP results between the HCC and chronic liver infection groups showed an area under the curve (AUC) of 0.794, with the best cutoff value of > 7.30 ng/mL, sensitivity of 76.5%, and specificity of 72.7% (Figure 5A). Meanwhile, the ROC curve assessing the validity of AFP for differentiating between the malignant and non-malignant groups showed an AUC of 0.854, with the best cutoff value of > 7.30, sensitivity of 76.5%, and specificity of 81.2% (Figure 5B).

Most patients had early-stage HCC, except for three patients. The full details of the radiological findings are presented in Table 6.

Table 4 Expression level of hsa-miR-519d-3p, *ACTB*, *RNU6*, and *SQSTM1* between the three study groups (mean ± SD)

Variable	HCC (*n* = 34)	Chronic liver infection (*n* = 11)	Control (*n* = 5)	F[1]	*P* value
Ct (ACTB)	30.65[a] ± 4.21	25.82[b] ± 2.31	27.34[b] ± 2.00	7.69	0.001 HS
Ct (RNU6)	36.03 ± 2.92	36.36 ± 2.82	38.55 ± 0.96	1.78	0.18 NS
Ct (miR-519d)	30.08[a] ± 3.00	33.61[b] ± 2.78	38.05[c] ± 1.08	20.48	< 0.001 HS
mRNA-*SQSTM1*	36.14 ± 3.17	34.89 ± 2.30	38.38 ± 1.86	2.48	0.10 NS
DCT (miR-519d)	−5.95[a] ± 1.98	−2.75[b] ± 0.89	−0.50[c] ± 0.40	31.17	< 0.001 HS
DDCT (miR-519d)	−5.59[a] ± 1.98	−2.39[b] ± 0.89	−0.14[c] ± 0.40	31.17	< 0.001 HS
RQ (miR-519d)[2]	41.94[a] ± 18.25–139.10	5.98[b] ± 3.14–8.28	1.17[c] ± 1.16–1.21	28.46	< 0.001 HS
DCT (*SQSTM1*)	5.49[a] ± 1.83	9.07[b] ± 0.70	11.04[c] ± 0.58	41.08	< 0.001 HS
DDCT (*SQSTM1*)	−5.51[a] ± 1.83	−1.93[b] ± 0.70	0.04[c] ± 0.58	41.08	< 0.001 HS
RQ (*SQSTM1*)[2]	33.91[a] ± 14.83–132.51	3.68[b] ± 2.28–5.50	0.84[c] ± 0.76–0.99	32.54	< 0.001 HS

[1]One-way analysis of variance (a, b post-hoc test).
[2]Kruskal–Wallis test (median and interquartile range).
[a]$P < 0.05$.
[b]$P < 0.01$.
[c]$P < 0.001$.
Ct: Threshold cycle; HS: Highly significant; NS: Non-significant.

Table 5 Alpha-fetoprotein laboratory results in the three subgroups

Variable	HCC (*n* = 34), median (IQR)	Chronic liver disease (*n* = 11), median (IQR)	Control (*n* = 5), median (IQR)		*P* value
AFP[1] (ng/mL) by ELISA	62.60[a] (8.20–600.80)	3.50[b] (2.50–20.00)	0.70[c] (0.50–1.00)	19.17	< 0.001 HS

[1]Kruskal–Wallis test (median and interquartile range).
[a]$P < 0.05$.
[b]$P < 0.01$.
[c]$P < 0.001$.
AFP: Alpha-fetoprotein; ELISA: Enzyme-linked immunosorbent assay; HCC: Hepatocellular carcinoma; HS: Highly significant; IQR: Interquartile range.

DISCUSSION

Our results suggest that hsa-miR-519d-3p is upregulated in the serum of the HCC group compared with the chronic liver disease and healthy control groups, with high sensitivity and specificity as a diagnostic marker. Similar results were observed by Fornari *et al*[18], where the miRNA was upregulated and considered an HCC oncogene. The study linked our target miRNA to DNA hypomethylation and p53, both of which are responsible for cell death and apoptosis. However, a recent study by Zhang *et al*[19] has linked miR-519d to the adenosine monophosphate-activated protein kinase pathway in HCC cells, regulating cellular energy metabolism by controlling the Ras-related protein (Rab10)[19]. A recent study has raised the hope of inducing autophagy in hepatoma cells by the administration of metformin through the activation of the mechanistic target of rapamycin pathway[20]. Alternatively, patients with colorectal cancer had improved survival and lower metastasis with upregulated miR-519d-3p by regulating trophinin-associated protein[11].

This study on serum mRNA of *SQSTM1* revealed significant upregulation of its serum level in the HCC group as compared to the levels in the chronic liver disease and healthy control groups. Thus, our results mean that mRNA of *SQSTM1* is upregulated in the serum of patients with HCC. This is compared to the findings of Xiang *et al*[21] who have found higher expression levels of the encoded protein p62 in hepatoma cells of patients with hepatitis B infection or those exposed to aflatoxin B1 [21], whereas our study population was mostly infected with hepatitis C virus.

Table 6 Clinical and radiological characteristics of hepatocellular carcinoma lesions.

Variable		mean ± SD
Child–Pugh score		6.76 ± 1.44
		n **(%)**
Cirrhosis	Cirrhosis	27 (79.4)
	No cirrhosis	7 (20.6)
BCLC stage	Stage A (early)	31 (91.2)
	Stage D (late)	3 (8.8)
Child–Pugh classification	A	17 (50.0)
	B	14 (41.2)
	C	3 (8.8)
Average tumor size	> 3 cm	3 (8.8)
	< 3 cm	31 (91.2)

BCLC: Barcelona Clinic Liver Cancer; SD: Standard deviation.

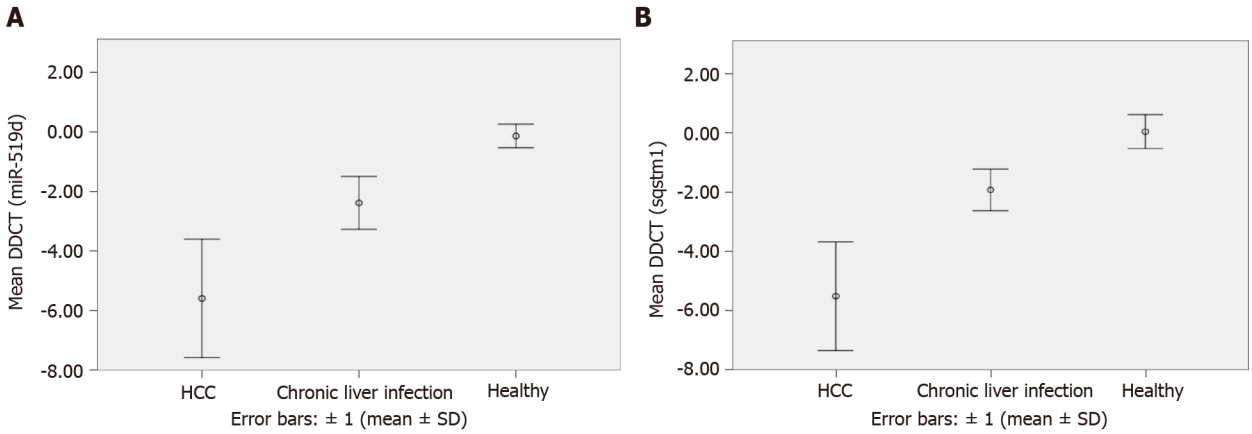

Figure 2 Box-plot figures showing the mean delta–delta threshold cycle in the new diagnostic biomarkers in different groups. A: Illustration of the mean delta–delta threshold cycle (DDCT) of the quantitative real-time polymerase chain reaction (qRT-PCR) results for hsa-miR-519d in the serum of the hepatocellular carcinoma (HCC), chronic liver infection, and control groups using error bars: ± 1 [mean ± standard deviation (SD)]; B: Illustration of the mean DDCT of the qRT-PCR results for mRNA of *SQSTM1* in the serum of the HCC, chronic liver infection, and control groups using error bars: ± 1 (mean ± SD). DDCT: Delta–delta threshold cycle; HCC: Hepatocellular carcinoma.

The *SQSTM1* gene is responsible for coding p62. This protein plays an important role as a receptor in selective autophagy, where specific cell organelles or proteins are degraded selectively by autophagosomes[22,23]. This ubiquitin-binding receptor protein is upregulated in early-stage HCC, as it is responsible for the maintenance of cancerous cells and their survival during stress[24].

In addition, our results show that hsa-miR-519d-3p is upregulated, synchronously with the upregulation of the mRNA of *SQSTM1*; this made us deduce that miRNA 519d stimulates the *SQSTM1* gene and increases the expression of its transcribed mRNA, not just increasing its translated protein level (p62) as previous studies have detected. In this study, we could not define the mechanism by which miR-519d-3p upregulates *SQSTM1*, but we have identified that the gene is upregulated at the transcriptional level, ***not*** at the post-transcriptional level. Besides, we can conclude that miR-519d-3p can affect autophagy and induce the progression of HCC through the targeted upregulation of *SQSTM1*.

In addition to these results, the sensitivity and specificity of hsa-miR-519d-3p, the mRNA of *SQSTM1*, and AFP were 91.2%–81.8%, 97.1%–100%, and 76.5%–72.7%, respectively. Also, the best cutoff values of the three parameters were ≥ 8.34 for miR-519d, ≥ 7.89 for the mRNA of *SQSTM1*, and ≥ 7.30 for AFP. Our results showed that miR-519d and the mRNA of *SQSTM1* showed higher sensitivity and specificity than

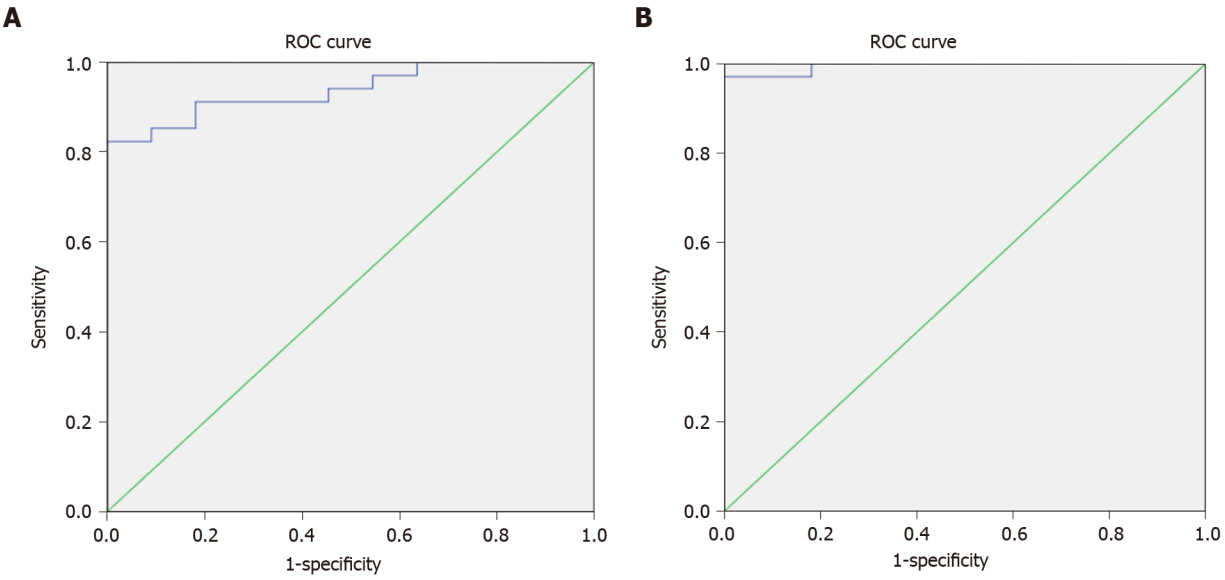

Figure 3 Receiver operating characteristic curves of the new diagnostic biomarkers studied to differentiate between hepatocellular carcinoma and chronic liver infection groups. A: Receiver operating characteristic (ROC) curve for assessing the validity of the RQ results of quantitative real-time polymerase chain reaction (qRT-PCR) for hsa-miR-519d in the serum to differentiate the hepatocellular carcinoma and chronic liver infection groups; B: ROC curve assessing the validity of the RQ results of qRT-PCR for mRNA of *SQSTM1* in the serum between hepatocellular carcinoma and chronic liver infection groups. ROC: Receiver operating characteristic.

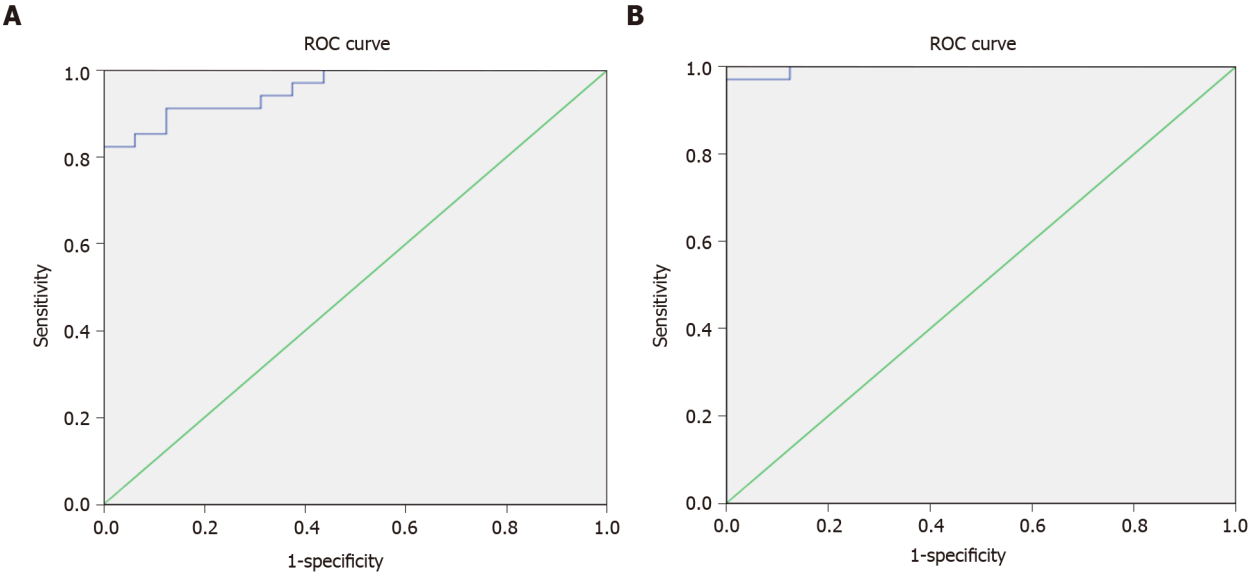

Figure 4 Receiver operating characteristic curves of the new diagnostic biomarkers studied to differentiate between the malignant and non-malignant groups. A: Receiver operating characteristic (ROC) curve assessing the validity of the RQ results of quantitative real-time polymerase chain reaction (qRT-PCR) for hsa-miR-519d in the serum among the malignant and non-malignant groups; B: ROC curve assessing the validity of the RQ results of qRT-PCR for mRNA of *SQSTM1* in the serum among the malignant and non-malignant groups. ROC: Receiver operating characteristic.

AFP, with better detection of early-stage HCC cases; thus can be used as diagnostic biomarkers for early HCC, improving the HCC outcome and prognosis. Moreover, when we compared the HCC group with the chronic liver disease group only or with the combined group of both patients with chronic liver disease and healthy subjects (malignant and non-malignant groups), we found similar results in both categories regarding hsa-miR-519d-3p, the mRNA of *SQSTM1*, and AFP.

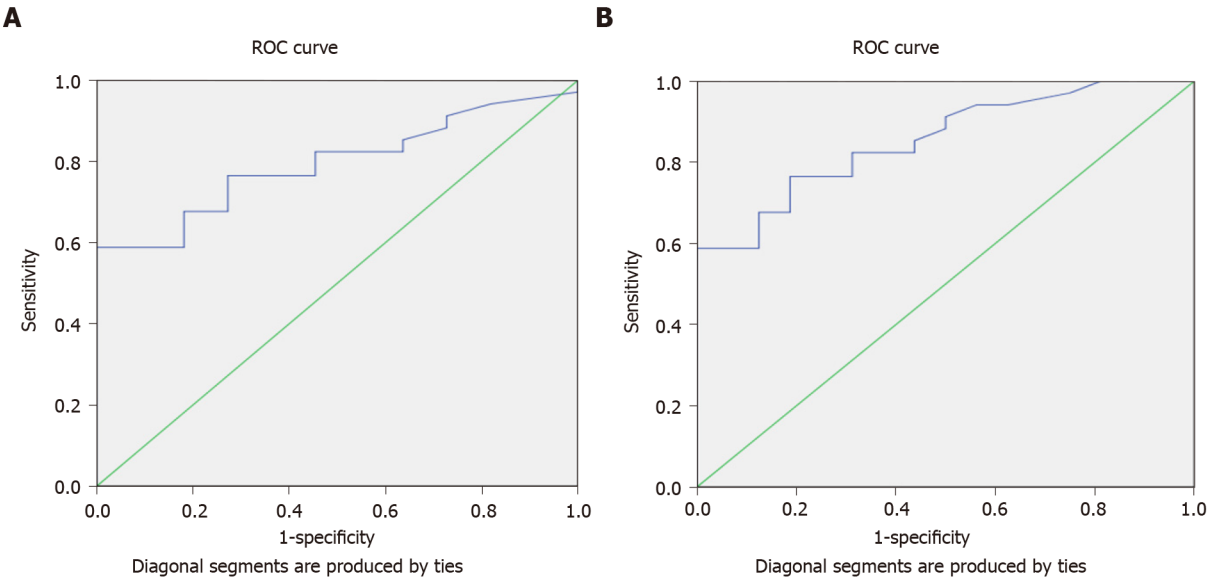

Figure 5 Receiver operating characteristic curves of the alpha-fetoprotein studied to differentiate between the hepatocellular carcinoma and chronic liver disease groups/malignant and non-malignant groups. A: Receiver operating characteristic (ROC) curve to assess the validity of alpha-fetoprotein (AFP) for the differentiation between the hepatocellular carcinoma and chronic liver disease groups; B: ROC curve assessing the validity of AFP for differentiating between the malignant and non-malignant groups. ROC: Receiver operating characteristic.

CONCLUSION

We are the first to establish a link between hsa-miR-519d-3p and the mRNA of *SQSTM1* in HCC. Hsa-miR-519d-3p and the mRNA of *SQSTM1* could be used in the diagnosis of HCC in its early stages. Further studies are needed to detect levels of miR-519d-3p and the mRNA of *SQSTM1* before and after various modalities of treatment to assess their ability to monitor treatment responses and detect recurrences. Multicentric studies with more variability to validate the use of miR-519d-3P and the mRNA of *SQSTM1* as diagnostic biomarkers of HCC on a wide scale are needed.

ARTICLE HIGHLIGHTS

Research background

Autophagy is one of the pathways affected in hepatocellular carcinoma (HCC). Genetic regulation of this pathway through the *SQSTM1* gene was established. Autophagy is responsible for the destruction of cellular components through lysosomal degradation. This process is responsible for cellular recycling and preservation. It protects from cancerous transformation, thus any imbalance in this mechanism will increase the risk of cancer.

Research motivation

We aimed to establish the genetic-epigenetic-phenotypic pathway related to the autophagic process in the pathogenesis of HCC and whether these studied biomarkers could be used as surrogate diagnostic markers for autophagy pathway in HCC.

Research objectives

We examined hsa-miR-519d microRNA effect on HCC and its association with the *SQSTM1* genetic marker. We also examined the sensitivity and specificity of those biomarkers in the diagnosis of early-stage HCC cases.

Research methods

This is an observational study. We evaluated the candidate biomarkers through bioinformatics, and after establishing a computational statistical relation, we proceeded with their clinical association through laboratory validation. We measured the genetic and epigenetic biomarkers in the serum samples taken from HCC patients, chronic liver disease patients, and healthy participants. We used reverse transcription-

polymerase chain reaction and quantitative reverse transcription-polymerase chain reaction.

Research results

We determined the sensitivity and specificity of each biomarker separately and combined as compared to the established alpha-fetoprotein (AFP) biomarker. We found that all the studied biomarkers in our study have better sensitivity and specificity than AFP, when used separately or combined, at the diagnosis of early-stage HCC.

Research conclusions

We could use the autophagy pathway biomarkers in the early-stage HCC diagnosis.

Research perspectives

More autophagy biomarkers could be examined using first *in silico* analysis then clinical laboratory confirmation. Combining computational and clinical validations in clinical studies could benefit the research process immensely.

REFERENCES

1 **Levine B**, Kroemer G. Biological Functions of Autophagy Genes: A Disease Perspective. *Cell* 2019; **176**: 11-42 [PMID: 30633901 DOI: 10.1016/j.cell.2018.09.048]

2 **Dikic I**, Elazar Z. Mechanism and medical implications of mammalian autophagy. *Nat Rev Mol Cell Biol* 2018; **19**: 349-364 [PMID: 29618831 DOI: 10.1038/s41580-018-0003-4]

3 **Chen X**, He Y, Lu F. Autophagy in Stem Cell Biology: A Perspective on Stem Cell Self-Renewal and Differentiation. *Stem Cells Int* 2018; **2018**: 9131397 [PMID: 29765428 DOI: 10.1155/2018/9131397]

4 **Mizushima N**, Komatsu M. Autophagy: renovation of cells and tissues. *Cell* 2011; **147**: 728-741 [PMID: 22078875 DOI: 10.1016/j.cell.2011.10.026]

5 **Vabulas RM**, Hartl FU. Protein synthesis upon acute nutrient restriction relies on proteasome function. *Science* 2005; **310**: 1960-1963 [PMID: 16373576 DOI: 10.1126/science.1121925]

6 **Schulte LA**, López-Gil JC, Sainz B Jr, Hermann PC. The Cancer Stem Cell in Hepatocellular Carcinoma. *Cancers (Basel)* 2020; **12** [PMID: 32183251 DOI: 10.3390/cancers12030684]

7 **Xu P**, Wu Q, Yu J, Rao Y, Kou Z, Fang G, Shi X, Liu W, Han H. A Systematic Way to Infer the Regulation Relations of miRNAs on Target Genes and Critical miRNAs in Cancers. *Front Genet* 2020; **11**: 278 [PMID: 32296462 DOI: 10.3389/fgene.2020.00278]

8 **Deng X**, Zhao Y, Wang B. miR-519d-mediated downregulation of STAT3 suppresses breast cancer progression. *Oncol Rep* 2015; **34**: 2188-2194 [PMID: 26238950 DOI: 10.3892/or.2015.4160]

9 **Hua KT**, Hong JB, Sheen YS, Huang HY, Huang YL, Chen JS, Liao YH. miR-519d Promotes Melanoma Progression by Downregulating EphA4. *Cancer Res* 2018; **78**: 216-229 [PMID: 29093007 DOI: 10.1158/0008-5472.CAN-17-1933]

10 **Jin Y**, Li Y, Wang X, Yang Y. Dysregulation of MiR-519d Affects Oral Squamous Cell Carcinoma Invasion and Metastasis by Targeting MMP3. *J Cancer* 2019; **10**: 2720-2734 [PMID: 31258780 DOI: 10.7150/jca.31825]

11 **Ye X**, Lv H. MicroRNA-519d-3p inhibits cell proliferation and migration by targeting TROAP in colorectal cancer. *Biomed Pharmacother* 2018; **105**: 879-886 [PMID: 30021381 DOI: 10.1016/j.biopha.2018.04.114]

12 **Martinelli R**, Nardelli C, Pilone V, Buonomo T, Liguori R, Castanò I, Buono P, Masone S, Persico G, Forestieri P, Pastore L, Sacchetti L. miR-519d overexpression is associated with human obesity. *Obesity (Silver Spring)* 2010; **18**: 2170-2176 [PMID: 20057369 DOI: 10.1038/oby.2009.474]

13 **Sánchez-Martín P**, Komatsu M. p62/SQSTM1 - steering the cell through health and disease. *J Cell Sci* 2018; **131** [PMID: 30397181 DOI: 10.1242/jcs.222836]

14 **Inami Y**, Waguri S, Sakamoto A, Kouno T, Nakada K, Hino O, Watanabe S, Ando J, Iwadate M, Yamamoto M, Lee MS, Tanaka K, Komatsu M. Persistent activation of Nrf2 through p62 in hepatocellular carcinoma cells. *J Cell Biol* 2011; **193**: 275-284 [PMID: 21482715 DOI: 10.1083/jcb.201102031]

15 **Pan J**, Lu C, Jun W, Wu Y, Shi X, Ding Y. The up-regulation of P62 Levels is associated with resistance of sorafenib in hepatocarcinoma cells. *Int J Clin Exp Pathol* 2019; **12**: 2622-2630 [PMID: 31934090]

16 **Puissant A**, Fenouille N, Auberger P. When autophagy meets cancer through p62/SQSTM1. *Am J Cancer Res* 2012; **2**: 397-413 [PMID: 22860231]

17 **Marrero JA**, Kulik LM, Sirlin CB, Zhu AX, Finn RS, Abecassis MM, Roberts LR, Heimbach JK. Diagnosis, Staging, and Management of Hepatocellular Carcinoma: 2018 Practice Guidance by the American Association for the Study of Liver Diseases. *Hepatology* 2018; **68**: 723-750 [PMID: 29624699 DOI: 10.1002/hep.29913]

18 **Fornari F**, Milazzo M, Chieco P, Negrini M, Marasco E, Capranico G, Mantovani V, Marinello J, Sabbioni S, Callegari E, Cescon M, Ravaioli M, Croce CM, Bolondi L, Gramantieri L. In

hepatocellular carcinoma miR-519d is up-regulated by p53 and DNA hypomethylation and targets CDKN1A/p21, PTEN, AKT3 and TIMP2. *J Pathol* 2012; **227**: 275-285 [PMID: 22262409 DOI: 10.1002/path.3995]

19 **Zhang YJ**, Pan Q, Yu Y, Zhong XP. microRNA-519d Induces Autophagy and Apoptosis of Human Hepatocellular Carcinoma Cells Through Activation of the AMPK Signaling Pathway *via* Rab10. *Cancer Manag Res* 2020; **12**: 2589-2602 [PMID: 32346312 DOI: 10.2147/CMAR.S207548]

20 **Gao C**, Fang L, Zhang H, Zhang WS, Li XO, Du SY. Metformin Induces Autophagy *via* the AMPK-mTOR Signaling Pathway in Human Hepatocellular Carcinoma Cells. *Cancer Manag Res* 2020; **12**: 5803-5811 [PMID: 32765083 DOI: 10.2147/CMAR.S257966]

21 **Xiang X**, Qin HG, You XM, Wang YY, Qi LN, Ma L, Xiang BD, Zhong JH, Li LQ. Expression of P62 in hepatocellular carcinoma involving hepatitis B virus infection and aflatoxin B1 exposure. *Cancer Med* 2017; **6**: 2357-2369 [PMID: 28941211 DOI: 10.1002/cam4.1176]

22 **Emanuele S**, Lauricella M, D'Anneo A, Carlisi D, De Blasio A, Di Liberto D, Giuliano M. p62: Friend or Foe? *Int J Mol Sci* 2020; **21** [PMID: 32708719 DOI: 10.3390/ijms21145029]

23 **Lamark T**, Svenning S, Johansen T. Regulation of selective autophagy: the p62/SQSTM1 paradigm. *Essays Biochem* 2017; **61**: 609-624 [PMID: 29233872 DOI: 10.1042/EBC20170035]

24 **Umemura A**, He F, Taniguchi K, Nakagawa H, Yamachika S, Font-Burgada J, Zhong Z, Subramaniam S, Raghunandan S, Duran A, Linares JF, Reina-Campos M, Umemura S, Valasek MA, Seki E, Yamaguchi K, Koike K, Itoh Y, Diaz-Meco MT, Moscat J, Karin M. p62, Upregulated during Preneoplasia, Induces Hepatocellular Carcinogenesis by Maintaining Survival of Stressed HCC-Initiating Cells. *Cancer Cell* 2016; **29**: 935-948 [PMID: 27211490 DOI: 10.1016/j.ccell.2016.04.006]

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ORIGINAL ARTICLE

Observational Study

Determination of "indeterminate score" measurements in lean nonalcoholic fatty liver disease patients from western Saudi Arabia

Yasir Mohammed Khayyat

ORCID number: Yasir Mohammed Khayyat 0000-0002-8344-2028.

Author contributions: Khayyat YM conceived of and designed the study, collected the data, and wrote the article, providing final approval of the manuscript to be published.

Institutional review board statement: The Institutional Review Board of International Medical Centre, Jeddah, Saudi Arabia provided approval for this study (IRB No. 2019-11-215).

Informed consent statement: The requirement for consent was waived considering that there was no more than minimal risk to the subjects related to performance of FibroScan and blood tests measurements. The waiver was ensured to not adversely affect the rights and welfare of the subjects, in which tests performed were already completed, regardless of the research.

Conflict-of-interest statement: The author declares having no conflicts of interest related to this study and its publication.

Data sharing statement: The data that support the findings of this study are available from the corresponding author upon

Yasir Mohammed Khayyat, Department of Medicine, Umm Al Qura University, Makkah 13578, Saudi Arabia

Yasir Mohammed Khayyat, Department of Medicine, International Medical Centre, Jeddah 21451, Saudi Arabia

Corresponding author: Yasir Mohammed Khayyat, FACP, FRCP (C), MBChB, Associate Professor, Department of Medicine, Umm Al Qura University, Al Abdiyah District, Makkah 13578, Saudi Arabia. ymkhayyat@uqu.edu.sa

Abstract

BACKGROUND

Noninvasive measures to estimate liver fibrosis in lieu of biopsy in nonalcoholic liver disease (NAFLD) can broadly differentiate high *vs* low degrees of condition extent. However, an "indeterminate score" necessitates further clinical investigation and biopsy becomes essential, highlighting the need for identification of other noninvasive factors with accuracy for this midlevel extent and its prognosis. Lean NAFLD cases are of particular interest regarding this issue, as they present as otherwise healthy, and will benefit greatly from the less invasive assessment.

AIM

To estimate the agreement of two noninvasive assessment tools in lean NAFLD patients, and assess factors related to indeterminate scores.

METHODS

Ultrasound-diagnosed NAFLD patients, without sign of other chronic liver disease (n = 1262), were enrolled from a tertiary private medical centre between 2016-2019. After grouping by body mass index (obese, overweight, and lean), each participant underwent FibroScan. NAFLD fibrosis score (NFS) was used for subclassification (lower, higher, and indeterminate). No patient underwent liver biopsy. The kappa statistic was used to assess inter-rater agreement between the three groups on liver fibrosis degree assessed *via* FibroScan and NFS. Indeterminate score among the three groups was assessed to identify factors that predict its determination.

RESULTS

The NAFLD study cohort was composed of lean (159/1262, 12.6%), overweight (365/1262, 29%) and obese (737/1262, 58.4%) individuals. The lean patients were

reasonable request.

Country/Territory of origin: Saudi Arabia

Specialty type: Gastroenterology and hepatology

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification
Grade A (Excellent): 0
Grade B (Very good): 0
Grade C (Good): C
Grade D (Fair): 0
Grade E (Poor): 0

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significantly younger (49.95 ± 15.3 years, $P < 0.05$), with higher serum high density lipoprotein (52.56 ± 16.27 mg/dL, $P < 0.001$) and lower prevalences of type 2 diabetes mellitus, hypertension and hyperlipidaemia. All groups showed a predominance of lower fibrosis degree. The lean NAFLD patients showed a significantly lower NFS ($P < 0.001$). Degree of agreement between FibroScan and NFS was fair between the lean and obese NAFLD categories, and moderate in the overweight category. NFS was predictive of indeterminate score. Age was a factor among all the body mass index (BMI) categories; other associated factors, but with less strength, were serum alanine aminotransferase in the overweight category and BMI in the obese category.

CONCLUSION
Lean NAFLD patients showed lower degree and prevalence of liver fibrosis by NFS; however, follow-up biopsy is still needed.

Key Words: Nonalcoholic fatty liver disease; Liver fibrosis; Liver biopsy; Obesity; Overweight; Lean

Core Tip: Nonalcoholic fatty liver disease (NAFLD) has emerged as a leading cause of chronic liver disease and its complications. Evaluation of fibrosis in NAFLD is of the utmost importance to early application of targeted intervention. The utilization of liver biopsy has diminished, due to patient unacceptance, sampling error, and availability of noninvasive measures of fibrosis. In this study of NAFLD cases, lean patients showed a relatively healthy metabolic profile, lower fibrosis degree and less frequent "indeterminate score" than overweight and obese patients, among which increased age and serum alanine aminotransferase level were predictive factors for determination.

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INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is a growing cause of liver-related mortality which, in recent decades, has surpassed other known causes of chronic liver diseases. It is now considered in the differential diagnoses of both overweight and lean individuals, in association with a well-established panel of metabolic abnormalities. Traditionally, the NAFLD diagnosis has been made by transabdominal ultrasound and its extent determined by the invasive assessment method of percutaneous liver biopsy. This method, despite its accuracy in staging of fibrosis, is still limited by sampling error and a hazardous risk profile of procedure-related complications, regardless of whether the approach is targeted or non-targeted[1].

Visceral obesity was long considered the sole reason for suspicion of underlying NAFLD; however, it is now recognized that lean individuals develop NAFLD. Several inflammatory cytokines have been linked to the potent effect of visceral obesity and its effects on liver fibrosis, such as the NACHT, LPR and PYD-domain containing proteins (NALPs)[2] and on hypoadiponectemia (as well as its role in liver fibrosis)[3]. The reported incidence of NAFLD among the general population is 12.1%, and within that population, lean individuals account for 40.8% and their cases do not represent healthy or benign forms of the condition[4,5]. The lean NAFLD cases add a remarkable burden to the overall landscape of NAFLD. As such, the increased clinical awareness and research focus has led to generation of novel noninvasive tests based upon mathematical modelling, serum biomarkers and liver stiffness transient elastography, providing safe alternative assessment tools by which to evaluate liver fibrosis in lieu of biopsy[6]. Such tests can be applied by specialists and non-specialists alike, partic-

ularly for the primary staging of NAFLD[7]. They have been demonstrated to have good performance, with high negative predictive values compared to liver biopsy. They are also particularly informative for NAFLD patients with high risk of advanced fibrosis, through repeated assessment by transient elastography that provides good accuracy of prediction of liver and non-liver related mortality[8].

These less invasive methods of assessment, however, are limited by uncertainty regarding the evaluation of a category of cases that falls between the low and high grades of fibrosis; such cases are scored as "indeterminate" and that label prompts further evaluation by liver biopsy (simultaneously highlighting the limited utility of the noninvasive methods early in the disease process)[9]. Complicating this situation is the fact that the increasing emergence of lean NAFLD cases has in turn increased the demand for noninvasive testing. The study described herein was, thus, designed to first determine the prevalence of indeterminate scored cases among a representative group of lean NAFLD patients, then to comparatively assess findings from bedside transient elastography or FibroScan, and ultimately to identify factors that may predispose lean NAFLD patients to obtaining an indeterminate score by noninvasive liver fibrosis tools.

MATERIALS AND METHODS

Subjects

This study was conducted at a tertiary hospital, between 2016 and 2019. Patients at least 15 years of age who received diagnosis of NAFLD (based on findings from imaging studies in accordance with ultrasonography criteria of fatty liver[10]) and those presenting components of metabolic syndrome (*i.e.* type 2 diabetes mellitus, hypertension, hyperlipidaemia, central obesity) were recruited. Patients were denied study enrolment if they were under 15-years-old, showed evidence of concurrent active medical disease that is known to impair liver function or of other secondary causes of chronic liver disease, had incomplete data, died during the study recruitment period, or refused participation in the study. Patient data collected upon enrolment included general medical history, liver disease-related history [covering other causes of chronic liver disease, such as risk factors for acquiring viral hepatitis (hepatitis B and hepatitis C virus)], medications (including over-the-counter and herbal remedies), active alcohol use or abuse, and recreational drug use. All enrolled patients were directly assessed for other causes of chronic liver disease, including hemochromatosis, Wilson's disease, and alpha 1 antitrypsin clinical manifestations, as well as autoimmune liver diseases, including autoimmune hepatitis, primary biliary cirrhosis, primary sclerosing cholangitis, and hepatic vascular disease. All enrolled patients underwent complete physical examination, yielding anthropometric data on height and weight [by standard measurement protocols, used to assess body mass index (BMI)] as well as data on stigmata of chronic liver disease.

FibroScan and NAFLD fibrosis score

Each enrolled patient was fasted for 3 h and then subjected to FibroScan assessment using FibroScan 502 Touch instrument (Echosens©, Paris, France). A medium probe was applied when the skin capsule distance was ≤ 2.5 cm and an XL probe for ≥ 2.5 cm. For each patient, a median score was calculated from the values obtained from 10 successful scans performed at a single localized area.

For each enrolled patient, NAFLD fibrosis score (NFS)[11] was calculated by the following formula: -1.675 + 0.037 × age (in years) + 0.094 × BMI (as kg/m^2) + 1.13 × IFG/diabetes (with yes = 1, no = 0) + 0.99 × aspartate aminotransferase/alanine aminotransferase ratio - 0.013 × platelet count (as × 10^9/L) - 0.66 × albumin (as g/dL).

BMI categorization

After exclusion of other causes of chronic liver disease, the enrolled patients were divided into the following three groups according to their BMI: obese (BMI ≥ 30); overweight (BMI: 25-30); and lean (BMI ≤ 25). The noninvasive parameters of liver fibrosis were used to classify the BMI cohorts into low and high degree of liver fibrosis categories[12-14], with the former assigned to patients with FibroScan values < 7.9 kPa and NFS < –1.455 and the latter assigned to patients with FibroScan values > 9.5 kPa and NFS > 0.675; "indeterminate" was assigned for liver fibrosis when the measurement values fell between the low and high categorizations.

Laboratory parameters

All enrolled patients received testing for liver chemistry panel (after 4-6 h of fasting), serum glycosylated haemoglobin, and serum fasting lipid profile. Adherence to diabetic, hypertension and lipid lowering medications were verified through interviews with the patient interviews and/or immediate family relatives, as well as hospital dispensing records.

Statistical analysis

All statistical analyses were performed with SPSS software (version 26.0; IBM Corp., Armonk, NY, United States). Descriptive statistics and frequencies were calculated. Group differences were examined using one-way analysis of variance or its nonparametric equivalent, the Kruskal-Wallis test. In terms of post-hoc tests, Bonferroni correction was applied. Relationships between categorical variables were analysed with the chi-square test of independence. The kappa statistic was used to assess inter-rater agreement between the three groups on liver fibrosis degree assessed *via* FibroScan and NFS. Lastly, prediction of indeterminate NFS was determined by binary logistic regression modelling, with a P-value of < 0.005 indicating statistical significance. The statistical methods used and data interpretation were verified by an external biostatistician.

Ethical statements

The study was conducted in accordance with the Declaration of Helsinki, and all procedures were approved by the Ethics Committee of International Medical Centre (Approval No. 2019-11-115).

RESULTS

Study groups and categories

A total of 1753 patients were recruited during the study period, with 1262 meeting the criteria for enrolment and inclusion in the final analysis. A total of 491 patients had been excluded for the following reasons: incomplete data (n = 103); chronic hepatitis B (n = 185); chronic hepatitis C (n = 71); underwent weight management surgery (n = 66); active neoplastic disorders (n = 11); coexisting medical conditions known to cause liver function test alterations (n = 33); use of hepatotoxic medications(n = 8); and death during the study recruitment period (n = 13).

The entire study cohort was comprised of 159 lean NAFLD patients (12.6%), 365 overweight NAFLD patients (29.0%), and 737 obese NAFLD patients (58.4%). Tables 1 and 2 summarize the metabolic parameters and diseases among the three groups. The lean NAFLD group was of significantly younger age than the overweight and obese groups (P = 0.012).

Metabolic diseases

As shown in Table 1, the lean NAFLD group showed lower serum glycated haemoglobin (*i.e.* HbA1c) and higher serum high density lipoprotein (*i.e.* HDL) than either the overweight or obese NAFLD groups. The prevalence of various metabolic diseases differed significantly between the three BMI groups. Hyperlipidaemia was more prevalent in the overweight group (n = 205) and the obese group (n = 457) than in the lean group (n = 76, $P < 0.001$). Hypertension was also more prevalent in the overweight group (n = 144) and the obese group (n = 333) than in the lean group (n = 50, P = 0.002). Type 2 diabetes mellitus was more prevalent and to a much greater extent in the obese group (n = 405) compared to the overweight group (n = 171, $P < 0.001$) and lean group (n = 50, $P < 0.001$).

Noninvasive assessments

Transient elastography by FibroScan showed the three BMI groups to have a predominance of lower fibrosis measurements (F0-F2, *vs* higher fibrosis measurements of F3-F4) (Figure 1). In contrast, the NFS showed a significant difference between the three groups, with the lean group showing lower scores for patients in both the lower and higher fibrosis categories compared to that seen in the overweight group (P = 0.041) and the obese group ($P < 0.001$). Additionally, when the overweight group was compared with the obese group, the NFS was found to be significantly lower for the former ($P < 0.001$) (Table 2).

Table 1 Metabolic parameters in the groups classified by body mass index

Variable	Lean	Overweight	Obese	P[1]
	mean ± SD	mean ± SD	mean ± SD	
Age in yr	49.95 ± 15.34	51.34 ± 14.33	53.34 ±13.43	0.012[2]
BMI	23.14 ± 1.95	27.70 ± 1.71	35.38 ± 4.62	0.174
HbA1c, %	6.07 ± 1.41	6.51 ± 1.61	6.46 ± 1.39	0.290
ALT in U/L	37.14 ± 66.48	32.52 ± 32.16	30.73 ± 30.72	0.924
AST in U/L	28.30 ± 23.81	26.44 ± 26.96	25.04 ± 20.91	0.093
GGT in U/L	60.40 ± 81.59	56.61 ± 81.28	57.58 ± 95.50	0.141
ALKP in U/L	89.56 ± 52.69	79.77 ± 43.69	82.73 ± 38.86	0.132
Total bilirubin in mg/dL	0.74 ± 1.43	0.81 ± 1.61	0.63 ± 1.08	0.227
Direct bilirubin in mg/dL	0.35 ± 0.60	0.40 ± 1.06	0.29 ± 0.65	0.679
Total cholesterol in mg/dL	182.07 ± 48.19	172.69 ± 49.50	175.03 ± 47.37	0.222
LDL in mg/dL	118.84 ± 42.12	114.81 ± 42.00	115.38 ± 41.05	0.022
TG in mg/dL	118.69 ± 79.73	135.74 ± 88.66	132.65 ± 88.56	0.140
HDL in mg/dL	52.56 ± 16.27	47.30 ± 16.96	48.49 ± 16.50	< 0.001
FibroScan, kPa	7.43 ± 7.87	7.01 ± 8.39	8.12 ± 9.49	0.174
NFS	-2.74 ± 3.13	-2.11 ± 2.25	-1.14 ± 2.13	0.290

[1]Pairwise comparison using Bonferroni correction, with *P*-value of < 0.05 indicating statistical significance.
[2]Comparison using Kruskal-Wallis test, with *P*-value of < 0.05 indicating statistical significance.
ALKP: Alkaline phosphatase; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; BMI: Body mass index; GGT: Gamma-glutamyl transferase; HbA1c: Glycated haemoglobin; HDL: High density lipoprotein; LDL: Low density lipoprotein; NFS: Nonalcoholic fatty liver disease fibrosis score.

Upon evaluation of agreement between the noninvasive measures studied (FibroScan and NFS), the lean and obese groups showed fair agreement and the overweight group showed moderate agreement (Table 3).

Factors predicting "indeterminate scores"

In order to predict the possible factors that may predict an indeterminate score when NFS is used in patients with NAFLD and to compare them between the different BMI groups, single-predictor binary regression analysis was carried out with age, BMI, sex, HbA1c, AST, ALT, gamma-glutamyl transferase, alkaline phosphatase, total bilirubin, direct bilirubin, total cholesterol, low density lipoprotein, HDL, hyperlipidaemia, diabetes mellitus, and hypertension considered as independent variables (Table 4). Increasing age was found to be a statistically significant predictive factor for obtaining an indeterminate score when the NFS measurement of liver fibrosis was used. Similarly, elevated serum ALT and BMI values were found to be predictive of obtaining an indeterminate score when the NFS was used for overweight and obese groups, respectively.

DISCUSSION

The findings from this study reflect real-life data for NAFLD cases of various BMI classes and help to distinguish the distinctive metabolic phenotypes of each, providing particular insight into the lean NAFLD cases that represent a growing cohort worldwide. The lean NAFLD cases in this study were relatively young compared to other BMI groups and their phenotypic profile was closer to that of healthy individuals (in terms of having lower serum HbA1c, higher serum HDL, and less prevalence of type 2 diabetes mellitus, hypertension and hyperlipidaemia). Also, the lean group showed an overall lower fibrosis stage as measured by both FibroScan and NFS. The prevalence of cases yielding an indeterminate score was highest among the obese group (32%), followed by the overweight group (24.4%) and lean group (18.9%).

Table 2 Frequency of demographic features, metabolic diseases and noninvasive fibrosis assessment findings in the study cohort

Variable	Lean	Overweight	Obese	P[1]
Sex				0.002
Female	61 (38.4%)	142 (38.9%)	359 (48.7%)	
Male	98 (61.6%)	223 (61.1%)	378 (51.3%)	
Hyperlipidaemia				< 0.001
Absent	76 (47.8%)	130 (35.6%)	235 (31.9%)	
Present	76 (47.8%)	205 (56.2%)	457 (62.0%)	
DM				< 0.001
Non-diabetic	103 (64.8%)	171 (46.8%)	294 (39.9%)	
Diabetic	50 (31.4%)	171 (46.8%)	405 (55.0%)	
HTN				0.002
Normotensive	103 (64.8%)	198 (54.2%)	366 (49.7%)	
Hypertensive	50 (31.4%)	144 (39.5%)	333 (45.2%)	
NFS reference				< 0.001
F0-F2	85 (53.5%)	173 (47.4%)	256 (34.7%)	
F3-F4	5 (3.1%)	16 (4.4%)	84 (11.4%)	
Indeterminate score	30 (18.9%)	89 (24.4%)	237 (32.2%)	

[1]Comparison was done using chi-square test of significance, with *P*-value of < 0.05 indicating statistical significance. DM: Diabetes mellitus; HTN: Hypertension; NFS: Nonalcoholic fatty liver disease fibrosis score.

Table 3 Agreement between FibroScan and nonalcoholic fatty liver disease fibrosis score among body mass index categories

BMI class	Category	NFS < -1.455	NFS > 0.676	Agreement, κappa
Lean	Low fibrosis	72	1	0.37[c]
	High fibrosis	10	4	
Overweight	Low fibrosis	151	8	0.43[c]
	High fibrosis	9	8	
Obese	Low fibrosis	212	40	0.38[c]
	High fibrosis	30	38	

κappa: Kappa statistic used with [c]*P* < 0.001. BMI: Body mass index; NFS: Nonalcoholic fatty liver disease fibrosis score.

Upon assessment of agreement between these two modalities, the degree of agreement ranged between fair to moderate.

With the increased recognition of the importance of precision medicine in general and increased popular use of treatment algorithms in NAFLD, a proper noninvasive assessment method for liver fibrosis is needed. Indeed, advanced diagnostic methods are emerging. Transient elastography is a bedside test, easily applicable, and cost effective, with the added benefit of patient acceptance. It has been adopted clinically by non-specialist health care providers for initial assessment of liver fibrosis[15,16]. However, the drawbacks and imprecision of this technique include attenuation of the elastic shear waves by visceral obesity and subcutaneous tissues, leading to a failure rate of 3%-16%[17]. Technological enhancement of transient elastography has been made by the use of an XL probe to measure shear waves at a lower degree of fibrosis, yielding negative predictive value of 89% and specificity of 78%; nevertheless, increased BMI still carries the potential for discordance (odds ratio: 9)[14]. Since that advancement, a plethora of other noninvasive tests have been developed to overcome a variety of other obstacles using a combination of blood parameters entered into

Table 4 Logistic regression analysis for predictors of indeterminate score according to body mass index class within nonalcoholic fatty liver disease cohort

Variable	Lean			Overweight			Obese		
	OR	95%CI	*P*	OR	95%CI	*P*	OR	95%CI	*P*
Age	1.07	1.02, 1.13	0.009[b]	1.04	1.01, 1.08	0.016	1.03	1.02, 1.05	< 0.001[b]
HbA1c	1.28	0.84, 1.95	0.257	1.08	0.85, 1.36	0.541			
BMI						1.04	1.00, 1.08	.030	1.04
ALT				0.98	0.96, 0.99	0.011	1.00	0.99, 1.00	0.169
Hyperlipidaemia				0.75	0.31, 1.84	0.536	1.01	0.64, 1.57	0.981
LDL				0.99	0.98, 1.00	0.161			
DM	0.63	0.17, 2.30	0.484	0.55	0.21, 1.39	0.204	0.99	0.65, 1.50	0.946
HTN	0.61	0.19, 1.96	0.406	1.34	0.61, 2.91	0.464	0.77	0.51, 1.18	0.232

[b]*P* < 0.01. ALT: Alanine aminotransferase; BMI: Body mass index; DM: Diabetes mellitus; HbA1c: Glycated haemoglobin; HTN: Hypertension; LDL: Low density lipoprotein; OR: Odds ratio.

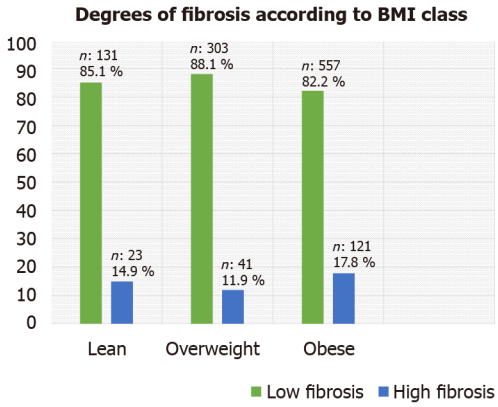

Figure 1 Grades of liver fibrosis among body mass index classified groups based on FibroScan measurements. BMI: Body mass index.

mathematical models, including direct biological and indirect markers of liver function and fibrosis[6].

Waist circumference and assessment of visceral obesity has been considered as another option to assess the degree of liver fibrosis. It is applied by means of a bedside clinical measurement of the visceral adiposity index (commonly known as the VAI); albeit, that its measurement is reportedly more robust with more advanced stages of fibrosis[18-21]. Using radiological modalities, abdominal ultrasound with assessment of the abdominal wall fat index (commonly known as the AFI)[22], and computed tomography scan with assessments of visceral fat[23], visceral adipose tissue[24] or visceral-to-subcutaneous abdominal fat ratio[25] are able to predict advanced steatohepatitis and liver fibrosis. Moreover, bioelectrical impedance estimated visceral fat (commonly known as BIA)[26] is able to predict histologically advance steatohepatitis and fibrosis.

This study found a combination of transient elastography (FibroScan) and NFS measurements in different BMI classes among individuals with predominantly lower fibrosis degree (accounting for > 80% of each BMI class). The lean NAFLD group of patients, in particular, showed fair agreement of the two tools within a lower category of fibrosis, compared to the moderate agreement shown among the overweight and obese groups. The literature includes reports of different strategies to increase the chance of proper assessment and accuracy. For example, repeat transient elastography is especially useful for when a higher degree of fibrosis is being measured (> 7.9 kPa); as shown by Chow *et al*[27], this strategy increased accuracy and subsequent normalization of the measurements in up to one-third of the patients examined. Combining FibroScan with other measures has also been shown to further increase accuracy. A

novel two-step approach to determine fibrosis in patients with high and indeterminate scores obtained with use of NFS followed by transient elastography measurement as found to minimize the need for liver biopsy compared to the use of either test alone [12]. In a Latin study by Perez-Gutiérrez *et al*[28] that correlated NFS to biopsy-based grading of liver fibrosis using Brunt criteria, up to 46% of the patients with indeterminate score showed no liver fibrosis; hence, this group would benefit from careful follow-up and possibly repeat liver biopsy.

Factors that affect interpretation of noninvasive assessment data were investigated in this study as well. A German multicentre study (known as the FLAG study) on ultrasound-based diagnosis of NAFLD in conjunction with several noninvasive assessment measures determined differences between the various noninvasive assessments of fibrosis; when groups of no-fibrosis, indeterminate score and advanced fibrosis were compared, the predictive factors were identified as increased age, waist circumference, serum AST, serum gamma-glutamyl transferase, serum ferritin, and type 2 diabetes mellitus[29]. Another study found type 2 diabetes mellitus to adversely affect the accuracy of the noninvasive parameters investigated [*i.e.* HEPASCORE, AST to platelet ratio index (the APRI) and FIB-4 tests] by down-staging their fibrosis assessment measures[30]. Similar studies have been carried out with real-life situation design. An example of such is a multi-European study that reported indeterminate scores for FIB-4 tests, ranging between 25%-30% among different NAFLD groups at primary care centres[9]. Considering the literature collectively, mitigation of liver fibrosis assessment without resorting to liver biopsy may be achieved by a combination of FibroScan measurement, NFS[12,31], serum M30 (a caspase that is cleaved to form K18 fragments that are released from apoptotic hepatocytes into the blood, where they can be detected by the M30 enzyme linked-immunosorbent assay), and APRI score[32]. Indeed, the increased accuracy achieved with this combination of tests ultimately minimized the need for liver biopsy.

In the study presented herein, patient-related characteristics, serum test results and metabolic diseases were assessed to identify potential predictive factors that may anticipate obtainment of an "indeterminate score" from NFS. Increased age and elevated serum ALT were found to increase the likelihood of need for liver biopsy. Cichoz-Lach *et al*[33] from Poland reported a similar statistically significant diagnosis of liver fibrosis in patients with indeterminate scores (constituting 30.9% of their cohort) upon analysis of NFS and BARD scores with the predictive factors of increased age, BMI > 30, and high ALT/AST ratio. In the present study, the relatively large study population provided new information of the burden of NAFLD in the region (Saudi Arabia) and the small contribution of lean NAFLD.

Importantly, lean NAFLD has long been considered as more prevalent in Asian countries. In this study, however, upon classifying NAFLD patients by BMI, we see a population prevalence of obesity similar to that in western populations; this also suggests greater generalizability of the region-specific data. Despite the fact that there was a predominantly lower degree of fibrosis in our study population, agreement was found between transient elastography and NFS. It is arguable that lean individuals may have less technical limitation for acquiring transient elastography measurement in their lean body configuration, however they still may score indeterminate score of fibrosis which subsequently impairs a precise estimation and leaves the need for liver biopsy. This limitation related to the low extent of liver fibrosis (and thus availability for the technology to detect) is an issue the merits further study. Additionally, long-term follow-up of patients with indeterminate score by NFS is needed in order to elucidate the prognosis of this measurement.

CONCLUSION

For lean NAFLD patients, noninvasive tools are valid for assessing liver fibrosis, subject to the same limitations as with obese NAFLD patients. Indeterminate score obtained by NFS is still an issue, with possible need for a subsequent histological-based assessment of liver fibrosis through invasive procedure (*i.e.* biopsy). Future studies can build upon this knowledge through efforts to determine the best follow-up strategy for such cases.

ARTICLE HIGHLIGHTS

Research background

Nonalcoholic fatty liver disease (NAFLD) is progressively surpassing other aetiologies of chronic liver disease, with its prevalence increasing worldwide. Earlier intervention was advocated to manage cases of less extensive fibrosis before they progress, and this process will involve the conventional invasive detection method of liver biopsy. Due to the increasing emergence of non-obese NAFLD, which is also called lean NAFLD, the need for further study of its phenotype has been recognized and related findings are expected to open new avenues for more accurate detection of fibrosis.

Research motivation

Since lean NAFLD patients are phenotypically healthy, their metabolic syndrome profile is normal. The expected degree of liver fibrosis among these cases is unknown. However, it is well recognized that use of the available noninvasive assessment tools for fibrosis in NAFLD yields a proportion of cases with "indeterminate score" who may require further assessment by liver biopsy.

Research objectives

To identify lean NAFLD characteristics distinguishing from obese NAFLD in terms of the degree of liver fibrosis using noninvasive assessment tools. Additionally, to study predictive factors that may predispose to obtainment of an indeterminate score, which may then be taken into consideration for decision-making on further affirmative evaluation by liver biopsy.

Research methods

NAFLD patients were categorized based on body mass index into lean, overweight and obese groups. Each group underwent assessment by the noninvasive tools of FibroScan and NAFLD fibrosis score (NFS). Group data based upon the subsequent subcategorizations of fibrosis degree (*i.e.* low, high and indeterminate) was applied to regression analysis to identify factors predictive of obtaining the indeterminate score.

Research results

A total of 1753 patients were recruited and 1262 of these were included in the final analysis. According to body mass index, the patients were grouped as lean (159, 12.6%), overweight (365, 29%) or obese (737, 58.4%). Lower fibrosis score was predominant within all three weight groups. Kappa statistical analysis of the FibroScan and NFS data indicated that lean and obese NAFLD cases had fair agreement between the two tools, while overweight NAFLD cases had moderate agreement. Logistic binary regression analysis performed for predictive factors of the indeterminate score obtained by NFS indicated age as a predictive factor in all three weight groups, and serum alanine aminotransferase and body mass index value as predictive in the overweight and obese groups, respectively.

Research conclusions

The lean NAFLD group showed a metabolic profile similar to healthy individuals but having a lower degree of fibrosis than their overweight and obese counterparts. The limitation of indeterminate score by NFS among obese NAFLD patients is similar to that with the lean NAFLD group; unfortunately, this is not explained by the fact that lean body mass index patients receive a more precise measurement of fibrosis than their obese counterparts. Factors that play a role in lean NAFLD patients obtaining an indeterminate score may be applied to overweight and obese counterparts; these being age and serum alanine aminotransferase of the patients.

Research perspectives

Considering lean individuals as a latent undiagnosed group among NAFLD cases, efforts to understand and properly evaluate their underlying liver fibrosis still requires systematic consideration. From the perspective of aiming to apply less invasive tools for clinical assessment of liver fibrosis, further data are needed to ascertain the benefits and limitations of the available noninvasive tools, in order to design an approach for accurate assessment of fibrosis in this newly recognized NAFLD group.

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REFERENCES

1 **Maheux A**, Purcell Y, Harguem S, Vilgrain V, Ronot M. Targeted and non-targeted liver biopsies carry the same risk of complication. *Eur Radiol* 2019; **29**: 5772-5783 [PMID: 31076864 DOI: 10.1007/s00330-019-06227-3]
2 **Mehta R**, Neupane A, Wang L, Goodman Z, Baranova A, Younossi ZM. Expression of NALPs in adipose and the fibrotic progression of non-alcoholic fatty liver disease in obese subjects. *BMC Gastroenterol* 2014; **14**: 208 [PMID: 25512222 DOI: 10.1186/s12876-014-0208-8]
3 **Yoneda M**, Iwasaki T, Fujita K, Kirikoshi H, Inamori M, Nozaki Y, Maeyama S, Wada K, Saito S, Terauchi Y, Nakajima A. Hypoadiponectinemia plays a crucial role in the development of nonalcoholic fatty liver disease in patients with type 2 diabetes mellitus independent of visceral adipose tissue. *Alcohol Clin Exp Res* 2007; **31**: S15-S21 [PMID: 17331160 DOI: 10.1111/j.1530-0277.2006.00281.x]
4 **VanWagner LB**, Armstrong MJ. Lean NAFLD: A not so benign condition? *Hepatol Commun* 2018; **2**: 5-8 [PMID: 29404505 DOI: 10.1002/hep4.1143]
5 **Ye Q**, Zou B, Yeo YH, Li J, Huang DQ, Wu Y, Yang H, Liu C, Kam LY, Tan XXE, Chien N, Trinh S, Henry L, Stave CD, Hosaka T, Cheung RC, Nguyen MH. Global prevalence, incidence, and outcomes of non-obese or lean non-alcoholic fatty liver disease: a systematic review and meta-analysis. *Lancet Gastroenterol Hepatol* 2020; **5**: 739-752 [PMID: 32413340 DOI: 10.1016/S2468-1253(20)30077-7]
6 **Nallagangula KS**, Nagaraj SK, Venkataswamy L, Chandrappa M. Liver fibrosis: a compilation on the biomarkers status and their significance during disease progression. *Future Sci OA* 2018; **4**: FSO250 [PMID: 29255622 DOI: 10.4155/fsoa-2017-0083]
7 **Broussier T**, Lannes A, Zuberbuhler F, Oberti F, Fouchard I, Hunault G, Cales P, Boursier J. Simple blood fibrosis tests reduce unnecessary referrals for specialized evaluations of liver fibrosis in NAFLD and ALD patients. *Clin Res Hepatol Gastroenterol* 2020; **44**: 349-355 [PMID: 31422033 DOI: 10.1016/j.clinre.2019.07.010]
8 **Kamarajah SK**, Chan WK, Nik Mustapha NR, Mahadeva S. Repeated liver stiffness measurement compared with paired liver biopsy in patients with non-alcoholic fatty liver disease. *Hepatol Int* 2018; **12**: 44-55 [PMID: 29372507 DOI: 10.1007/s12072-018-9843-4]
9 **Alexander M**, Loomis AK, Fairburn-Beech J, van der Lei J, Duarte-Salles T, Prieto-Alhambra D, Ansell D, Pasqua A, Lapi F, Rijnbeek P, Mosseveld M, Avillach P, Egger P, Kendrick S, Waterworth DM, Sattar N, Alazawi W. Real-world data reveal a diagnostic gap in non-alcoholic fatty liver disease. *BMC Med* 2018; **16**: 130 [PMID: 30099968 DOI: 10.1186/s12916-018-1103-x]
10 **Khov N**, Sharma A, Riley TR. Bedside ultrasound in the diagnosis of nonalcoholic fatty liver disease. *World J Gastroenterol* 2014; **20**: 6821-6825 [PMID: 24944472 DOI: 10.3748/wjg.v20.i22.6821]
11 **Angulo P**, Hui JM, Marchesini G, Bugianesi E, George J, Farrell GC, Enders F, Saksena S, Burt AD, Bida JP, Lindor K, Sanderson SO, Lenzi M, Adams LA, Kench J, Therneau TM, Day CP. The NAFLD fibrosis score: a noninvasive system that identifies liver fibrosis in patients with NAFLD. *Hepatology* 2007; **45**: 846-854 [PMID: 17393509 DOI: 10.1002/hep.21496]
12 **Chan WK**, Nik Mustapha NR, Mahadeva S. A novel 2-step approach combining the NAFLD fibrosis score and liver stiffness measurement for predicting advanced fibrosis. *Hepatol Int* 2015; **9**: 594-602 [PMID: 25788185 DOI: 10.1007/s12072-014-9596-7]
13 **Wong VW**, Vergniol J, Wong GL, Foucher J, Chan HL, Le Bail B, Choi PC, Kowo M, Chan AW, Merrouche W, Sung JJ, de Lédinghen V. Diagnosis of fibrosis and cirrhosis using liver stiffness measurement in nonalcoholic fatty liver disease. *Hepatology* 2010; **51**: 454-462 [PMID: 20101745 DOI: 10.1002/hep.23312]
14 **Wong VW**, Vergniol J, Wong GL, Foucher J, Chan AW, Chermak F, Choi PC, Merrouche W, Chu SH, Pesque S, Chan HL, de Lédinghen V. Liver stiffness measurement using XL probe in patients with nonalcoholic fatty liver disease. *Am J Gastroenterol* 2012; **107**: 1862-1871 [PMID: 23032979 DOI: 10.1038/ajg.2012.331]
15 **Tapper EB**, Sengupta N, Hunink MG, Afdhal NH, Lai M. Cost-Effective Evaluation of Nonalcoholic Fatty Liver Disease With NAFLD Fibrosis Score and Vibration Controlled Transient Elastography. *Am J Gastroenterol* 2015; **110**: 1298-1304 [PMID: 26303130 DOI: 10.1038/ajg.2015.241]
16 **Tapper EB**, Hunink MG, Afdhal NH, Lai M, Sengupta N. Cost-Effectiveness Analysis: Risk Stratification of Nonalcoholic Fatty Liver Disease (NAFLD) by the Primary Care Physician Using the NAFLD Fibrosis Score. *PLoS One* 2016; **11**: e0147237 [PMID: 26905872 DOI: 10.1371/journal.pone.0147237]
17 **Festi D**, Schiumerini R, Marzi L, Di Biase AR, Mandolesi D, Montrone L, Scaioli E, Bonato G, Marchesini-Reggiani G, Colecchia A. Review article: the diagnosis of non-alcoholic fatty liver disease -- availability and accuracy of non-invasive methods. *Aliment Pharmacol Ther* 2013; **37**: 392-400 [PMID: 23278163 DOI: 10.1111/apt.12186]

18 **Ercin CN**, Dogru T, Genc H, Celebi G, Aslan F, Gurel H, Kara M, Sertoglu E, Tapan S, Bagci S, Rizzo M, Sonmez A. Insulin Resistance but Not Visceral Adiposity Index Is Associated with Liver Fibrosis in Nondiabetic Subjects with Nonalcoholic Fatty Liver Disease. *Metab Syndr Relat Disord* 2015; **13**: 319-325 [PMID: 26011302 DOI: 10.1089/met.2015.0018]

19 **Díez-Rodríguez R**, Ballesteros-Pomar MD, Calleja-Fernández A, González-De-Francisco T, González-Herráez L, Calleja-Antolín S, Cano-Rodríguez I, Olcoz-Goñi JL. Insulin resistance and metabolic syndrome are related to non-alcoholic fatty liver disease, but not visceral adiposity index, in severely obese patients. *Rev Esp Enferm Dig* 2014; **106**: 522-528 [PMID: 25544409]

20 **Petta S**, Amato MC, Di Marco V, Cammà C, Pizzolanti G, Barcellona MR, Cabibi D, Galluzzo A, Sinagra D, Giordano C, Craxì A. Visceral adiposity index is associated with significant fibrosis in patients with non-alcoholic fatty liver disease. *Aliment Pharmacol Ther* 2012; **35**: 238-247 [PMID: 22117531 DOI: 10.1111/j.1365-2036.2011.04929.x]

21 **Vongsuvanh R**, George J, McLeod D, van der Poorten D. Visceral adiposity index is not a predictor of liver histology in patients with non-alcoholic fatty liver disease. *J Hepatol* 2012; **57**: 392-398 [PMID: 22521350 DOI: 10.1016/j.jhep.2012.03.013]

22 **Fukuda K**, Seki Y, Ichihi M, Okada T, Hirata A, Kogita S, Sawai Y, Igura T, Tsugawa M, Imai Y. Usefulness of ultrasonographic estimation of preperitoneal and subcutaneous fat thickness in the diagnosis of nonalcoholic fatty liver disease in diabetic patients. *J Med Ultrason (2001)* 2015; **42**: 357-363 [PMID: 26576787 DOI: 10.1007/s10396-015-0615-7]

23 **Francque S**, Verrijken A, Mertens I, Hubens G, Van Marck E, Pelckmans P, Michielsen P, Van Gaal L. Visceral adiposity and insulin resistance are independent predictors of the presence of non-cirrhotic NAFLD-related portal hypertension. *Int J Obes (Lond)* 2011; **35**: 270-278 [PMID: 20661251 DOI: 10.1038/ijo.2010.134]

24 **Yu SJ**, Kim W, Kim D, Yoon JH, Lee K, Kim JH, Cho EJ, Lee JH, Kim HY, Kim YJ, Kim CY. Visceral Obesity Predicts Significant Fibrosis in Patients With Nonalcoholic Fatty Liver Disease. *Medicine (Baltimore)* 2015; **94**: e2159 [PMID: 26632897 DOI: 10.1097/MD.0000000000002159]

25 **Jung CH**, Rhee EJ, Kwon H, Chang Y, Ryu S, Lee WY. Visceral-to-Subcutaneous Abdominal Fat Ratio Is Associated with Nonalcoholic Fatty Liver Disease and Liver Fibrosis. *Endocrinol Metab (Seoul)* 2020; **35**: 165-176 [PMID: 32207277 DOI: 10.3803/EnM.2020.35.1.165]

26 **Hernández-Conde M**, Llop E, Carrillo CF, Tormo B, Abad J, Rodriguez L, Perelló C, Gomez ML, Martínez-Porras JL, Puga NF, Trapero-Marugan M, Fraga E, Aracil CF, Panero JLC. Estimation of visceral fat is useful for the diagnosis of significant fibrosis in patients with non-alcoholic fatty liver disease. *World J Gastroenterol* 2020; **26**: 6658-6668 [PMID: 33268953 DOI: 10.3748/wjg.v26.i42.6658]

27 **Chow JC**, Wong GL, Chan AW, Shu SS, Chan CK, Leung JK, Choi PC, Chim AM, Chan HL, Wong VW. Repeating measurements by transient elastography in non-alcoholic fatty liver disease patients with high liver stiffness. *J Gastroenterol Hepatol* 2019; **34**: 241-248 [PMID: 29890010 DOI: 10.1111/jgh.14311]

28 **Pérez-Gutiérrez OZ**, Hernández-Rocha C, Candia-Balboa RA, Arrese MA, Benítez C, Brizuela-Alcántara DC, Méndez-Sánchez N, Uribe M, Chávez-Tapia NC. Validation study of systems for noninvasive diagnosis of fibrosis in nonalcoholic fatty liver disease in Latin population. *Ann Hepatol* 2013; **12**: 416-424 [PMID: 23619258 DOI: 10.1016/S1665-2681(19)31004-X]

29 **Hofmann WP**, Buggisch P, Schubert L, Dikopoulos N, Schwenzer J, Muche M, Felten G, Heyne R, Ingiliz P, Schmidt A, Stein K, Wedemeyer H, Berg T, Wiegand J, Lammert F, Zeuzem S, Schattenberg JM. The Fatty Liver Assessment in Germany (FLAG) cohort study identifies large heterogeneity in NAFLD care. *JHEP Rep* 2020; **2**: 100168 [PMID: 32964201 DOI: 10.1016/j.jhepr.2020.100168]

30 **Bertot LC**, Jeffrey GP, de Boer B, MacQuillan G, Garas G, Chin J, Huang Y, Adams LA. Diabetes impacts prediction of cirrhosis and prognosis by non-invasive fibrosis models in non-alcoholic fatty liver disease. *Liver Int* 2018; **38**: 1793-1802 [PMID: 29575516 DOI: 10.1111/liv.13739]

31 **Drolz A**, Wehmeyer M, Diedrich T, Piecha F, Schulze Zur Wiesch J, Kluwe J. [Combination of NAFLD Fibrosis Score and liver stiffness measurement for identification of moderate fibrosis stages (II & III) in non-alcoholic fatty liver disease]. *Z Gastroenterol* 2018; **56**: 43-50 [PMID: 29316577 DOI: 10.1055/s-0043-124956]

32 **Mahadeva S**, Mahfudz AS, Vijayanathan A, Goh KL, Kulenthran A, Cheah PL. Performance of transient elastography (TE) and factors associated with discordance in non-alcoholic fatty liver disease. *J Dig Dis* 2013; **14**: 604-610 [PMID: 23859493 DOI: 10.1111/1751-2980.12088]

33 **Cichoż-Lach H**, Celiński K, Prozorow-Król B, Swatek J, Słomka M, Lach T. The BARD score and the NAFLD fibrosis score in the assessment of advanced liver fibrosis in nonalcoholic fatty liver disease. *Med Sci Monit* 2012; **18**: CR735-CR740 [PMID: 23197236 DOI: 10.12659/MSM.883601]

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ORIGINAL ARTICLE

Observational Study

Managing liver transplantation during the COVID-19 pandemic: A survey among transplant centers in the Southeast United States

Adalberto Jose Gonzalez, Nikhil Kapila, Emmanuel Thomas, Antonio Pinna, Andreas Tzakis, Xaralambos Bobby Zervos

ORCID number: Adalberto Jose Gonzalez 0000-0001-8108-5402; Nikhil Kapila 0000-0001-5551-4234; Emmanuel Thomas 0000-0003-1416-3903; Antonio Pinna 0000-0001-6523-3858; Andreas Tzakis 0000-0001-8077-2315; Xaralambos Bobby Zervos 0000-0001-6783-0525.

Author contributions: Gonzalez AJ and Kapila N wrote the initial manuscript; Thomas E, Pinna A, Tzakis A, and Zervos XB devised the study design and questionnaire and edited the manuscript.

Institutional review board statement: The study did not require approval by the Cleveland Clinic Florida IRB as it was a survey study and did not involve patient data.

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Adalberto Jose Gonzalez, Department of Gastroenterology, Cleveland Clinic Florida, Weston, FL 33324, United States

Nikhil Kapila, Antonio Pinna, Andreas Tzakis, Xaralambos Bobby Zervos, Department of Transplant, Cleveland Clinic Florida, Weston, FL 33331, United States

Emmanuel Thomas, Schiff Center for Liver Diseases, University of Miami Miller School of Medicine, Miami, FL 33136, United States

Emmanuel Thomas, Sylvester Cancer Center, University of Miami Miller School of Medicine, Miami, FL 33136, United States

Corresponding author: Xaralambos Bobby Zervos, DO, Doctor, Department of Transplant, Cleveland Clinic Florida, 2950 Cleveland Clinic Blvd, Weston, FL 33331, United States. zervosx@ccf.org

Abstract

BACKGROUND

The coronavirus disease-2019 (COVID-19) pandemic has had a profound worldwide impact. Indeed, it has led to a vast decrease in organ transplantation, including liver transplants (LT). There is little data regarding adjustments made by LT centers as a response to the COVID-19 pandemic.

AIM

To assess the experience of LT centers in the United States during the pandemic.

METHODS

We performed an observational survey study from May 11, 2020 to June 5, 2020. We sent out a 13 question survey to 15 LT centers across the southeastern United States.

RESULTS

Eleven LT centers responded to the survey. We found that (11/11) 100% of transplant centers made adjustments because of the COVID-19 pandemic. At least 50% of transplant centers had at least one transplant recipient infected with COVID-19. To adjust, greater than 50% of centers performed fewer LT, 100% of patients were tested for COVID-19, and most centers implemented a virtual

and revised according to the STROBE statement checklist of items.

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platform.

CONCLUSION

The COVID-19 pandemic greatly affected liver transplantation in the southeastern United States. It was evident that a concerted effort was made by LT centers to protect their patients and employees from COVID-19 but also to continue the life-saving procedure of LT in this sick patient population. Further studies are needed to assess how LT centers around the world managed the pandemic in order to learn strategies to continue life-saving procedures in this patient population.

Key Words: COVID-19; Liver transplantation; Survey; Telemedicine; Immunosuppression; Solid organ transplantation

Core Tip: The coronavirus disease-2019 (COVID-19) pandemic tremendously affected solid organ transplantation around the world, but little information has been published regarding adaptation from transplant centers. We performed a survey study of 11 Liver transplant (LT) centers in the southeastern United States. 100% of transplant centers made adjustments. COVID-19 testing of transplant candidates, virtual clinic visits, and use of remote allocation of staff were among the most commonly utilized strategies. These strategies can be advantageously used in LT centers in the future. We recommend contingency plans be in place in case of future unprecedented states of emergency.

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INTRODUCTION

The coronavirus disease-2019 (COVID-19) pandemic brought forth new challenges for transplant centers in countries all around the world. Concern for the safety of transplant donors, recipients and hospital staff, in addition to a scarcity of hospital resources allocated to organ transplantation, led to a steep decline in the number of transplanted organs worldwide[1].

In the early stages of the pandemic, limited guidance was offered to liver transplant (LT) centers in regards to the appropriate policies and practices of proceeding with transplantation. To date, there is little data regarding adjustments made by LT centers in response to the COVID-19 pandemic. In this study, we assess the impact of COVID-19 on LT centers early in the pandemic and the adjustments that these centers made in the setting of an unprecedented crisis.

MATERIALS AND METHODS

We performed an observational, survey-based study using a 13-question survey (Figure 1). The questionnaire (Table 1) was created and distributed using an emailed link to Qualtrics (Provo, UT). The questionnaire included both automatic and fill in responses. The technical functionality and ease of use of the electronic questionnaire had been tested before sending out the questionnaire. We identified transplant hepatologists from 15 LT centers in the Southeast United States. Contact information of transplant hepatologists was obtained from a database maintained by the Southeastern division of the American Liver Foundation. Participants were not compensated. Survey participants were informed of the survey details *via* electronic mail. On May 11, 2020, the questionnaire was sent *via* electronic mail. The deadline to respond to the questionnaire was June 5, 2020. Only questionnaires that were entirely completed were

Table 1 Questionnaire

Questionnaire
1 What percentage of your office staff is working remotely?
2 What percentage of your visits is now virtual?
3 How many transplants have been performed in the last 2 mo?
4 What percentage of your donors is screened for COVID-19?
5 What percentage of your candidates is screened for COVID-19?
6 Do you have a dedicated COVID-free ICU space?
7 Is there a current MELD cut-off for new evaluations to occur?
8 Are you currently rotating providers in teams to minimize exposure?
9 Are you flying out for donors?
10 Is there direct communication with UNOS regarding operations of your program?
11 What is the comparison of liver transplants in the past 2 mo to the same time frame in 2019?
12 How many of your transplanted patients contracted the COVID-19 virus?
13 What were the outcomes of those infected?

COVID-19: Coronavirus disease-2019; ICU: Intensive care unit; MELD: Model for end stage liver disease; UNOS: United Network for Organ Sharing.

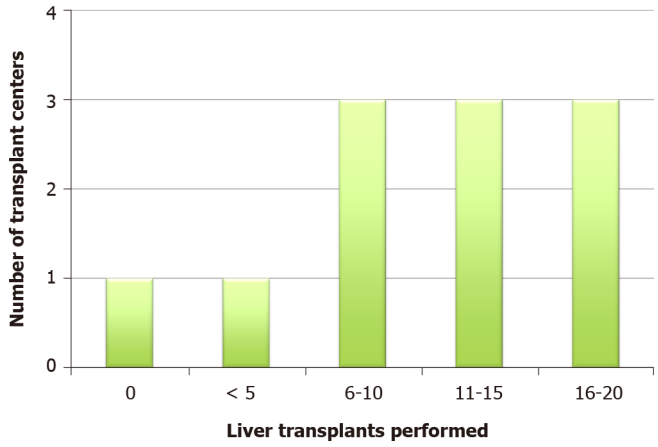

Figure 1 Number of transplants in the preceding 2 mo.

analyzed. The CHERRIES guidelines were used to further describe the methodology and results of our survey.

Results of the questionnaire were analyzed using statistics of central tendency. All data analyses were conducted using SAS version 9.4 (Cary, NC). As this was a survey study without the review of specific patient data, IRB approval was not obtained.

RESULTS

Study population

Of the 15 transplant centers, 11 (73.3%) responded to the questionnaire. All of the centers are academic-based institutions. Nine different cities in 6 different states across the southeastern United States were represented. Ten (91%) of the transplant centers had a dedicated COVID-free space in the intensive care unit (ICU).

Effect of the COVID-19 pandemic on liver transplant centers

Most participating centers performed at least 11 transplants during the preceding 8 wk (Figure 1), ranging from 0 to 20 transplants. Five of 11 centers performed less than 10

transplants. Compared to the previous year, 6 (55%) centers performed less LTs (Figure 2). This included a single center where LT services were stopped altogether. Six (55%) centers had at least 1 recipient infected with COVID-19. During the study period, the mean number of infected transplant recipients per center was 1.8.

Response by liver transplant centers

All centers routinely tested donors and recipients for COVID-19. During the study period, 58% of clinic visits were conducted virtually, and all centers reported at least some degree of telehealth medicine (Figure 3). On average, 73% of each transplant center's staff was assigned to work remotely. Transplant centers attempted to minimize exposure and institutions rotated 72.7% of their providers to minimize exposure. Less than half (45%) of transplant centers had a model for end stage liver disease (MELD) cut-off. For those centers that implemented a cut-off, 25 was the median MELD (Figure 4). All 5 centers that used a MELD cut-off performed less transplants than the year prior. More than half (55%) of the centers continued to fly to procure organs. Centers that continued to fly out for donors performed an average of 15 transplants compared to 9 transplants in centers that stopped flying out for donors. Fifty-five percent of centers had direct communication with United Network for Organ Sharing (UNOS). The centers that did not communicate with UNOS also did not fly out for organs and performed fewer transplants on average (8 *vs* 12).

DISCUSSION

The COVID-19 pandemic presented transplant centers with the unique challenge of providing potential life-saving therapy in the midst of an unprecedented public health crisis. Although several studies have investigated the effects of COVID-19 on rates of transplantation and outcomes in LT recipients[2-5], few have assessed the policy adjustments that centers were forced to implement[6]. To our knowledge, our study is the first to study the early effects of the COVID-19 pandemic, specifically on liver transplant centers, and the steps taken by these centers to provide care to their patients.

The response rate to our survey was at 73%. A recent study that surveyed clinicians on practices and policies at abdominal transplant programs in the United States found a similarly high response rate of 79.3%[6]. This suggests that transplant physicians have a keen interest to improve their understanding and adjust their practice in the midst of the COVID-19 pandemic. At the time of our study, there was limited guidance on appropriate practices and policies for LT programs during the pandemic. In fact, it was not until the third week of April 2020 that the American Association for Study of Liver Disease released a consensus statement from a panel of experts that offered guidance on management during the pandemic[7]. Nearly half of the surveyed centers maintained direct communication with UNOS for guidance[8]. Considering the magnitude of the pandemic and the many challenges that LT programs were therefore forced to manage, we expected more programs to have been in communication with UNOS for guidance during this unprecedented period.

Over the past year, several studies[1] have shown decreases in all types of solid organ transplantation due to the COVID-19 pandemic similar to our findings. The decrease in transplantation is due to many reasons including a paucity of supplies, limited ICU space[6], decreased nursing and medical staff, and the uncertainty of post-transplant care and immunosuppression during the pandemic[9,10]. The majority (90.9%) of centers in our study continued performing LT, albeit often at a limited capacity, thus highlighting the importance of continuing these life-saving procedures. A single center ceased performing all LT. It was also the only center without a dedicated, COVID-free space in the ICU, thus underscoring the tremendous impact that limited resources had on transplant centers during the pandemic. Due to concerns for safety and limited resources, nearly half of centers stopped flying for organ procurement and made use of locally available donors. This may serve as a future impetus for an increased focus on local organ donations.

The safety of liver transplant recipients and hospital staff has been an area of concern since the onset of the COVID-19 pandemic. Nearly 3% of people that have been infected with COVID-19 are healthcare workers[11]. Additionally, several studies have shown that COVID-19 infection rate may be higher in LT recipients, although outcomes are similar when compared to the general population[3,5]. During the study period, a majority (55%) of centers reported at least one transplant recipient with COVID-19 infection. No center reported a COVID-19 related mortality; however, since

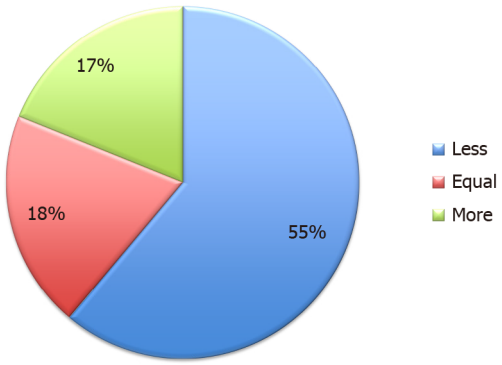

Figure 2 Comparison of liver transplants in 2020 compared to 2019.

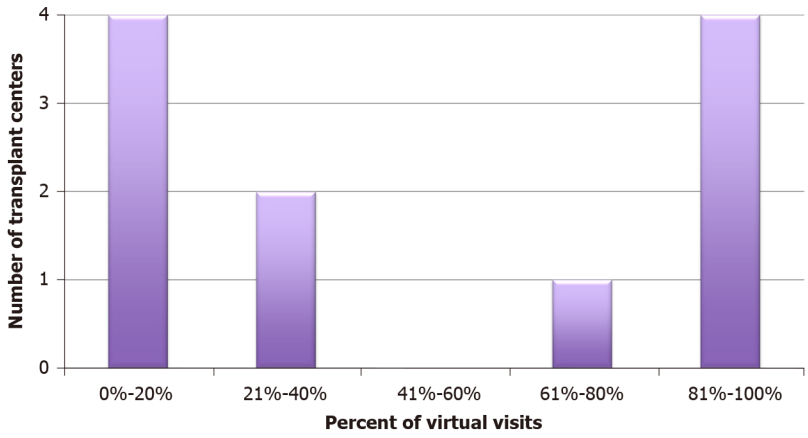

Figure 3 Percent of virtual visits.

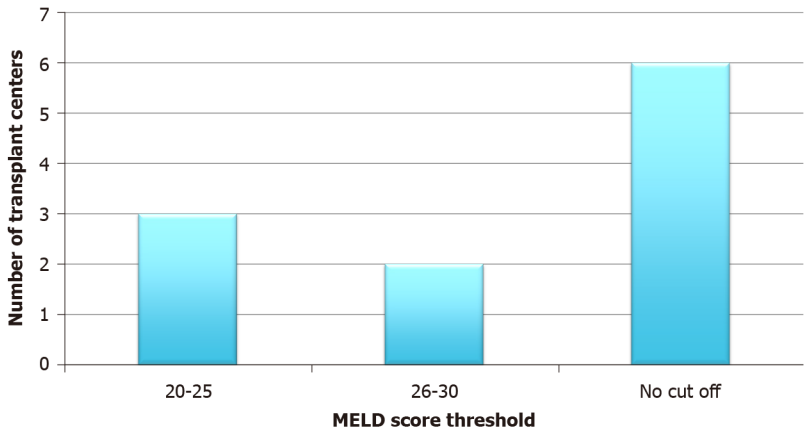

Figure 4 Model for end stage liver disease score cut-off for new evaluation. MELD: Model for end stage liver disease.

the survey was conducted the number of patients infected and the mortality is likely to have changed.

At the onset of the pandemic, transplant centers took steps to ensure the safety of liver transplant staff and recipients. Some of the interventions put in place included testing all LT candidates and donors for COVID-19, utilizing a virtual visit platform, and rotating staff to work remotely. Similar to what was reported in other studies[12, 13], all centers used telemedicine to some capacity. Transplant centers may have been better equipped to adapt to telemedicine due the basic infrastructure that is required for normal operations. Our survey shows that the pandemic changed centers' approach to telemedicine. Though imperfect in many ways, telemedicine has

broadened the reach of transplant programs and has given patients increased access to transplant providers[13].

Our study adds to the growing data[6,14,15] regarding the management and policies of LT during the COVID-19 pandemic. Our study provides a unique perspective to the practice of transplant centers in the Southeast United States, which was a "hotspot" for COVID-19, albeit after the initial wave that affected the New York City region. Also, we had a high response rate to our survey, allowing us to better understand the practices in the majority of centers in the region.

We had several limitations to our study. The primary limitation was the sample size with the inclusion of 11 transplant centers. Though the number of centers was limited, our goal was to highlight the practices of a unique region in the United States. Our survey was only distributed to transplant hepatologists and did not include surgeons and other transplant staff that may have offered more perspective on their centers' practices. Although the peak of the pandemic has passed, this study is a learning opportunity and an encouragement to develop contingency plans for possible future public health emergencies. Finally, due to the nature of the study, there is the possibility of recall bias.

CONCLUSION

COVID-19 changed the practice of medicine across the world, and in our study, we highlight how COVID-19 affected LT practices in the Southeast United States. Our study offers a unique perspective to how individual transplant centers adapted their practice and created their own strategies in response to the COVID-19 public health emergency, despite the lack of clear guidelines. Moving forward, the transplant community should use this experience as an important learning opportunity and as a chance to develop contingency plans for future public health emergencies, natural disasters, and other emergency situations. This may be in the form of specific preemptive guidelines, emergency committees, and resources for communication. These strategies are imperative to continue efficiently performing these life-saving procedures, even during unprecedented situations.

ARTICLE HIGHLIGHTS

Research background

The coronavirus disease-2019 (COVID-19) pandemic greatly affected liver transplant (LT) centers. This is the first study to investigate the effects of COVID-19 specifically on LT centers and the adjustments made by them to provide care to their patients.

Research motivation

There is limited data on policy adjustments made by LT centers during the pandemic. Our findings can help guide transplant centers during future health care emergencies but also to encourage the development of contingency plans for possible future public health emergencies.

Research objectives

Our main aim was to assess the experience of southeastern United States LT centers during the COVID-19 pandemic. Specifically, we wanted to see how the pandemic affected LT centers and the adjustments made by the centers. We were able to realize these objectives.

Research methods

We performed an observation, survey-based study using a 13-question survey. The survey was sent *via* electronic mail to 15 LT centers across the Southeastern United States.

Research results

Eleven of fifteen LT centers responded. 100% of centers made adjustments during the COVID-19 pandemic. Greater than 50% of centers performed fewer LTs. 100% of patients were tested for COVID-19, and most centers implemented a virtual platform.

Research conclusions

LT centers varied in their policy adjustments during the COVID-19 pandemic. This was likely due to the lack of clear guidelines. Going forward, the transplant community should use this experience as an important learning opportunity and galvanize contingency plans for possible future public health emergencies.

Research perspectives

Future studies should assess the most effective way to establish and implement clear guidelines to continue liver transplantation during emergency situations. Future studies should also assess which policy adjustments made during the COVID-19 pandemic were safest and most effective in continuing liver transplantation.

REFERENCES

1 **Loupy A**, Aubert O, Reese PP, Bastien O, Bayer F, Jacquelinet C. Organ procurement and transplantation during the COVID-19 pandemic. *Lancet* 2020; **395**: e95-e96 [PMID: 32407668 DOI: 10.1016/S0140-6736(20)31040-0]

2 **Sahin TT**, Akbulut S, Yilmaz S. COVID-19 pandemic: Its impact on liver disease and liver transplantation. *World J Gastroenterol* 2020; **26**: 2987-2999 [PMID: 32587443 DOI: 10.3748/wjg.v26.i22.2987]

3 **Bhoori S**, Rossi RE, Citterio D, Mazzaferro V. COVID-19 in long-term liver transplant patients: preliminary experience from an Italian transplant centre in Lombardy. *Lancet Gastroenterol Hepatol* 2020; **5**: 532-533 [PMID: 32278366 DOI: 10.1016/S2468-1253(20)30116-3]

4 **Becchetti C**, Zambelli MF, Pasulo L, Donato MF, Invernizzi F, Detry O, Dahlqvist G, Ciccarelli O, Morelli MC, Fraga M, Svegliati-Baroni G, van Vlierberghe H, Coenraad MJ, Romero MC, de Gottardi A, Toniutto P, Del Prete L, Abbati C, Samuel D, Pirenne J, Nevens F, Dufour JF; COVID-LT group. COVID-19 in an international European liver transplant recipient cohort. *Gut* 2020; **69**: 1832-1840 [PMID: 32571972 DOI: 10.1136/gutjnl-2020-321923]

5 **Colmenero J**, Rodríguez-Perálvarez M, Salcedo M, Arias-Milla A, Muñoz-Serrano A, Graus J, Nuño J, Gastaca M, Bustamante-Schneider J, Cachero A, Lladó L, Caballero A, Fernández-Yunquera A, Loinaz C, Fernández I, Fondevila C, Navasa M, Iñarrairaegui M, Castells L, Pascual S, Ramírez P, Vinaixa C, González-Dieguez ML, González-Grande R, Hierro L, Nogueras F, Otero A, Álamo JM, Blanco-Fernández G, Fábrega E, García-Pajares F, Montero JL, Tomé S, De la Rosa G, Pons JA. Epidemiological pattern, incidence, and outcomes of COVID-19 in liver transplant patients. *J Hepatol* 2021; **74**: 148-155 [PMID: 32750442 DOI: 10.1016/j.jhep.2020.07.040]

6 **Boyarsky BJ**, Po-Yu Chiang T, Werbel WA, Durand CM, Avery RK, Getsin SN, Jackson KR, Kernodle AB, Van Pilsum Rasmussen SE, Massie AB, Segev DL, Garonzik-Wang JM. Early impact of COVID-19 on transplant center practices and policies in the United States. *Am J Transplant* 2020; **20**: 1809-1818 [PMID: 32282982 DOI: 10.1111/ajt.15915]

7 **Fix OK**, Hameed B, Fontana RJ, Kwok RM, McGuire BM, Mulligan DC, Pratt DS, Russo MW, Schilsky ML, Verna EC, Loomba R, Cohen DE, Bezerra JA, Reddy KR, Chung RT. Clinical Best Practice Advice for Hepatology and Liver Transplant Providers During the COVID-19 Pandemic: AASLD Expert Panel Consensus Statement. *Hepatology* 2020; **72**: 287-304 [PMID: 32298473 DOI: 10.1002/hep.31281]

8 COVID-19 Resources for Organ Transplants and Donations. [cited 25 March 2021]. In: UNOS. Available from: https://unos.org/covid/

9 **Forns X**, Navasa M. Liver transplant immunosuppression during the covid-19 pandemic. *Gastroenterol Hepatol* 2020; **43**: 457-463 [PMID: 32646657 DOI: 10.1016/j.gastrohep.2020.06.003]

10 **Ridruejo E**, Soza A. The liver in times of COVID-19: What hepatologists should know. *Ann Hepatol* 2020; **19**: 353-358 [PMID: 32425991 DOI: 10.1016/j.aohep.2020.05.001]

11 **CDC COVID-19 Response Team**. Characteristics of Health Care Personnel with COVID-19 - United States, February 12-April 9, 2020. *MMWR Morb Mortal Wkly Rep* 2020; **69**: 477-481 [PMID: 32298247 DOI: 10.15585/mmwr.mm6915e6]

12 **Bokolo AJ**. Exploring the adoption of telemedicine and virtual software for care of outpatients during and after COVID-19 pandemic. *Ir J Med Sci* 2021; **190**: 1-10 [PMID: 32642981 DOI: 10.1007/s11845-020-02299-z]

13 **Smith AC**, Thomas E, Snoswell CL, Haydon H, Mehrotra A, Clemensen J, Caffery LJ. Telehealth for global emergencies: Implications for coronavirus disease 2019 (COVID-19). *J Telemed Telecare* 2020; **26**: 309-313 [PMID: 32196391 DOI: 10.1177/1357633X20916567]

14 **Strauss AT**, Boyarsky BJ, Garonzik-Wang JM, Werbel W, Durand CM, Avery RK, Jackson KR, Kernodle AB, Baker T, Snyder J, Segev DL, Massie AB. Liver transplantation in the United States during the COVID-19 pandemic: National and center-level responses. *Am J Transplant* 2021; **21**: 1838-1847 [PMID: 33107180 DOI: 10.1111/ajt.16373]

15 **Raveh Y**, Simkins J, Vianna R, Tekin A, Nicolau-Raducu R. A Less Restrictive Policy for Liver Transplantation in Coronavirus Disease 2019 Positive Patients, Based Upon Cycle Threshold Values. *Transplant Proc* 2021; **53**: 1126-1131 [PMID: 33610305 DOI: 10.1016/j.transproceed.2021.01.035]

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ORIGINAL ARTICLE

Prospective Study

Accuracy of virtual chromoendoscopy in differentiating gastric antral vascular ectasia from portal hypertensive gastropathy: A proof of concept study

Ahmad M Al-Taee, Mark P Cubillan, Alice Hinton, Lindsay A Sobotka, Alex S Befeler, Christine Y Hachem, Hisham Hussan

ORCID number: Ahmad M Al-Taee 0000-0002-2930-533X; Mark P Cubillan 0000-0001-8776-3796; Alice Hinton 0000-0003-4505-4021; Lindsay A Sobotka 0000-0003-1052-2067; Alex S Befeler 0000-0002-4898-5625; Christine Y Hachem 0000-0002-2779-7940; Hisham Hussan 0000-0002-8646-8370.

Author contributions: Hussan H, Befeler AS, and Hachem CY performed the conceptualization and methodology; Al-Taee AM and Cubillan MP contributed to the data collection; Hinton A performed the data analysis; Al-Taee AM, Cubillan MP, and Sobotka LA contributed to writing-original draft preparation; all authors contributed to writing, reviewing and editing.

Institutional review board statement: The study protocol was approved by the Saint Louis University Institutional Review Board.

Informed consent statement: The study protocol, patient's rights and obligations were reviewed with eligible patients and informed consent was obtained from all participants.

Ahmad M Al-Taee, Division of Gastroenterology and Hepatology, NYU Langone Health, New York, NY 10016, United States

Mark P Cubillan, Department of Internal Medicine, Saint Louis University, St Louis, MO 63110, United States

Alice Hinton, Division of Biostatistics, The Ohio State University, Columbus, OH 43210, United States

Lindsay A Sobotka, Hisham Hussan, Division of Gastroenterology, Hepatology, and Nutrition, The Ohio State University, Columbus, OH 43210, United States

Alex S Befeler, Christine Y Hachem, Division of Gastroenterology and Hepatology, Saint Louis University, St Louis, MO 63110, United States

Corresponding author: Ahmad M Al-Taee, MD, Academic Fellow, Division of Gastroenterology and Hepatology, NYU Langone Health, 530 First Ave, HCC 4G, New York, NY 10016, United States. ahmad.al-taee@nyulangone.org

Abstract

BACKGROUND

Accurate detection of gastric antral vascular ectasia (GAVE) is critical for proper management of cirrhosis-related gastrointestinal bleeding. However, endoscopic diagnosis of GAVE can be challenging when GAVE overlaps with severe portal hypertensive gastropathy (PHG).

AIM

To determine the added diagnostic value of virtual chromoendoscopy to high definition white light for real-time endoscopic diagnosis of GAVE and PHG.

METHODS

We developed an I-scan virtual chromoendoscopy criteria for diagnosis of GAVE and PHG. We tested our criteria in a cross-sectional cohort of cirrhotic adults with GAVE and PHG when high-definition white light endoscopy (HDWLE) diagnosis was in doubt. We then compared the accuracy of I-scan *vs* HDWLE alone to

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histology.

RESULTS
Twenty-three patients were included in this study (65.2% Caucasians and 60.9% males). Chronic hepatitis C was the predominant cause of cirrhosis (43.5%) and seven adults (30.4%) had confirmed GAVE on histology. I-scan had higher sensitivity (100% *vs* 85.7%) and specificity (75% *vs* 62.5%) in diagnosing GAVE compared to HDWLE. This translates into a higher, albeit not statistically significant, accuracy of I-scan in detecting GAVE compared to HDWLE alone (82% *vs* 70%). I-scan was less likely to lead to an accurate diagnosis of GAVE in patients on dialysis ($P < 0.05$) and in patients with elevated creatinine ($P < 0.05$). I-scan had similar accuracy to HDWLE in detecting PHG.

CONCLUSION
This pilot work supports that virtual chromoendoscopy may obviate the need for biopsies when the presence of GAVE is in doubt. Larger studies are needed to assess the impact of virtual chromoendoscopy on success of endoscopic therapy for GAVE.

Key Words: Portal hypertensive gastropathy; Gastric antral vascular ectasia; Virtual chromoendoscopy; Endoscopy

Core Tip: Gastric antral vascular ectasia (GAVE) and portal hypertensive gastropathy (PHG) are two causes of GI bleeding in cirrhosis. Gastric biopsies, which are the gold standard to differentiate the two conditions, may be contraindicated given coagulopathy or thrombocytopenia in cirrhosis. We developed virtual chromoendoscopy (I-scan) criteria for diagnosis of GAVE and PHG. We tested our criteria in a prospective cohort of cirrhotic adults with GAVE and PHG when high-definition white light endoscopy (HDWLE) diagnosis was doubtful. We compared accuracy of I-scan *vs* HDWLE to histology. Compared to HDWLE, I-scan demonstrated superior performance for real-time diagnosis of PHG and GAVE in cirrhosis.

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INTRODUCTION

Gastric antral vascular ectasia (GAVE) and portal hypertensive gastropathy (PHG) account for up to 10% of causes of gastrointestinal bleeding in patients with cirrhosis [1-3]. Management of GAVE is aimed at temporizing bleeding with endoscopic therapy. In contrast, management of PHG is targeted at reducing portal pressure with pharmacologic agents and portosystemic shunting[1-3]. As a result, accurate diagnosis is critical for optimal treatment of GAVE- and PHG-related bleeding[4,5]. Endoscopically, GAVE often manifests as red stripes radiating away from the pylorus commonly referred to as "watermelon stomach" but can also present in a more diffuse, 'honeycomb' pattern[6-8]. Alternatively, PHG usually involves the mucosa in the gastric fundus and body and is characterized by four main features: A mosaic-like pattern, presence of red point lesions, cherry red spots and black brown spots[9]. Despite their typical appearance, distinguishing between GAVE and PHG can be challenging with endoscopy alone as advanced PHG can have similar endoscopic features to GAVE.

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While endoscopic appearance can suggest the diagnosis, gastric biopsies are the current gold standard for differentiating PHG from GAVE. Biopsies may be contraindicated given coagulopathy or thrombocytopenia that are commonly seen with cirrhosis[10,11]. Recently, there has been an increasing interest in the use of digital chromoendoscopy for real-time optical diagnosis of various gastrointestinal pathologies[12]. Utilizing narrow band imaging (Olympus, Tokyo, Japan), Hayashi and Saeki[13], demonstrated that PHG had obscured collecting venules (CVs) and intramucosal hemorrhage as opposed to partial and marked dilation of the capillaries surrounding the gastric pits in patients with GAVE[13]. Achim *et al*[12] demonstrated that the I-scan virtual chromoendoscopy (Pentax, Tokyo, Japan) has an increased sensitivity in the diagnosis of PHG when compared with white light endoscopy[12]. Building on these studies, we aimed to compare the sensitivity, specificity and accuracy of I-scan to high-definition white light endoscopy (HDWLE) in distinguishing between GAVE and PHG. Our main hypothesis is that I-scan virtual chromoendoscopy is more sensitive and specific than HDWLE at diagnosing GAVE when compared to gastric biopsy.

MATERIALS AND METHODS

Study participants

A cross-sectional cohort study was conducted at Saint Louis University-affiliated hospitals in St. Louis Missouri between July 17, 2012 and July 8, 2013. Inclusion and exclusion criteria are highlighted in Figure 1. All adult patients with cirrhosis undergoing an upper endoscopy were considered candidates for this study. Cirrhosis was confirmed on liver biopsy or clinically coupled with laboratory tests (*e.g.* serum albumin less than 3.0 g/dL or blood platelet counts less than 150000 mm^3) and radiologic evidence of cirrhosis. Patients were excluded from the study if GAVE or PHG were absent or had a characteristic endoscopic appearance that could be clearly diagnosed without biopsy. We also excluded pregnant women or if a gastric biopsy did not confirm the diagnosis of GAVE or PHG. The study protocol was approved by the Saint Louis University Institutional Review Board. The study protocol, patient's rights and obligations were reviewed with eligible patients and informed consent was obtained from all participants.

Development of the diagnostic criteria for GAVE and PHG

To create our diagnostic criteria, the author HH prospectively obtained I-scan pictures of the gastric mucosa when endoscopically evaluating classic PHG and GAVE in consenting adults with cirrhosis who underwent esophagogastroduodenoscopy (EGD). Upon review of the images and building on prior studies by Hayashi and Saeki [13] and Achim *et al*[12], the author HH created an I-scan criteria for diagnosis of GAVE and PHG. Gastric pits are usually round, pink, and surrounded by the subepithelial capillary network that drain into CVs. When PHG is present, there is pit edema and capillary engorgement on I-scan which manifests as the snake-skin appearance on HDWLE (Figure 2A). Similarly, CVs appear as dilated star-like dark-red spots with defined borders while intramucosal hemorrhage are typically lighter in color and have a hazier border compared to venules on I-scan (Figure 2B and C). In contrast, the classic appearance of GAVE on I-scan was defined as presence of capillary ectasia characterized by bright red spots with defined borders (Figure 2D)[12, 13]. Additional examples of our PHG and GAVE under HDWLE and I-scan are in Figures 3 and 4. Participating endoscopists were then provided with a PowerPoint presentation explaining the visual appearance of GAVE and PHG with I-scan.

Pre-endoscopic evaluation

Prior to endoscopy, the following data were obtained from the patient once deemed to be eligible for this study: Age, gender, race, history of gastrointestinal bleeding in the past 3 mo, use of certain medications (non-steroidal anti-inflammatory drugs, aspirin, anticoagulants, iron tablets, or beta blockers), alcohol use, and the presence of ascites or lower extremity edema on exam.

Endoscopic examination and specimen collection

All patients underwent an EGD similar to endoscopic evaluation performed in most clinical settings. Upper endoscope (models EG-3470K, EG-2990I, EG-3490K, and EG-2790K) developed by Pentax (Tokyo, Japan) were utilized in this study. Under direct

Figure 1 Study design. PHG: Portal hypertensive gastropathy; GAVE: Gastric antral vascular ectasia; HDWL: High definition white light endoscopy.

visualization, the esophagus was intubated and the endoscope was advanced to the stomach. The gastric mucosa was first inspected using HDWLE for mucosal findings suggestive of GAVE and/or PHG. Patients who had abnormal gastric mucosal findings concerning for GAVE and/or PHG in whom the diagnosis was not certain utilizing HDWLE given lack of classic features underwent further evaluation with I-scan. Areas of abnormal gastric mucosa were carefully examined for 30 to 60 s utilizing HDWLE and the endoscopist determined the following: Visual diagnosis (PHG or GAVE), confidence level about diagnosis (high or low), location (antrum, antrum/body, antrum/body/fundus, antrum/fundus, fundus, or body), PHG severity (mild, moderate, or severe), GAVE appearance (stripped, diffuse, punctate, past previous treatment), stigmata of recent bleeding, and presence of varices. High quality photos were taken. After HDWLE exam was completed, I-scan mode and electronic magnification (× 2) were activated. The tip of the scope was positioned about 2 cm away from the mucosa for careful examination. The endoscopist determined the following: Visual diagnosis (PHG or GAVE), confidence level about diagnosis (high or low), and presence of certain features on I-scan (pit edema, dilated capillaries, dilated venules, or intramucosal hemorrhage). High quality photos were taken. At completion of the visual inspection, biopsies of the abnormal gastric mucosa for histologic confirmation were taken using a standard biopsy forceps (Boston Scientific, Marlborough, MA).

Post-endoscopic evaluation

Biopsy specimens were examined by a gastrointestinal pathologist using hematoxylin and eosin as well as special stains to establish the diagnosis. Pathologist commented on the presence of edema, vascular ectasia, acute and/or chronic inflammation, reactive epithelial cells, smooth fibers, microthrombi, hyalinosis, metaplasia, CD31 and

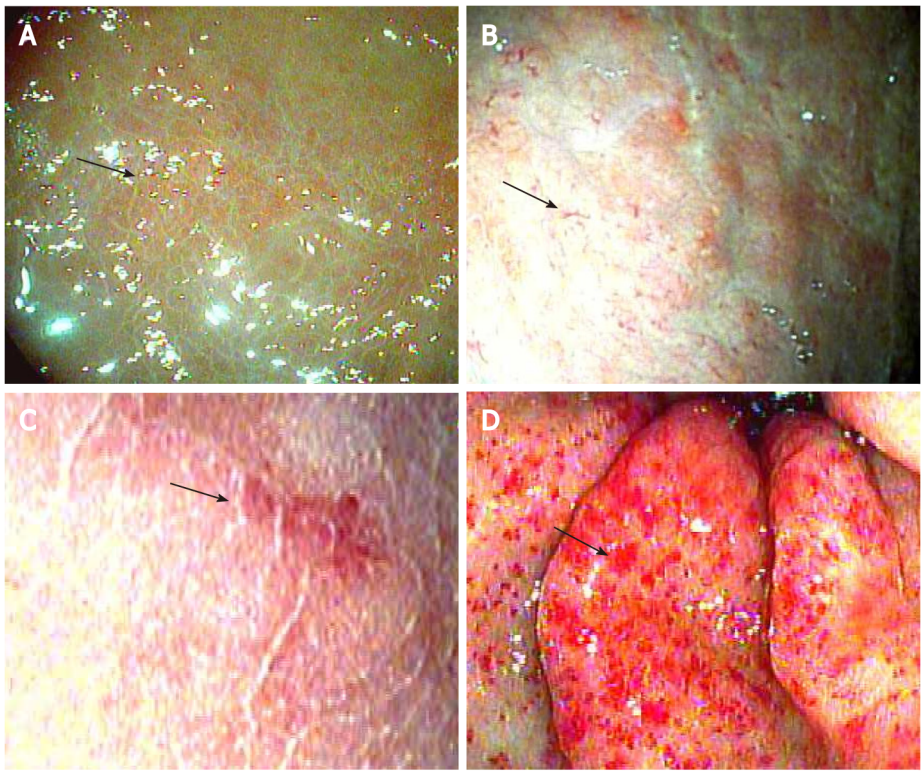

Figure 2 Portal hypertensive gastropathy. A: I-scan with pit edema/capillary engorgement; B: Dilated collecting venules under magnification; C: Intramucosal hemorrhage under magnification; D: Gastric antral vascular ectasia on I-scan defined as presence of capillary ectasia.

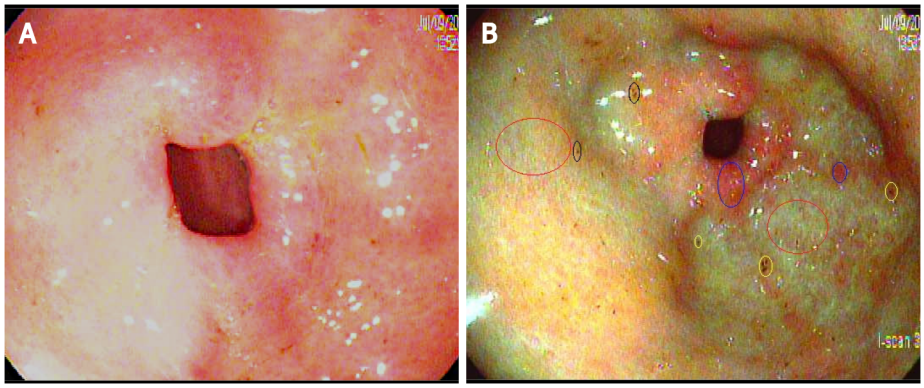

Figure 3 Portal hypertensive gastropathy under high definition white light endoscopy and I-scan Pit edema (red circles), intramucosal hemorrhage (yellow circles), capillary congestion (blue circles), and dilated venules (black circle). A: High definition white light endoscopy; B: I-scan.

CD61 positivity, and pathologic diagnosis. According to Westerhoff *et al*[14], staining for CD61 and CD31 has improved diagnostic accuracy of GAVE and PHG compared to H&E staining[14].

Statistical analysis

Analyses were performed with SAS 9.4 (SAS Institute Inc., Cary, NC). To characterize the ability of HDWLE and I-scan to diagnose GAVE and PHG, sensitivities and specificities were calculated. Further, the percent accuracy of HDWLE and I-scan in diagnosing GAVE and PHG was compared with Fisher exact tests. Categorical data was summarized with frequencies and percentages while continuous data was summarized with medians and interquartile ranges (IQR). Differences between patients with correct and incorrect I-scan diagnoses of PHG were assessed through the use of Fisher exact tests or Wilcoxon rank-sum tests, as appropriate. Differences between patients with a correct and incorrect I-scan diagnosis of GAVE were analyzed

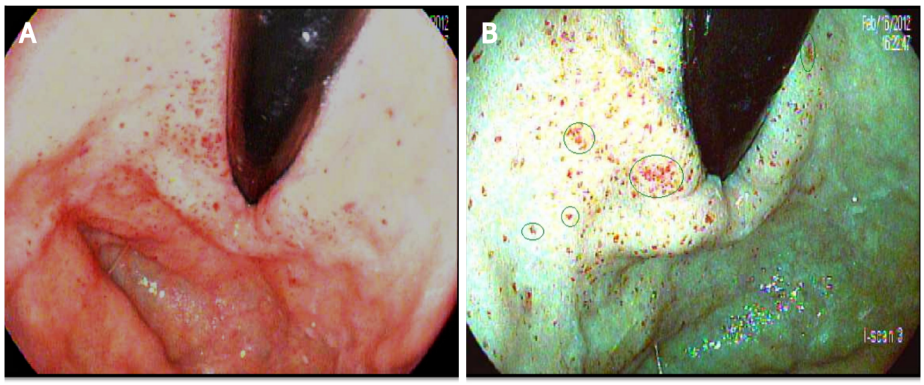

Figure 4 Gastric antral vascular ectasia under high-definition white light and I-scan dilated capillaries (green circles). A: High-definition white light; B: I-scan.

similarly. All statistical tests were evaluated at the α = 0.05 significance level.

Ethics statement

The study protocol was approved by the Saint Louis University Institutional Review Board. The study protocol, patient's rights and obligations were reviewed with eligible patients and informed consent was obtained from all participants.

RESULTS

Patient characteristics

A total of 25 patients met the initial inclusion criteria and were eligible to participate. Two patients were subsequently excluded given biopsies did not show GAVE or PHG. Baseline characteristics of the study cohort including medications and laboratory analysis are summarized in Table 1. The majority of the patients included in this study were Caucasian (65.2%), male (60.9%) and had chronic hepatitis C causing cirrhosis (43.5%). None of the patients were prescribed anticoagulation or antiplatelet agents other than aspirin (31.8%). Median blood work for included patients included hemoglobin 10.6 g/dL, platelets 125000 *per* mm^3, INR 1.1 and creatinine 1.0 mg/dL. The majority of patients underwent an upper endoscopy for management of esophagogastric varices (73.9%). Some patients already had some form of therapy for portal HTN including TIPS (8.7%), history of liver transplantation (13%) or use of beta blockers (45.5%).

Comparing HDWLE and I-scan for diagnosis of GAVE and PHG

Seven adults (30.4%) had confirmed GAVE on histology. HDWLE had a sensitivity of 85.7% and specificity of 62.5% in diagnosing GAVE compared to a sensitivity of 100% and 75% specificity utilizing our I-scan criteria (examples of GAVE and PHG under I-scan are in Supplementary Figures 1 and 2). As a result, utilizing HDWLE alone, the diagnosis of GAVE was accurately made in 69.57% (n = 16) of cases compared to 82.61% (n = 19) when utilizing I-scan technology (P = 0.491; Fisher exact test Table 2). In contrast, HDWLE has a sensitivity of 93.8% and a 75% specificity in diagnosing PHG compared to a sensitivity of 87.5% and specificity of 71.4% utilizing I-scan (accuracy of 82.61% with or without I-scan, P = 1.000 as in Table 3). I-scan was more likely to make an incorrect diagnosis of PHG in patients with alcoholic cirrhosis, alcohol use, or in patients with lower bilirubin levels while a better diagnosis of PHG was made antrum using I-scan when the antrum is involved (P < 0.05) (Supplementary Table 1). I-scan was more likely to make an incorrect diagnosis of GAVE if the patient was on dialysis or an elevated creatinine (P < 0.05) (Supplementary Table 2). Other factors including age, gender, race, ascites, presence of varices, or laboratory findings were no significant.

Table 1 Summary of the patient population

	Overall (*n* = 23)	
Age (median, IQR), *n* (%)	60	
Male	14	60.9
Caucasian	15	65%
Etiology of cirrhosis		
Alcohol (EtOH)	3	13.0
Granulomatous hepatitis	1	4.4
HBV	1	4.4
HCV	10	43.5
HCV, EtOH	1	4.4
Nonalcoholic steatohepatitis	6	26.1
Primary sclerosing cholangitis	1	4.4
Liver biopsy	10	43.5
Liver transplantation	3	13.0
Portal hypertension on imaging	17	73.9
TIPS	2	8.7
Cirrhosis on CT/US	23	100.0
History of connective tissue disease	1	4.4
Dialysis	2	8.7
Endoscopy suite, *n* (%)		
Reason for EGD		
Anemia	1	4.4
GI Bleed	4	17.4
Varices	18	78.2
Anticoagulation	0	0.0
Alcohol use in the past 15 d	5	21.7
ASA in the past 15 d	7	31.8
NSAIDS use in the past 15 d	0	0.0
Plavix	0	0.0
Beta blockers	10	45.5
Labs within 3 mo Pre EGD[1]	**median**	**IQR**
Hemoglobin	10.6	9.5–13.3
Mean corpuscular volume	89.2	87.0–90.5
Platelet count	126.5	68.0–152.0
INR	1.1	1.1–1.2
Serum sodium	139.0	137.0–142.0
Alanine aminotransferase	30.0	25.0–54.0
Aspartate aminotransferase	50.0	32.0–79.0
Total bilirubin	1.6	1.2–2.6
Alkaline phosphatase	108.0	85.0–134.0
Serum albumin	3.2	2.4–3.4
Ferritin	74.3	5.0–2458.0

Creatinine	1.0	0.70–1.47
Labs within 4-8 wk after EGD[1]	**median**	**IQR**
Hemoglobin	11.4	8.9–12.8
Mean corpuscular volume	87.9	84.8–91.6
Platelet count	117.0	63.0–166.0
INR	1.2	1.1–1.3
Serum sodium	140.0	137.0–142.0
Alanine aminotransferase	31.0	21.0–42.0
Aspartate aminotransferase	44.0	29.0–68.0
Total bilirubin	1.2	0.9–1.9
Alkaline phosphatase	132.0	79.0–185.0
Serum albumin	3.0	2.6–3.3
Ferritin	197.4	63.0–199.0
Creatinine	1.0	0.70–1.50

[1]Reference ranges: Hemoglobin 12-15.5 g/dL, mean corpuscular volume 83-11 fL, platelet count 150-400 K/mm^3, INR 0.9-1.2, serum sodium 134-145 mEq/L, alanine aminotransferase 0-61 U/L, aspartate aminotransferase 5-34 U/L, total bilirubin 0.2-1.2 mg/dL, alkaline phosphatase 40-150 U/L, serum albumin 3.4-5 g/dL, ferritin 12-200 ng/mL, and creatinine 0.7-1.3 mg/dL.
CT: Computed tomography; US: Ultrasound; EGD: Esophagogastroduodenoscopy; NSAIDS: Non-steroidal anti-inflammatory drugs; INR: International normalized ratio; IQR: Interquartile ranges; HCV: Hepatitis C; HBV: Hepatitis B.

Table 2 Comparison of white light and I-scan to the gold standard biopsy for diagnosis of gastric antral vascular ectasia

		Biopsy		
		No GAVE	GAVE	
White Light	No GAVE	10	1	Sensitivity: 85.7%
	GAVE	6	6	Specificity: 62.5%
I-Scan	No GAVE	12	0	Sensitivity: 100%
	GAVE	4	7	Specificity: 75.0%

GAVE: Gastric antral vascular ectasia.

Table 3 Comparison of white light and I-scan to the gold standard biopsy for diagnosis of portal hypertensive gastropathy

		Biopsy		
		No PHG	PHG	
White Light	No PHG	4	1	Sensitivity: 93.8%
	PHG	3	15	Specificity: 57.1%
I-Scan	No PHG	5	2	Sensitivity: 87.5%
	PHG	2	14	Specificity: 71.4%

PHG: Portal hypertensive gastropathy.

DISCUSSION

In this pilot study, I-scan with magnification demonstrated a trend towards superior overall performance characteristics for real-time visual diagnosis of PHG and GAVE compared to HDWLE in patients with cirrhosis and ambiguous findings on endoscopic evaluation. This novel method may allow for an accurate, real time

diagnosis in multiple critical clinical situations, such as when biopsy is contraindicated or when more urgent decisions regarding endoscopic management of gastrointestinal bleeding is needed. Therefore, I-scan should be considered a valuable diagnostic tool in such challenging clinical scenarios, although further prospective evaluation is needed.

The superiority of I-scan compared to HDWLE can be contributed to I-scan's ability to provide real-time structural and vascular enhancement of HDWLE images. I-scan image processing involves three algorithms: Surface enhancement (SE), contrast enhancement (CE), and tone enhancement (TE). SE improves the delineation of the examined mucosa by accentuating blood vessels. CE can sharpen the appearance of surface vessels and enhance the visualized details of mucosa surface texture. TE accentuates mucosal patterns and vascular structures to aid in lesion characterization. These enhancements significantly contribute to the endoscopist ability to perform an accurate diagnosis based on the endoscopic appearance which is noted in this study when comparing the ability for the endoscopist to accurately diagnose GAVE based on visual appearance of the gastric mucosa. The utilization of I-scan technology allowed for increased sensitivity and specificity when diagnosing GAVE compared to standard HDWLE. This translated into an accuracy of 82% for I-scan and 70% for HDLWE. While this finding was not statistically significant likely due to small sample size, it does show a trend towards statistical significance. A more recent study using Narrow Band Imaging showed an increased accuracy of virtual chromoendoscopy at diagnosing GAVE. However, our study relied on more extensive advanced imaging diagnosis criteria and used special stains to confirm GAVE[15].

The clinical implications of improved visual diagnosis of GAVE are significant. Utilizing I-scan with magnification may potentially obviate the need for obtaining biopsies when visual diagnosis of GAVE can be made using I-scan. This can be especially helpful in situations where obtaining biopsies is discouraged given coagulopathy or active gastrointestinal bleeding which are relatively common scenarios in patients with cirrhosis. An accurate, real time diagnosis allows the endoscopist to initiate definitive management for gastrointestinal bleeding in a timely manner instead of delaying to confirm diagnosis *via* pathology evaluation. Ultimately, we suspect this will improve patient outcomes and utilization of hospital resources. In addition, an accurate visual diagnosis can obliviate the need to obtain biopsy which will results significant cost savings.

Patients with alcoholic cirrhosis or alcohol use were less likely to have an accurate diagnosis of PHG, suggesting that alcohol may alter the gastric pit and vascular patterns leading to a difficult PHG diagnosis. Indeed, alcohol use is known to alter the upper gastrointestinal mucosa and lead to atrophy and inflammation[16]. In contrast, I-scan had better ability to diagnosis PHG in the antrum and which is the stomach location where GAVE usually appears. These findings highlights the ability of I-scan in making accurate diagnosis of GAVE *vs* PHG in the antrum which is critical for management. We do note that patient with an elevated creatinine, and on dialysis were more likely to have an incorrect diagnosis of GAVE utilizing I-scan technology. At this time, the association between renal dysfunction on incorrect diagnosis using I-scan remain unclear and may have only been noted in this study due to the small sample size or could be due to underlying edema leading to obscured diagnosis. These findings are novel and have not been noted in other studies evaluating the accuracy of I-scan technology in diagnosing gastrointestinal pathology.

In light of the emerging technologies in endoscopic imaging, the preservation and incorporation of valuable endoscopic innovations (PIVI) initiative was developed by the American Society for Gastrointestinal Endoscopy to set thresholds that any new technology should meet before it can replace the current practice of random biopsies. These thresholds have been described for diminutive colonic polyps[17] and Barrett's esophagus[18] but not for PHG or GAVE. This study shows promising results in utilizing I-scan technology to assist with accurate visual diagnosis. Despite the promising results notes in this study, there is limitation to this data. First, the small sample size may have affected the results and these results should be confirmed with a larger study prior to implementing into clinic practice. Given multiple endoscopist performed the procedures after a short PowerPoint presentation on the visual diagnosis of GAVE and PHG utilizing I-scan technology, there was likely some variability in endoscopist's diagnosis. Finally, we could not account for the learning curve leading to more accurate diagnosis for GAVE and PHG with HDWLE later in the study.

CONCLUSION

We conclude that, utilizing I-scan with magnification may obviate the need for biopsies when visual diagnosis of either PHG or GAVE can be made with high confidence. This pilot work supports the further evaluation of I-scan in these challenging clinical situations using a larger sample size and a follow up of outcomes in a randomized fashion.

ARTICLE HIGHLIGHTS

Research background

Gastric antral vascular ectasia (GAVE) and portal hypertensive gastropathy (PHG) are two not uncommon causes of upper gastrointestinal bleeding in patients with cirrhosis. While endoscopic appearance can suggest the diagnosis, gastric biopsies are the current gold standard for differentiating PHG from GAVE.

Research motivation

Distinguishing GAVE from PHG is important as the management is different for the two conditions. Obtaining gastric biopsies to diagnose GAVE and PHG may be contraindicated given coagulopathy or thrombocytopenia which are commonly seen with cirrhosis. Here we hypothesized that I-scan virtual chromoendoscopy is more sensitive and specific than high-definition white light endoscopy (HDWLE) at diagnosing GAVE when compared to gastric biopsy.

Research objectives

The main objective of this work was to determine the added diagnostic value of virtual chromoendoscopy to high definition white light for real-time endoscopic diagnosis of GAVE and PHG.

Research methods

We developed an I-scan virtual chromoendoscopy criteria for diagnosis of GAVE and PHG. We then tested these criteria in a prospective cohort of cirrhotic adults with GAVE and PHG when HDWLE diagnosis was in doubt. We then compared the accuracy of I-scan *vs* HDWLE alone compared to histology.

Research results

I-scan with magnification demonstrated superior overall performance characteristics for real-time visual diagnosis of PHG and GAVE compared to HDWLE in patients with cirrhosis and ambiguous findings on endoscopic evaluation.

Research conclusions

This novel finding allows for an accurate, real time diagnosis in multiple critical clinical situations, such as when biopsy is contraindicated or when more urgent decisions regarding endoscopic management of gastrointestinal bleeding is needed.

Research perspectives

Utilizing I-scan with magnification may obviate the need for biopsies when visual diagnosis of either PHG or GAVE can be made with high confidence. This pilot work supports the further evaluation of I-scan in these challenging clinical situations using a larger sample size and a follow up of outcomes in a randomized fashion.

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REFERENCES

1 **Qureshi K**, Al-Osaimi AM. Approach to the management of portal hypertensive gastropathy and

gastric antral vascular ectasia. *Gastroenterol Clin North Am* 2014; **43**: 835-847 [PMID: 25440929 DOI: 10.1016/j.gtc.2014.08.012]

2 **Tekola BD**, Caldwell S. Approach to the management of portal hypertensive gastropathy and gastric antral vascular ectasia. *Clin Liver Dis (Hoboken)* 2012; **1**: 163-166 [PMID: 31186879 DOI: 10.1002/cld.99]

3 **Pérez-Ayuso RM**, Piqué JM, Bosch J, Panés J, González A, Pérez R, Rigau J, Quintero E, Valderrama R, Viver J. Propranolol in prevention of recurrent bleeding from severe portal hypertensive gastropathy in cirrhosis. *Lancet* 1991; **337**: 1431-1434 [PMID: 1675316 DOI: 10.1016/0140-6736(91)93125-s]

4 **Gjeorgjievski M**, Cappell MS. Portal hypertensive gastropathy: A systematic review of the pathophysiology, clinical presentation, natural history and therapy. *World J Hepatol* 2016; **8**: 231-262 [PMID: 26855694 DOI: 10.4254/wjh.v8.i4.231]

5 **Patwardhan VR**, Cardenas A. Review article: the management of portal hypertensive gastropathy and gastric antral vascular ectasia in cirrhosis. *Aliment Pharmacol Ther* 2014; **40**: 354-362 [PMID: 24889902 DOI: 10.1111/apt.12824]

6 **Fuccio L**, Mussetto A, Laterza L, Eusebi LH, Bazzoli F. Diagnosis and management of gastric antral vascular ectasia. *World J Gastrointest Endosc* 2013; **5**: 6-13 [PMID: 23330048 DOI: 10.4253/wjge.v5.i1.6]

7 **Ito M**, Uchida Y, Kamano S, Kawabata H, Nishioka M. Clinical comparisons between two subsets of gastric antral vascular ectasia. *Gastrointest Endosc* 2001; **53**: 764-770 [PMID: 11375585 DOI: 10.1067/mge.2001.113922]

8 **Jabbari M**, Cherry R, Lough JO, Daly DS, Kinnear DG, Goresky CA. Gastric antral vascular ectasia: the watermelon stomach. *Gastroenterology* 1984; **87**: 1165-1170 [PMID: 6332757]

9 **Carpinelli L**, Primignani M, Preatoni P, Angeli P, Battaglia G, Beretta L, Bortoli A, Capria A, Cestari R, Cosentino F, Crotta S, Gerunda G, Lorenzini I, Maiolo P, Merighi A, Rossi A, Sangiovanni A, de Franchis R. Portal hypertensive gastropathy: reproducibility of a classification, prevalence of elementary lesions, sensitivity and specificity in the diagnosis of cirrhosis of the liver. A NIEC multicentre study. New Italian Endoscopic Club. *Ital J Gastroenterol Hepatol* 1997; **29**: 533-540 [PMID: 9513828]

10 **Sarin SK**, Sreenivas DV, Lahoti D, Saraya A. Factors influencing development of portal hypertensive gastropathy in patients with portal hypertension. *Gastroenterology* 1992; **102**: 994-999 [PMID: 1537536 DOI: 10.1016/0016-5085(92)90188-5]

11 **Payen JL**, Calès P, Voigt JJ, Barbe S, Pilette C, Dubuisson L, Desmorat H, Vinel JP, Kervran A, Chayvialle JA. Severe portal hypertensive gastropathy and antral vascular ectasia are distinct entities in patients with cirrhosis. *Gastroenterology* 1995; **108**: 138-144 [PMID: 7806035 DOI: 10.1016/0016-5085(95)90018-7]

12 **Achim AC**, Vesa SC, Dumitru E. The Efficacy of Virtual Chromoendoscopy in the Diagnosis of Portal Hypertensive Gastropathy. *J Gastrointestin Liver Dis* 2016; **25**: 289-293 [PMID: 27689191 DOI: 10.15403/jgld.2014.1121.253.chr]

13 **Hayashi S**, Saeki S. Endoscopic microvascular architecture of the portal hypertensive gastric muscosa on narrow band imaging. *Diges Endosc* 2007; 116 [DOI: 10.1111/j.1443-1661.2007.00701.x]

14 **Westerhoff M**, Tretiakova M, Hovan L, Miller J, Noffsinger A, Hart J. CD61, CD31, and CD34 improve diagnostic accuracy in gastric antral vascular ectasia and portal hypertensive gastropathy: An immunohistochemical and digital morphometric study. *Am J Surg Pathol* 2010; **34**: 494-501 [PMID: 20351488 DOI: 10.1097/PAS.0b013e3181d38f0a]

15 **Chang CY**, Chen PH, Hou MC, Chang WC, Yang TC, Hsin IF, Liao WC, Lee FY. Magnifying endoscopy with narrow-band image for diagnosing diffuse type of gastric antral vascular ectasia in cirrhotic patients. *Eur J Gastroenterol Hepatol* 2021; **33**: 495-500 [PMID: 32433425 DOI: 10.1097/MEG.0000000000001757]

16 **Bienia A**, Sodolski W, Luchowska E. The effect of chronic alcohol abuse on gastric and duodenal mucosa. *Ann Univ Mariae Curie Sklodowska Med* 2002; **57**: 570-582 [PMID: 12898897]

17 **Rex DK**, Kahi C, O'Brien M, Levin TR, Pohl H, Rastogi A, Burgart L, Imperiale T, Ladabaum U, Cohen J, Lieberman DA. The American Society for Gastrointestinal Endoscopy PIVI (Preservation and Incorporation of Valuable Endoscopic Innovations) on real-time endoscopic assessment of the histology of diminutive colorectal polyps. *Gastrointest Endosc* 2011; **73**: 419-422 [PMID: 21353837 DOI: 10.1016/j.gie.2011.01.023]

18 **Sharma P**, Savides TJ, Canto MI, Corley DA, Falk GW, Goldblum JR, Wang KK, Wallace MB, Wolfsen HC; ASGE Technology and Standards of Practice Committee. The American Society for Gastrointestinal Endoscopy PIVI (Preservation and Incorporation of Valuable Endoscopic Innovations) on imaging in Barrett's Esophagus. *Gastrointest Endosc* 2012; **76**: 252-254 [PMID: 22817781 DOI: 10.1016/j.gie.2012.05.007]

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ORIGINAL ARTICLE

Prospective Study

Non-alcoholic steatohepatitis in liver transplant recipients diagnosed by serum cytokeratin 18 and transient elastography: A prospective study

Alshaima Alhinai, Afsheen Qayyum-Khan, Xun Zhang, Patrick Samaha, Peter Metrakos, Marc Deschenes, Philip Wong, Peter Ghali, Tian-Yan Chen, Giada Sebastiani

ORCID number: Alshaima Alhinai 0000-0003-4928-3614; Afsheen Qayyum-Khan 0000-0001-8345-0346; Xun Zhang 0000-0002-1194-0140; Patrick Samaha 0000-0001-6104-2798; Peter Metrakos 0000-0002-6191-8136; Marc Deschenes 0000-0003-3966-1888; Philip Wong 0000-0002-3446-4116; Peter Ghali 0000-0001-5914-3231; Tian-Yan Chen 0000-0001-5724-7854; Giada Sebastiani 0000-0003-2655-8283.

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Alshaima Alhinai, Experimental Medicine, McGill University, Montreal H4A3J1, Canada

Afsheen Qayyum-Khan, Patrick Samaha, Marc Deschenes, Philip Wong, Tian-Yan Chen, Giada Sebastiani, Medicine, McGill University Health Centre, Montreal H4A3J1, Canada

Xun Zhang, Departments of Pediatrics and Epidemiology, McGill University, Montreal H4A3J1, Canada

Peter Metrakos, Cancer Research Program, The Research Institute of McGill University and The Research Institute of McGill University Health Center, Montreal H4A3J1, Canada

Peter Ghali, Medicine, University of Florida, Jacksonville, Florida 32218, United States

Corresponding author: Giada Sebastiani, MD, Associate Professor, Medicine, McGill University Health Centre, 1001 boulevard Decarie, Montreal H4A3J1, Canada.
giada.sebastiani@mcgill.ca

Abstract

BACKGROUND

Nonalcoholic fatty liver disease (NAFLD) and nonalcoholic steatohepatitis (NASH) seem common after liver transplantation.

AIM

To investigate incidence and predictors of NAFLD and NASH by employing noninvasive testing in liver transplant recipients, namely controlled attenuation parameter (CAP) and the serum biomarker cytokeratin 18 (CK-18). We also evaluated the diagnostic accuracy of CK-18 and CAP compared to liver histology.

METHODS

We prospectively recruited consecutive adult patients who received liver transplant at the McGill University Health Centre between 2015-2018. Serial measurements of CK-18 and CAP were recorded. NAFLD and NASH were diagnosed by CAP ≥ 270 dB/m, and a combination of CAP ≥ 270 dB/m with CK-18 > 130.5 U/L, respectively. Incidences and predictors of NAFLD and NASH were investigated using survival analysis and Cox proportional hazards.

Clinical trial registration statement: The study was registered at ClinicalTrials.gov (NCT03128918).

Informed consent statement: All patients provided their informed written consent prior to participation.

Conflict-of-interest statement: Deschenes M has served as an advisory board member for Merck, Janssen, Gilead; Wong P has acted as consultant for BMS, Gilead, Merck, Novartis; Sebastiani G has acted as speaker for Pfizer, Merck, Novonordisk, Novartis, Gilead and AbbVie, served as an advisory board member for Merck, Gilead, Pfizer, Allergan, Novonordisk, Intercept and Novartis and has received research funding from Merck and Theratec. All other authors have no conflicts of interest to declare.

Data sharing statement: According to stipulations of the patient consent form signed by all study participants, ethical restrictions imposed by our Institutional Ethics review boards (Institutional Ethics Review Board Biomedical B Research Ethics Board of the McGill University Health Centre), and legal restrictions imposed by Canadian law regarding clinical trials, anonymized data are available upon request. Please send data access requests to Sheldon Levy, Biomedical B (BMB) Research Ethics Board (REB) Coordinator Centre for Applied Ethics, 5100, boul. de Maisonneuve Ouest, 5th floor, Office 576, Montréal, Québec, H4A 3T2, Canada.

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RESULTS

Overall, 40 liver transplant recipients (mean age 57 years; 70% males) were included. During a median follow-up of 16.8 mo (interquartile range 15.6-18.0), 63.0% and 48.5% of patients developed NAFLD and NASH, respectively. On multivariable analysis, after adjusting for sex and alanine aminotransferase, body mass index was an independent predictor of development of NAFLD [adjusted hazard ratio (aHR): 1.21, 95% confidence interval (CI): 1.04-1.41; P = 0.01] and NASH (aHR: 1.26, 95%CI: 1.06-1.49; P < 0.01). Compared to liver histology, CAP had a 76% accuracy to diagnose NAFLD, while the accuracy of CAP plus CK-18 to diagnose NASH was 82%.

CONCLUSION

NAFLD and NASH diagnosed non-invasively are frequent in liver transplant recipients within the first 18 mo. Close follow-up and nutritional counselling should be planned in overweight patients.

Key Words: Nonalcoholic steatohepatitis; Nonalcoholic fatty liver disease; Controlled attenuation parameter; Cytokeratin 18; Overweight; Accuracy

Core Tip: This is the first prospective study using cytokeratin 18 in association with transient elastography with controlled association parameter to investigate nonalcoholic fatty liver disease (NAFLD) and nonalcoholic steatohepatitis (NASH) in liver transplant recipients. NAFLD and NASH diagnosed by non-invasive tests occur frequently in the first 18 mo from liver transplant. Overweight is the main risk factor. Non-invasive liver fibrosis markers have suboptimal accuracy.

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INTRODUCTION

In recent years, there has been a shift in the etiologies of liver diseases leading to liver transplantation (LT): Chronic hepatitis C is declining, while nonalcoholic fatty liver disease (NAFLD) is on the rise. NAFLD affects 25.24% of the general population globally, driven by the epidemic of metabolic conditions such as obesity and type 2 diabetes mellitus[1-3]. NAFLD is an umbrella term encompassing a spectrum of clinical and pathologic features characterized by a fatty overload involving over 5% of the liver weight in the absence of other causes of liver disease. It ranges from simple steatosis or nonalcoholic fatty liver (NAFL) to nonalcoholic steatohepatitis (NASH). Without treatment, NAFL can evolve to NASH, liver fibrosis and cirrhosis, eventually resulting in liver failure and hepatocellular carcinoma (HCC)[2,4]. NASH is now the second leading indication for liver transplant in North America and is projected to become the main indication in the next 10 years[5,6].

In contrast to alcoholic liver disease, the mitigation of NASH risk factors is not a requirement for transplant eligibility. Hence, risk factors for NASH may persist or worsen after LT, placing these recipients at risk for recurrence. *De novo* NASH in patients transplanted for other etiologies of liver disease can also occur due to excess of metabolic risk factors following LT, including type 2 diabetes mellitus, rapid weight gain, hypertension, hyperlipidemia. Immunosuppressive medications may also play a role, as both corticosteroids and calcineurin inhibitors promote diabetes, hypertension and hypercholesterolemia[7,8]. About 20% and 10% of LT recipients develop *de novo* NAFLD and NASH, respectively[8]. Recurrent NAFLD and NASH can be as frequent as 62% and 33%, respectively. NAFLD is a common occurrence within 6 mo, whereas

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the onset of NASH occurs in a period of 6 mo to 1 year in several studies[9]. Due to these reasons, LT recipients may require monitoring to detect changes to the liver graft and prevent hepatic failure and mortality. The majority of studies evaluating recurrent NAFLD and NASH in LT recipients have been of retrospective nature, with no serial monitoring. Hence, longitudinal, prospective data on the frequency of NAFLD and NASH are lacking in the first months following LT. Protocol biopsies have long been used to identify liver disease recurrence and guide management. However, liver biopsy is invasive, costly and prone to sampling error[10]. Recent non-invasive tools for the diagnosis of hepatic steatosis and fibrosis include the measurement of liver stiffness by transient elastography (TE) and the associated controlled attenuation parameter (CAP)[2,11-13]. The accuracy of TE for the diagnosis of liver graft fibrosis seems similar to the non-transplant population[14]. Few studies have investigated the accuracy of CAP in the post-transplant setting[15,16]. Serum cytokeratin 18 (CK-18) has been proposed for the non-invasive diagnosis of NASH. CK-18 is the major intermediate filament protein in the liver and one of the most prominent substrates of caspases during hepatocyte apoptosis. Apoptotic cell death of hepatocytes is associated with the release of caspase-cleaved CK-18 fragments into the bloodstream [17]. Apoptotic activity occurs in NASH but not in NAFL, as such the presence of CK-18 fragments in the blood may differentiate the two conditions[17-19]. In a meta-analysis of over 1600 patients, CK-18 predicted the presence of NASH with a pooled area under the curve (AUC) of 0.82[20]. One report suggests that CK-18 could also have a prognostic value in predicting one-year survival post-LT[21]. No study has employed CK-18 to diagnose NASH in LT recipients.

We prospectively investigated incidence and predictors of NAFLD and NASH diagnosed by TE with CAP and CK-18 in LT recipients within the first 18 mo post-transplantation. We also studied the diagnostic accuracy of non-invasive tests compared to paired liver biopsies performed as a part of clinical care.

MATERIALS AND METHODS

Study design and population

This was a prospective, longitudinal study conducted at a single site, the McGill University Health Center (MUHC) Solid Organ Transplant Unit, and it included all eligible and consecutive patients who underwent LT between March 2015 and June 2018. Since 1990, a computerized database on all LT recipients has been maintained into which demographic data, clinical diagnosis, laboratory results, and prescription information had been prospectively entered. In order to be included, patients had to fulfill the following criteria: Age > 18 years; patient and graft survival > 6 mo; a minimum follow-up of 1 year. Exclusion criteria were any of the following: LT due to chronic hepatitis C, genotype 3; patients who received liver grafts involving more than 10% steatosis; failure of TE with CAP examination or unreliable measurement at study entry. The immunosuppressive regimen used as a standard by the LT program is induction with anti-thymocyte globulin, tacrolimus and mycophenolate mofetil as maintenance immunosuppression and rapid prednisone taper. Overweight and obesity were defined as body mass index (BMI) > 25 and > 30 kg/m^2, respectively.

Ethics

The study was approved by the Research Ethics Board of the Research Institute of MUHC (code 15-002-MUHC) and was registered at ClinicalTrials.gov (NCT03128918). The study was conducted according to the Declaration of Helsinki and Good Clinical Practice guidelines. All patients provided their informed written consent prior to participation.

Study assessment

Study visits were scheduled at baseline, month 3, 6, 9, 12 and 18, for a total of 5 visits (Figure 1). The following parameters were collected at each study visit: BMI, laboratory tests for hematology, blood chemistry. The questionnaire Alcohol Use Disorders Identification Test (AUDIT-C) was administered[22]. TE with CAP measurement and plasma to measure CK-18 were also acquired at each study visit. TE examination was performed in patients fasting for at least 3 h using FibroScan 502 Touch (Echosens, Paris, France). The same two experienced operators performed all elastographic measurements. The standard M probe was used in all patients. The XL probe was used in cases of failure of TE with the M probe or if BMI > 30 kg/m^2. The following criteria were applied to define the result of TE as reliable: At least 10

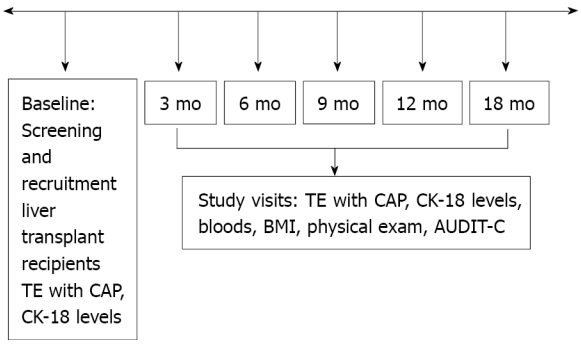

Figure 1 Study design showing baseline and study visit. AUDIT-C: Alcohol Use Disorders Identification Test; BMI: Body mass index; CAP: Controlled attenuation parameter; TE: Transient elastography; CK-18: Cytokeratin 18.

validated measurements and an interquartile range (IQR) < 30% of the median liver stiffness measurement (LSM)[23]. Available liver biopsies were used for the diagnostic accuracy study. Liver biopsy was performed at the discretion of the treating transplant hepatologist, as part of standard of care. All biopsies were obtained with a 16G Tru-Cut type needle and interpreted by two experienced liver pathologists. The stage of fibrosis was reported according to the Kleiner classification[24]. The NAFLD activity score (NAS) was calculated as the unweighted sum of the scores for steatosis (0-3), lobular inflammation (0-3) and hepatocellular ballooning (0-2). A diagnosis of NASH was made if NAS ≥ 5[24]. The CAP cut-off used for diagnosis of NAFLD was 270 dB/m, as recently reported in LT recipients[16]. Plasma stored at -80 °C was used for quantitative measurement of CK-18 levels by the Human cytokeratin ELISA kit (MJS Biolynx inc, Brockville Ontario, Canada). A cut-off of CK-18 > 130.5 U/L was used to indicate significant hepatocyte apoptosis, diagnostic for NASH when combined with CAP > 270 dB/m[25,26]. Liver fibrosis (stage ≥ 1 out of 4) was diagnosed as LSM ≥ 7.4 kPa[16]. The following simple serum fibrosis biomarkers were also computed: Hepatic steatosis index (HSI), defined as 8 × aspartate aminotransferase (AST)/alanine aminotransferase (ALT) + BMI (+ 2, if female; +2, if diabetes mellitus present)[27], fibrosis-4 (FIB-4), calculated as [age (years) × AST]/[platelet count (10^9/L) × ALT][28], and AST to platelet ratio (APRI), calculated as {[AST level/AST (upper limit of normal)]/platelet count (10^9/L) × 100}[29]. Liver fibrosis was defined as FIB-4 > 3.64 and APRI > 1, as previously described in the liver transplant setting[30].

Statistical analysis

The performance of the non-invasive tests to diagnose NAFLD, NASH and liver fibrosis was measured with the following: Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), accuracy, positive and negative likelihood ratios (LR^+ and LR^-, respectively). Correlation coefficients of TE with CAP with serum biomarkers were calculated using the Pearson correlation analysis. For the longitudinal analysis, baseline (study entry) corresponded to the day of LT. Patients were followed until March 2020 or were censored either when they developed the outcome or at their last study visit (18 mo post-LT). At each visit, complete medical history and physical examination were performed along with routine laboratory work-up. Standard diagnostic and therapeutic management following LT was offered during the follow-up. Continuous variables were expressed as mean (standard deviation), and categorical variables were presented as numbers (%). We estimated incidence rates of NAFLD and NASH by dividing the number of participants developing the outcome by the number of person-years (PY) of follow-up. Poisson count models were used to calculate CI for incidence rates. Multivariable time-dependent Cox regression models were constructed to assess predictors of the development of NAFLD and NASH and included covariates that were determined a priori to be clinically important and with a *P*-value < 0.1 on univariable analysis. The final model was adjusted for sex, BMI and ALT. Robust variance estimation was used in all Cox regression analyses to account for the correlation of data contributed by the same participant at multiple visits. We considered an association with the outcome significant when the 95%CI excluded one. We generated Kaplan-Meier curves to illustrate and compare the cumulative incidence of NAFLD and NASH in overweight *vs* normal weight patients. The log-rank test was used to evaluate differences among incidences. All tests were two-tailed and with a significance level of $\alpha = 0.05$. Statistical analysis was performed using STATA 15 (StataCorp LP, TX, United States).

RESULTS

After applying exclusion criteria, 40 LT recipients were included in this prospective study (Figure 2). The main demographic, clinical and biochemical characteristics of the study population at baseline are summarized in Table 1. Univariable analysis by outcome category of NAFLD and NASH is also reported. Overall, mean age was 57.3 years and 70% of patients were male. The most frequent indications for LT were NASH and HCC. Metabolic comorbidities were frequent, with overweight, type 2 diabetes mellitus and hypertension affecting 40%, 35% and 37.5% of the patients, respectively. Patients who developed NAFLD and NASH during the follow-up period were more frequently transplanted for NASH and on tacrolimus as immunosuppressant.

Diagnostic accuracy of non-invasive tests compared to liver histology and correlation between TE with CAP and serum biomarkers

During the study period, 35 liver biopsies (mean length ± SD: 1.7 ± 0.4 cm) from 24 patients were available. The median time between liver biopsies and non-invasive diagnostic testing was 38.6 ± 30 d. Table 2 shows the performance of non-invasive tests compared to liver histology. The diagnostic accuracy of CAP and HSI for NAFLD was 76% and 45.7%, respectively. The diagnostic accuracy of a combination of CAP ≥ 270 dB/m and CK-18 > 130.5 to diagnose NASH was 82%. The diagnostic accuracy of LSM, FIB-4 and APRI for liver fibrosis was low at 57.8%, 48.7% and 54.1%, respectively. There was a medium positive correlation between CAP and HSI of 0.4. There was a medium positive correlation between LSM and FIB-4 of 0.4, and a weak positive correlation between LSM and APRI of 0.1.

Incidence and predictors of NAFLD and NASH by CAP and CK-18

During a median follow-up of 16.8 mo (IQR: 15.6-18.0), 22 patients (63.0%) developed NAFLD (incidence rate: 71.0 per 100 PY, 95%CI: 45.0-78.0), and 17 patients (48.5%) developed NASH (incidence rate: 48.6 per 100 PY, 95%CI: 31.4-66.0). On multivariate Cox regression analysis, BMI was an independent predictor of both NAFLD (adjusted HR: 1.1, 95%: 1.0-1.2) and NASH (adjusted HR: 1.1, 95%CI: 1.0-1.3) (Table 3). To further elaborate on the effect of high BMI on the incidence of NAFLD and NASH, a hazard plot was performed and showed that overweight was a significant risk factor for both NAFLD and NASH (log-rank, $P < 0.01$, respectively) (Figure 3).

Changes in LSM, FIB-4 and APRI during follow-up

Given the low accuracy for the non-invasive fibrosis tests, we studied changes in LSM, FIB-4 and APRI during the follow-up. While the majority of patients had an LSM ranging from 2.5 to 15 kPa, there were patients who developed marked increases, and these were observed in the first six months of follow-up (Figure 4A). Similarly, while most of the patients had FIB-4 and APRI ranging from 1 to 2.5 and from 0.5 to 1.5, respectively, there were patients who developed marked increases during the first six months of follow-up (Figures 4B and 4C).

DISCUSSION

In this prospective study, we have shown that NAFLD and NASH diagnosed non-invasively are frequent occurrences in the first 18 mo from LT. Similar to results reported in previous retrospective studies, the majority of incident NAFLD and NASH in our population occurred within the first year of LT[31-33]. The main predictor of these events was high BMI, thus underlying the importance of controlling the weight beginning from the first 3 mo post-LT. We also showed that the diagnostic accuracy of non-invasive tests for NAFLD is good and similar to previously reported, while non-invasive fibrosis tests have low accuracy in the first months following LT. Finally, we first report the accuracy of the apoptotic biomarker CK-18 combined with CAP for the diagnosis of NASH.

We compared the performance of non-invasive tests to liver biopsy. We used a CAP cut-off ≥ 270 dB/m, as referenced by Siddiqui *et al*[16], and compared it to the presence of steatosis grade 0 *vs* 1-3 on liver biopsy. Our results showed a lower sensitivity (58% *vs* 74%), however the specificity (86% *vs* 87%), PPV (70% *vs* 78%) and NPV (79% *vs* 84%) were similar. The variations can be explained by the different population sizes, number of available liver biopsies and the timing of the study conducted within the

Table 1 Characteristics of patients at study entry

	Whole cohort	Patients who developed NAFLD	Patients who developed NASH
	n = 40	*n* = 22	*n* = 17
Age (yr)	57.3 ± 8.5	55.5 ± 9.2	56.3 ± 7.9
Male (%)	28 (70)	18 (82)	14 (82)
Ethnicity (%)			
Caucasian	32 (80)	19 (86)	15 (88)
Other (Asian, Black, Arab)	8 (20)	3 (14)	2 (11)
Etiology of liver disease (%)			
NASH	21 (52.5)	13 (52)	12 (70)
HCC	9 (22.5)	2 (9)	2 (12)
HCV (excluding genotype 3)	8 (20)	6 (27)	3 (18)
Alcoholic liver disease	1 (2.5)	1 (4.5)	0
Other	1 (2.5)	0	0
BMI (kg/m^2)	24.8 ± 4.6	26.2 ± 5.1	26.6 ± 4.5
BMI >25 (%)	18 (40)	14 (64)	12 (70)
Comorbidities (%)			
Diabetes	14 (35)	9 (41)	8 (47)
Hypertension	15 (37.5)	7 (32)	8 (47)
Dyslipidemia	6 (15)	6 (27)	5 (29)
MELD-Na Score	< 9	< 9	< 9
Laboratory			
AST (U/L)	27.6 ± 33	31.8 ± 41.2	34.5 ± 45.1
ALT (U/L)	32.8 ± 42.8	37.6 ± 52.6	40.6 ± 57.7
GGT (U/L)	177.5 ± 256.6	177.7 ± 271.4	188.1 ± 297.6
Bilirubin (μmol/L)	17 ± 15.9	18.2 ± 17.3	18 ± 18.2
INR	1.25 ± 1.39	1.05 ± 0.12	1.04 ± 1.3
Albumin (g/L)	39.6 ± 3.69	38.7 ± 4.3	39.4 ± 3.9
Platelets (10^9/L)	172.3 ± 86.9	185 ± 92.5	170.5 ± 93.6

ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; BMI: Body mass index; GGT: Gamma-glutamyl transpeptidase; HCV: Hepatitis C virus; HCC: Hepatocellular carcinoma; INR: International normalized ratio; MELD-Na: Model for end stage liver disease-sodium; NAFLD: Nonalcoholic fatty liver disease; NASH: Nonalcoholic steatohepatitis.

first 18 mo from LT. When HSI was compared to histology, it showed less accuracy than CAP as demonstrated before in other studies on non-LT populations[34,35]. Secondly, we used a combination of CK-18 > 130.5 with CAP ≥ 270 dB/m and compared it to the presence of NASH (NAS ≥ 5 or proven NASH) on liver histology. To our knowledge, this is the first study to use CK-18 to detect NASH in LT patients. Compared to one meta-analysis of over 1600 patients that assessed the accuracy of CK-18 (cut-off range: 121.6-380.2 U/L) in non-transplanted patients with NASH, our results are similar for both sensitivity (75% *vs* 78%) and specificity (83% *vs* 87%)[20]. Compared to another more recent meta-analysis of over 1400 patients that evaluated the diagnostic value of CK-18 for the diagnosis of NASH, our results also reported similar sensitivity (75% *vs* 75%), specificity (83% *vs* 77%), LR^+ (4.5 *vs* 3.3), and LR^- (0.3 *vs* 0.3)[36].

There are two interesting points. Firstly, our cut-off values of all the non-invasive biomarkers reported a higher NPV than PPV which could indicate that these tests are more efficient at ruling-out NAFLD, NASH and liver fibrosis rather than ruling-in these diseases, as previously described[16,37]. However, their ability to minimize the

Table 2 Diagnostic accuracy of non-invasive tests compared to liver histology (*N* = 35 from 24 patients)

	NAFLD		NASH	Liver fibrosis		
	CAP	HSI	CAP + CK-18	LSM	FIB-4	APRI
Sensitivity (%)	58	64.3	75	61.9	7.1	14.3
Specificity (%)	86	33	83	54.2	73.9	78.3
PPV (%)	70	39	37	54.2	14.3	28.6
NPV (%)	79	58	96	61.9	56.7	60
LR^+	4.28	0.96	4.5	1.35	0.27	0.66
LR^-	0.48	1.07	0.3	0.7	1.26	1.1
Accuracy (%)	76	45.7	82	57.8	48.7	54.1

APRI: Aspartate aminotransferase-to-Platelets Ratio Index; CAP: Controlled attenuation parameter; CK-18: Cytokeratin 18; FIB-4: Fibrosis 4 index; HSI: Hepatic steatosis index; LSM: Liver stiffness measurement; LR: Likelihood ratio; MELD-Na: Model for end stage liver disease-sodium; NAFLD: Nonalcoholic fatty liver disease; NASH: Nonalcoholic steatohepatitis; NPV: Negative predictive value; PPV: Positive predictive value.

Table 3 Risk factors for post-Liver Transplant development of nonalcoholic fatty liver disease and nonalcoholic steatohepatitis using univariate and multivariate Cox regression analysis

	NAFLD				NASH			
	Univariate analysis		Multivariate analysis		Univariate analysis		Multivariate analysis	
	HR (95%CI)	*P* value	aHR (95%CI)	*P* value	HR (95%CI)	*P* value	aHR (95%CI)	*P* value
Female sex (yes vs no)	0.6 (0.4-1.2)	0.1	0.9 (0.3-1.7)	0.5	0.6 (0.3-1.1)	0.1	0.9 (0.4-2.1)	0.8
Age (per year)	1.0 (0.9-1.0)	0.6			1.0 (0.9-1.0)	0.9		
BMI (per kg/m^2)	1.1 (1.0-1.2)	< 0.01	1.1 (1.0-1.2)	< 0.01	1.1 (1.0-1.2)	0.01	1.1 (1.0-1.3)	< 0.01
Diabetes (yes *vs* no)	1.7 (1.0-2.7)	0.02			1.3 (0.7-2.1)	0.3		
Dyslipidemia (yes *vs* no)	4.6 (1.7-12.8)	< 0.01			4.4 (1.5-13)	0.007		
ALT (per U/L)	1.0 (0.9-1.0)	0.09	1 (0.9-1.0)	0.3	1.0 (1.0-1.0)	0.03	1 (0.9-1.0)	0.1

aHR: Adjusted hazard ratio; ALT: Alanine aminotransferase; BMI: Body mass index; CI: Confidence interval; HR: Hazard ratio; NAFLD: Nonalcoholic fatty liver disease; NASH: Nonalcoholic steatohepatitis.

need for liver biopsy in this clinical setting still requires further validation. Secondly, while we combined CK-18 with CAP to diagnose NASH, our results are very closely related to those the two meta-analyses which used CK-18 alone to diagnose NASH. This makes us question the role of combining CAP with CK-18 to diagnose NASH. Two studies investigated the combined use of CK-18 with TE to detect fibrosis and found either no significant improvement or only some improvement in AUC by combining CK-18 and TE compared to using a single test[38,39]. Yet, other studies have shown that combining CK-18 with other biomarkers improves the accuracy to diagnose NASH[40,41]. Our analysis must be replicated in a larger sample using different combinations of biomarkers to better understand this.

Our results are comparable to a recent cross-sectional study by Mikolasevic *et al*[15] which reported a prevalence of liver steatosis of 68.6% and severe liver steatosis of 46.8% in LT recipients using CAP and LSM. Our incidence rates are also comparable to previously published meta-analyses and retrospective studies, while minor variations are most likely due to the difference in populations, the cut-off values to define steatosis/NAFLD and NASH, and the absence of the use of CK-18 as a diagnostic tool in those studies[15,31-33]. On multivariate Cox regression analysis, high BMI was the main risk factor for the development of NAFLD and NASH in patients post LT, conceding with results from previous studies[15,31]. Obesity is an independent risk factor for the development of NAFLD and NASH and can occur or continue to be present even during the first months post-LT. Indeed, other studies have shown that the maximum weight gain occurs in the first year post LT mainly because of the use of

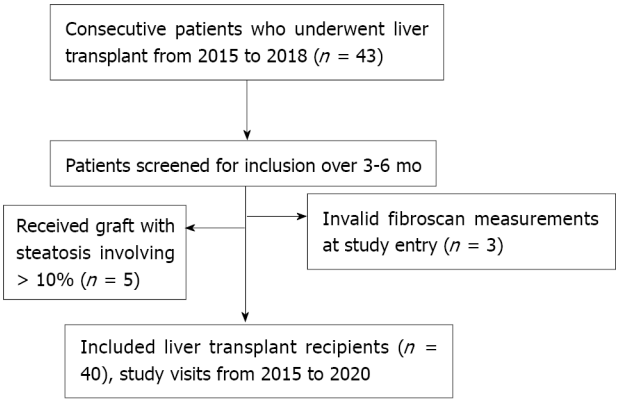

Figure 2 Flow chart displaying the selection of study participants. Of 48 consecutive patients undergoing liver transplant, 3 were excluded because of invalid TE examination and 5 because they received a liver graft with steatosis involving > 10% of hepatocytes. TE: Transient elastography.

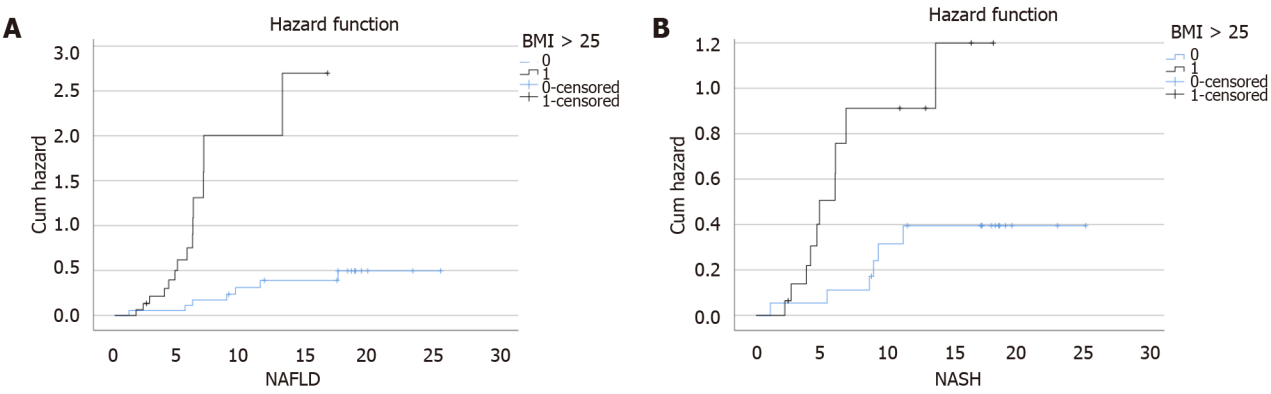

Figure 3 Hazard ratio by body mass index category in nonalcoholic fatty liver disease (log-rank: *P* < 0.0001) and in nonalcoholic steatohepatitis (log-rank: *P* = 0.009). BMI: Body mass index; NAFLD: Nonalcoholic fatty liver disease; NASH: Nonalcoholic steatohepatitis.

immunosuppressive medications[42,43]. Type 2 diabetes mellitus and dyslipidemia were significant risk factors on univariate analysis, also in line with previous results [15]. The presence of these risk factors poses a risk for the development of fatty deposits in the graft and progression to NAFLD and NASH. Therefore, strategies must be implemented both before and after LT to control and prevent the progression of liver disease. These strategies include weight reduction with a low carbohydrate diet and performing regular exercise, avoiding alcohol and smoking, controlling of comorbid metabolic diseases, and controlling immunosuppression medications post-LT.

We also reported a low performance of non-invasive fibrosis tests during the first 18 mo following LT. Similar findings have been reported previously in post-LT patients with HCV recurrence. El-Meteini *et al*[44] concluded that TE and APRI were not correlated with the degree of fibrosis in liver biopsy done at 3 mo post-LT in 31 patients. Other studies reported a poor diagnostic accuracy of APRI and FIB-4 compared to liver biopsy for the presence of advanced fibrosis post-LT[45,46]. Indeed, some of our patients experienced an important variation in LSM, FIB-4 and APRI particularly during the first 6 mo post-LT. This could be due to several reasons. Inflammation due to congestion or cholestasis is common post-LT and could be one reason for the inaccuracy of fibrosis tests. Fluctuations in liver enzymes and platelets during the first 6 mo may also account for these findings as LT recipients have started receiving and adjusting their immunosuppressive medications. Since a majority of our liver recipients were overweight, this could have interfered with the LSM results[47]. Since our study and the previous studies were performed on small cohorts, a conclusion regarding the accuracy of non-invasive fibrosis tests cannot be made.

There are limitations to our study. The sample size was small which could have interfered with the interpretation of the results. Nevertheless, our incidence rates and predictors are similar to previous retrospective studies[15,31-33]. Additionally, not all patients had available liver biopsy to compare with non-invasive tests. Only 24 out of

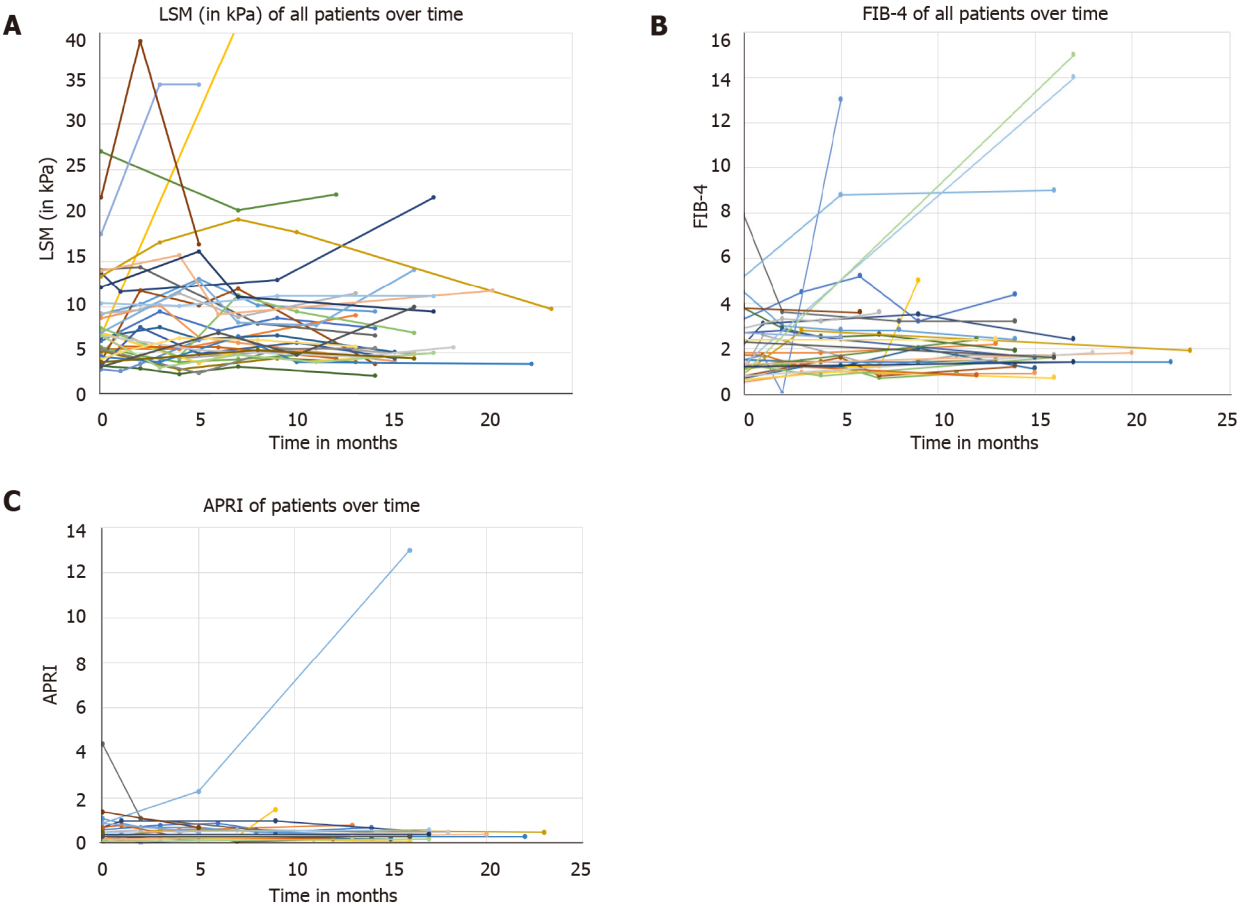

Figure 4 Spaghetti plot of changes. A: Spaghetti plot of changes in liver stiffness measurement during study period; B: Spaghetti plot of changes in fibrosis-4 during study period; C: Spaghetti plot of changes in aspartate aminotransferase-to-Platelets Ratio Index. APRI: Aspartate aminotransferase-to-Platelets Ratio Index; FIB-4: Fibrosis-4; LSM: Liver stiffness measurement.

40 patients required liver biopsy during follow up therefore the comparison was only possible in these patients, for a total of 35 liver biopsies. Regardless of this, the results obtained from our study provide a rationale for the use of non-invasive tests to frequently monitor this patient population, which could not be feasible with liver biopsy, and can be viewed as an opportunity for larger studies to be done on this topic. Another limitation of our study is that CK-18 is not currently a routine test, as such its application to clinical practice should be further explored. The median study length was 16.8 mo, so in the future we plan to continue following these patients for a longer duration by monitoring CAP scores and re-occurrence of steatosis.

CONCLUSION

In conclusion, our study showed that LT recipients have a high risk of developing NAFLD and NASH during the first 18 mo following LT, mainly driven by high BMI. While CAP and CK-18 are promising non-invasive tools for diagnosing NAFLD and NASH, LSM and other fibrosis biomarkers are not reliable tests in detecting liver fibrosis in the first month post-transplant. Larger scale, long-term data on the use of non-invasive tests is needed to determine their accuracy to diagnose and monitor disease progression, as well as their prognostic value. These data may result in the implementation of non-invasive tests and optimization of surveillance.

ARTICLE HIGHLIGHTS

Research background

Nonalcoholic fatty liver disease (NAFLD) is a major indication for liver transplant (LT)

globally. NAFLD and nonalcoholic steatohepatitis (NASH) may occur after LT.

Research motivation

Studies on the incidence of NASH and NAFLD in the first months following LT are limited.

Research objectives

This work aimed to determine the incidence of NASH and NAFLD in the first 18 mo following LT by means of non-invasive diagnostic tests. It also aimed to investigate the diagnostic accuracy of these non-invasive tests compared to liver histology.

Research methods

Consecutive adult patients who received LT at a single center were recruited between 2015-2018. Serial measurements of the biomarker cytokeratin 18 (CK-18) and controlled attenuation parameter (CAP) were recorded. NAFLD and NASH were diagnosed by CAP ≥ 270 dB/m, and a combination of CAP ≥ 270 dB/m with CK-18 > 130.5 U/L, respectively. Incidence and predictors of NAFLD and NASH were investigated using survival analysis.

Research results

During a median follow-up of 16.8 mo, 63% and 48.5% of 40 LT recipients developed NAFLD and NASH, respectively. The diagnostic accuracy for NAFLD and NASH was 76% and 82%, respectively.

Research conclusions

NAFLD and NASH diagnosed by CAP and CK-18 are frequent in LT recipients within the first 18 mo.

Research perspectives

To improve post-transplant outcomes, close follow-up with non-invasive tests and metabolic counselling could be considered.

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REFERENCES

1 **Marchesini G**, Brizi M, Bianchi G, Tomassetti S, Bugianesi E, Lenzi M, McCullough AJ, Natale S, Forlani G, Melchionda N. Nonalcoholic fatty liver disease: a feature of the metabolic syndrome. *Diabetes* 2001; **50**: 1844-1850 [PMID: 11473047 DOI: 10.2337/diabetes.50.8.1844]

2 **Ratziu V**, Bellentani S, Cortez-Pinto H, Day C, Marchesini G. A position statement on NAFLD/NASH based on the EASL 2009 special conference. *J Hepatol* 2010; **53**: 372-384 [PMID: 20494470 DOI: 10.1016/j.jhep.2010.04.008]

3 **Williams CD**, Stengel J, Asike MI, Torres DM, Shaw J, Contreras M, Landt CL, Harrison SA. Prevalence of nonalcoholic fatty liver disease and nonalcoholic steatohepatitis among a largely middle-aged population utilizing ultrasound and liver biopsy: a prospective study. *Gastroenterology* 2011; **140**: 124-131 [PMID: 20858492 DOI: 10.1053/j.gastro.2010.09.038]

4 **Chalasani N**, Younossi Z, Lavine JE, Charlton M, Cusi K, Rinella M, Harrison SA, Brunt EM, Sanyal AJ. The diagnosis and management of nonalcoholic fatty liver disease: Practice guidance from the American Association for the Study of Liver Diseases. *Hepatology* 2018; **67**: 328-357 [PMID: 28714183 DOI: 10.1002/hep.29367]

5 **Mikolasevic I**, Filipec-Kanizaj T, Mijic M, Jakopcic I, Milic S, Hrstic I, Sobocan N, Stimac D, Burra P. Nonalcoholic fatty liver disease and liver transplantation - Where do we stand? *World J Gastroenterol* 2018; **24**: 1491-1506 [PMID: 29662288 DOI: 10.3748/wjg.v24.i14.1491]

6 **Wong RJ**, Aguilar M, Cheung R, Perumpail RB, Harrison SA, Younossi ZM, Ahmed A. Nonalcoholic steatohepatitis is the second leading etiology of liver disease among adults awaiting liver transplantation in the United States. *Gastroenterology* 2015; **148**: 547-555 [PMID: 25461851 DOI: 10.1053/j.gastro.2014.11.039]

7 **Marchetti P**, Navalesi R. The metabolic effects of cyclosporin and tacrolimus. *J Endocrinol Invest* 2000; **23**: 482-490 [PMID: 11005276 DOI: 10.1007/BF03343761]

8 **Seo S**, Maganti K, Khehra M, Ramsamooj R, Tsodikov A, Bowlus C, McVicar J, Zern M, Torok N. De novo nonalcoholic fatty liver disease after liver transplantation. *Liver Transpl* 2007; **13**: 844-847 [PMID: 17029282 DOI: 10.1002/lt.20932]

9 **Patil DT**, Yerian LM. Evolution of nonalcoholic fatty liver disease recurrence after liver transplantation. *Liver Transpl* 2012; **18**: 1147-1153 [PMID: 22740341 DOI: 10.1002/lt.23499]

10 **Sebastiani G**, Gkouvatsos K, Plebani M. Non-invasive assessment of liver fibrosis: it is time for laboratory medicine. *Clin Chem Lab Med* 2011; **49**: 13-32 [PMID: 20961196 DOI: 10.1515/CCLM.2011.001]

11 **European Association for the Study of the Liver**. . EASL Clinical Practice Guidelines: management of hepatitis C virus infection. *J Hepatol* 2011; **55**: 245-264 [PMID: 21371579 DOI: 10.1016/j.jhep.2011.02.023]

12 **Sherman M**, Shafran S, Burak K, Doucette K, Wong W, Girgrah N, Yoshida E, Renner E, Wong P, Deschênes M. Management of chronic hepatitis B: consensus guidelines. *Can J Gastroenterol* 2007; **21** Suppl C: 5C-24C [PMID: 17568823]

13 **Shiha G**, Sarin SK, Ibrahim AE, Omata M, Kumar A, Lesmana LA, Leung N, Tozun N, Hamid S, Jafri W, Maruyama H, Bedossa P, Pinzani M, Chawla Y, Esmat G, Doss W, Elzanaty T, Sakhuja P, Nasr AM, Omar A, Wai CT, Abdallah A, Salama M, Hamed A, Yousry A, Waked I, Elsahar M, Fateen A, Mogawer S, Hamdy H, Elwakil R; Jury of the APASL Consensus Development Meeting 29 January 2008 on Liver Fibrosis With Without Hepatitis B or C. Liver fibrosis: consensus recommendations of the Asian Pacific Association for the Study of the Liver (APASL). *Hepatol Int* 2009; **3**: 323-333 [PMID: 19669358 DOI: 10.1007/s12072-008-9114-x]

14 **Barrault C**, Roudot-Thoraval F, Tran Van Nhieu J, Atanasiu C, Kluger MD, Medkour F, Douvin C, Mallat A, Zafrani ES, Cherqui D, Duvoux C. Non-invasive assessment of liver graft fibrosis by transient elastography after liver transplantation. *Clin Res Hepatol Gastroenterol* 2013; **37**: 347-352 [PMID: 23318116 DOI: 10.1016/j.clinre.2012.11.003]

15 **Mikolasevic I**, Hauser G, Mijic M, Domislovic V, Radic-Kristo D, Krznaric Z, Razov-Radas M, Pavic T, Matasin M, Filipec Kanizaj T. Assessment of Steatosis and Fibrosis in Liver Transplant Recipients Using Controlled Attenuation Parameter and Liver Stiffness Measurements. *Can J Gastroenterol Hepatol* 2021; **2021**: 6657047 [PMID: 33628759 DOI: 10.1155/2021/6657047]

16 **Siddiqui MS**, Idowu MO, Stromberg K, Sima A, Lee E, Patel S, Ghaus S, Driscoll C, Sterling RK, John B, Bhati CS. Diagnostic Performance of Vibration-Controlled Transient Elastography in Liver Transplant Recipients. *Clin Gastroenterol Hepatol* 2021; **19**: 367-374 [PMID: 32272251 DOI: 10.1016/j.cgh.2020.03.067]

17 **Feldstein AE**, Wieckowska A, Lopez AR, Liu YC, Zein NN, McCullough AJ. Cytokeratin-18 fragment levels as noninvasive biomarkers for nonalcoholic steatohepatitis: a multicenter validation study. *Hepatology* 2009; **50**: 1072-1078 [PMID: 19585618 DOI: 10.1002/hep.23050]

18 **Diab DL**, Yerian L, Schauer P, Kashyap SR, Lopez R, Hazen SL, Feldstein AE. Cytokeratin 18 fragment levels as a noninvasive biomarker for nonalcoholic steatohepatitis in bariatric surgery patients. *Clin Gastroenterol Hepatol* 2008; **6**: 1249-1254 [PMID: 18995215 DOI: 10.1016/j.cgh.2008.07.016]

19 **Feldstein AE**, Alkhouri N, De Vito R, Alisi A, Lopez R, Nobili V. Serum cytokeratin-18 fragment levels are useful biomarkers for nonalcoholic steatohepatitis in children. *Am J Gastroenterol* 2013; **108**: 1526-1531 [PMID: 23752877 DOI: 10.1038/ajg.2013.168]

20 **Musso G**, Gambino R, Cassader M, Pagano G. Meta-analysis: natural history of non-alcoholic fatty liver disease (NAFLD) and diagnostic accuracy of non-invasive tests for liver disease severity. *Ann Med* 2011; **43**: 617-649 [PMID: 21039302 DOI: 10.3109/07853890.2010.518623]

21 **Lorente L**, Rodriguez ST, Sanz P, Pérez-Cejas A, Padilla J, Díaz D, González A, Martín MM, Jiménez A, Barrera MA. Prognostic Value of Serum Caspase-Cleaved Cytokeratin-18 Levels before Liver Transplantation for One-Year Survival of Patients with Hepatocellular Carcinoma. *Int J Mol Sci* 2016; **17** [PMID: 27618033 DOI: 10.3390/ijms17091524]

22 **Higgins-Biddle JC**, Babor TF. A review of the Alcohol Use Disorders Identification Test (AUDIT), AUDIT-C, and USAUDIT for screening in the United States: Past issues and future directions. *Am J Drug Alcohol Abuse* 2018; **44**: 578-586 [PMID: 29723083 DOI: 10.1080/00952990.2018.1456545]

23 **Tapper EB**, Loomba R. Noninvasive imaging biomarker assessment of liver fibrosis by elastography in NAFLD. *Nat Rev Gastroenterol Hepatol* 2018; **15**: 274-282 [PMID: 29463906 DOI: 10.1038/nrgastro.2018.10]

24 **Kleiner DE**, Brunt EM, Van Natta M, Behling C, Contos MJ, Cummings OW, Ferrell LD, Liu YC, Torbenson MS, Unalp-Arida A, Yeh M, McCullough AJ, Sanyal AJ; Nonalcoholic Steatohepatitis Clinical Research Network. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. *Hepatology* 2005; **41**: 1313-1321 [PMID: 15915461 DOI: 10.1002/hep.20701]

25 **Arab JP**, Hernández-Rocha C, Morales C, Vargas JI, Solís N, Pizarro M, Robles C, Sandoval D, Ponthus S, Benítez C, Barrera F, Soza A, Riquelme A, Arrese M. Serum cytokeratin-18 fragment levels as noninvasive marker of nonalcoholic steatohepatitis in the chilean population. *Gastroenterol Hepatol* 2017; **40**: 388-394 [PMID: 28359548 DOI: 10.1016/j.gastrohep.2017.02.009]

26 **Benmassaoud A**, Ghali P, Cox J, Wong P, Szabo J, Deschenes M, Osikowicz M, Lebouche B, Klein MB, Sebastiani G. Screening for nonalcoholic steatohepatitis by using cytokeratin 18 and transient elastography in HIV mono-infection. *PLoS One* 2018; **13**: e0191985 [PMID: 29381754 DOI: 10.1371/journal.pone.0191985]

27 **Lee JH**, Kim D, Kim HJ, Lee CH, Yang JI, Kim W, Kim YJ, Yoon JH, Cho SH, Sung MW, Lee HS.

Hepatic steatosis index: a simple screening tool reflecting nonalcoholic fatty liver disease. *Dig Liver Dis* 2010; **42**: 503-508 [PMID: 19766548 DOI: 10.1016/j.dld.2009.08.002]

28 **Shah AG**, Lydecker A, Murray K, Tetri BN, Contos MJ, Sanyal AJ; Nash Clinical Research Network. Comparison of noninvasive markers of fibrosis in patients with nonalcoholic fatty liver disease. *Clin Gastroenterol Hepatol* 2009; **7**: 1104-1112 [PMID: 19523535 DOI: 10.1016/j.cgh.2009.05.033]

29 **Wai CT**, Greenson JK, Fontana RJ, Kalbfleisch JD, Marrero JA, Conjeevaram HS, Lok AS. A simple noninvasive index can predict both significant fibrosis and cirrhosis in patients with chronic hepatitis C. *Hepatology* 2003; **38**: 518-526 [PMID: 12883497 DOI: 10.1053/jhep.2003.50346]

30 **Imai H**, Kamei H, Onishi Y, Ishizu Y, Ishigami M, Goto H, Ogura Y. Diagnostic Usefulness of APRI and FIB-4 for the Prediction of Liver Fibrosis After Liver Transplantation in Patients Infected with Hepatitis C Virus. *Transplant Proc* 2018; **50**: 1431-1436 [PMID: 29705278 DOI: 10.1016/j.transproceed.2018.03.005]

31 **Chayanupatkul M**, Dasani DB, Sogaard K, Schiano TD. The Utility of Assessing Liver Allograft Fibrosis and Steatosis Post-Liver Transplantation Using Transient Elastography With Controlled Attenuation Parameter. *Transplant Proc* 2021; **53**: 159-165 [PMID: 32434740 DOI: 10.1016/j.transproceed.2020.02.160]

32 **Karlas T**, Kollmeier J, Böhm S, Müller J, Kovacs P, Tröltzsch M, Weimann A, Bartels M, Rosendahl J, Mössner J, Berg T, Keim V, Wiegand J. Noninvasive characterization of graft steatosis after liver transplantation. *Scand J Gastroenterol* 2015; **50**: 224-232 [PMID: 25429378 DOI: 10.3109/00365521.2014.983156]

33 **Saeed N**, Glass L, Sharma P, Shannon C, Sonnenday CJ, Tincopa MA. Incidence and Risks for Nonalcoholic Fatty Liver Disease and Steatohepatitis Post-liver Transplant: Systematic Review and Meta-analysis. *Transplantation* 2019; **103**: e345-e354 [PMID: 31415032 DOI: 10.1097/TP.0000000000002916]

34 **Chon YE**, Jung KS, Kim SU, Park JY, Park YN, Kim DY, Ahn SH, Chon CY, Lee HW, Park Y, Han KH. Controlled attenuation parameter (CAP) for detection of hepatic steatosis in patients with chronic liver diseases: a prospective study of a native Korean population. *Liver Int* 2014; **34**: 102-109 [PMID: 24028214 DOI: 10.1111/liv.12282]

35 **Xu L**, Lu W, Li P, Shen F, Mi YQ, Fan JG. A comparison of hepatic steatosis index, controlled attenuation parameter and ultrasound as noninvasive diagnostic tools for steatosis in chronic hepatitis B. *Dig Liver Dis* 2017; **49**: 910-917 [PMID: 28433586 DOI: 10.1016/j.dld.2017.03.013]

36 **He L**, Deng L, Zhang Q, Guo J, Zhou J, Song W, Yuan F. Diagnostic Value of CK-18, FGF-21, and Related Biomarker Panel in Nonalcoholic Fatty Liver Disease: A Systematic Review and Meta-Analysis. *Biomed Res Int* 2017; **2017**: 9729107 [PMID: 28326329 DOI: 10.1155/2017/9729107]

37 **Xiao G**, Zhu S, Xiao X, Yan L, Yang J, Wu G. Comparison of laboratory tests, ultrasound, or magnetic resonance elastography to detect fibrosis in patients with nonalcoholic fatty liver disease: A meta-analysis. *Hepatology* 2017; **66**: 1486-1501 [PMID: 28586172 DOI: 10.1002/hep.29302]

38 **Ergelen R**, Akyuz U, Aydin Y, Eren F, Yilmaz Y. Measurements of serum procollagen-III peptide and M30 do not improve the diagnostic accuracy of transient elastography for the detection of hepatic fibrosis in patients with nonalcoholic fatty liver disease. *Eur J Gastroenterol Hepatol* 2015; **27**: 667-671 [PMID: 25923941 DOI: 10.1097/MEG.0000000000000342]

39 **Rosso C**, Caviglia GP, Abate ML, Vanni E, Mezzabotta L, Touscoz GA, Olivero A, Marengo A, Rizzetto M, Bugianesi E, Smedile A. Cytokeratin 18-Aspartate396 apoptotic fragment for fibrosis detection in patients with non-alcoholic fatty liver disease and chronic viral hepatitis. *Dig Liver Dis* 2016; **48**: 55-61 [PMID: 26514735 DOI: 10.1016/j.dld.2015.09.008]

40 **Cao W**, Zhao C, Shen C, Wang Y. Cytokeratin 18, alanine aminotransferase, platelets and triglycerides predict the presence of nonalcoholic steatohepatitis. *PLoS One* 2013; **8**: e82092 [PMID: 24324749 DOI: 10.1371/journal.pone.0082092]

41 **Pirvulescu I**, Gheorghe L, Csiki I, Becheanu G, Dumbravă M, Fica S, Martin S, Sarbu A, Gheorghe C, Diculescu M, Copăescu C. Noninvasive clinical model for the diagnosis of nonalcoholic steatohepatitis in overweight and morbidly obese patients undergoing bariatric surgery. *Chirurgia (Bucur)* 2012; **107**: 772-779 [PMID: 23294957]

42 **Krasnoff JB**, Vintro AQ, Ascher NL, Bass NM, Paul SM, Dodd MJ, Painter PL. A randomized trial of exercise and dietary counseling after liver transplantation. *Am J Transplant* 2006; **6**: 1896-1905 [PMID: 16889545 DOI: 10.1111/j.1600-6143.2006.01391.x]

43 **Richards J**, Gunson B, Johnson J, Neuberger J. Weight gain and obesity after liver transplantation. *Transpl Int* 2005; **18**: 461-466 [PMID: 15773968 DOI: 10.1111/j.1432-2277.2004.00067.x]

44 **El-Meteini M**, Sakr M, Eldorry A, Mohran Z, Abdelkader NA, Dabbous H, Montasser I, Refaie R, Salah M, Aly M. Non-Invasive Assessment of Graft Fibrosis After Living Donor Liver Transplantation: Is There Still a Role for Liver Biopsy? *Transplant Proc* 2019; **51**: 2451-2456 [PMID: 31358454 DOI: 10.1016/j.transproceed.2019.01.197]

45 **Kabbany MN**, Conjeevaram Selvakumar PK, Guirguis J, Rivas J, Akras Z, Lopez R, Hanouneh I, Eghtesad B, Alkhouri N. Accuracy of Noninvasive Fibrosis Scores in Predicting the Presence of Fibrosis in Patients after Liver Transplantation. *Exp Clin Transplant* 2018; **16**: 562-567 [PMID: 28952917 DOI: 10.6002/ect.2016.0340]

46 **Kamphues C**, Lotz K, Röcken C, Berg T, Eurich D, Pratschke J, Neuhaus P, Neumann UP. Chances and limitations of non-invasive tests in the assessment of liver fibrosis in liver transplant patients. *Clin Transplant* 2010; **24**: 652-659 [PMID: 19925459 DOI: 10.1111/j.1399-0012.2009.01152.x]

47 **Wong GL**, Wong VW, Chim AM, Yiu KK, Chu SH, Li MK, Chan HL. Factors associated with unreliable liver stiffness measurement and its failure with transient elastography in the Chinese population. *J Gastroenterol Hepatol* 2011; **26**: 300-305 [PMID: 21261720 DOI: 10.1111/j.1440-1746.2010.06510.x]

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CASE REPORT

Rare primary mature teratoma of the liver: A case report

Yury A Kovalenko, Yury O Zharikov, Yana V Kiseleva, Anton B Goncharov, Tatyana V Shevchenko, Beslan N Gurmikov, Dmitry V Kalinin, Alexey V Zhao

ORCID number: Yury A Kovalenko 0000-0001-9879-6403; Yury O Zharikov 0000-0001-9636-3807; Yana V Kiseleva 0000-0002-0009-9245; Anton B Goncharov 0000-0002-3528-036X; Tatyana V Shevchenko 0000-0003-4643-0252; Beslan N Gurmikov 0000-0001-5958-3608; Dmitry V Kalinin 0000-0001-6247-9481; Alexey V Zhao 0000-0002-0204-8337.

Author contributions: Kovalenko YA is the coordinator, project management, patient management, paper reviewer and editor, senior author; Zharikov YO contributed to the surgical brigade, intraoperative protocol preparation and proofreading; Kiseleva YV drafted the primary report, performed data collection; Goncharov AB contributed to the surgical brigade, patient management; Shevchenko TV performed data collection, surgical brigade; Gurmikov BN performed data collection, clinical assessment; Kalinin DV drafted the primary report, patient consultant, prepared the figures, and reviewed the paper; Zhao AV drafted the primary report, clinical assessment, patient management, and reviewed the paper.

Informed consent statement: Informed written consent was obtained from the patients for publication of this report and any accompanying images.

Yury A Kovalenko, Anton B Goncharov, Tatyana V Shevchenko, Beslan N Gurmikov, Alexey V Zhao, Department of Surgical Oncology and Chemotherapy, A.V. Vishnevsky National Medical Research Center of Surgery of the Russian Ministry of Healthcare, Moscow 115093, Russia

Yury O Zharikov, Department of Human Anatomy, Sechenov First Moscow State Medical University (Sechenov University), Moscow 119048, Russia

Yana V Kiseleva, International School "Medicine of the Future", Sechenov First Moscow State Medical University (Sechenov University), Moscow 119048, Russia

Dmitry V Kalinin, Pathology Department, A.V. Vishnevsky National Medical Research Center of Surgery of the Russian Ministry of Healthcare, Moscow 115093, Russia

Corresponding author: Yury O Zharikov, PhD, MBA, Associate Professor, Department of Human Anatomy, Sechenov First Moscow State Medical University (Sechenov University), 8-2 Trubetskaya Street, Moscow 119048, Russia. dr_zharikov@mail.ru

Abstract

BACKGROUND

Primary liver teratoma is an extremely rare tumor usually affecting children under the age of 3 years. Specific signs of teratoma on ultrasound, computed tomography (CT) or magnetic resonance imaging are lacking, which makes morphology the only diagnostic tool. Misdiagnosis of a mature teratoma may lead to excessive liver resection, whereas misdiagnosis of an immature teratoma may result in spread, causing a life-threatening condition. Consequently, a careful tumor examination is important, and the rarest types of tumors must be accounted for.

CASE SUMMARY

We describe a 52 years old female who presented with a solid mass in the left liver lobe. Contrast-enhanced CT and magnetic resonance imaging (MRI) revealed a round, heterogeneous lesion containing a number of fluid areas and areas of calcification in the middle, and the provisional diagnosis was cholangiocarcinoma. The patient underwent resection of liver segment I. Immunohistochemistry analysis of the resected lesion indicated thyroid follicular epithelium; however, the thyroid gland was intact. 10 years prior to presentation the patient underwent a surgery due to mature teratoma of the right ovary, nevertheless the tumor was benign and could not spread to the liver, in addition teratoma of the liver was also benign. This led to the final diagnosis of primary mature liver teratoma.

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CONCLUSION

Primary hepatic teratoma, including heterotopia of the thyroid gland in the liver, is an extremely rare condition in adults that needs to be considered in the differential diagnosis of solid-cystic neoplasms in the liver and cholangiocarcinoma. This case adds to the limited literature on the patient presentation, clinical workup and management of liver teratomas.

Key Words: Case report; Primary liver teratoma; Ectopic thyroid gland tissue; Mature teratoma; Epidermoi cyst

Core Tip: Primary liver teratoma is an extremely rare tumor. This condition in adults needs to be considered in the differential diagnosis of solid-cystic neoplasms in the liver and cholangiocarcinoma. A careful tumor examination is important, and the rarest types of tumors must be accounted for to allow the diagnosis of heterotopia of the thyroid gland in the liver.

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INTRODUCTION

Teratoma is a rare germ cell tumor (GCT) that comprises at least two of three germ cell layers, the ectoderm, mesoderm and endoderm, and affects both children and adults. Teratomas primarily affect gonadal tissues, as the origin of these tumors is primordial germ cells, which migrate from the allantois to the fetal gonads during the first week of fetal life[1]. Thus, teratomas may also occur along the migration path of primordial germ cells, which can remain in midline extragonadal sites[2]. Consequently, the liver is an extremely rare site for primary teratomas, with an incidence of approximately 1% of all teratomas. Most patients with hepatic teratoma are children under the age of 3 years[3]. Nevertheless, primary or secondary teratomas of the liver can lead to serious health issues and can be a life-threatening condition that claims a comprehensive diagnosis and well-timed therapy. Therefore, our case report and review aim to collect scarce information about hepatic teratomas.

Classification of teratomas

Depending on the differentiation degree of their components, teratomas are classified as mature and immature[1]. Immature teratomas have a tendency for rapid growth, malignant transformation, and metastasis within adults; therefore, the prognosis is very poor[2].

Mature teratomas can be cystic, solid and mixed. According to the reported cases, cystic teratomas of the liver are the most common within mature teratomas. Mature cystic teratomas of the liver represent a mostly unilocular cystic cavity that may have septation and/or calcification and comprise mature elements derived from 3 cell layers, such as thyroid tissue, tooth enamel, hairs, skin, bone, fat, cartilage, neural tissue, or epithelium. The most commonly mature cystic teratomas affect the ovaries; however, approximately 1% of these lesions are found in the liver, usually within females in the right liver lobe[4-6]. The shape and size of mature cystic teratomas on gross appearance are not unique and vary significantly; thus, the largest reported lesion dimensions were 21 cm× 18 cm× 12 cm, and the weight was 1837 g[7]. The symptoms of mature cystic liver teratoma are nonspecific and conditioned by mechanical pressure of the growing tumor, including abdominal distension, constipation, fever, loss of appetite, abdominal pain, a sense of fullness in the right upper quadrant, vomiting, *etc*.[3,8]. Cases of asymptomatic mature teratoma have also been reported[9,10]. Rahmat *et al*[11] described a 46 years old male who presented

with cholangitis caused by a primary benign teratoma of the liver measuring 5.0 cm x 6.5 cm x 8.0 cm and compressing a common bile duct. Despite their high degree of differentiation, cystic teratomas can transform to malignant tumors and harbor other neoplasms; therefore, complete surgical removal is an optimal treatment that can be followed by chemotherapy if necessary[5,12]. Recently, Ramkumar *et al*[13] reported a case of a primary mature teratoma rupture accompanied by acute abdominal pain in a 65 years old female. Surgical removal of the tumor was performed after liquid and antibiotic therapy, and areas of necrosis and hemorrhage were found on histopathology[13].

The differentiation degree of the components of immature teratomas is low, and these tumors may involve any type of tissue, although neurogenic elements are the most common. On histopathology, these teratomas can also be divided into predominantly cystic, solid, and solid with multiple cysts and may contain areas of necrosis and hemorrhage. Immature teratomas tend to show rapid growth with liver capsule invasion and metastasis[2]. Primary immature hepatic teratomas are extremely rare. To the best of our knowledge, only 3 case reports have been published in the English literature up to 2021. The liver is also a rare site of teratoma metastasis; however, secondary immature teratomas are more frequent[14,15]. The symptoms of immature liver teratoma have been described in a few case reports and include pain and sensation of fullness in the right upper quadrant, fatigue, sweating, nausea, vomiting, and weight loss[2,16]. Malek-Hosseini *et al*[16] reported the largest immature liver teratoma, measuring 27 cm in diameter, and the patient recovered completely through surgery with a good follow-up. Immature liver teratomas lead to an elevation in AFP levels, whereas mature teratomas cannot produce AFP; thus, AFP is usually utilized for the differential diagnosis; nevertheless, AFP elevation does not necessarily occur [14,17]. The treatment of immature teratomas includes adjuvant chemotherapy and complete resection of the primary tumor and every metastasis whenever possible[18]. Nonresectable hepatic teratomas require liver transplantation[19].

Diagnosis of hepatic teratomas

The main diagnostic tools for liver teratoma detection are contrast-enhanced CT and MRI, which can show the size, shape, and structure of the tumor and its position related to adjacent elements and organs. CT scans can reveal areas of calcification in teratomas, whereas MRI scans are not sensitive to calcium[3]. Cho *et al*[20] revealed the high sensitivity of attenuation correction CT (AC-CT) acquired during ^{18}F-FDG PET-CT in the diagnosis of immature ovarian teratomas, as their components show significant ^{18}F-FDG uptake. Thus, ^{18}F-FDG PET-CT may be a useful diagnostic tool[20]. Serum AFP, LDH, hCG, CEA, and liver enzymes may be elevated in some patients[2]. However, the final diagnosis of teratoma can be made based only on the histopathology of the tumor samples[9].

Growing teratoma syndrome

Teratomas are usually treated with surgery and chemotherapy. However, metastatic teratomas of nonseminomatous germ cell tumors (NSGCTs) may not respond to chemotherapy and become significantly enlarged even after the original tumor is removed and serum tumor markers (AFP, beta-HCG) and LDH return to normal. This condition is known as *growing teratoma syndrome* (GTS). This syndrome is uncommon, and its etiology and pathogenesis are still unclear; consequently, the diagnosis may be delayed, and the patient's prognosis may become poor[21]. There are two dominant theories on the pathogenesis of GTS: (1) Chemotherapy leads to the survival and subsequent thriving of mature components, whereas immature components are highly sensible; and (2) Chemotherapy results in DNA damage and transformation of the immature teratoma to a mature teratoma[22]. Hiester *et al*[23] suggested a model of GTS development, according to which these tumors comprise meroclones derived from holoclones under chemotherapy. The authors termed these cells "teratoma-forming transit-amplifying cells (TF-TACs)"[23].

GTS should be suspected in every patient with a growing tumor and normal tumor marker levels after chemotherapy of the original NSGCT[21]. The most common sites of original NSGCTs are the ovaries and testis, whereas metastasis usually affects the retroperitoneum; nevertheless, cases of GTS from liver metastasis have also been reported. The common features of the described patients included young age (22 and 24 years old), multiple metastatic deposits among the entire liver, retroperitoneal lymph nodes and kidney from testicular tumors, and elevated AFP levels. Interestingly, the liver teratomas were mature, and there was no evidence of malignancy. Both patients underwent radical orchiectomy, nephrectomy, retroperitoneal lymphadenectomy and chemotherapy, and AFP levels returned to normal.

However, the liver teratomas continued to grow, confirming the GTS diagnosis, and patients were accepted for liver transplantation (LT). After LT, there was no evidence of teratoma recurrence[24,25]. O'Reilly *et al*[22] presented the first case of GTS in a primary liver teratoma in a 22 years old female. AFP levels were elevated (over 18000 cm before chemotherapy) and significantly decreased thereafter, whereas the tumor continued to enlarge up to 31.4 cm x 25.4 cm x 42.1 cm, and GTS was suspected. The patient was discharged after right hepatectomy and resection of the right mediastinal and diaphragmatic metastases, and there was no evidence of teratoma recurrence after 18 mo[22]. Growing teratomas of the liver may cause a disturbance in vital function either by the mechanical compression of contiguous organs and vessels or by hepatic failure; moreover, the incidence of GTS-related malignancy is 2%-8%. As these tumors do not respond to chemo- or radiotherapy, such patients should undergo complete surgical removal of the teratomas, as incomplete resection has a higher rate of tumor recurrence[23].

CASE PRESENTATION

Chief complaints

A 52 years old woman was referred to our hospital by a specialist at the diagnostic center after a solid tumor was detected in the left lobe of the liver with ultrasound (US).

History of present illness

US revealed that the lesion measured 118 mm x 93 mm in size with sharp edges, a heterogeneous and hyperechoic parenchyma and areas of calcification. The patient did not have any complaints associated with this lesion.

History of past illness

The patient underwent right oophorectomy 10 years prior to presentation due to an epidermoid cyst (mature teratoma), and no chemo- or radiotherapy was assigned because the tumor was benign. Apart from that, the medical and family histories were unremarkable.

Personal and family history

Personal and family history is not burdened.

Physical examination

During the general examination, no abnormalities were detected.

Laboratory examinations

The laboratory assessment also did not reveal any pathological findings. The tumor markers CA 19-9 and AFP were not elevated (< 2.5 U/mL and 4.61 U/mL, respectively).

Imaging examinations

Subsequent US with color flow mapping (CFM) revealed moderate vascularization of the lesion and compression of the left portal vein, left hepatic artery and left hepatic vein. Subsequent CT and MRI revealed a heterogeneous lesion 111 mm x 109 mm x 97 mm in size with a round shape containing a number of fluid areas sized from 5 to 12 mm and areas of calcification in the middle of the tumor. The distal intrahepatic bile ducts were dilated, and the inferior vena cava was compressed (Figures 1 and 2). With reference to the CT and MRI scans, the provisional diagnosis was formulated as cholangiocarcinoma of the left hepatic lobe.

MULTIDISCIPLINARY EXPERT CONSULTATION

The histological examination suggested biliary hamartoma, but the lack of bilirubin in the cells lining the cavity did not allow us to exclude lymphangioma or follicular cancer (Figure 3). To reveal the true nature of the tumor and exclude a malignancy, immunohistochemical tests were performed. They demonstrated focal positive expression of thyroglobulin (clone 2H11+6 E1), TTF-1 (clone 8G7G3/1), and galectin-3

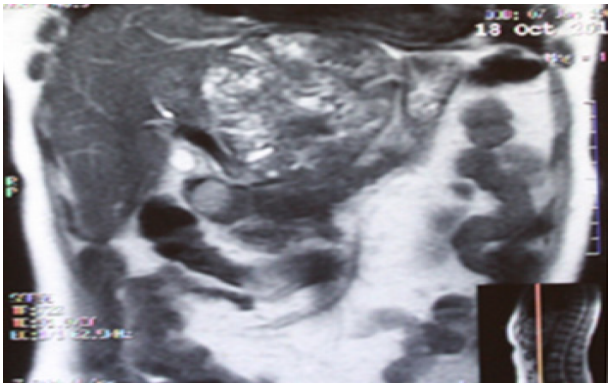

Figure 1 Magnetic resonance imaging of the abdomen: Ill-defined contrast-enhancing, multilobulated cystic lesion involving segments II, III, VI and VIII.

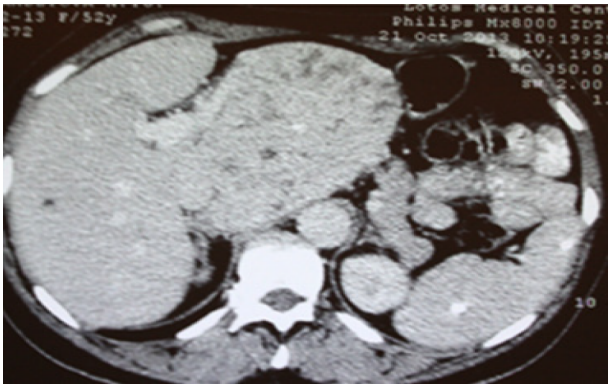

Figure 2 Abdominal computed tomography with contrast enhancement: Tumor invades segment I of the liver (longitudinal section). Ill-defined contrast-enhancing, multilobulated cystic lesion involving segments II, III, VI and VIII.

(clone 9C4), overexpression of cytokeratin 8 and 18 (clones B22.1 + B23.1) and negative expression of CD34 (clone QBEnd/10). The immunophenotype corresponded to the thyroid follicular epithelium. In the postoperative period, we performed ultrasonography, which did not show thyroid gland malignancy and the patient had no endocrine problems.

FINAL DIAGNOSIS

According to the gross appearing, histology and immunohistophenotype the ectopic thyroid gland in the liver (mature teratoma) was finally evident in the patient.

TREATMENT

The patient underwent resection of segment I with the surrounding tumor hepatic parenchyma, D1 Lymphadenectomy and cholecystectomy. The intraoperative inspection revealed an increase in the left liver lobe due to the well-defined encapsulated inhomogeneous tumor in the first segment of the liver (14 cm x 13 cm x 13 cm), crushing atrophied segments 2 and 3 (Figures 4 and 5). The consistency of the tumor was soft, and on its surface, there were twisted veins.

OUTCOME AND FOLLOW-UP

The postoperative period was uneventful. Considering the benign nature of teratoma no complementary treatment was indicated. The patient was discharged from the

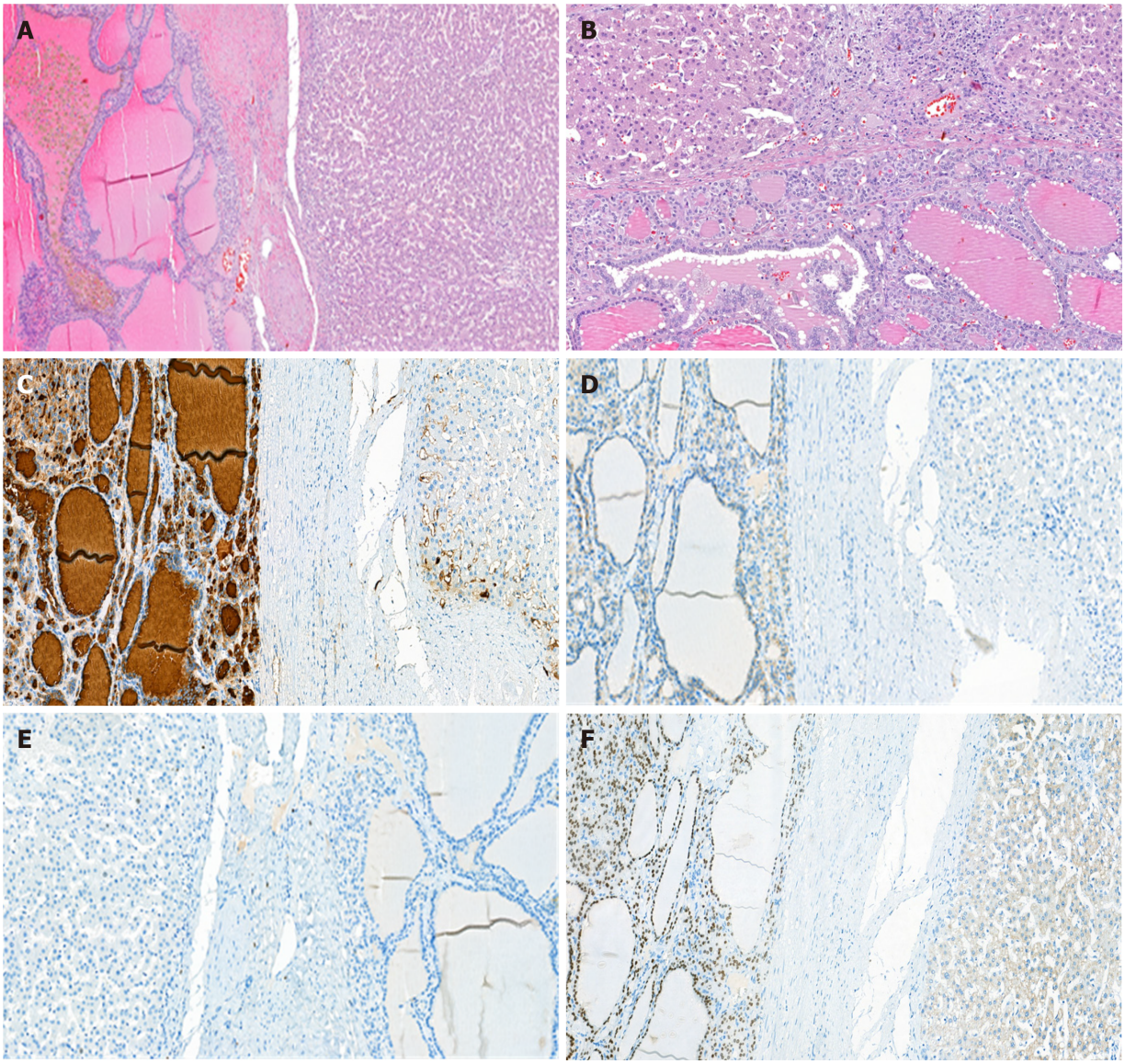

Figure 3 Pathology findings of liver mass. A: Microscopic appearance - the liver node, with shaped borders, is formed from cavities of different sizes filled with eosinophilic fluid, resembling a colloid (100×); B: Cubic single-layered epithelium lining the cavities (200×). Along the apical surface of the cells, there are characteristic vacuoles in the thick colloid; C: Epithelium labeled with anti-thyroglobulin (2H11 + 6 E1) revealing the thyroid origin (200×); D: Membrane CD56 reveals the neuroendocrine nature of tumor cells (200×); E: A single cell within a tumor node labeled with Ki67, the same as the adjacent normal liver (200×); F: Nuclear TTF-1 immunostaining also suggests a thyroid and thyroid-derived tumor origin (200×).

hospital on the 8th day after the operation. Eight years after operation the patient has no complaints, no evidence of teratoma recurrence nor newly formed teratomas were revealed during CT examination in 2021.

DISCUSSION

Hepatic teratoma is rare; to the best of our knowledge, only a small number of case reports exist in the literature (Table 1), and no liver-specific treatment guidelines have been established[5]. The successful treatment of an ectopic thyroid gland in the liver, confirmed by morphological and immunohistochemical tests, described herein was very difficult to correctly diagnose preoperatively due to the highly variable instrumental visualization of the tumor and clinical manifestations of this disease. We managed to find only one similar case of hepatic teratoma in the reviewed literature [26].

The patient's medical history provided no evidence of teratoma in thyroid gland tissue. Before the results of the morphological and immunohistochemical tests became available, the patient was considered to have perihilar cholangiocarcinoma. Bearing in

Table 1 Primary liver teratoma case reports

Ref.	Patient age	Diagnosis	Liver lobe	Treatment	Follow-up
Madan *et al*[8]	34, female	Mature cystic teratoma	Right	Complete resection	Uneventful
Watanabe *et al*[27]	20, female	N/A	Right	Complete resection	N/A
Winter *et al*[28]	61, female	Mature Teratoma	Right	N/A	N/A
Martin *et al*[29]	53, female	Mature cystic teratoma	Right	Complete resection	Uneventful
Nirmala *et al*[6]	36, female	Mature teratoma	Right	Complete resection	Uneventful
O'Reilly *et al*[22]	22, female	Immature teratoma	Right	Complete resection, chemotherapy	Uneventful
Certo *et al*[10]	27, female	Mature teratoma	N/A	Complete resection	N/A
Jaklitsch *et al*[7]	27, female	Mature cystic teratoma	N/A	Complete resection	Uneventful
Cöl *et al*[2]	21, female	Immature teratoma	Right	Complete resection, chemotherapy	Recurrence, death
Xu *et al*[30]	34, male	Immature teratoma	Right	Complete resection, chemotherapy	Recurrence, death
Han *et al*[31]	46, male	Mature cystic teratoma	Quadrant	Complete resection	Uneventful

N/A: Not available.

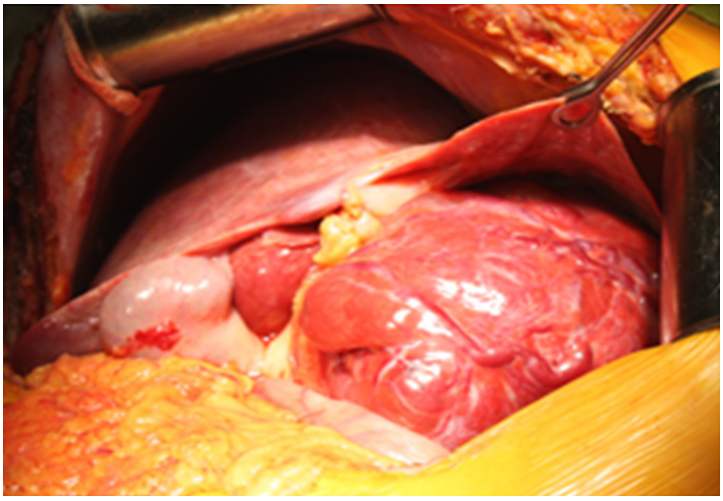

Figure 4 Intraoperative image. Tumor invades segment I of the liver, atrophied left hepatic lobe.

mind the state of our patient, we initially planned hepatectomy with a reconstruction biliary tract live-saving procedure.

The immunohistochemical test results demonstrated thyroid follicular epithelium as a result of the focal positive expression of thyroglobulin (clone 2H11+6 E1), TTF-1 (clone 8G7G3/1), and galectin-3 (clone 9C4), overexpression of cytokeratin 8 and 18 (clones B22.1 + B23.1) and negative expression of CD34 (clone QBEnd/10). This clinical case clearly demonstrates the diagnostic challenge of patients presenting with heterotopia of the thyroid gland in the liver simulating perihilar cholangiocarcinoma. Only a comprehensive examination by clinical, biochemical, and radiological methods makes tumor detection possible and allows the identification of such rare conditions. The diagnostic challenges of this condition can be met with the mass-forming type of cholangiocarcinoma. A proper preoperative evaluation, surgical treatment and preparation facilitate positive treatment outcomes.

The patient underwent ovariectomy due to an epidermoid cyst (mature teratoma) of the right ovary 10 years prior to the detection of the hepatic tumor. Unfortunately, micrographs of the lesion were not available. The ovarian teratoma had no signs of malignancy; therefore, no chemotherapy or radiotherapy was indicated. Nevertheless, hepatic teratomas are not metastases from ovarian teratomas, as mature ovarian teratomas cannot spread. Hepatic teratoma is sometimes misdiagnosed as an immature ovarian teratoma if malignant; however, in the current case, the lesion had

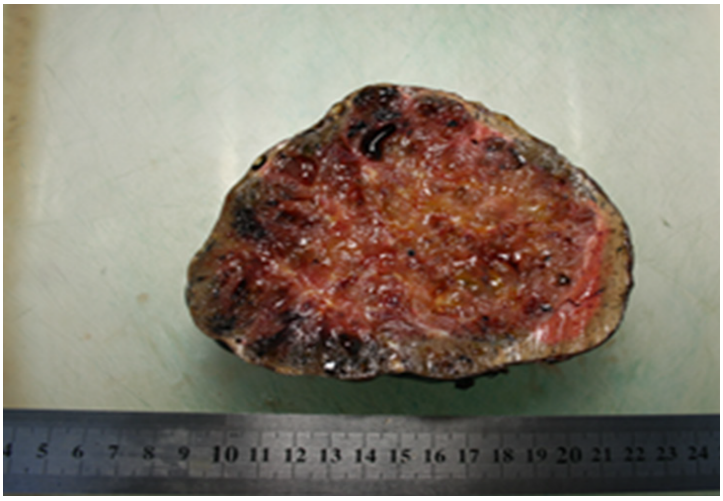

Figure 5 Macroscopic appearance - on the sections, a liver node with areas of reddish-yellow and brown color, with many cavities filled with a brown gelatinous liquid. There are also whitish-gray strands within the tumor.

no signs of malignancy. Consequently, the patient was diagnosed with metachronous teratomas of the right ovary and liver.

In summary, we present an exceedingly rare clinical presentation of heterotopia of the thyroid gland in the liver in an adult patient who underwent surgical resection. The clinical workup included a CT scan, with confirmation of the diagnosis of hepatic teratoma on histopathology. Resection remains the mainstay of treatment.

CONCLUSION

Heterotopia of the thyroid gland in the liver is an extremely rare condition in adults that needs to be considered in the differential diagnosis of solid-cystic neoplasms in the liver and cholangiocarcinoma. Surgical resection remains the mainstay of management, and risk stratification based on histology should determine postoperative surveillance. This case adds to the limited literature on the patient presentation, clinical workup, and management of liver teratomas.

REFERENCES

1 **Peterson CM**, Buckley C, Holley S, Menias CO. Teratomas: a multimodality review. *Curr Probl Diagn Radiol* 2012; **41**: 210-219 [PMID: 23009771 DOI: 10.1067/j.cpradiol.2012.02.001]

2 **Cöl C**. Immature teratoma in both mediastinum and liver of a 21-Year-old female patient. *Acta Med Austriaca* 2003; **30**: 26-28 [PMID: 12558563 DOI: 10.1046/j.1563-2571.2003.02024.x]

3 **Gupta R**, Bansal K, Manchanda V, Gupta R. Mature cystic teratoma of liver. *APSP J Case Rep* 2013; **4**: 13 [PMID: 24040591]

4 **Ahmed A**, Lotfollahzadeh S. Cystic teratoma. In: StatPearls. StatPearls Publishing. 2020. Available from: http://www.ncbi.nlm.nih.gov/books/NBK564325/

5 **Krainev AA**, Mathavan VK, Klink D, Fuentes RC, Birhiray R. Resection of a mature cystic teratoma of the liver harboring a carcinoid tumor. *J Surg Case Rep* 2018; **2018**: rjy279 [PMID: 30397434 DOI: 10.1093/jscr/rjy279]

6 **Nirmala V**, Chopra P, Machado NO. An unusual adult hepatic teratoma. *Histopathology* 2003; **43**: 306-308 [PMID: 12940789 DOI: 10.1046/j.1365-2559.2003.01675.x]

7 **Jaklitsch M**, Sobral M, deFigueiredo AAFP, Martins A, Marques HP. Rare giant: mature cystic teratoma in the liver. *J Surg Case Rep* 2019; **2019**: rjz347 [PMID: 31832137 DOI: 10.1093/jscr/rjz347]

8 **Madan M**, Arora R, Singh J, Kaur A. Mature cystic teratoma of the liver in an adult female. *Indian J Pathol Microbiol* 2010; **53**: 872-873 [PMID: 21045458 DOI: 10.4103/0377-4929.72022]

9 **Silva DS**, Dominguez M, Silvestre F, Calhim I, Daniel J, Teixeira M, Ribeiro V, Davide J. Liver teratoma in an adult. *Eur Surg* 2007; **39**: 372-375 [DOI: 10.1007/s10353-007-0370-0]

10 **Certo M**, Franca M, Gomes M, Machado R. Liver teratoma. *Acta Gastroenterol Belg* 2008; **71**: 275-279 [PMID: 18720943]

11 **Rahmat K**, Vijayananthan A, Abdullah B, Amin S. Benign teratoma of the liver: a rare cause of

cholangitis. *Biomed Imaging Interv J* 2006; **2**: e20 [PMID: 21614237 DOI: 10.2349/biij.2.3.e20]
12 **Lee SY**, Jang MH, Koo YJ, Lee DH. Undifferentiated carcinoma arising in ovarian mature cystic teratoma: a case report and literature review. *Int J Clin Exp Pathol* 2020; **13**: 1750-1754 [PMID: 32782700]
13 **Ramkumar J**, Best A, Gurung A, Dufresne AM, Melich G, Vikis E, MacKenzie S. Resection of ruptured hepatic teratoma in an adult. *Int J Surg Case Rep* 2018; **53**: 414-419 [PMID: 30567058 DOI: 10.1016/j.ijscr.2018.11.032]
14 **Shannon NB**, Chan NHL, Teo MCC. Recurrence of immature ovarian teratoma as malignant follicular carcinoma with liver and peritoneal metastasis 22 years after completion of initial treatment. *BMJ Case Rep* 2017; **2017** [PMID: 29066646 DOI: 10.1136/bcr-2017-219665]
15 **Byun JC**, Choi IJ, Han MS, Lee SC, Roh MS, Cha MS. Soft tissue metastasis of an immature teratoma of the ovary. *J Obstet Gynaecol Res* 2011; **37**: 1689-1693 [PMID: 21651648 DOI: 10.1111/j.1447-0756.2011.01553.x]
16 **Malek-Hosseini SA**, Baezzat SR, Shamsaie A, Geramizadeh B, Salahi R, Salahi H, Lotfi M. Huge immature teratoma of the liver in an adult: a case report and review of the literature. *Clin J Gastroenterol* 2010; **3**: 332-336 [PMID: 26190492 DOI: 10.1007/s12328-010-0183-8]
17 **Paradies G**, Zullino F, Orofino A, Leggio S. Rare extragonadal teratomas in children: complete tumor excision as a reliable and essential procedure for significant survival. Clinical experience and review of the literature. *Ann Ital Chir* 2014; **85**: 56-68 [PMID: 23165250]
18 **Pietzak EJ**, Assel M, Becerra MF, Tennenbaum D, Feldman DR, Bajorin DF, Motzer RJ, Bosl GJ, Carver BS, Sjoberg DD, Sheinfeld J. Histologic and Oncologic Outcomes Following Liver Mass Resection With Retroperitoneal Lymph Node Dissection in Patients With Nonseminomatous Germ Cell Tumor. *Urology* 2018; **118**: 114-118 [PMID: 29704586 DOI: 10.1016/j.urology.2018.04.009]
19 **Oh D**, Yi NJ, Song S, Kim OK, Hong SK, Yoon KC, Ahn SW, Kim HS, Kim H, Kim HY, Kang HJ, Lee M, Lee KB, Lee KW, Suh KS. Split liver transplantation for retroperitoneal immature teratoma masquerading as hepatoblastoma. *Pediatr Transplant* 2017; **21** [PMID: 28714114 DOI: 10.1111/petr.13025]
20 **Cho A**, Kim SW, Choi J, Kang W, Lee JD, Yun M. The additional value of attenuation correction CT acquired during 18F-FDG PET/CT in differentiating mature from immature teratomas. *Clin Nucl Med* 2014; **39**: e193-e196 [PMID: 23989446 DOI: 10.1097/RLU.0b013e3182a20d5c]
21 **Kataria SP**, Varshney AN, Nagar M, Mandal AK, Jha V. Growing Teratoma Syndrome. *Indian J Surg Oncol* 2017; **8**: 46-50 [PMID: 28127182 DOI: 10.1007/s13193-016-0568-3]
22 **O'Reilly D**, Alken S, Fiore B, Dooley L, Prior L, Hoti E, Fennelly D. Growing Teratoma Syndrome of the Liver in a 22-Year-Old Female. *J Adolesc Young Adult Oncol* 2020; **9**: 124-127 [PMID: 31545120 DOI: 10.1089/jayao.2019.0081]
23 **Hiester A**, Nettersheim D, Nini A, Lusch A, Albers P. Management, Treatment, and Molecular Background of the Growing Teratoma Syndrome. *Urol Clin North Am* 2019; **46**: 419-427 [PMID: 31277736 DOI: 10.1016/j.ucl.2019.04.008]
24 **Kapoor V**, Ferris JV, Rajendiran S. Growing teratoma syndrome of the liver: treatment with living related donor liver transplantation. *AJR Am J Roentgenol* 2003; **181**: 839-841 [PMID: 12933491 DOI: 10.2214/ajr.181.3.1810839]
25 **Eghtesad B**, Marsh WJ, Cacciarelli T, Geller D, Reyes J, Jain A, Fontes P, Devera M, Fung J. Liver transplantation for growing teratoma syndrome: report of a case. *Liver Transpl* 2003; **9**: 1222-1224 [PMID: 14586885 DOI: 10.1053/jlts.2003.50238]
26 **Strohschneider T**, Timm D, Worbes C. [Ectopic thyroid gland tissue in the liver]. *Chirurg* 1993; **64**: 751-753 [PMID: 8222936]
27 **Watanabe I**, Kasai M, Suzuki S. True teratoma of the liver--report of a case and review of the literature--. *Acta Hepatogastroenterol (Stuttg)* 1978; **25**: 40-44 [PMID: 636740]
28 **Winter TC**, Freeny P. Hepatic teratoma in an adult. Case report with a review of the literature. *J Clin Gastroenterol* 1993; **17**: 308-310 [PMID: 8308217 DOI: 10.1097/00004836-199312000-00009]
29 **Martin LC**, Papadatos D, Michaud C, Thomas J. Best cases from the AFIP: liver teratoma. *Radiographics* 2004; **24**: 1467-1471 [PMID: 15371619 DOI: 10.1148/rg.245035209]
30 **Xu AM**, Gong SJ, Song WH, Li XW, Pan CH, Zhu JJ, Wu MC. Primary mixed germ cell tumor of the liver with sarcomatous components. *World J Gastroenterol* 2010; **16**: 652-656 [PMID: 20128038 DOI: 10.3748/wjg.v16.i5.652]
31 **Han SY**. Dermoid cyst of the liver. Report of a case. *Am J Roentgenol Radium Ther Nucl Med* 1970; **109**: 842-843 [PMID: 5451888 DOI: 10.2214/ajr.109.4.842]

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