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ABOUT COVER

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World Journal of Hepatology (*World J Hepatol*, *WJH*, online ISSN 1948-5182, DOI: 10.4254), is a peer-reviewed open access academic journal that aims to guide clinical practice and improve diagnostic and therapeutic skills of clinicians.

WJH covers topics concerning liver biology/pathology, cirrhosis and its complications, liver fibrosis, liver failure, portal hypertension, hepatitis B and C and inflammatory disorders, steatohepatitis and metabolic liver disease, hepatocellular carcinoma, *etc*. Priority publication will be given to articles concerning diagnosis and treatment of hepatology diseases. The following aspects are covered: Clinical diagnosis, laboratory diagnosis, *etc*.

We encourage authors to submit their manuscripts to *WJH*. We will give priority to manuscripts that are supported by major national and international foundations and those that are of great basic and clinical significance.

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REVIEW

Hepatocellular carcinoma in non-cirrhotic liver: A comprehensive review

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Abstract

Hepatocellular carcinoma (HCC) is the most common type of primary liver cancer, which in turns accounts for the sixth most common cancer worldwide. Despite being the 6th most common cancer it is the second leading cause of cancer related deaths. HCC typically arises in the background of cirrhosis, however, about 20% of cases can develop in a non-cirrhotic liver. This particular subgroup of HCC generally presents at an advanced stage as surveillance is not performed in a non-cirrhotic liver. HCC in non-cirrhotic patients is clinically silent in its early stages because of lack of symptoms and surveillance imaging; and higher hepatic reserve in this population. Interestingly, F3 fibrosis in non-alcoholic fatty liver disease, hepatitis B virus and hepatitis C virus infections are associated with high risk of developing HCC. Even though considerable progress has been made in the management of this entity, there is a dire need for implementation of surveillance strategies in the patient population at risk, to decrease the disease burden at presentation and improve the prognosis of these patients. This comprehensive review details the epidemiology, risk factors, clinical features, diagnosis and management of HCC in non-cirrhotic patients and provides future directions for research.

Key words: Hepatocellular carcinoma; Non-cirrhotic liver; Hepatitis B; Hepatitis C; Risk factors; Clinical features; Diagnostic modalities; Management strategies; Future directions

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Core tip: Hepatocellular carcinoma (HCC) is the 2nd leading cause of cancer related deaths. Majority of HCC arise in a cirrhotic liver, however, 20% of cases can develop in non-cirrhotic liver. This comprehensive review focuses on risk factors, clinical features, diagnostic modalities, management strategies and future directions for HCC in non-cirrhotic liver.

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INTRODUCTION

Primary liver cancer is the sixth most common cancer worldwide, 90% of which are hepatocellular carcinoma (HCC)[1,2]. HCC is the second leading cause of cancer-related deaths worldwide[3]. Although, most of the cases occur in developing countries, its incidence in developed countries has increased recently[4]. HCC typically arises in the setting of cirrhosis however; approximately 20% of HCC's have been known to develop in a non-cirrhotic liver[5,6]. This sub-group of HCC often presents at advanced stages because surveillance is not performed in a non-cirrhotic liver. Fibrolamellar carcinoma (FLC), a rare variant of HCC also occurs without any background cirrhosis or hepatitis[6,7]. This review discusses the epidemiology, risk factors, clinical features, diagnosis and management of HCC in non-cirrhotic patients as well as provides future direction for research in this population.

LITERATURE SEARCH

A comprehensive literature search was performed and research papers regarding non-cirrhotic HCC and related literature was analyzed to prepare this review article. Special emphasis was placed on research related to the diagnosis and management of non-cirrhotic HCC in the last 5 yr.

EPIDEMIOLOGY

Worldwide, liver cancer is the fifth most common cancer in men and the ninth in women. It is the 2nd leading cause of cancer death in men and the sixth leading among women, with about 745500 deaths in 2012. In the United States, there is expected to be an estimated 42220 new cases and 30200 death cases of liver and intrahepatic bile duct carcinomas in 2018[8,9]. HCC is a little over 2 times more likely in men over women, the incidence being 5.5 per 100000 in male and 2 per 100000 in female in the United States[8]. Non-cirrhotic HCC has a bimodal age distribution, peaking during the 2nd and 7th decade of life[5]. The FLC variant comprises 1%-9% of all HCC and accounts for less than 1% of HCC in the United States[10,11]. Overall, there is a lack of significant data on HCC that arises in non-cirrhotic liver. Given that HCC is one of the fastest growing cause of cancer-related deaths and has a survival rate of less than 12% in the United States, there is a need for further research to explore the epidemiology of non-cirrhotic HCC[8].

RISK FACTORS

Non-alcoholic fatty liver disease /Non-alcoholic steatohepatitis

Non-alcoholic fatty liver disease (NAFLD) comprises of a spectrum that includes isolated steatosis, non-alcoholic steatohepatitis (NASH, hepatic inflammation and cell death), fibrosis and cirrhosis (Table 1). With the growing obesity epidemic, NAFLD has become the most common liver disorder in the United States. A strong association

Table 1 Incidence of different risk factors for hepatocellular carcinoma in cirrhotic and non-cirrhotic liver in various studies

Parameter	Study	No (NC)	No (CL)	Percentage (NC)	Percentage (CL)	Mean Incidence (NC)	Mean Incidence (CL)
Alcohol	Schütte *et al*[16]	14	285	15%	50%	21.77%	30%
	Nzeako *et al*[52]	25	130	7%	28%		
	Trevisani *et al*[4]	15	105	16.50%	30%		
	Techathuvanan *et al*[153]	26	48	36%	34%		
	Chang *et al*[154]	98	99	44%	44.50%		
	Grazi *et al*[155]	29	67	21.50%	23%		
	Shim *et al*[28]	22	22	12.40%	4.10%		
HBV	Nzeako *et al*[52]	17	43	5%	9.30%	30.60%	41.65%
	Trevisani *et al*[4]	10	81	10%	22.55		
	Techathuvanan *et al*[153]	5	26	10.60%	32%		
	Chang *et al*[154]	142	150	64.30%	69%		
	Grazi *et al*[155]	17	46	12.60%	14.90%		
	Shim *et al*[28]	105	443	59%	83.30%		
	Kew *et al*[156]	NR	NR	52.80%	60.50%		
HCV	Trevisani *et al*[4]	48	76	15%	76%	14.36%	44.18%
	Chang *et al*[154]	39	57	18.10%	27.40%		
	Grazi *et al*[155]	33	182	24.40%	56.80%		
	Shim *et al*[28]	13	27	7.30%	5.10%		
	Albeldawi *et al*[157]	6	107	7%	55.70%		
NASH	Schütte *et al*[16]	6	37	6.45%	6.48%	NA	NA
Cigarette smoking	Schütte *et al*[16]	55	231	40%	47.60%	28.37%	32.10%
	Trevisani *et al*[4]	16	70	20%	28%		
	Techathuvanan *et al*[153]	4	5	4.20%	2.80%		
	Chang *et al*[154]	110	111	49.30%	50%		
Diabetes Mellitus	Schütte *et al*[16]	59	368	71.20%	83.70%	40.73%	46.40%
	Shim *et al*[28]	27	84	15.20%	15.80%		
	Albeldawi *et al*[157]	29	113	35.80%	39.70%		
Family History	Techathuvanan *et al*[153]	9	7	12.30%	4.90%	13.85%	9.60%
	Chang *et al*[154]	32	30	15.40%	14.30%		
Cryptogenic	Schütte *et al*[16]	53	65	57%	11.38%	39.15%	9.44%
	Shim *et al*[28]	38	40	21.30%	7.50%		

HBV: Hepatitis B virus; HCV: Hepatitis C virus; NASH: Non-alcoholic steatohepatitis; NC: Non-cirrhotic liver; CL : Cirrhotic liver.

has been reported between fatty liver disease and HCC in non-cirrhotic livers[12,13]. NAFLD-related HCC has been acknowledged as a growing burden in this country[14,15]. It has also been recognized as the most common etiology in new HCC cases, likely due to the recent advances in viral hepatitis, especially hepatitis C virus (HCV) treatment[15]. In a study performed by Schütte *et al*[16] the etiology of majority of HCC in non-cirrhotic liver was related to metabolic syndrome (MS). The causes of non-cirrhotic HCC is shown in Figure 1.

NAFLD, with or without NASH is the hepatic manifestation of MS and predisposes to HCC in non-cirrhotic patients[17]. Type 2 diabetes mellitus and obesity that is associated with NAFLD and MS leads to the release of multiple pro-inflammatory cytokines like TNF-alpha, IL-6, leptin and resistin and decreased amounts of adiponectin. These processes favor the development of hepatic steatosis and inflammation within the liver and subsequently precede the development of HCC[12]. Even though type 2 diabetes and obesity have both been implicated as independent risk factors for HCC, studies that establish a clear link to HCC in non-cirrhotic livers are scarce[18-20]. Not only MS and obesity but being overweight is also associated with higher risk of HCC. Overweight and obesity increase HCC prevalence in general population and especially in hepatitis B virus (HBV) and HCV patients[21]. Other features of MS like hypertension and dyslipidemia have not been extensively studied

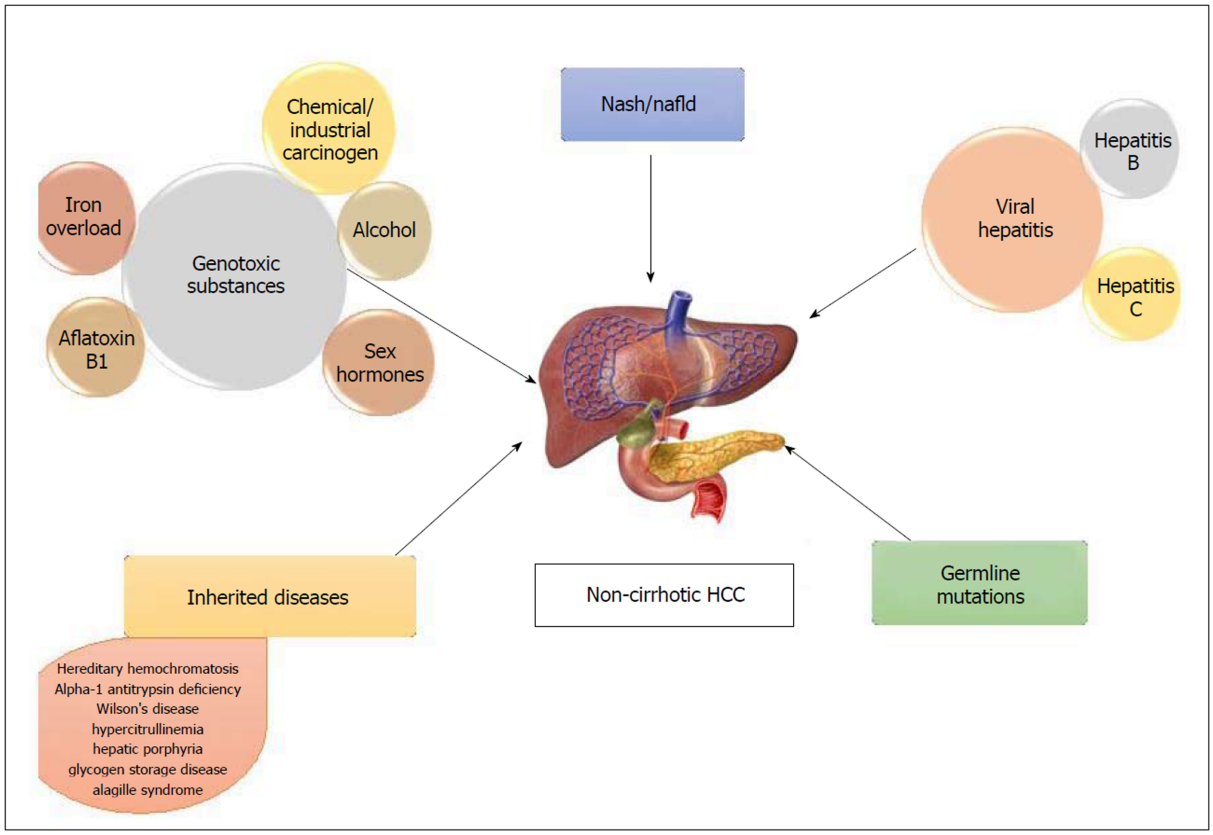

Figure 1 Causes of non-cirrhotic hepatocellular carcinoma. HCC: Hepatocellular carcinoma.

for the linkage. A recent Australian study comparing HCC characteristics between cirrhotic and non-cirrhotic NAFLD found that apart from the presence or absence of cirrhosis, the fibrosis stage was the only patient characteristic that conferred a worse prognosis as this was related to the size of the tumors (P = 0.03)[22].

Viral hepatitis

30% of HBV-related HCC occurs in non-cirrhotic patients[23] (Figure 2). HBV, a partially double stranded DNA virus is able to integrate into the host cell and acts as a mutagenic agent causing secondary chromosomal rearrangement and increasing genomic instability. In addition, transactivation of genes by the regulatory protein HBx is known to increase cell proliferation, deregulate cell cycle control and interfere with DNA repair and apoptosis[24]. Certain risk factors in chronic hepatitis B patients in turn impart a higher risk for non-cirrhotic HCC. BCP T1762/A1764 mutation and high viral loads have been reported to be strong viral factors and independent predictors of HCC in non-cirrhotic patients who are chronic HBV carriers[25,26]. Older patients with chronic Hepatitis B had a higher annual incidence of non-cirrhotic HCC compared to cirrhotic patients; 1.1% per year in men and 0.3%-0.4% per year in women greater than the age of 55[27]. African American and Asian race has also been associated with a higher incidence of HCC in non-cirrhotic chronic hepatitis B patients[27]. About half of the cryptogenic HCC cases have occult Hepatitis B infection defined by the presence of HBV DNA in the liver or blood without HBsAg[28].

HCC in chronic Hepatitis C patients primarily occurs in the background of cirrhosis that is related to a necroinflammatory state with tissue damage, regeneration, repair and fibrosis[29,30]. HCV being a single stranded RNA virus cannot integrate with the host genome due to the absence of a DNA intermediate. However, it still possesses direct oncogenic potential although lower compared to HBV; with several of its gene products capable of contributing to carcinogenesis[4,31]. The core protein can alter cell regulation *via* enhanced telomerase activity[31]. Non-structural proteins like NS3, NS4B and NS5A can potentially induce carcinogenesis through interactions with cellular promoters and proteins[32]. The incidence of HCC in non-cirrhotic HCV patients ranges from 4.4%-10.6%[32]. Its role as a major risk factor is suggested by the development of HCC even after eradication of the virus[33]. In treatment naïve HCV patients, male gender, advanced age, persistently elevated aminotransferases, high gamma-glutamyl transferase levels, hepatic steatosis, diabetes and alcohol abuse have

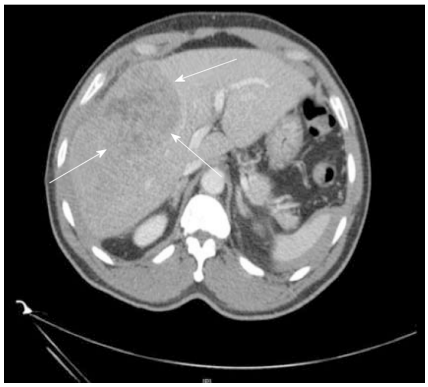

Figure 2 Computed tomography image of a 64-yr-old male with hepatitis B. No cirrhosis found to have large 9 cm mass in right lobe (arrows) on CT abdomen done for abdominal pain.

all been shown to increase the risk for non-cirrhotic HCC[34,35]. In patients with sustained virologic response after chronic HCV treatment, those who had diabetes mellitus and increased Fibrosis-4 index were at a higher risk. Studies have also shown increased risk of HCC in patients with hepatitis C and F3 fibrosis. Apart from hepatitis C, this increased risk is noted in even patients with hepatitis B and NAFLD who have F3 fibrosis[22,25,27,36]. The exact pathophysiology behind this still remains to be elucidated.

Genotoxic substances

Alcohol: Several studies have shown heavy alcohol intake in patients with non-cirrhotic HCC, however most of these were not statistically significant[4]. Alcohol may not be a major cause in non-cirrhotic patients, but it should still be treated as a serious risk factor for the development of HCC. This is related to its direct genotoxic effect in the development of HCC mediated through endotoxin production, oxidative stress and inflammation[37]. Heavy alcohol intake in the setting of other risk factors like chronic HCV and diabetes mellitus may potentiate the oxidative stress and free radical damage; leading to rapid progression to HCC[38-40].

Aflatoxin B1: Aflatoxin B1 (AFB1) is an extremely potent hepatocarcinogen that is a secondary metabolite produced by fungi, *Aspergillus flavus* and *Aspergillus parasiticus*. They are typically found in tropical and sub-tropical regions of the world in which grains such as rice stored in hot humid conditions promote growth of these toxin-producing fungi[41]. Most cases occur in sub-Saharan Africa, Southeast Asia and China where HBV is highly prevalent. However, its incidence in the United States is extremely low; 0.003 in HbsAg negative and 0.08 in HbsAg-positive patients[42]. In addition, its burden in non-cirrhotic individuals is unknown. AFB1 is metabolized by the P450 enzymes in the liver to generate an epoxide, which binds to DNA and induces mutation of the p53 tumor suppressor gene[24,43]. Like cirrhotic patients, non-cirrhotic patients with chronic HBV are also at a higher risk for aflatoxin-mediated HCC[44,45].

Iron overload: Secondary iron overload is seen in patients with hematological disease like myelodysplastic syndrome, chronic anemia and polytransfusion[46-48]. The notion that increased liver iron stores in the liver cause HCC in non-cirrhotic patient has been present for at least three decades and there are several case reports that have highlighted the role of excess iron as a potential carcinogen[48,49]. Iron-induced carcinogenesis could be related to the production of reactive oxygen species *via* Fenton reaction inducing oxidative stress. This in turn promotes protein and DNA modification, blunts immune response by impairing T cell proliferation against tumor transformed cells and induces of p53 mutations[50,51].

Miscellaneous: Chemical and industrial carcinogens like nitrosamines, azo dyes, aromatic amines, vinyl chloride, organic solvents, pesticides and arsenic have been known to induce carcinogenesis in non-cirrhotic liver[52,53]. Studies have shown KRAS mutations in vinyl chloride-induced HCC and HRAS mutations in methylene chloride-induced liver tumors[23]. Polycyclic aromatic hydrocarbons derived from red meat, cigarette smoking, and environmental pollutants also increase the risk of HCC by forming DNA adducts[53-55].

Sex hormones: Several case reports have established a link between chronic anabolic

androgen steroid abuse and non-cirrhotic HCC in young professional bodybuilders[56-58]. "Adenoma-carcinoma sequence" or "*de novo* carcinoma development" are the two proposed mechanisms for carcinogenesis[56]. Oral contraceptive (OCs) is also a known risk factor for the development of non-cirrhotic HCC[59,60]. In a retrospective case series of 26 white women aged under 50 who developed HCC in a non-cirrhotic liver, women who used OCs for 8 or more years had a 4.4-fold increased risk ($P < 0.01$)[59]. Both anabolic steroids and estrogen undergo significant enterohepatic circulation and slow biliary excretion, which increases intrahepatic concentrations and causes direct toxicity[59].

Inherited diseases

Hereditary hemochromatosis and alpha-1 antitrypsin deficiency are inherited diseases in which HCC occurs frequently without cirrhosis[61-65]. Acute hepatic porphyrias which include 3 autosomal dominant disorders: Acute intermittent porphyria (AIP), variegate porphyria (VP) and hereditary coproporphyria (HCP) has also been considered as a cause of non-cirrhotic HCC[66]. Overproduction of aminolevulinic acid and/or porphobilinogen overproduction and excretion has been implicated as a cause of hepatic carcinogenesis in AIP, but the mechanism is poorly understood in VP and HCP[67].

Hypercitrullinemia, a hereditary urea cycle disorder is associated with hepatocarcinogenesis in non-cirrhotic liver likely *via* citrulline-mediated promotion effect on hepatocyte proliferation[68].

Wilson's disease, an autosomal recessive disorder of copper metabolism is also implicated as cause of HCC in scattered case reports. The mechanism is related to copper mediated stimulation of fibroblast growth factor-2 and copper induced stabilization of hypoxia-inducible-factor-1 alpha, which causes expression of genes that promote angiogenesis[69,70]. Even though HCC prevalence in Wilson's disease is lower than other inherited forms of liver disease, it remains a significant risk factor for development of HCC in non-cirrhotic liver as well.

Glycogen storage disorders (GSD) are a group of inherited metabolic disorders characterized by the accumulation of excessive abnormal glycogen in liver, muscle or both. HCC typically occurs without cirrhosis in glycogen storage disease type 1 (GSD-1) *via* adenoma-carcinoma sequence[62]. HCC in GSD type III is extremely rare and generally occurs in the background of cirrhosis, however Oterdoom *et al*[71] reported the first documented case in a non-cirrhotic.

Alagille syndrome, an autosomal dominant disease that causes significant neonatal jaundice and cholestasis in older children has been shown in case reports to cause non-cirrhotic HCC[72,73].

Hepatic vascular disease like Budd-Chiari syndrome and nodular regenerative hyperplasia have been associated with non-cirrhotic HCC in case reports, however the mechanism of hepatocarcinogenesis in the absence of cirrhosis is unknown[74,75].

Germline mutations

Studies establishing associations between germline mutations and non-cirrhotic HCC have been scarce. In a recent study by Donati *et al*[76], germline mutations in telomerase reverse transcriptase gene (*hTERT*) were associated with shorter telomere lengths and progression of NAFLD to HCC in non-cirrhotic liver. Future studies may identify such germline mutations, which would allow for closer surveillance in these high-risk individuals.

Hepatic adenoma

Hepatic adenomas (HA) is a benign liver tumor that carries a small risk for the development of HCC[60]. The risk of malignant transformation is controversial and has been heavily debated based on available past research studies. Two studies that analyzed available literature, estimated this risk to be approximately 5%[77,78]. Studies on HA in non-cirrhotic livers have been scarce and those that exist have limitations related to overestimation of the risk and biased reviews of resected cases[79,80]. Nonetheless, there is compelling evidence in the literature to reserve resection of adenomas for adenoma diameter > 5 cm with telangiectatic or unclassified subtypes and male sex[77,78,81].

CLINICAL FEATURES

HCC in non-cirrhotic patients is clinically silent in its early stages because of lack of symptoms and surveillance imaging; and higher hepatic reserve in this population[82]. The median age of these patients is 69 yr however, the FLC variant commonly occurs in adolescents and young adults, ranging from 10-35 yr at presentation[11,16].

Unfortunately, these tumors are often found in advanced stages with approximately 25% of non-cirrhotic HCC presenting with extra-hepatic metastasis[16]. When symptoms do occur, they arise due to large tumor burdens from its insidious progression. The most common presenting symptom is abdominal pain (52%). Other symptoms include abdominal distention, weight loss, malaise, anorexia, fatigue, chronic diarrhea, jaundice, chest pain and fever of unknown origin[4,83,84]. Non-cirrhotic HCC can also present in the form of paraneoplastic syndrome of hypercalcemia or hypoglycemia[82,85]. FLC has been known to present with gynecomastia, recurrent deep vein thrombosis, Budd-Chiari syndrome, non-bacterial thrombotic endocarditis, fulminant liver failure or encephalopathy[10].

DIAGNOSIS

Serum alpha-fetoprotein

Alpha-fetoprotein (AFP), a serum glycoprotein is a commonly used tumor marker for HCC[86]. Due to its poor sensitivity, the American Association for the Study of Liver Disease (AASLD) guidelines suggests surveillance with ultrasound with or without AFP in cirrhotic patients. In non-cirrhotic HCC, the sensitivity of AFP further decreases with elevation less common compared to cirrhotic HCC (31%-67% *vs* 63%-84%). AFP levels in majority of patients with the FLC-variant HCC are normal[87,88]. Levels > 400 ng/mL are essentially diagnostic for non-cirrhotic HCC and are equally prevalent in both groups[89]. This implies that elevated AFP levels may suggest a HCC, but normal levels should never be used to exclude the diagnosis, especially in a patient with high-risk factors. AFP levels however, may have a role in tumor surveillance and prognosis.

Des-gamma-carboxyprothrombin

Des-gamma-carboxyprothrombin (DCP) also known as PIVKA II (protein induced by vitamin K absence) is produced by malignant hepatocytes and its relationship to HCC has been known since 1984[90,91]. DCP has been reported to be more sensitive and specific than AFP for the diagnosis of HCC with a cutoff of > 40 mAU/mL[92]. However, its role in the detection of small tumors and early HCC is unclear as various studies have utilized different cutoff values for DCP and AFP[92-94]. Moreover, it has never been studied in non-cirrhotic patients. DCP might be the answer for early HCC diagnosis in non-cirrhotic patients along with monitoring treatment response and recurrence. Perhaps DCP could compliment AFP as evidenced by improved sensitivities emphasized in several studies[91,93]. Future studies should be directed towards establishing a relationship between elevated DCP and non-cirrhotic HCC.

Imaging

The radiological appearance of HCC in cirrhotic and non-cirrhotic patients is very similar, except HCC in non-cirrhotic livers frequently present as a solitary mass with or without satellite lesions, are much larger in tumor size and are often seen with a central scar[95].

Ultrasonography: Ultrasonography (US) is a non-invasive test that allows determining the size, location, morphology and vascular involvement of the lesion. The appearance of HCC on US is variable and non-specific ranging from hypo or hyperechoic lesions with or without heterogeneity or necrotic areas. This imaging modality is limited in the detection of tumors < 2 cm and tumors in a liver base with a heterogeneous diffuse nodular pattern[82,84].

Contrast-enhanced US (CEUS) could be valuable diagnostic tool because the dye allows its use in patients with nephropathies and those with known adverse reactions to other contrast agents[82]. CEUS shows a typical HCC vascular pattern, although inconsistently when compared to computed tomography (CT) and magnetic resonance imaging (MRI) especially for tumors < 2 cm[96-99]. There is a need for such studies in non-cirrhotic HCC, but for now its role in diagnosis is limited. However, it may have a role in guiding biopsies and monitoring tumor response to treatment with anti-angiogenic properties[100,101].

Computed tomography: CT scan can make the diagnosis of HCC with a high degree of confidence, hence proper technique and contrast administration is crucial for an accurate assessment (Figure 3)[82]. The main diagnostic criteria include hypervascularization on the arterial "wash-in" phase and washout during portal phase of enhancement, which is similar in cirrhotic and non-cirrhotic livers[102,103]. It often presents as a single large well-circumscribed encapsulated hypoattenuating lesion on unenhanced CT, with other tumor features like fat involvement, foci of

hemorrhage and necrotic areas more common in non-cirrhotic HCC[104]. The FLC variant may show a similar pattern on contrast enhanced imaging as the classic non-cirrhotic HCC and often demonstrates internal calcifications, central scars and a discontinuous capsule[105].

Magnetic resonance imaging: MRI is superior to CT for the diagnosis of HCC (Figure 4). Its appearance on T1 sequences varies depending on the degree of fibrosis, necrosis and fat but it more commonly presents as a hypointense lesion. Its appearance on T2 is variable as well but it is commonly a hyperintense lesion. Intracellular fat accumulation, which is present in 10%-17% of non-cirrhotic HCC and 36% of well-differentiated HCC is easier to detect on MRI compared to CT/US and signifies a better prognosis[82,84]. The gadolinium enhancement shows a similar pattern on MRI as described in contrast enhanced CT[82]. About 50% of non-cirrhotic HCC have a central scar that is detectable by MRI[99]. The FLC variant is hypointense on T1, hyperintense on T2 and heterogenous after gadolinium enhancement[106]. The central scar that is frequently seen in this subtype can be hypo or hyperintense on T2 sequences depending on the presence of necrosis and altered vascularity[107]. The differentiation between HCC and other benign liver lesions (focal nodular hyperplasia and hepatocellular adenoma) on MRI has been challenging in a non-cirrhotic liver often requiring unnecessary interventions for histopathological proof. In conclusion, T1 hypointensity, T2 hypo or hyperintensity, lack of central tumor enhancement and presence of satellite lesions are independent imaging factors, with a combined specificity of 98%, can allow MRI guided diagnosis of HCC in non-cirrhotic liver with a high level of confidence[107].

Percutaneous liver biopsy

Histological diagnosis *via* liver biopsy may only be necessary if imaging studies are inconclusive for being compatible with HCC[108-110]. The AASLD does not recommend biopsy for lesions > 1 cm if two different imaging studies yield concordant findings[108]. When performed, they are done *via* transabdominal technique under CT or US guidance with varying degrees of sensitivity (66%–93% based on tumor size) and 100% specificity and positive predictive value[109]. Liver biopsy may be needed in patients who are not candidates for curative resection, to establish diagnosis for the purpose of systemic therapy or transplantation[109].

MANAGEMENT

Surgical Resection

Surgical resection is the treatment of choice for HCC in non-cirrhotic liver and is considered equally safe in non-cirrhotic and cirrhotic patients[111-113]. Since clinical staging systems like Okuda/Barcelona-Clinic Liver Cancer associated with underlying cirrhosis are not relevant in these patients, primary tumor features are utilized for staging and prognosis. Patients that are typically not candidates for surgical resection are those that have extrahepatic spread of their disease or anatomical constraints related to the tumor. Majority of the patients require a major hepatic resection, often with advanced surgical techniques for inferior vena cava or diaphragmatic resection; or total vascular exclusion[114]. These surgeries are feasible due to the preserved liver function and low perioperative mortality when compared to cirrhotic livers[114]. Common complications associated with such resections include intra-abdominal collections and liver insufficiency[115]. Perioperative morbidity and mortality is low when compared to the cirrhotic liver, 29.5% and 2.7% respectively[115]. Impressive postoperative survival rates of 96%, 87% and 68% after 1, 3 and 5-yr respectively have been reported in patients with tumors without vascular invasion; factors such as portal vein thrombosis, lymph node involvement and tumor recurrence are associated with poor outcomes[112,113].

Tumor recurrence

Tumor recurrence is the major cause of death in non-cirrhotic livers with HCC since no effective post-operative adjuvant chemotherapy exists[116,117]. The recurrence rate of HCC in non-cirrhotic liver is extremely high after surgical resection. Taking into account the best reported figures, the 1, 3 and 5-yr disease free survival rate is 79%, 58% and 54% respectively. Repeat hepatectomy is feasible in these patients due to normal liver function, which allows for good regenerative capacity. A mean time recurrence of 31 mo with a 61% 5-yr survival and 25% 10-yr survival after a first repeat hepatectomy has been reported for non-cirrhotic patients[116]. A good functioning liver also allows for repeat second and third hepatectomies in these patients and can be considered equally safe and effective when compared to the

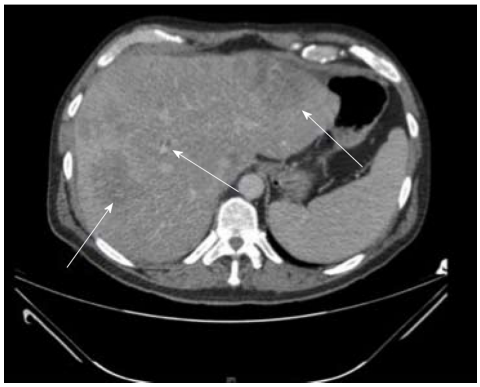

Figure 3 Computed tomography image of a 55-yr-old male with no significant past medical history found to have multifocal hepatocellular carcinoma in the right lobe of liver on imaging done for abdominal pain and jaundice.

first[84]. However, when surgical resections fail to control recurrent disease or repeat tumors are considered unresectable, patients may need to be evaluated for liver transplantation (LT).

LT

Historically, LT was not routinely recommended in non-cirrhotic HCC due to the lack of precise guidelines or selection criteria for this patient population. Very early reports and studies have demonstrated high tumor recurrences and dismal survival outcomes. A systematic review of all reported cases of LT in non-cirrhotic patients from 1966-1988 reported a 5-yr survival rate of 11.2% for HCC and 39.4% for the FLC variant[118]. McPeake *et al*[119] reported a 40% recurrence rate for lesions 4-8 cm and 78% in lesions > 8 cm. However, Mergental *et al*[120] reported a 5-yr survival of 50%-70% after analysis of literature and European Liver Transplant Registry with median tumor sizes of 8 cm. This study recommends that Milan criteria should not be used to exclude patients with non-cirrhotic HCC and identified extrahepatic spread, gross vascular or lymph node involvement, multiple tumors and tumor recurrence < 1 yr after resection as predictors of poor outcome after LT. Based on these findings, an international consensus conference report recommended LT in patients with non-resectable HCC or in patients who experience intrahepatic recurrence after surgical resection; provided these patients have no macrovascular invasion or extrahepatic spread[121]. Decaens *et al*[122] highlighted the need for prospective studies addressing pre-LT imaging for tumor characteristics, response to treatment performed and kinetics of tumor progression during the waiting period and rate of dropout from tumor progression. It will be interesting to note the recurrence and overall survival (OS) of these patients in future studies after improved patient selection and advances in perioperative management and surgical care.

Systemic therapy

Sorafenib is an Food and Drug Administration (FDA) approved oral multi-tyrosine kinase inhibitor, which is the first line therapy for patients with advanced HCC[123,124]. It is indicated in patients who are not deemed surgical or transplant candidates with preserved liver function. It inhibits tumor growth and has anti-proliferative, anti-angiogenic and pro-apoptotic features[125]. The most common side effects reported include diarrhea and hand-foot skin reactions[126]. Sorafenib has demonstrated survival benefit in cirrhotic HCC, however no studies have formally evaluated outcomes in patients with a non-cirrhotic liver. Given the similarity of angiogenic characteristics between cirrhotic and non-cirrhotic HCC, Sorafenib could have a role in the management of advanced HCC in non-cirrhotic patients[127]. Recently, the FDA approved two new systemic drugs for advanced HCC. Regorafenib, a multikinase inhibitor, and Nivolumab, a PD-1 (programmed cell death protein 1) inhibitor have shown survival benefit in patients who had disease progression despite treatment with Sorafenib[128,129].

Systemic chemotherapy with other agents has been ineffective and has resulted in sub-optimal outcomes in cirrhotic advanced HCC. This has been largely due to the underlying cirrhosis with altered drug metabolism, which can lead to serious toxicity requiring either decrease in the dose or discontinuation[130,131]. However, non-cirrhotic HCC patients with a healthy liver may be able to tolerate these agents. Edeline *et al*[132] in their study had a 52% disease control rate with ECC (epirubicin, cisplatin and 5-

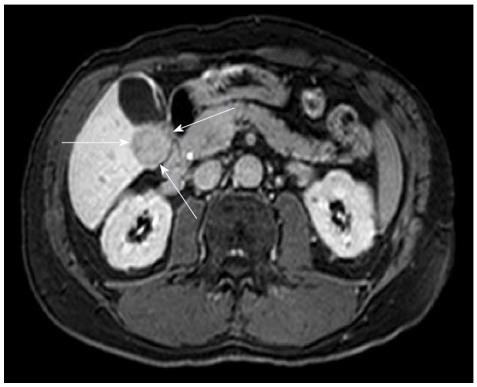

Figure 4 Magnetic resonance imaging (e-THRIVE_BH AX 15 min delay) of 61-yr-old male with hepatitis C virus, without cirrhosis showing a 2.8 cm × 3 cm mass lesion in segment 5 consistent with hepatocellular carcinoma.

flurouracil) or ECF (epirubicin, cisplastin and capecitabine). Romano *et al*[130] demonstrated partial response to Docetaxel with long-term survival and without severe toxicity in 3 patients. Gras *et al*[133] reported a complete response with GEMOX chemotherapy without a 5-yr relapse after discontinuation in a patient with advanced FLC-HCC in a non-cirrhotic liver. These reports highlight the need for further trials to explore the use of systemic chemotherapy to improve prognosis of patients with advanced non-cirrhotic HCC. Systemic chemotherapy may result in downsizing of the tumor allowing such patients to be candidates for curative surgical resection or liver transplant.

There have been few studies that have evaluated the combination of Sorafenib with other systemic chemotherapeutic agents in non-cirrhotic advanced HCC. In patients who experience recurrences after LT, combination of Sorafenib and mTOR inhibitor in conjunction with locoregional treatments improved survival, with 1 and 5-year survival rates of 82% and 33% respectively[134]. This could open potential avenues for research for combination chemotherapy and loco-ablative techniques like radiofrequency ablation (RFA), transarterial chemoembolization (TACE) and radio-embolization commonly employed in cirrhotic HCC; in the management of non-cirrhotic HCC.

Loco-ablative therapies and selective internal radiation therapy

Local ablation therapies like RFA and TACE are considered first line treatment options for unresectable HCC in cirrhotic patients. These techniques along with selective internal radiation therapy (SIRT) have been employed for down staging of tumors and control progression[135,136]. Unfortunately, their role in non-cirrhotic HCC has not been established in studies likely due to the rarity of the disease, benefit of other treatment modalities, poor prognosis and high tumor recurrence. However, for metastatic disease after prior hepatectomy, RFA has been associated with improved progression free survival and OS when compared to transcatheter therapy[135].

A recent case report published by Mafeld *et al*[136] showed a 94% reduction in tumor size 7 mo after SIRT with Yttrium-90 for unresectable FLC-HCC after which the patient underwent a curative surgical resection. Studies establishing SIRT as a standard of care in regular non-cirrhotic HCC may be difficult given its high 90-d morbidity; complications and lack of long term follow up data in the current case[136]. However, SIRT should be considered for the FLC-variant given limited treatment options for unresectable disease and its grave prognosis.

PROGNOSIS AND SURVEILLANCE

Survival of patients with HCC in non-cirrhotic liver mainly depends on tumor related factors such as tumor size, existence of satellite lesions, lack of tumor capsule, vascular invasion, grading, incomplete resection, HBV infection and the amount of intraoperative blood transfusions[117,137-139]. Poor prognostic factors that affected the OS rate in patients undergoing surgical resection include the need for blood transfusion and advanced age > 65. Factors that affected the recurrence free survival rate included the presence of multiple tumors[117]. FLC-HCC has a 70% 5-yr survival following surgical resection whereas for unresectable disease, the 5-yr survival rate is 0-5% with a median survival of 12 mo[140]. The number of tumors and vascular invasion are

considered poor prognostic factors in this variant; the 3-yr recurrence free survival rate is 9% in patients with vascular invasion and 35% without[140].

AFP levels could be better suited as prognostic indicators. Burnett *et al*[141] used AFP staging based on four levels: < 10 ng/mL, 10 to 150 ng/mL, 150 to 500 ng/mL and > 500 ng/mL; and found these to be appropriate predictors of prognosis in non-cirrhotic HCC. Witjes *et al*[89] showed that elevated pre-operative AFP levels were associated with worse outcomes and high recurrence rates. Using an AFP cut off of 9 ng/mL, they demonstrated 1 and 3-yr survival rates of 53% and 21% respectively in patients with high AFP and 86% and 75% in patients with low AFP.

The need for effective surveillance needs to be addressed given the high tumor recurrence rate. The most common and significant issue raised is the delayed presentation of HCC in non-cirrhotic patients. There is a further need to direct research towards alternative cost-effective surveillance strategies and risk factor profiling to identify this high-risk population, decrease the tumor burden upon presentation and improve survival outcomes. Fu *et al*. reported a high association between high relative telomere length (RTL) and risk of HCC in non-cirrhotic chronic hepatitis B patients. Future studies could expand the role of RTL in serum DNA as a non-invasive biomarker for surveillance in non-cirrhotic HCC[142]. Wang *et al*[143] developed a prognostic scoring system of HCC after hepatectomy based on 11 independent risk factors and classified patients into low, intermediate and high-risk groups with an 80, 27 and 6-mo recurrence free survival respectively in each group. Such categorization is perhaps what is required in non-cirrhotic patients. Alkaline phosphatase (ALP) has also been reported as an independent risk factor that affects recurrence-free and survival rates[139]. High ALP levels could have a potential role in predicting HCC recurrence in non-cirrhotic patients and could be part of guidelines that establish risk for tumor recurrence. Further studies are necessary however, to validate the use of such scoring systems. This might allow for a more cost-effective and economic surveillance in high and intermediate risk groups.

FUTURE CONSIDERATIONS

microRNA

microRNAs (miRNAs) are endogenous non-coding 21-23 nucleotide RNAs that are involved in post-transcriptional regulation and thus play an important role in almost all main cellular pathways including regulation of major tumor-related genes in carcinogenesis[144,145]. miRNAs are also involved in iron metabolism through regulation of genes that control iron homeostasis in hepatocytes[146]. Dysregulation of miRNAs can lead to iron overload which can lead to the generation of reactive oxygen species and cause oxidative stress which damages DNA, lipids and proteins[147]. miRNA could serve as important diagnostic and prognostic biomarkers in non-cirrhotic HCC. Koh *et al*[148] identified 16 miRNAs that displayed significant change in expression non-tumor and HCC tissues in non-cirrhotic livers. Analysis of miRNA in the serum is an exciting prospect for the diagnosis and/or prognosis of non-cirrhotic HCC.

Early and unique changes in circulating miRNA in the serum could potentially allow it to be a biomarker for the early detection of non-cirrhotic HCC. In a study performed by Zhang *et al*[149], a 3 mi-RNA panel comprising of miR-92-3p, miR-107, and miR-3126-5p was equally effective as AFP for diagnosis of early HCC. Furthermore, the combination of miRNA panel and AFP had higher sensitivity and specificity than AFP alone, especially in patients with early HCC or low-level AFP. However, the study did not specify presence or absence of cirrhosis in the patient cohort. miRNA could also serve as an important prognostic marker in non-cirrhotic liver. Dysregulation of certain miRNA has been associated with poor disease-free survival after liver resection of HCC[145]. Another study showed low levels of miRNA (miR-181a-5p) and poor disease control after Sorafenib therapy[150]. Again, these studies did not comment on background liver cirrhosis. In a recent study by Mei *et al*[151] cirrhotic and non-cirrhotic HCC patients were found to have 41 differentially expressed miRNAs. Specifically, two miRNAs (mir-149 and mir-1296) were strongly associated with non-cirrhotic HCC and influenced the TMN tumor staging. Furthermore, increased mir-149 was associated with increased post-operative survival in non-cirrhotic HCC. Huang *et al*[152] developed 5-panel mi-RNA that capable of assessing risk in HCC patients. The hope is that future studies could identify a similar miRNA signature for non-cirrhotic HCC patients. Hence, miRNAs are an exciting future research prospect and could perhaps be the solution for improved diagnosis, surveillance and prognosis along with therapeutic management of advanced non-cirrhotic HCC.

CONTRIBUTIONS

The current review should help make significant contributions to research progress for HCC in non-cirrhotic liver. It highlights the major risk factors implicated in the development of HCC in a non-cirrhotic liver; especially NAFLD/NASH. It also brings to attention other rare risk factors that would further assist clinicians in decreasing the incidence of cryptogenic HCC. The review has also attempted to drive research towards finding alternative means of diagnosing non-cirrhotic HCC early by exploring other tumor markers like DCP as well as improve surveillance and monitoring of these patients. Finally, the review placed special emphasis on the management of non-cirrhotic HCC by incorporating the latest literature, which would allow researcher to explore other potential avenues especially systematic chemotherapy and loco-ablative techniques.

CONCLUSION

HCC in a non-cirrhotic liver is a complex disease phenomenon with risk factors, pathogenesis, clinical features, management and prognosis that are distinct from the cirrhotic counterpart. Even though considerable progress has been made in the management of this entity, there is a dire need for implementation of surveillance strategies in the patient population at risk, to decrease the disease burden at presentation and improve the prognosis of these patients. The hope is that this review sparks further research to close the knowledge gap and uncover answers to questions that have puzzled experts for decades.

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REVIEW

Treatment of primary sclerosing cholangitis in children

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Abstract

Primary sclerosing cholangitis (PSC) is a rare disease of stricturing and destruction of the biliary tree with a complex genetic and environmental etiology. Most patients have co-occurring inflammatory bowel disease. Children generally present with uncomplicated disease, but undergo a variable progression to end-stage liver disease. Within ten years of diagnosis, 50% of children will develop clinical complications including 30% requiring liver transplantation. Cholangiocarcinoma is a rare but serious complication affecting 1% of children. Ursodeoxycholic acid and oral vancomycin therapy used widely in children as medical therapy, and may be effective in a subset of patients. Gamma glutamyltransferase is a potential surrogate endpoint for disease activity, with improved survival in patients who achieve a normal value. Endoscopic retrograde cholangiopancreatography is a necessary adjunct to medical therapy to evaluate mass lesions or dominant strictures for malignancy, and also to relieve biliary obstruction. Liver transplantation remains the only option for patients who progress to end-stage liver disease. We review special considerations for patients before and after transplant, and in patients with inflammatory bowel disease. There is presently no published treatment algorithm or guideline for the management of children with PSC. We review the evidence for drug efficacy, dosing, duration of therapy, and treatment targets in PSC, and provide a framework for endoscopic and medical management of this complex problem.

Key words: Liver transplant; Pediatric; Ursodeoxycholic acid; Oral vancomycin; Oral vancomycin therapy; Endoscopic retrograde cholangiopancreatography

Core tip: This review provides an evidence-based framework for endoscopic and medical management of children with primary sclerosing cholangitis.

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INTRODUCTION

Primary sclerosing cholangitis (PSC) is a chronic inflammatory disease characterized by cholestasis and progressive stricturing and destruction of the intrahepatic and extrahepatic biliary tree. PSC is rare in the general pediatric population, with an incidence and prevalence of 0.2 and 1.5 cases per 100000 children, respectively. PSC is common in children with inflammatory bowel disease (IBD), affecting at least 10% of children with ulcerative colitis[1].

The etiology of PSC is complex and involves both genetic and environmental factors. Multiple abnormalities along the "gut-liver axis" have been identified including defects in: immune regulation, hepatobiliary protection mechanisms, bile acid metabolism, microbiome and intestinal permeability. Patients undergo a variable progression through hepatobiliary fibrosis, cirrhosis, and end-stage liver disease (ESLD) with a greatly increased risk for cholangiocarcinoma (CCA). In pediatrics most cases of PSC initially present without complications. Fewer than 5% have ESLD or dominant biliary strictures (DS) at diagnosis. Within ten years of diagnosis, 50% of children will develop clinical complications including 30% requiring liver transplantation (LT)[2].

There is presently no medical therapy to delay the progression of liver disease or the onset of clinical complications in PSC. The disease is recognized as having one the largest unmet needs in hepatology[3]. Ursodeoxycholic acid (UDCA) and oral vancomycin therapy (OVT) are used widely in children but the slowly progressive nature of PSC has hindered adequately-powered clinical trials. Advanced endoscopy plays an important role in palliation of PSC, with endoscopic retrograde cholangiopancreatography (ERCP) often being necessary to stent and balloon dilate biliary strictures. LT remains the only option for PSC patients with ESLD[4]. Here we review common and emerging treatment strategies for PSC in children, and their role in management based on recent literature.

URSODEOXYCHOLIC ACID

One aspect of PSC pathogenesis appears to be an abnormal bile acid pool[5-7]. HydroPHOBIC bile acids may be hepatotoxic, and high concentrations present in PSC appear to be cytotoxic within the biliary tree. PSC patients may lack an effective "bicarbonate umbrella" buffer layer between cholangiocytes and the biliary lumen[8], compounding this effect. UDCA is a hydroPHILIC bile acid with cytoprotective effects that is readily absorbed orally. UDCA increases levels of hydroPHILIC bile acids in bile[9,10] and decreases histocompatibility antigen display by hepatocytes[11]. UDCA is effective for adults with primary biliary cholangitis, another immune-mediated disease targeting bile ducts[12,13]. Its role in PSC is controversial however.

Clinical trials have consistently shown that UDCA is more effective than placebo in lowering serum alkaline phosphatase, a potential surrogate marker of disease activity in PSC[14-18]. No benefit to patient survival in treated vs. untreated patients has been shown however. In one trial of high-dose UDCA (25-30 mg/kg/d), outcomes were worse in treated cases prompting early cessation to avoid patient harm[19]. Ultimately the lack of a clear survival benefit and the potential for harm at certain doses prompted the American Association for the Study of Liver Diseases to recommend against the use of UDCA in PSC[20]. Controversy remains however since even the largest trials to date have been substantially under-powered[21] which may be why they failed to show a survival benefit. Further complicating the challenge for clinicians, in a prospective study evaluating the effects of UDCA withdrawal from chronically treated PSC patients, deterioration in serum liver tests occurred and patients reported increased pruritus[22]. Experts feel there is at least some role for a six month therapeutic trial of UDCA, with continuation of the drug in patients with a substantial biochemical response[23]. There are no practice guidelines for PSC in pediatrics, and UDCA is prescribed chronically in over 80% of patients with PSC[2].

The largest retrospective analysis of PSC outcomes showed that survival was

similar in UDCA-treated and untreated children[24]. Patients who normalized their gamma-glutamyltransferase (GGT) however (achieving a level of < 50 IU/L by one year), or reduced it from baseline by at least 75%, had improved survival compared to patients who did not. This survival benefit was similar whether a patient normalized GGT on UDCA treatment or spontaneously with no treatment. Untreated patients who achieved biochemical normalization had similar survival to UDCA-treated patients. Retrospective studies and post-hoc analyses of clinical trial data in adults have consistently shown that patients who fully normalize serum alkaline phosphatase (ALP) have dramatically better survival outcomes[25-29]. Even in the clinical trial suggesting that high-dose UDCA was detrimental overall[16], the subset of patients who normalized their ALP on treatment experienced favorable survival and no complications[21].

While there is a ceiling on the appropriate dose of UDCA, with 25-30 mg/kg/d demonstrating harm[30], there is not agreement on what constitutes a minimum effective dosage. Unpublished data from the Pediatric PSC Consortium suggest that 15 mg/kg/d is the most common dosage used in children. Doses as low as 9 mg/kg/d effectively reduced ALP and GGT in a small pediatric series[31]. In a randomized trial comparing three doses of UDCA, patients in a "low dose" 10 mg/kg UDCA group experienced statistically significant reduction in ALP and GGT over two years, similar to the response seen in the "standard" 20 mg/kg and "high" 30mg/kg dose groupings. With capsule strength limitations, the actual dose received in the "low dose" group was 7.4-13.2 mg/kg/d[15]. A just-completed, NIH-funded pediatric study of UDCA withdrawal showed that liver biochemistry was normal in patients entering the study at 13 mg/kg/d of UDCA or greater. A randomized-controlled trial of UDCA at doses of 13-15 mg/kg/d showed significant reduction in ALP and aspartate aminotransferase over one year[14]. It appears that any UDCA doses as low as 7 mg/kg/d may be reasonable with the most data supportive of approximately 13-23 mg/kg/d.

PSC patients are heterogeneous, and differences between patients who do or do not normalize biochemistry on UDCA are presently unknown. There are likely several factors: Earlier disease stage (before DS or extensive hepatic fibrosis have set in for instance), genotypic or phenotypic differences, or the presence of specific changes in a patient's microbiome or bile acid pool that are amenable to UDCA therapy in some but not all patients. Regardless, there are clearly patients who are responsive to UDCA, and it seems reasonable and safe to attempt a treatment trial at low or medium doses (approximately 15-25 mg/kg/d). In our experience, the maximal effect of UDCA on serum biochemistry is achieved within 8-12 wk, often sooner. This seems a reasonable length of time to try UDCA therapy. Long-term therapy should be reserved for those who show a dramatic biochemical response and normalization of GGT, or for rare children with substantial pruritus that resolves with treatment.

Approximately one third of children with PSC will normalize their serum biochemistry spontaneously. This is especially true in patients who were asymptomatic and identified only *via* screening bloodwork. These patients appear to undergo such changes frequently, possibly due to presence of an earlier stage of the disease where the inflammatory process waxes and wanes. Sorting out which UDCA-responders truly require lifelong therapy is difficult. The rate of disease progression in pediatrics, regardless of treatment with UDCA or not, is low and thus there is little urgency to initiate UDCA immediately nor is there a necessity to continue the medicine indefinitely. Patients can reasonably wait for two serial GGT values > 50, separated by 2-3 mo before initiating therapy, to reduce the incidence of treatment for highly fluctuating enzymes that spontaneously normalize. A recent clinical trial evaluated UDCA withdrawal from children with PSC who had been on chronic therapy with normal biochemistry. Upon complete withdrawal of the medication for 12 wk, 15/22 patients (68%) did not have a flare (GGT > 100) including 7/22 (32%) who maintained GGT < 29[32]. To prevent unnecessary chronic medication use, it is reasonable to attempt therapeutic withdrawal with regular monitoring of serum biochemistry to ensure each child truly needs chronic UDCA.

ORAL VANCOMYCIN THERAPY

The gut microbiome has been implicated in PSC pathogenesis[33-37]. The interaction between host immunity and dysbiosis remains poorly understood however. PSC patients are known to have reduced bacterial diversity and microbiome profiles that are distinct from healthy controls and from patients with isolated IBD. Enterococcus, Fusobacterium and Lactobacillus species are over-represented in the stool of PSC patients. An operational taxonomic unit of the Enterococcus genus was associated

with elevated serum ALP levels, a disease severity marker in adult patients[38]. Even the oral microbiome is abnormal in PSC, with dysbiosis shown in the saliva[39]. Because of this, several antimicrobial agents have been used and studied in the treatment of PSC including rifaximin[40], tetracycline[41], minocycline[42] and metronidazole[43,44], with mixed results. OVT has gained the most traction in pediatric PSC on the basis of positive effects noted in a small, uncontrolled case series of 14 patients[45]. We approach OVT for PSC with hope, based on many promising (but unpublished) personal anecdotes from patient and clinicians, and also caution, given the paucity of published data and lack of any large, controlled clinical trials.

Vancomycin works against gram positive bacteria by inhibiting cross-linking of cell wall substrates. When given orally, the drug has minimal systemic absorption[46]. While the drug is potent against clostridium difficile and other gram positive organisms within the gastrointestinal tract, vancomycin may also function as an immunomodulator. OVT use in children with PSC was shown to increase transforming growth factor beta levels and peripheral T-regulatory cell counts[47]. OVT is presently used in at least 7% of patients with PSC. Practice patterns at different centers vary widely. Most commonly OVT is reserved for select patients with persistently elevated biochemical markers who failed trials of UDCA. At some centers however, OVT is used as primary therapy in virtually all new PSC patients, regardless of biochemical markers[48]. There is immense interest in this therapy amongst the patients, parents, and medical providers. Damman *et al*[4] provided an excellent review of the promising but small body of published evidence that OVT may be an effective therapy for PSC. Two randomized pilot trials in adults showed efficacy in reducing serum markers of cholestasis over 12 wk in patients receiving 125 mg or 250 mg four times daily[44,49]. Metronidazole was also effective for most endpoints however, and a placebo response was seen for virtually all markers of cholestasis.

Pediatric data is limited to two small case series, published from the same group. Each contains 14 pediatric PSC patients, six of whom were described in both series[45,47]. Vancomycin was administered at 50 mg/kg/d (maximum 1500 mg daily), divided into three doses. In the original publication, after 1-2 mo of therapy, all patients had lower GGT: 9/14 (64%) normalized GGT to 50 or below but 5/14 (36%) did not, including all four patients noted to be cirrhotic before treatment. Bilirubin, an important marker of long-term prognosis even when mildly elevated[2], was not improved in any patient[45]. GGT increased when OVT was stopped, and decreased again once OVT was resumed. In the second series, highly subjective and nonspecific improvements were noted in histology and/or cholangiography in all patients after at least 3 mo of therapy. In patients with PSC-IBD, transforming growth factor beta levels increased, and subsets of T regulatory white blood cells increased on OVT[47]. Most patients were treated continuously and indefinitely. No patients were described to develop ESLD or DS. Mean follow-up time was short at less than a year and a half however. The vast majority of children will not experience liver complications within that timeframe, regardless of therapy. In a large cohort of children with PSC, only 6% of patients progressed to ESLD or DS within one and a half years, while 94% did not[2], making it difficult to infer any relative causality of OVT preventing adverse liver outcomes in these combined series of 22 unique patients.

OVT is a promising potential therapy for PSC, but more data is needed. Important questions remain as to the optimal dose, its efficacy in different stages of hepatic and biliary fibrosis, its efficacy in patients with or without IBD, and whether it should be used as primary or as salvage therapy. Although vancomycin-resistant enterococcus (VRE) has not yet been described in PSC patients with history of chronic OVT exposure, it is unclear how much surveillance testing has been done. It seems inevitable that VRE may become a problem with more widespread, long-term use. Many of these questions will be addressed in a multicenter, randomized-controlled trial that is presently being planned in children. Until more data is available, we recommend somewhat judicious use of the drug, limiting OVT to non-cirrhotic PSC patients who failed an 8-12 wk trial of UDCA, with persistently elevated GGT. Based on the case series and anecdotal data, patients will respond within 8-12 wk, often sooner. Those patients who experience a brisk reduction and ultimate normalization of GGT within this time period can be considered for longer-term therapy with at least semi-annual evaluation for vancomycin-resistant enterococcus. The length of safe treatment with OVT is presently undefined, and providers should approach this on a case-by-case basis with patients and families. OVT is not a panacea. In cases where serum biochemistry does not respond to OVT within 12 wk, we recommend cessation of the drug. Patients in this category may well be cirrhotic. Careful monitoring for the sequelae of ESLD is necessary, and pre-transplant workup should begin in patients with portal hypertension.

MANAGEMENT OF IBD AND THE ROLE OF IMMUNOSUPPRESSION FOR PSC

Most children with PSC have IBD[2,50]. The two disorders share pathophysiologic mechanisms along the "gut-liver axis"[51]. The hypothesized pathogenic mechanism for PSC include an inappropriate and dysregulated immune response in genetically susceptible individuals[52-54], defects in the normal mechanisms that protect the liver from the toxicity of bile acids[5-7], a pathogenic distortion in the fecal microbiome leading to the accumulation of toxic bile acid species and a subsequent inappropriate inflammatory response[33-37], migration of gut-activated mucosal lymphocytes to the liver[55], disruption of the intestinal epithelial barrier due to inflammation[56-58], and an inappropriate inflammatory response to bacterial products and toxic bile acids delivered to the liver through the inflamed gut *via* the portal vein[59-61].

It is unknown whether treating a patient's IBD and inducing a sustained endoscopic remission also treats the patient's PSC. There are no prospective assessments of whether patients with PSC-IBD in sustained endoscopic or histologic IBD remission have improvement in liver biochemistry, imaging or histopathology. A PSC-IBD phenotype, independent of the IBD disease activity, tends to impart a favorable long-term prognosis of ductular disease. Children with PSC-IBD are nearly half as likely to experience a liver complication in follow-up compared to those with PSC alone[2]. Likewise, when PSC occurs concomitantly with IBD, the intestinal disease course is generally milder than in patients with IBD without PSC[62]. Presently it is not understood why when both diseases co-occur they tend to behave more mildly than when either is present individually. Over time PSC and IBD activity tend to wax and wane independently from one another[51]. Liver biochemistry abnormalities are not correlated with IBD activity in general, whether from PSC or another source[63]. Fecal calprotectin and GGT measurements over time were not correlated in children[64], and the severity of ductular disease was not strongly associated with the severity of colitis in adults[65]. Patients who underwent colectomy prior to a PSC diagnosis had a better liver prognosis however[66], implying at least some aspect of colonic disease that drives biliary disease. Longer duration of IBD, even after colectomy, was associated with a greater risk of CCA[67]. Even after LT for PSC on aggressive immunosuppression, exacerbation of existing IBD is common[68,69]. One quarter of patients without known IBD before transplant for PSC will develop it de-novo after transplant[70].

Immunosuppressive and biologic medications, independent of their effect on concomitant IBD or Autoimmune hepatitis (AIH), have not shown a benefit for PSC. Small pilot trials have been performed in adult PSC patients with a variety of agents including: Prednisone[71], budesonide[72,73], methotrexate[74,75], mycophenolate mofetil[76,77], tacrolimus[78], infliximab[79], and etanercept[80], with none demonstrating particular efficacy. When biologic agents are used to treat IBD in PSC-IBD patients, there is no benefit to the liver. Aspartate aminotransferase, alanine aminotransferase, bilirubin, elastography and cholangiography were not improved on adalimumab, infliximab or vedolizumab in PSC-IBD patients[55,81]. Of note, IBD patients without known PSC who were treated with vedolizumab were more likely to develop PSC than those treated with anti-tumor necrosis factor agents, an effect that was particularly pronounced in those with a Crohn disease phenotype[82]. Pediatric studies of immunosuppression for PSC are limited, but also show a lack of efficacy. Azathioprine and prednisone in a prospective study of children with PSC-AIH overlap failed to halt progression of ductular disease[83]. Separate data suggest that PSC-IBD patients may be less-tolerant of azathioprine, with a higher rate of adverse hepatobiliary or pancreatic reactions[84]. Retrospective data show infliximab is not helpful for PSC in children with PSC-IBD[85]. PSC and PSC-AIH patients have similar long-term survival despite most of the latter group receiving systemic immunosuppression[2].

Ultimately the IBD in PSC-IBD should be treated to a goal of deep remission per standards in that field, to prevent bowel surgery and colorectal malignancy. Treatment of IBD does not consistently improve PSC however. Clinicians should be aware that PSC-IBD patients are at risk of subclinical endoscopic and histologic disease activity, even when symptom free and even after colectomy[86,87]. PSC-IBD patients have an approximately four times greater risk of colorectal cancers compared to patients with IBD alone[88]. While rarely seen in children with IBD in general, the increased risk likely applies to pediatric patients as well. In the absence of formal screening guidelines, annual or biannual surveillance colonoscopy seems warranted, especially in teenage patients or those with several years of active colonic disease. Fluorescein-enhanced, probe-based confocal laser endomicroscopy (pCLE) and chromoendoscopy to obtain targeted biopsies from areas suspicious for dysplasia

were far superior to sequential random biopsies using routine screening endoscopy. In one study of screening endoscopy in adults with PSC-IBD, 90% of dysplastic biopsies were discovered on pCLE *vs* only 10% randomly[89]. Pediatric providers are far less experienced than their adult counterparts in colonoscopic screening for dysplasia and polyps, and most have no knowledge of pCLE and chromoendoscopy. We recommend collaboration with adult endoscopists for cancer screening when possible, particularly at critical points in care such as prior to LT.

NEW MEDICAL THERAPIES AND FUTURE DIRECTIONS

Not all patients respond to UDCA or OVT, but new therapies are constantly under investigation. The future seems bright for children and families dealing with PSC. After no large clinical trials in pediatric PSC for decades, three different multicenter clinical trials enrolling pediatric patients are in the late stages of planning for OVT a combination agent HTD1801, and sulfasalazine. There are several active areas of investigation in the field with promising clinical data from adults with PSC. Norursodeoxycholic acid is a synthetic homologue of UDCA which may enhance the protective bicarbonate buffer between cholangiocytes and bile acids in the biliary lumen. A dose-dependent improvement in ALP and GGT was seen in a 12-wk phase 2 adult multicenter trial with a good safety profile[7], and a phase III trial is ongoing. Obeticholic acid is an activator of the farnesoid X receptor, central to bile acid metabolism and homeostasis, currently approved to treat adults with primary biliary cholangitis[90]. A phase II trial of obeticholic acid is underway in adults with PSC. Inhibition of bile acid reuptake in the ileum is also being explored as a therapeutic mechanism[91].

Since PSC has a multifactorial etiology, the possibility of combination therapy with multiple agents has been explored. A combination of all-trans retinoic acid, an inhibitor of bile acid synthesis previously shown to have some benefit in adults with PSC[92], with the anti-inflammatory chemokine receptor antagonist cenicriviroc shown to have benefit in non-alcoholic steatohepatitis[93], demonstrated synergy in treating an animal model of cholestasis[94]. When metronidazole[43] or all-trans retinoic acid[92] were combined with UDCA, biochemical improvements were demonstrated compared to UDCA alone. Personalized medicine with combination therapy tailored to an individual patient's microbiome, metabolome, bile acid pool and genome may be the future of PSC therapy.

ENDOSCOPIC THERAPY

Close collaboration with an advanced endoscopist, skilled in ERCP is necessary when managing children with PSC. While once used as a diagnostic tool to visualize the focal stricturing and saccular dilation of bile ducts that produce the classic "beaded" appearance of PSC, advancements in magnetic resonance cholangiopancreatography have largely replaced its role in initial PSC diagnosis. ERCP is now mainly a tool to relieve biliary obstruction related to strictures, and to obtain tissue or brushings when CCA is suspected.

The first successful ERCP in a 3.5-mo old child utilizing an adult duodenoscope was reported in 1976[95]. Since then, multiple studies have demonstrated the feasibility, safety and utility of ERCP in the pediatric population with reported success and complication rates comparable to those quoted in the adult literature[96-100]. Pediatric expertise with ERCP is limited however. Pediatric-trained advanced endoscopists are currently practicing at very few institutions in the world, thus necessitating close collaboration with adult GI colleagues for most providers. To ensure optimal care for pediatric patients in adult medical centers it is necessary to establish these relationships in advance of when a PSC patient needs a procedure. Teams should establish protocols to ensure safe sedation of children in an adult hospital, to ensure insurance coverage for patients, and to plan for transportation of patients to and from procedures.

Pediatric and adult patients undergoing ERCP for PSC are at significantly higher risk than the normal population for post-ERCP bacterial cholangitis. Cholangitis in these patients may be life threatening. Prophylactic antibiotics per the American College of Gastroenterology PSC guidelines show antibiotic therapy as a rationale precaution to prevent post-ERCP cholangitis although this is a conditional recommendation based on low quality of evidence. Typically pre-operative antibiotics will be started and followed by a 3-5 d course using either a quinolone or cephalosporin, although to date no prospective studies have determined the best

antibiotic regimen[101].

The main role of ERCP is dealing with DS which is defined in adult patients as a common bile duct measuring < 1.5 mm in diameter at is most narrow site or a hepatic duct measuring less than < 1.0 mm in diameter[102]. There is no standard definition of DS in pediatrics, though these definitions are likely applicable to all but the smallest children. For most patients with DS, endoscopic intervention can relieve the cholangitis and associated pruritus. Balloon dilation and deployment of intraductal stents are two available options, with balloon dilation generally favored. Balloon dilation of DS has been shown to prolong development of ESLD in adult PSC patients[103]. A recent European multicenter randomized trial (DILSTENT) compared balloon dilation *vs* stenting for DS and found that short-term stenting was not superior to balloon dilation. In addition, stenting was associated with far more frequent treatment-related serious adverse events including: post ERCP pancreatitis, cholangitis and cholecystitis[104,105]. Routine stenting is not currently recommended. Many patients require multiple repeated ERCP sessions to achieve stricture palliation.

ERCP is the key tool for diagnosis of CCA. Of adult PSC patients with DS, 26% ultimately developed CCA[19]. CCA is far rarer in children than in adults with PSC. It developed in fewer than 1% of children in a large series, who were all over age 15 with large duct involvement[2]. CCA should be considered in all PSC patients with a DS. The typical presenting symptoms of CCA are due to obstruction of the biliary tree including worsening jaundice, pruritus, clay colored stool, and dark urine. Unexplained chronic abdominal pain and weight loss in a child with PSC, even in the absence of jaundice, should prompt evaluation as well. In a small case series, 2 of 4 teenage male patients with CCA had pulmonary metastasis at the time of diagnosis, raising the possibility of a more rapidly progressive phenotype in children[106]. In adult patients with PSC, screening liver ultrasound and serum CA 19-9 are recommended every 6-12 mo[20]. These recommendations can reasonably be applied to children over aged 15, with abnormalities being referred to ERCP.

The ability to diagnose CCA with ERCP is limited. Endoscopic techniques commonly used to detect malignancy with DS include brushings for cytology, fluorescence in situ hybridization (FISH) and biopsies for pathology. The specificities of these tests are very high, however even when combined, the sensitivities approach only 60% thus having a low negative predictive value and making them inadequate as a sole means to exclude CCA[107,108]. Cholangioscopy is being increasingly utilized to directly visualize the ductal lumen and target biopsies to areas of irregular mucosa, increasing yield over blind sampling[109]. Combining cytology, FISH and cholangioscopy raises the sensitivity and specificity of ERCP for CCA to more than 90%[102,108].

MANAGEMENT OF PRURITUS

Although relatively common in adult PSC patients, pruritus is rare in children. Chronic pruritus was noted in only 4/120 (3%) children in a large single-center series[50]. In patients with ESLD from PSC with severe biliary stricturing present, the associated pruritus can impair sleep and have a major impact on quality of life. Intractable pruritus can be an indication for LT. The etiology of pruritus among cholestatic liver disease, including PSC, is not well understood. The accumulation of bile acids, increased endogenous opioid production, and elevations in lysophosphatidic acid levels, are some of the better-understood mechanisms for the development of pruritus. Data on currently available medications for pruritus is largely anecdotal, and each of several options is effective for only a proportion of patients.

Among patients with PSC presenting with pruritus, initial evaluation for the presence of a DS should be pursued. Endoscopic treatment may be necessary before the use of medical therapy. Formal treatment guidelines for medical therapy of pruritus in adult PSC have recommended bile acid sequestrants, antibiotics such as rifampin, opioid antagonists including naloxone, and selective serotonin reuptake inhibitors such as sertraline, though the strength of evidence for many of these therapies is lacking[20]. Limited case reports have reported mixed results with the use of probiotics[110], ondansetron[111], dronabinol[112], phototherapy, plasmapheresis and dialysis[113]. UDCA has a role in pruritus management as previously mentioned[23], but not all patients respond. Treatment is tailored to the individual patient, often requiring multiple trials of different agents to achieve control of symptoms.

Recently, two European groups have shown significant improvement in pruritus with bezafibrate in patients with PBC who were poor responders to UDCA[114,115]. The FITCH study (http://www.clinicaltrials.gov, NCT02701166) is an ongoing phase 3,

multicenter, double-blind, randomized placebo-controlled trial to evaluate the effect of bezafibrate among those with primary biliary cholangitis or primary/secondary sclerosing cholangitis. Studies of maralixibat (http://www.clinicaltrials.gov, NCT02061540) and curcumin (http://www.clinicaltrials.gov, NCT02978339) are active as well.

TREATMENT TARGETS AND ENDPOINTS

The validation of biomarkers as surrogate endpoints is needed in pediatric PSC. Presently there is no accepted surrogate endpoint that reliably predicts clinical outcomes in PSC. There is no formal agreement on which biochemical, radiographic or histologic markers represent the best way to prove remission or stratify a patient as "low-risk" or "high-risk" for progression to liver outcomes. Validation of a surrogate marker of disease activity is critical for clinical trial design as well. Since PSC progresses slowly, over years or even decades, it is preferable to power a large clinical trial to show normalization of a biomarker over six months, rather than a reduction in clinical events over 5-10 years.

In 2014, the International Primary Sclerosing Cholangitis Study Group initiated a Delphi process to identify candidate surrogate endpoints. ALP, vibration controlled transient elastography (VCTE), liver histology, ALP and liver histology in combination, and serum total bilirubin were chosen for future study and validation[116]. Childhood-onset PSC was not specifically considered in this Delphi process however, and ALP is not a useful biomarker for pediatric liver disease. Normal ALP varies widely in children and adolescents. Values of over 500 IU/L are normal in boys and girls aged 12-13 due to rapid growth and bone turnover[117,118], and values in the thousands are normal in young children with benign transient hyperphosphatasemia[119]. Measurement of liver-specific isozyme levels of ALP is not routine in clinical practice. GGT levels are instead measured routinely in pediatric clinical practice. GGT has no source from bone, which avoids the confounding effect of skeletal growth seen with ALP.

There is increasing evidence of the utility of GGT as a candidate surrogate endpoint in pediatric PSC. GGT at diagnosis of PSC in children correlates with long-term outcomes, but ALP does not[2]. GGT response paralleled other markers including ALP in a clinical trial of norursodeoxycholic acid[7]. GGT reduction at one-year predicted long-term outcome in pediatric PSC[24]. ALP and GGT elevation in PSC represent a similar phenomenon of cholestasis and relative biliary obstruction and inflammation. As described earlier, when ALP normalizes in adults with PSC, prognosis is excellent[21]. GGT normalization thus seems to be the most practical treatment target for children. The larger the reduction in GGT better the overall prognosis, with a > 75% reduction representing the best response. Patients who normalize GGT to less than 50 IU/L have the best prognosis overall[24]. More research is needed to determine the optimal GGT response in a shorter timeframe. In practice, clinical experience suggests that an optimal GGT response is seen within 8-12 wk, with patients reaching a nadir of potential GGT at that point, with little to no further improvement with ongoing therapy.

Serum bilirubin has prognostic utility in pediatric PSC[2], but the prevalence of DS and marked elevations of bilirubin in children is low, making it a less useful biomarker to monitor treatment when compared to adults. Not enough is known about VCTE in pediatric liver diseases to support its use in monitoring treatment. While VTCE measurements likely improve when a patient responds to a particular therapy, the time course of a measureable effect is presently unknown. Similarly, histopathology may be useful to follow relative fibrosis and inflammation over time, but liver biopsy is invasive, and the time course of improvement on a therapy is unknown. Several other biomarkers correlate with PSC prognosis when done at the time of diagnosis in adults. The enhanced liver fibrosis score is a noninvasive measurement of three serum byproducts of hepatic fibrogenesis. In PSC, enhanced liver fibrosis score at diagnosis is a strong predictor of prognosis[120]. This tool is not widely available clinically and there are not yet data in children. MRI elastography and MRI cholangiography are areas of active research in pediatric PSC[121], and may serve as an additional study to prove "deep remission" of the biliary tree on therapy when done at diagnosis and repeated 1-2 years later.

LIVER TRANSPLANTATION

LT is the only effective therapy for PSC that has progressed to cirrhosis with end stage

liver disease. PSC is one of the leading indications for LT, accounting for 5% of all LT in the United States[122]. Outcomes after transplantation for PSC are favorable and comparable to other pediatric liver disorders with > 90% patient and graft survival at 5 years[123,124].

Complications of portal hypertension and end stage liver disease such as: Recurring variceal bleeding, abdominal ascites, bacterial peritonitis and hepatic encephalopathy are the most common clinical reason to consider LT transplantation for PSC. The Model for End Stage Liver Disease (MELD) score is a useful prognostic marker in PSC. Similar to other liver diseases, pediatric PSC patients who meet minimal listing criteria should benefit from LT. Unfortunately, many children with PSC are more ill than their MELD score reflects. Chronic pruritus can be particularly intractable and debilitating in ESLD from PSC which negatively impacts quality of life and school performance. Within the United States organ allocation system, hepatologists may need to appeal to regional review boards for additional allocation priority beyond a patient's MELD score in such cases. Adolescent patients with PSC frequently experience much longer wait times pre-LT compared to other pediatric liver conditions. Long wait times are one reason patients with PSC are more likely to receive living related donation LT[125]. Preliminary data from the pediatric PSC consortium suggests transplant evaluation be undertaken for adolescent patients with MELD > 10 given need for transplant in the next 1-2 years[126]. A MELD > 20 has shown significantly decreased short-term survival after LT[127] in a retrospective study. This was most likely due to the patient becoming more ill pre-LT or the MELD score poorly reflecting the actual disease severity. For these reasons, early LT evaluation and referral after developing complications of cirrhosis such as esophageal varices is warranted, even if the MELD score does not appear excessively elevated.

Hepatobiliary cancer is an uncommon indication for LT in children, but these cases do occur. Approximately 1% of pediatric-onset PSC patients will develop CCA, primarily in teenage males. Neoadjuvant chemoradiation followed by LT offers favorable outcomes for select patients[128], possibly better than outcomes achieved with primary surgical resection without transplantation. There were no confirmed cases of hepatocellular carcinoma in a large cohort of pediatric PSC patients[2], but theoretically this risk exists with any chronic liver disease. Consultation with a referral center experienced in LT for hepatobiliary cancers is strongly recommended to assist with surgical planning, prior to undergoing any resection operation locally.

LT in PSC has unique perioperative considerations. Many patients have recurring bouts of bacterial cholangitis prior to transplant, with repeated or chronic exposures to broad spectrum antibiotics. Multidrug resistant organisms are of particular concern. Additionally, due to high rates of co-existing IBD and autoimmune hepatitis in children with PSC, many patients enter transplantation on immunosuppressive medications creating additional risk for opportunistic and atypical infections. With increasing use of OVT for PSC in children, care should be taken to screen patients for VRE. Infectious disease consultation is recommended to determine optimal perioperative antimicrobial prophylaxis.

Management of IBD surrounding LT for PSC is complex. No immunosuppression protocol has proven to be the most effective at controlling IBD pre-LT and accepted IBD management is most commonly recommended. Similarly, choice of immunosuppression regimen post-LT is complex. No single regimen is more effective. Cyclosporine and azathioprine were noted to have protective effects for IBD post-LT for PSC, whereas mycophenolate and tacrolimus were detrimental[129]. Aminosalicylates may provide a protective benefit from IBD recurrence. Nevertheless, despite significant immunosuppression post-LT, > 50% of patients will have active IBD disease warranting additional therapy[130]. Furthermore, no consensus exists regarding optimal timing of colectomy related to LT. While data suggests colectomy pre-LT may prevent PSC recurrence, adequate data to suggest routine colectomy in all patients does not exist[131]. Timing of colectomy should be personalized with factors such as severity of portal hypertension and severity of underlying IBD taken into consideration. The presence of dysplastic lesions in the colon is an absolute indication for colectomy prior to LT.

Transplantation can occur with deceased and living donors with similar success. Some data suggest living-related donation may offer superior survival[132]. This may be related to shorter wait-times and being less ill at the time of transplant, or because of immunologic similarities and lower rates of PSC recurrence. Roux-en-Y choledochojejunostomy and duct-to-duct biliary anastomosis showed similar one-year patient and graft survival in a meta-analysis[133]. PSC patients have an increased risk of vascular thrombosis after LT[134] and require careful postoperative observation and anticoagulant prophylaxis.

PSC frequently reoccurs (rPSC) in the transplanted liver. rPSC is diagnosed when PSC-like ductal lesions and cholestasis occur six months or more after transplant. Care

must be taken to exclude ductular lesions from vascular complications (hepatic artery stenosis or thrombosis), anastomotic biliary strictures, and CMV infections. rPSC occurs in 16% of adult transplant patients at a median of 6-years[135]. In children, the five-year recurrence risk after LT for PSC is 23%. Graft survival after recurrence is poor: 53% after five years[136]. The underlying etiology is unknown, but associated risk factors include younger age at PSC diagnosis and/or transplant, the coexistence of IBD, and thymoglobulin induction[137]. One study demonstrated rituximab may prevent disease recurrence, including with ABO-incompatible LT donation[138]. Prevention of rPSC requires ongoing study as factors such as colectomy (noted above) and the optimal induction and maintenance immunosuppressive regimens.

After LT, rates of colorectal carcinoma are particularly high[139]. Colorectal cancers occur in nearly 20% of all transplant recipients during follow-up. LT patients with IBD need annual endoscopic surveillance for colorectal cancer.

CONCLUSION

There are presently no specific guidelines for the treatment of children with PSC. Data is limited and much work needs to be done to identify a consistently effective therapy and to define the best surrogate biomarkers for treatment response. At least some patients respond to UDCA or OVT, and the vast majority of children with PSC already try one or both of these therapies. In an effort to offer providers and patients a framework for a standardized approach to treatment we suggest the following evidence-based treatment algorithm, detailed in Figure 1, to be updated as more data becomes available in the coming years.

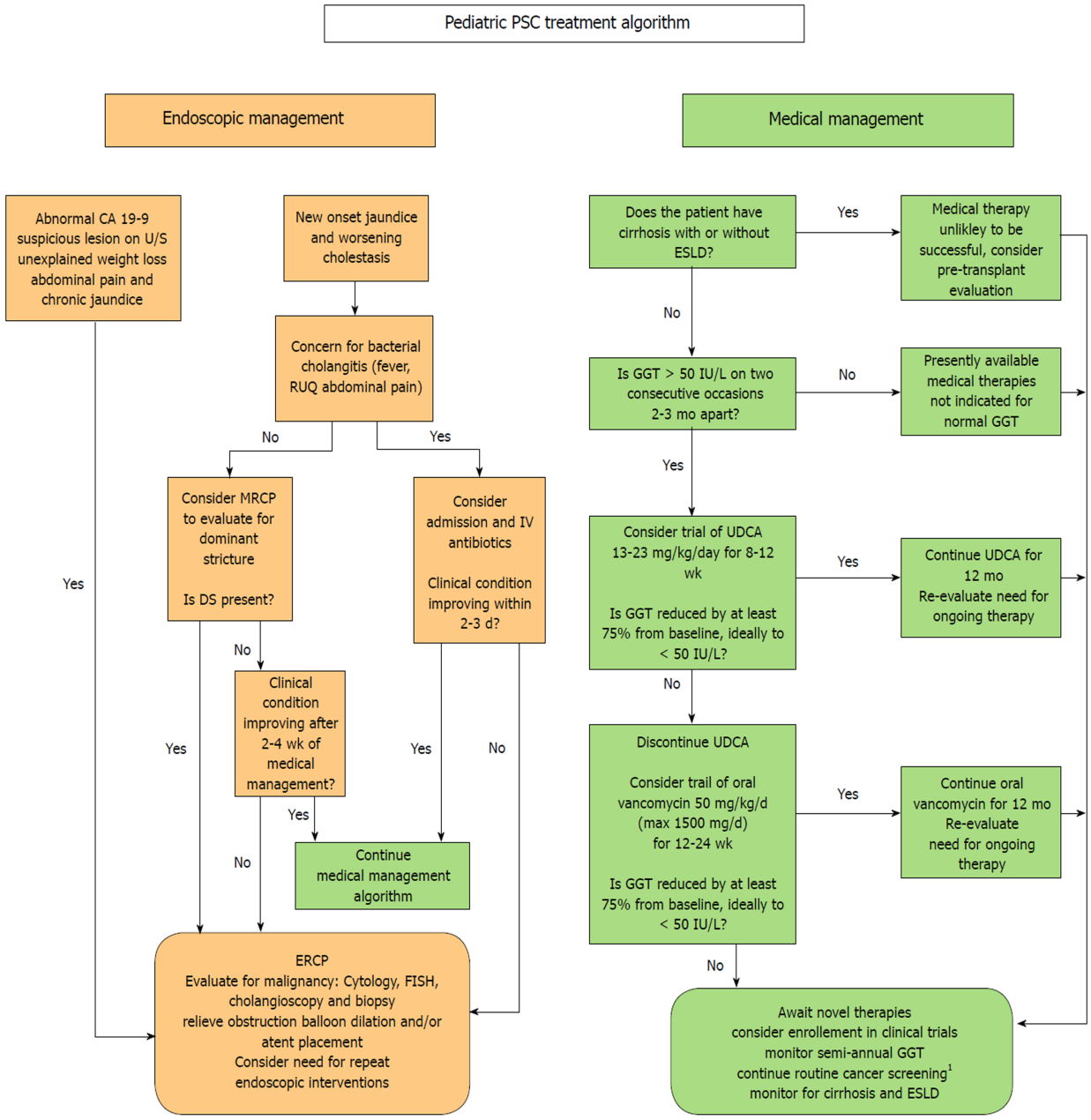

Figure 1 Endoscopic and medical management algorithm for pediatric primary sclerosing cholangitis.[1]consider CA 19-9 and abdominal ultrasound every 6-12 mo for all patients over 15 and surveillance colonoscopy every 1-2 yr for patients with primary sclerosing cholangitis-inflammatory bowel disease.

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REVIEW

Hepatitis in slaughterhouse workers

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Abstract

Slaughterhouse workers (SHW) are at increased risk of hepatitis which can occur due to different organisms and should be investigated for viral, bacterial, and parasitic organisms. Slaughter house personnel including butchers are at a higher risk of infections from cuts and blood-letting, with the possible risk of the transmission of blood-borne pathogens to their colleagues. The objective of this review is to evaluate the common etiologies of hepatitis in SHW which will assist in the assessment of these patients presenting with transaminitis. Types of Microorganisms causing hepatitis with their reservoirs, routes of transmission, laboratory diagnosis, clinical features, treatment options and preventive strategies are included in this review. Proper investigation and awareness is of utmost importance as it causes significant financial constraints derived from workers health cost and from livestock production losses when the disease is confirmed. The work up is essential because infected workers might be a source of infections to other colleagues, family and the consumers.

Key words: Hepatitis; Slaughterhouse workers; Liver infections; Transaminitis; Occupational safety; Abattoir

Core tip: Butchers and other personnel of slaughterhouse belong to a high-risk occupation and are at increased risk of transmissible diseases. This group of patients presenting to the healthcare providers with hepatitis require extensive work up to find the causative agent. In this review article, we have searched a list of organisms associated with hepatitis in slaughterhouse workers. We have also proposed an algorithm for the evaluation and management of hepatitis in these workers. It is critical to work up

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hepatitis in these infective patients because they might be a source of transmissible diseases to their colleagues, family members and consumers.

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INTRODUCTION

Slaughterhouse workers (SHW) are at a higher risk of infectious hepatitis that can be multifactorial and should be evaluated for viral, bacterial, and parasitic organisms. Viral infections are commonly sustained by certain reservoirs *e.g.*, the hepatitis B virus (HBV) has been found in gorillas, monkeys and cattle[1,2]. Slaughter house personnel including butchers are at a higher risk of infections from cuts and blood-letting, with the possible risk of the transmission of blood-borne pathogens to their colleagues[2,3].

The objective of this review is to evaluate the common etiologies of hepatitis in SHW that will be helpful for the assessment of the patients coming with hepatitis. It causes significant financial constraints derived from workers health cost and from livestock production losses if the disease is confirmed. This is also certainly important, as, the infected workers might be a source of infections to other colleagues, family, and the consumers.

COMMON ETIOLOGIES OF HEPATITIS IN SHW

Viral infections

HBV: Several cases of HBV infection have been reported in the SHW. This not only affects their colleagues but also their families[4]. HBV can be transmitted parenterally, perinatally, sexually, and horizontally. Horizontal transmission occurs *via* open wounds and by saliva, which is an important concern for the SHW as studies have reported infection in the SHW *via* this transmission[5].

SHW is also a high-risk group for HBV infections like the surgeons and blood donors *etc*. Many studies have compared rates of HBV infection among the high-risk groups, including the SHW, in the hospital and community based locations and found that HBV infection was quite higher in the community than reported in the hospital cases[6]. Butchers are exposed to the public during encounters of sale; also, infection of the cattle from the SHW is a concern, which can affect the community. SHW should be considered high-risk population for HBV infection, like health care workers and be recommended HBV vaccination[3]. A recent update by American Association for the Study of Liver Diseases in regard to the high-risk who should be screened for HBV infection , identifies the slaughter house workers at risk , in view of the blood or the body fluid exposure and requires post-exposure prophylaxis[7].

Hepatitis E virus (HEV): HEV infection in humans is usually rare in developed countries but is more frequent in many developing countries[8,9]. Majority of HEV infections are unremarkable and self-limiting but can lead to acute liver failure in immunosuppressed patients[10,11].

HEV is reported in the wild boar, camels, cows and goats. In addition to the fecal-oral route, consumption of contaminated water, raw or undercooked animal tissues or organs such as liver can be a source of HEV infection[12-14].

Rift Valley fever virus (RVF): RVF virus is an RNA virus responsible for causing significant illness both in humans and animals[15]. Mostly the patients are asymptomatic or have mild flu like disease, but small percentages of patients develop a life-threatening illness with ocular disease, hepatitis, encephalitis or hemorrhagic fever[16,17].

Humans are mostly affected by having contact with the blood and the fluids of the animals infected with RVF virus during slaughtering, taking care of sick animals or during the animal birth. Therefore, SHW and cattlemen are at an increased for the infection due to direct exposure to the infected animals[18,19].

Bacterial infections

Q fever: Q fever is a zoonotic disease caused by the bacterium, *Coxiella burnetii*. The disease was first seen in Australia among the meat packers and mentioned as "abattoir fever"[20]. The organism has extensive and worldwide reservoir, mostly ungulates, can be transmitted in the urine, feces, milk and parturition products of infected animals[21]. Humans dealing with animals are mostly infected after the inhalation of contaminated aerosols in the air[22].

The disease may present acutely with a wide range of symptoms like fever, pneumonia, hepatitis and different neurologic manifestations ranging from simple headache to meningitis, encephalitis or both. Chronic disease can manifest later, after an initial infection, as endocarditis, or chronic fatigue syndrome[21,23]. Many outbreaks have been reported in the different parts of the world. Wilson *et al*[24] investigated the largest outbreak of Q fever in Scotland reporting about 110 cases, which occurred in the setting of the co-located slaughterhouse. In Korea, Chu *et al*[25] also reported the seroconversion of about 10.2% among SHW for Q fever. In their research, the critical risk factor was the contact with cattle blood especially around the mouth. Esmaeili *et al*[26] found a higher seroprevalence among butchers and SHW in Iran in a large cross-sectional study. The total seroprevalence of Q fever among subjects in the study was 22.5%.

Salmonellosis: Typhoid fever, a common infectious disease, typically manifests as acute systemic disease involving multiple body organs, and difficult to distinguish clinically from other infections. The liver can also be affected resulting in hepatomegaly and transaminitis. Although, acute hepatitis due to salmonellosis is a rare entity, delay in the treatment leads to increase mortality[27]. This is a very common foodborne illness and associated with contaminated poultry meat and pork[28]. Multiple studies through the world have evaluated the risks factors causing the transmission of salmonella to the humans[29,30]. SHW dealing with the poultry directly are at a higher risk for getting infected.

Campylobacter jejuni*:Campylobacter jejuni*, a gram-negative bacterium, is responsible for causing a major food borne gastroenteritis in humans[31]. It also causes a variety of extra-intestinal manifestations including meningitis, hepatitis, gram negative bacteremia and cardiac complications[32]. The common mode of transmission to humans include eating and handling of contaminated poultry[33]. Broiler flocks are infected in the poultry houses. Many studies have discussed factors responsible for the infection of the broiler flocks[34-36].

Leptospirosis: Leptospirosis is a zoonotic disease caused by spirochetes belonging to the genus Leptospira. Different domestic animals host this bacterium and include cattle, pigs, and sheep[37]. Humans are infected through the broken skin or exposure to contaminated water and soil from infected urine of animals[38]. SHW are increasingly exposed to Leptospira species and have noted to have the higher seroprevalence values twice those of other non-risk occupations[39,40]. The identified factors leading to increasing prevalence in SHW are smoking, drinking at work, and poor hygiene at work[38,41]. Most of the infected cases are mild but more severe clinical spectrum of leptospirosis include hepatitis, and Weil's disease with renal failure and jaundice[42]. Esmaeilli *et al* did a serological survey of leptospirosis among different population groups in Iran. The major risk factors associated with higher prevalence included eating hare meat and exposure to dead animals[43].

Bovine tuberculosis: Although, an uncommon cause of hepatitis in slaughter workers, *Mycobacterium bovis* (*M. Bovis*) is transmitted from animals to humans, either through the ingestion of animal products or through the airborne inhalation of spores[44]. Both domestic and domesticated animals are infested by *M. Bovis* and include cattle, sheep, pigs, goats, cats, dogs and horses[45]. The disease spectrum is similar to that of Mycobacterium tuberculosis and includes fever, night sweats, and weight loss. The other symptoms result, depending on the tissue of the body infected by the organism[46].

Brucellosis: Brucellosis, a zoonotic bacterial illness affects both animals and humans worldwide. It spreads systemically and mainly affects the lymph nodes, liver, spleen and bone marrow. The intracellular location of the bacterium is responsible for chronic infections. It commonly infects the gastrointestinal tract, but brain, nerves, GU, skin and hepatobiliary systems are also involved[47-49]. Brucella hepatitis usually occurs in the chronic granulomatous form with mild transaminitis, but acute cases have also been reported[49-51]. Brucellosis is transmitted to humans *via* the intake of contaminated milk products or during physical contact with infected tissues of the animals or inhalation of contaminated aerosols[52,53]. SHW, shepherds, veterinary

doctors, meat packing staff and lab staff are at an increased risk for the infection, due to increased exposure to the contaminated tissues[53].

***Clostridium perfringens* (*C. perfringens*):** *C. perfringens* cause food poisoning from food contaminated with the organism such as eating undercooked meats. Toxin mediated illness is usually self-limited and cause abdominal pain, nausea and diarrhea and last for about 6-24 h[54]. The worst form of illness is gas gangrene. Gas gangrene with *C. perfringens* typically presents with necrosis of the soft tissue, gas production, and septicemia. It rarely involves internal solid viscera like liver, kidneys, heart, *etc*. Hepatic infection possibly results from the extension of infection into the biliary tree and then into the liver[55,56]. Immunocompromised patients, such as liver transplant patients, are at an increased risk of infection with *C. perfringens*[55,57]. Very few cases of *C. perfringens* causing allograft failure are reported in the literature[58-60]. The disease has also been reported in the animals especially broiler and may be a source of transmission to the immunocompromised SHW[61].

Chlamydia psittaci: Ornithosis is a bacterial disease caused by Chlamydia psittaci and transmitted from infected parrots, pigeons, sparrows and many other bird species. The patients usually inhale the infected organism in the form of aerosolized respiratory secretions or dried feces or contact from the infected tissues of the birds[62,63]. The common symptoms of Psittacosis include influenza-like illness but can worsen to severe pneumonia and other non-respiratory health problems. Transaminitis with hepatomegaly and jaundice has been reported in the literature[62,64].

Parasitic infections

Echinococcosis: Echinococcosis is a zoonotic parasitic infection caused by larval form of different species belonging to the Echinococcus tapeworms[65]. Cystic echinococcosis represents a persistent zoonosis and one of the etiologies of parasitic hepatitis. Humans are mostly infected *via* ingesting parasitic eggs excreted within the feces of the definitive hosts, resulting in the development of cysts, primarily in the lungs and liver. This causes damage as they enlarge resulting in hepatitis and pneumonitis[65]. Some cases are reported in France and Moldova but the disease is likely present worldwide[66,67].

The prevalence in South America ranges from 20%-95% in some areas[68]. Although intermediate hosts are variable, the common ones include sheep, goats, pigs, camels, horses, and cattle [65]. Human Liver is mainly affected by the sheep strain (G1) resulting in echinococcal cysts[69].

Toxoplasma gondii: Toxoplasma gondii is a common parasitic infection with varied clinical presentations. The disease ranges from symptomless stage to a wide spectrum of clinical presentation ranging from fever and lymphadenopathy to multi-organ involvement including hepatitis, encephalomyelitis or myocarditis[70,71]. Toxoplasma gondii is commonly transmitted *via* drinking water or eating undercooked/raw meat contaminated with tissue cysts[72]. Many studies have reported an increased prevalence of infection in the SHW[70,73].

Trichinosis: Trichinosis is a parasitic infection transmitted commonly by ingestion of partially cooked/uncooked or raw pork contaminated with the cysts or larvae[74]. The incubation period is variable from a few days to weeks depending on the stage of the transmission (enteral phase or parenteral phase). The disease has acute and chronic phases. The earlier manifestations of trichinellosis include gastrointestinal upset with diarrhea along with, fever, muscle aches and persists while the larvae migrate throughout the body[74]. Larval tissue penetration and migration in the body is responsible for immune-mediated inflammatory reaction resulting in eosinophilia. Severe illness causes cardiac, neurological, hepatic manifestations and thromboembolic disease. Hepatic involvement is rare but has been reported in the literature[75,76].

Fungal infections

Candidiasis and other fungal infections can be transmitted to the SHW but rarely cause hepatitis in an immunocompetent patient. The possibility of hepatitis is usually in the patient with systemic candidiasis or severe sepsis due to candidiasis.

APPROACH FOR THE EVALUATION AND MANAGEMENT OF THE PATIENTS WITH HEPATITIS WORKING IN THE SLAUGHTER HOUSE

There is a wide array of the etiologies causing hepatitis in the patients working in the slaughter house (Table 1). The initial evaluation of hepatitis should guide whether it is an isolated presentation, or a manifestation of the systemic illness. The predominant hepatitis presentation is usually viral in etiology which includes HBV or HEV. Management is usually observant in acute disease, requires clinical monitoring to evaluate for possible liver failure. As per current guideline , the anti-viral medication are served for the fulminant presentation. The inquiry regarding the prevalent livestock diseases in the community can help in diagnosis. The type of the animal exposure will assist in identifying the causative organism. This is discussed in Figure 1.

The systemic manifestation should be evaluated for meningoencephalitis, renal failure, pancytopenia, pulmonary infiltrates, splenomegaly, cardiac diseases, skin and soft tissue infections, lymphadenopathy and muscular diseases. Management (anti-microbial) largely varies if the infection is bacterial or parasitic, hence the initial serology and imaging studies should guide to differentiate between the etiologies. Some bacterial diseases present as acute systemic illness and are usually from the atypical organism. Thus, empiric treatment with doxycycline may be considered while awaiting the bacterial serology. The bacterial infections, like bovine tuberculosis, will have chronic onset and will need thorough evaluation before consideration of anti-tuberculosis treatment. The parasitic etiology can be suspected based on characteristic imaging finding like the echinococcal cysts in liver or lungs. The other parasitic causes can be ruled out based on the serum serology and stool examination for the ova and parasites. Figure 2 elaborates a proposed algorithm for the assessment and management of slaughter house workers presenting with hepatitis.

CONCLUSION

This review concludes that SHW are high-risk occupational group for hepatic infections and there should be regular screening tests against the transmissible infections. All SHW should be instructed to see medical attention as soon as they there an event that might lead to transmission of disease. This is especially important for the workers directly involved in animal slaughtering. Individuals involved in transportation and handling of animal residues, or inspection the carcasses may be at a lower risk.

Table 1 Various microorganisms responsible for causing hepatitis among slaughterhouse workers along with their reservoirs, routes of transmission, diagnostic tools, clinical features treatment and preventive strategies

Microorganisms	Reservoirs of infection	Routes of transmission	Laboratory diagnosis	Common clinical features	Treatment options	Preventive strategies
HBV	(1) Gorillas; (2) Chimpanzees; and (3) Cows[77]	(1) Parenterally; (2) Perinatally; (3) Sexually; and (4) Horizontally[5]	Serology: (1) HBsAg; (2) HBeAg; (3) Anti-HBc IgM; (4) Anti-HBc IgG; (5) Anti-HBe; and (6) HBV DNA[77]	(1) Constitutional symptoms; (2) Anorexia; (3) Nausea; (4) Vomiting; (5) Low-grade fever; (6) Myalgia; (7) Disordered gustatory acuity and smell; (8) RUQ pain; (9) Hepatic encephalopathy; (10) Ascites; (11) Gastrointestinal bleeding; and (12) Coagulopathy[77]	(1) NtRTIs: (a) Tenofovir; and (b) Adefovir; (2) NRTIs: (a) Entecavir; (b) Elbivudine; (c) Lamivudine; and (3) PEG- interferon -a 2a, interferon alfa-2b[78]	(1) Pre-exposure vaccination; and (2) Post exposure prophylaxis with vaccination and immunoglobulin's depending on clinical status[3]
HEV	(1) Wild boar; (2) Camels; (3) Cows; (4) Goats; and (5) Pigs[13]	Fecal-oral route[14]	Anti-HEV IgM[14]	(1) Prodromal-phase; (2) Myalgia; (3) Arthralgia; (4) Fever; (5) Anorexia; (6) Nausea/vomiting; (7) Weight loss; (8) Right upper quadrant pain; (9) Icteric-phase; (10) Jaundice; (11) Dark urine; (12) Light-colored stools; (13) Pruritus; and (14) Right upper quadrant tenderness and hepatomegaly[12,14]	(1) Mostly are self-limited; and (2) Current treatment options: (a) Ribavirin; (b) Pegylated interferon for chronic infection in immune-compromised[79]	(1) Hygiene; and (2) Recombinant vaccines have demonstrated efficacy against HEV. (Available in China)[79]
RVF	Livestock[15]	(1) Contact with the blood and the fluids of the animals; and (2) Infected mosquitoes[15]	(1) Both IgM and IgG antibodies are specific to RVF virus; and (2) PCR of the antigens[17]	Mostly the patients are asymptomatic or have mild flu like disease, but a small percentage of patients may develop life threatening illness with ocular disease, hepatitis, encephalitis or hemorrhagic fever[16,17]	(1) Most human cases of RVF are mild and self-limiting; and (2) A specific treatment for RVF has not established[80]	(1) Avoid contact with blood, body fluids, or tissues of infected animals and protecting themselves against mosquitoes and other bloodsucking insects; and (2) Use of mosquito repellents and bed nets are two effective methods[80]
Q fever	(1) Domestic mammals, especially ungulates (cattle, sheep, and goats); and (2) Also has been found in wild mammals, birds, and arthropods[21]	(1) Transmitted *via* the urine, feces, milk and parturition products of infected animals; and (2) Aerosolized breathing in dust that has been contaminated by infected animal feces, urine, milk, and birth products[22]	(1) Serologic testing with PCR in the early stages of acute illness[81]; and (2) A fourfold rise in IgG antibody titer between acute and convalescent samples	(1) Acute: (a) Fever; (b) Pneumonia; (c) Hepatitis; and (d) Neurologic manifestations ranging from a simple headache to meningitis, encephalitis or both; and (2) Chronic infection: (a) Endocarditis; and (b) Chronic fatigue syndrome[21,23]	(1) Acute illness: Self-limited but 2 wk of doxycycline recommender; and (2) Chronic Q fever: Requires several months of antibiotics with a combination of antibiotics including doxycycline and hydroxychloroquine[81]	(1) Avoiding contact with animals, especially while animals are giving birth; and (2) Do not consume raw milk or raw milk products[81]

Salmonella	Intestinal tracts of humans and other animals, including poultry, other birds, amphibians, and reptiles[82]	Foodborne illness associated with contaminated poultry meat and pork[28-30]	(1) Serotyping and DNA fingerprinting; (2) Blood cultures; (3) PCR using H1-d primers; and (4) The Widal test or Typhidot for serology is rarely used now[82]	(1) Systemic disease involving multiple body organs; and (2) Liver can also be affected resulting in hepatomegaly and transaminitis[27,28]	Antibiotics based on sensitivities[82]	(1) Do not eat or drink foods containing raw eggs, or raw (unpasteurized) milk; and (2) Wash hands, kitchen work surfaces, and utensils with soap and water immediately after they have been in contact with raw meat or poultry[82]
Campylobacter jejuni	Wildlife reservoirs: (a) Wild birds species include migratory birds—ranes, ducks, geese and seagulls; and (b) Rodents and insects[83]	(1) Eating and handling of contaminated poultry, water and milk; and (2) Contact through the feces of a dog or cat[33]	(1) Serological diagnosis with ELISA; (2) PCR to detect *Campylobacter jejuni* in stool; and (3) Detection of antigens in stool specimens[84]	(1) Food borne gastroenteritis; (2) Extra intestinal manifestations; (3) Meningitis; (4) Hepatitis; (5) Bacteremia; and (6) Cardiac complications[31,32]	(1) Azithromycin and Fluoroquinolones; and (2) Antimicrobial susceptibility testing is essential before treatment[84]	Good hygiene[84]
Leptospirosis	(1) Domestic animals; (2) Cattle; (3) Pigs; and (4) Sheep[37]	Humans are infected through the broken skin or exposure to contaminated water and soil from infected urine of animals[38]	(1) DNA PCR; (2) Urine is the most reliable body fluid to study because the urine contains leptospires paired; (3) Antileptospira antibodies; and (4) MAT of acute and convalescent serum specimens[85]	(1) Most of the infected cases are mild; (2) Severe disease; (2) Hepatitis; and (3) Weil's disease with renal failure and jaundice[42]	(1) Mild leptospirosis; (2) Doxycycline; (3) Ampicillin; (4) Amoxicillin; (5) Severe leptospirosis; (6) Intravenous penicillin G; (7) Third generation cephalosporin *i.e.* cefotaxime and ceftriaxone; and (8) Alternative regimens include ampicillin, amoxicillin, or erythromycin[86]	Good hygiene[42]
Bovine tuberculosis	(1) Both domestic and domesticated animals; (2) Cattle; (3) Sheep; (4) Pigs; (5) Goats; (7) Cats; (8) Dogs; and (9) Horses[45]	(1) Animals to humans; (2) Ingestion of animal products; and (3) Airborne inhalation of spores[44]	(1) AFB staining; (2) Mycobacterial cultures; (3) Molecular testing for mycobacterial DNA; (4) TST; and (5) IGRAs[87]	(1) Fever; (2) Night sweats; (3) Weight loss; and (4) The other symptoms depend on the tissue of the body infected by the organism[46]	Two months of isoniazid, rifampin, and ethambutol , followed by seven months of isoniazid and rifampin[46]	(1) Immunization with BCG vaccine; and (2) Treatment of latent infection[88]
Brucellosis	(1) Domestic animals; (2) *B. abortus* in cattle; (3) *B. melitensis* in sheep, goats, and camels; and (4) *B. suis* in swine[47,48]	(1) Intake of contaminated milk products; (2) Physical contact with infected tissues of the animals; and (3) Inhalation of contaminated aerosols[48]	(1) Cultures; (2) Serology; and (3) PCR[48]	(1) It commonly infects the GI tract but brain, nerves, GU, skin and hepatobiliary systems can also be involved; and (2) Brucella hepatitis usually occurs in the chronic granulomatous form with mild transaminitis but acute cases have been reported also[48-50]	(1) Combination of antibiotics is used; (2) Doxycycline and streptomycin; and (3) Based on the severity and location of infection, multiple combinations and longer durations are needed[89]	(1) Vaccination of domestic livestock, serologic testing, quarantine of herds, and slaughter of infected animals; (2) Protection of slaughterhouse workers; (3) Separated areas for killing from other processing areas; (4) Use of protective clothing and disinfectants; (5) Control of air circulation; and (6) Pasteurization of milk[89]

C. perfringens	(1) Soil; (2) Water; (3) Air; (4) Feces of healthy and infected individuals; (5) Gastrointestinal tract of humans and animals; and (7) Variety of dehydrated and processed foods[90]	(1) Food Poisoning; (2) Ingestion of large number of *C. perfringens* vegetative cells present in the contaminated food; (3) Gas gangrene; and (4) Contamination of wounds with dirt or any foreign material contaminated with *C. perfringens*[90]	(1) Cultures; (2) Imaging of the infected sites; and (3) Tissue biopsy with culture and Gram stain[90]	(1) Gastroenteritis[54,55]; (2) Gas gangrene; (3) Necrosis of the soft tissue; (4) Septicemia; (5) It rarely involves internal solid viscera like liver, kidneys, heart, *etc*.; and (6) Hepatic infection possibly results from the extension of infection into the biliary tree and then into the liver[56]	(1) Treatment of gas gangrene; (2) Surgical debridement; (3) Antibiotic therapy; and (4) Supportive measures[90]	(1) Protective materials like Lab coat, gloves and eye protection when dealing with infected materials[90]; and (2) Vaccination of non-immunized trauma individuals
Chlamydia psittaci	(1) Infected birds; (2) Parrots; (3) Pigeons; and (4) Sparrows[91]	(1) Aerosolized respiratory secretions; (2) Dried feces; and (3) Contact from the infected tissues of the birds[62,63]	(1) Complement fixation; (2) Micro immunofluorescence; and (3) PCR[91]	(1) Mild disease; (2) Influenza-like illness; (3) Severe disease; (4) Pneumonia; (5) Transaminitis with hepatomegaly and jaundice; and (6) Cardiac involvement[62,64]	(1) Antibiotics; (2) Tetracycline; and (3) Macrolides[91]	Follow precautions when handling and cleaning birds and cage[91]
Echinococcosis	(1) Sheep; (2) Goats; (3) Pigs; (4) Camels; (5) Horses; (6) Cattle; and (7) Human Liver is mainly affected by the sheep strain (G1) resulting in echinococcal cysts[65,69]	Ingestion of Echinococcal eggs excreted within the feces of the definitive host[92]	(1) Indirect hemagglutination test; (2) ELISA; and (3) Imaging tests for the location and size of the cysts[92]	(1) Development of cysts, primarily in the lungs and liver; and (2) This causes damage as they enlarge resulting in hepatitis and pneumonitis[65]	(1) Surgical removal of intact cysts; (2) Chemotherapy; (3) Albendazole and/or Praziquantel; (4) Chemotherapy is recommended 4 wk before, and for 1 mo after the surgery; and (5) Percutaneous aspiration, injection, re-aspiration is used in patients with inoperable intra-parenchymatous cysts[92]	Protective materials like Lab coat, gloves and eye protection when dealing with infected materials[92]
Toxoplasma gondii	(1) The definitive hosts are cats; and (2) The intermediate hosts are warm-blooded animals, including most mammals and birds[93]	(1) Consumption of poorly-cooked infected meat; (2) Ingestion of water, food, or milk contaminated with oocysts; (3) Inhalation of aerosols containing oocysts; (4) Contact with sand or soil contaminated by cat feces; (5) Transmission is also possible through blood transfusions and organ transplants; and (6) Transplacental if mother is infected[72]	(1) Positive serology for IgM and IgG antibodies; and (2) PCR[93]	(1) Fever; (2) Lymphadenopathy; (3) Multiorgan involvement; (4) Hepatitis; (5) Encephalomyelitis; and (6) Myocarditis[70,71]	(1) Spiramycin; (2) Can be taken by women in their first trimester to prevent transplacental transmission; (3) Sulfadiazine and folinic acid; and (4) For pregnant women in their third trimester[93]	(1) Protective materials like Lab coat, gloves and eye protection when dealing with infected materials[92]; and (2) Testing for toxoplasmosis in females before pregnancy[93]
Trichinosis	Different species are found world-wide in carnivorous and omnivorous animals like bears, foxes, walruses, hyenas, Pigs and cougars[94]	Ingestion of partially cooked/ uncooked or raw pork contaminated with the cysts or larvae[69]	(1) ELISAs; (2) Indirect immunofluorescence; (3) Latex agglutination; (4) Western blot; (5) Muscle biopsy; and (6) PCR[94]	(1) Diarrhea; (2) Fever; (3) Muscle aches; (4) Cardiac, neurological, and thromboembolic disease; and (5) Hepatic involvement is rare but has been reported in the literature[69]	(1) Albendazole; and (2) Mebendazole[94]	Consumption of properly cooked meat[94]

PCR: Polymerase chain reaction; ELISAs: Enzyme-linked immunosorbent assays; NtRTIs: Nucleotide analog reverse-transcriptase inhibitors; NRTIs: Nucleoside analog reverse-transcriptase inhibitors; MAT: Microscopic agglutination test; BCG: Bacillus Calmette-Guérin; TST: Tuberculin skin test; IGRAs; Interferon-gamma release assays; HBV: Hepatitis B virus; RVF: Rift Valley fever virus; HEV: Hepatitis E virus; *C. Perfringens*: *Clostridium Perfringens*.

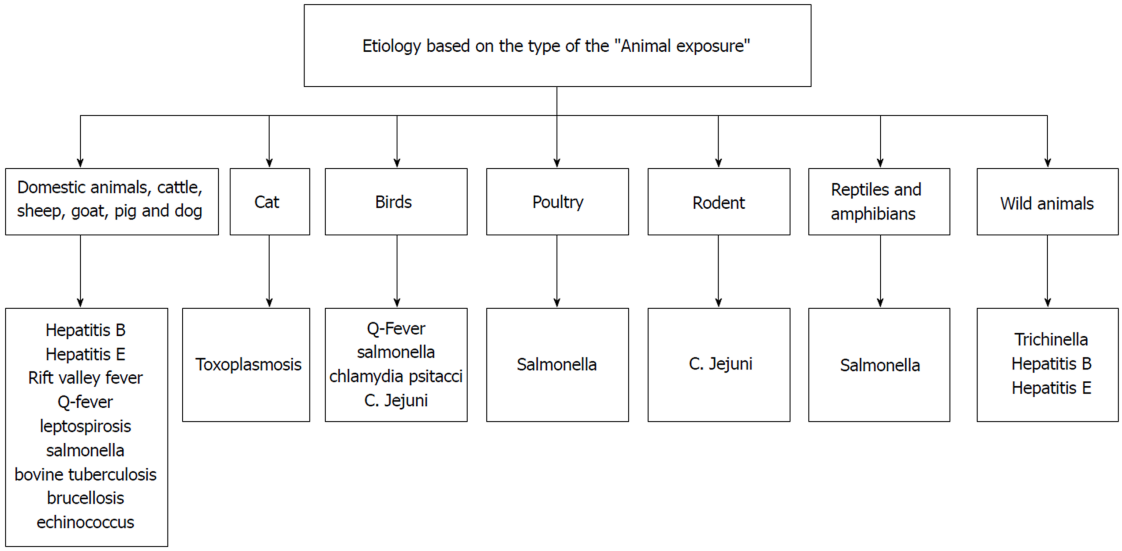

Figure 1 Etiology of hepatitis in slaughter house workers based on the type of the animal exposure.

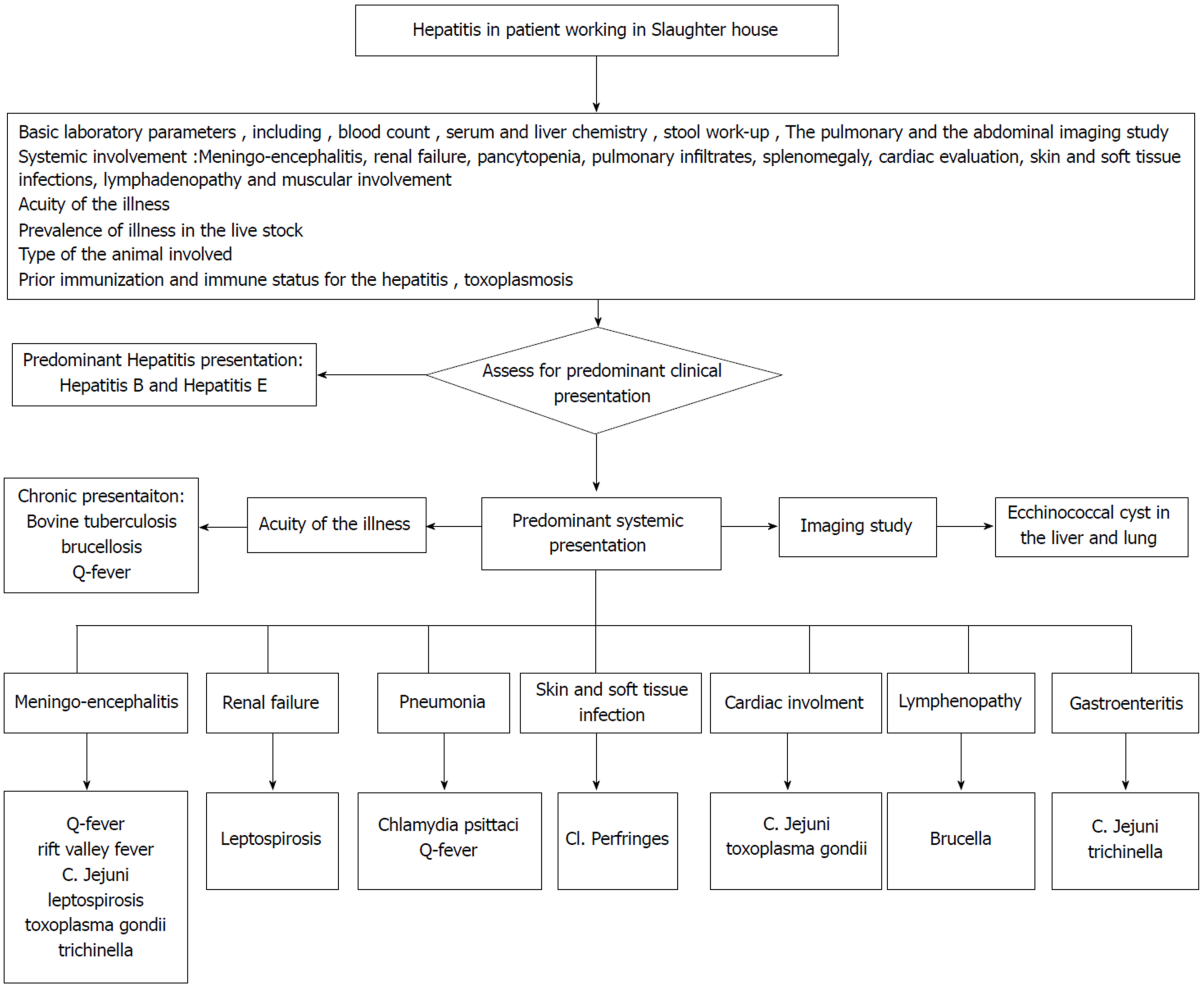

Figure 2 Algorithm for the evaluation and management of hepatitis in the patients working in Slaughterhouses.

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REVIEW

Serum biomarkers and risk of hepatocellular carcinoma recurrence after liver transplantation

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Abstract

Liver transplantation (LT) is the only potentially curative treatment for selected patients with cirrhosis and hepatocellular carcinoma (HCC) who are not candidates for resection. When the Milan criteria are strictly applied, 75% to 85%of 3- to 4-year actuarial survival rates are achieved, but up to 20% of the patients experience HCC recurrence after transplantation. The Milan criteria are based on the preoperative tumor macromorphology, tumor size and number on computed tomography or magnetic resonance imaging that neither correlate well with posttransplant histological study of the liver explant nor accurately predict HCC recurrence after LT, since they do not include objective measures of tumor biology. Preoperative biological markers, including alpha-fetoprotein, des-gamma-carboxiprothrombin or neutrophil-to-lymphocyte ratio and platelet-to-lymphocyte ratio, can predict the risk for HCC recurrence after transplantation. These biomarkers have been proposed as surrogate markers of tumor differentiation and vascular invasion, with varied risk magnitudes depending on the defined cutoffs. Different studies have shown that the combination of one or several biomarkers integrated into prognostic models predict the risk of HCC recurrence after LT more accurately than Milan criteria alone. In this review, we focus on the potential utility of these serum biological markers to improve the performance of Milan criteria to identify patients at high risk of tumoral

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recurrence after LT.

Key words: Hepatocellular carcinoma; Liver transplantation; Recurrence; Selection criteria; Prognostic score; Biomarker; Alpha-fetoprotein; Systemic inflammatory marker

Core tip: The Milan criteria for liver transplantation have improved survival of patients with small hepatocellular carcinoma (HCC), but up to 20% of patients still experience HCC recurrence after transplantation. Microvascular invasion and tumors with poor histologic grade of differentiation are the most important risk factors for HCC recurrence, but they are evidenced after surgery on explant pathology examination. Several surrogate pretransplant biomarkers, directly related with tumor biology or systemic inflammation markers conditioning tumor progression, have been suggested to identify, alone or integrated in pretransplant prognostic scores, patients at high risk of HCC recurrence after liver transplantation.

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INTRODUCTION

Liver transplantation (LT) is the best treatment option for selected patients with cirrhosis and small hepatocellular carcinoma (HCC) who are not candidates for resection. Mazzaferro *et al*[1] proposed the Milan criteria in 1996 for selection of patients with HCC for LT (defined as single lesion ≤ 5 cm, up to three separate lesions with none larger than 3 cm, no evidence of gross vascular invasion, and no regional nodal or distant metastases), and since then they have been applied worldwide. Patients fulfilling these criteria achieve similar survival rates as patients with LT without malignancies, of about 75% to 85% at 3 and 4 years respectively[2]. However, albeit that the Milan criteria are considered too restrictive and limiting for the transplantation option, HCC recurrence develops after LT in up to 20% of the patients[1-3], having adverse negative impact on patient survival. A poor histologic grade of differentiation, presence of vascular invasion, nodule size of > 5 cm, lymph nodes metastases and bilobar tumor involvement are classically associated with an increased risk of HCC recurrence after LT.

The Milan criteria are based on the preoperative tumor macromorphology on computed tomography or magnetic resonance imaging, that neither correlate well with posttransplant histologic study of the liver explant[4,5] nor accurately predict HCC recurrence after LT, since they do not include objective measures of tumor biology. In fact, small HCC may present biological aggressive features with unfavorable post-LT outcome, while other patients with HCC beyond Milan criteria but fulfilling the University of California San Francisco (UCSF) criteria[6] or the Up-to-7 criteria[7] could have a low risk of HCC recurrence in the presence of favorable tumor biology and could benefit from LT.

Liver biopsy is still the gold standard for determining the molecular biology of the tumor, its behavior and invasive characteristics. Some centers deny LT to patients with poorly differentiated tumors on needle biopsy, irrespective of number and size of tumoral nodules, and they have reported an excellent overall survival and low recurrence rates after LT even in patients exceeding Milan criteria[8-10]. However, preoperative biopsy often underestimates poorly differentiated tumors and does not accurately predict microvascular invasion, when compared with the final specimen examination after liver resection or LT[11,12]. Due to these limitations and because of the risk of needle tract tumor seeding, preoperative biopsy is not currently recommended for routine HCC evaluation; although, it is still needed in patients with atypical radiological features and in doubtful cases.

Preoperative biological markers can predict the risk for recurrence after transplantation. Biological markers can be categorized as: (1) serum markers directly

related with tumor biology, such as alpha-fetoprotein (AFP) and des-gamma-carboxyprothrombin (DCP); or (2) systemic inflammation markers, such as neutrophil-to-lymphocyte ratio (NLR) and platelet-to-lymphocyte ratio (PLR) conditioning tumor progression. In this review, we focus on the utility of these serum biological markers to improve the performance of Milan criteria for predicting recurrence after LT for HCC.

SERUM BIOLOGICAL MARKERS RELATED WITH TUMOR BIOLOGY

AFP

AFP is a 67-kDa glycoprotein that is produced by the liver in early fetal life. In adults, AFP production is restricted to a variety of liver tumors, including HCC, because of the dedifferentiation of hepatocytes. First considered a reliable marker for HCC diagnosis, at present the joined committees of the European Association for the Study of the Liver (commonly known as EASL) and the European Organization for Research and Treatment of Cancer (commonly known as EORTC) consider AFP testing as suboptimal for routine screening of early HCC (2B)[13]. In fact, about 80% of small HCC (< 2 cm) do not show high levels of serum AFP[14,15]. In the other hand, AFP level can be increased in patients with chronic liver disease, with a degree of hepatocytes regeneration in absence of malignancy[16].

Nevertheless, AFP is a surrogate marker of tumor differentiation and vascular invasion[17-20] and has proven a useful biomarker to identify patients at a higher risk for HCC recurrence[21], with varied risk magnitudes depending on the defined AFP cutoffs[22-33]. AFP has been integrated into several prognostic models for predicting recurrence after LT for HCC, by combining AFP level with tumor size and number, at different cutoffs for each variable (Table 1). Integration of AFP into the selection criteria was first proposed for patients receiving living donor (LD) LT in Asian countries, since the fast-track to LDLT may result in inclusion of patients with biologically aggressive HCC.

In the score proposed by Yang *et al*[34] patients were awarded between 1 and 4 points for each feature, with three different cutoffs: tumor size of 3, 5 and 6.5 cm; tumor number = 1, 3 and 5; and, AFP of 20, 200 and 1000 ng/mL. With a maximum score of 12 points, patients with a score ≥ 7 were considered as nontransplantable patients. In contrast, the Hangzhou criteria[35] consider transplantable patients as those with well or moderately differentiated HCC and having a total tumor diameter of > 8 cm and AFP of < 400 ng/mL. A large study conducted in 6487 patients registered in the Scientific Registry of Transplant Recipients database[36] showed that total tumor volume of ≤ 115 cm^3 and pretransplant AFP of ≤ 400 ng/mL identified patients at low risk of HCC recurrence after LT more effectively than both the Milan and UCSF criteria. This prognostic score has been validated both retrospectively in Poland[37] and prospectively in a multicenter study carried out in Canada, Switzerland and the United Kingdom[38].

The Liver Transplantation French Study Group developed and validated a prognostic model, known as the AFP model, for predicting recurrence after LT that combines AFP level, tumor size and number, at different cutoffs for each variable[17]. Tumor size was assigned: 0, 1 or 4 points when the largest tumor size was ≤ 3 cm, between 3-6 cm or ≥ 6 cm respectively; 0 or 2 points for ≤ 3 nodules or ≥ 4 nodules; and, AFP level added 0, 2 or 3 points for AFP ≤ 100, between 100-1000 or > 1000 ng/mL respectively; with a maximum score of 9 points. A cutoff of 2 points classified patients at low or high risk for HCC recurrence after LT. Thus, AFP > 1000 ng/mL provides enough points for excluding patients from LT whatever the size and number of nodules.

The AFP model better discriminated patients at high and low risk than Milan criteria. This model identifies patients within Milan criteria but with high risk of 5-year HCC recurrence as those having AFP > 1000 ng/mL (37.7% *vs* 13.3%), while patients beyond Milan criteria but with AFP < 100 have a low risk of HCC recurrence (14.4% *vs* 47.6%). Indeed, this model has been officially adopted in the liver allocation policy in France since 2013. This score has been validated in a single center from Spain[39] and in two multicenter studies, respectively from Italy[20] and Latin America[40], with similar results. Moreover, the AFP model has also been validated in a cohort of 400 patients with LDLT from Korea, in whom this model showed an improvement in predicting no HCC recurrence but not the occurrence of HCC recurrence[41].

All these models have been proved successful for selecting patients beyond the Milan criteria who will achieve similar outcomes to patients within Milan criteria.

Table 1 Main selection criteria for liver transplantation including alpha-fetoprotein

Reference	Country	*n*	AFP cutoff, ng/mL	Criteria	Validated in
Yang *et al*[34], 2007	Korea	63	20, 200 and 1000	Tumor number, tumor size and AFP level with different cutoffs	
Zheng *et al*[35], 2008	China	195	400	Hangzhou criteria: (1) TTD ≤ 8 or (2) TTD > 8, well or moderately differentiated and AFP < 400	
Toso *et al*[36], 2009	SRTR database	6487	400	TTV/AFP criteria for overall survival after LT: TTV ≤ 115cm^3 and AFP ≤ 400	Validated for recurrence after LT: Grat *et al*[37], 2013; Toso *et al*[38],2015.
Duvoux *et al*[17], 2012	France	537 (training cohort); 435 (validation cohort)	100 and 1000	AFP model: tumor number, tumor size and AFP level with different cutoffs	Varona *et al*[39], 2015; Notarpaolo *et al*[20], 2017; Piñero *et al*[40], 2016; Rhu *et al*[41], 2018
Lai *et al*[45], 2012	Italy	158	400	AFP-TTD criteria: TTD < 8 cm and AFP < 400	
Grąt *et al*[42], 2014	Poland	101	100	Warsaw criteria: (I) fulfillment of Milan criteria; or (II) Up-to-7 or UCSF criteria and AFP < 100	Piñero *et al*[43], 2016; Grat *et al*[44], 2017
Kim *et al*[46], 2014	Korea	180	1000	Samsung criteria: Up to 7 tumors ≤ 6 cm, and AFP ≤ 1000	

AFP: Alpha-fetoprotein; LT: Liver transplantation; SRTR: Scientific Registry of Transplant Recipients; TTD: Total tumor diameter; TTV: Total tumor volume; UCSF: University of California San Francisco.

Also, in a recent study[42] evaluating the role of AFP as predictor of HCC recurrence with respect to the fulfillment of Milan, UCSF or Up-to-7 criteria, patients beyond Milan criteria but within UCSF or Up-to-7 and with AFP < 100 ng/mL had a minimal risk of HCC recurrence after LT, criteria that have been validated in other studies[43,44].

Albeit AFP has proved to be a useful biomarker for identifying patients at a higher risk for HCC recurrence, there is no consensus about the best cutoff value to be considered. While different cutoffs have been proposed in several scores[17,34], other criteria include a sole cutoff at 400 ng/mL[35,36,45] or 1000 ng/mL[46]. Also, serial measurements of AFP (accounting for AFP variations) have been considered to better reflect the dynamic variations in the tumor biological behavior than a cutoff value of AFP level in a single assessment. Progression of AFP level while on the waiting list exceeding 15 ng/mL per mo[47,48], 50 ng/mL per mo[49] or 0.1 ng/mL per d[50] have been suggested as strong predictors of HCC recurrence after LT. In contrast, Grąt *et al*[42] found AFP > 100 ng/mL to better identify patients at risk of HCC recurrence than AFP slope.

DCP

Increased levels of DCP or prothrombin induced by vitamin K absence or antagonist II (PIVKA-II) are found in patients with HCC[51-43]. This abnormal form of prothrombin, produced during malignant transformation of hepatocytes, induces expression of angiogenic factors such as endothelial growth factor receptor and vascular endothelial growth factor (VEGF)[54,55]. Up-regulation of DCP has been found to correlate with the degree of malignancy of HCC, as DCP-positive tumors are characterized by increased likelihoods of intrahepatic metastasis, capsule infiltration, and portal venous invasion[56,57]. Moreover, the DCP-positive and AFP-negative tumors are more aggressive, for high risk of recurrence after treatment, since they are usually larger tumors with a poor grade of differentiation and vascular invasion[58,59].

DCP has been suggested as a stronger predictor of HCC recurrence after LT than AFP[57,60] and some centers from Asia have proposed the combined use of DCP level with tumor number and/or size in selection of candidates for LDLT with or without consideration of the AFP value (Table 2). The Kyoto criteria[61] and the Kyushu criteria[62] have been retrospectively and prospectively validated in the same centers where these scores were proposed[63-65]. Patients beyond Milan criteria but meeting Kyoto criteria had similar recurrence rate as patients within Milan criteria[61], while

Kyushu criteria was more powerful than UCSF, Tokyo and Kyoto criteria in predicting HCC recurrence[65].

Other centers have proposed different scores combining AFP and DCP levels with different cutoffs for both serum biomarkers that have improved Milan criteria for selection of patients at higher risk of HCC recurrence after LT. The A-P level criteria[66] included AFP ≤ 200 ng/mL "and" DCP ≤ 100 AU/mol, while the A-P 200 criteria[67] considered AFP ≤ 200 ng/mL "or" DCP ≤ 200 AU/molto identify patients at lower risk of HCC recurrence. Kim *et al*[68] found AFP > 150 ng/mL and DCP > 100 AU/mol to be associated with a higher risk of HCC recurrence after LT.

Lee *et al*[69] from Seoul, Korea developed and validated a model to predict recurrence after LDLT for HCC beyond the Milan criteria. Using a multivariate Cox proportional hazard model, the authors derived the model of recurrence after LT (commonly known as MoRAL) score using serum levels of AFP and DCP. Patients with a low MoRAL score (≤ 314.8) and no extrahepatic metastasis, even though their tumors exceeded the Milan criteria, had a lower tumor recurrence risk than patients within the Milan criteria with a high MoRAL score (> 314.8). Finally, the only study carried out in a non-Asiatic center found AFP ≥ 250 ng/mL and DCP ≥ 7.5 ng/mL to be associated with a higher risk of HCC recurrence[70], and added predictive information to the Milan criteria [hazard ratio (HR): 4.5 *vs* 2.6 with Milan criteria alone].

SYSTEMIC INFLAMMATION MARKERS

NLR and PLR

Two inflammation markers, the NLR and the PLR, have an important role in predicting outcome in several malignancies and have been associated with HCC recurrence after LT. Both the NLR and the PLR measure the proportion of peripheral blood neutrophils or platelets, respectively, to lymphocytes.

The link between NLR and liver malignancies was first demonstrated by Halazun *et al*[71] in patients who underwent surgery for colorectal liver metastasis. Same authors also reported that patients within Milan criteria and NLR ≥ 5 had a poorer recurrence-free survival than those with NLR < 5 (25% *vs* 75%) and proposed a pre-LT score for HCC recurrence after LT including NLR and tumor size > 3 cm (C-statistics: 0.74)[72]. Since then, NLR has been identified as an independent risk factor for HCC recurrence, along with microvascular invasion and/or tumor size and number in some studies[73-77], but not in others[78-80]. A recent systematic review by Najjar *et al*[81] and a meta-analysis by Xu *et al*[82] showed that elevated NLR is associated with a lower recurrence-free survival after LT (pooled HR: 3.77, 95%CI: 2.01-7.06) and with vascular invasion. Because of the different NLR cutoffs considered in the studies included in the meta-analysis (ranging from 2.6 to 6), Xu *et al*[82] recommend a cutoff NLR value of 4.

The prognostic significance of PLR for HCC recurrence after LT has been less extensively studied than that of NLR, but in a recent systematic review and meta-analysis including 899 patients from five studies, high PLR was associated with a significant increase of HCC recurrence after LT[83]. However, this association must be taken in consideration with great caution since a moderate level of heterogeneity was found among the studies included. In a recent study by Xia *et al*[84], PLR failed to predict HCC recurrence in patients meeting Milan criteria, but the 5-year recurrence-free survival in patients with HCC beyond Milan criteria but within Hangzhou criteria (total tumor diameter of ≤ 8 cm or > 8 cm, well or moderately differentiated and pretransplant AFP of < 400 ng/mL and PLR < 120) was comparable to the figure for patients within Milan criteria (73.3% *vs* 72.8%).

Han *et al*[85] also found that PLR was associated with HCC recurrence after LT, but interestingly a stronger association was found when considering the absolute platelet count. HCC recurrence rate after LT was higher in patients with platelet count of 75 × 10^9/L or greater at the day before surgery compared to patients with platelet count lower than 75 × 10^9/L (28.2% *vs* 13.2%). Moreover, the proportion of poorly differentiated tumors, microvascular invasion and bile duct invasion were higher in patients with platelet count of 75 × 10^9/L or greater. In the experience of those authors, the incorporation of platelet count at 75×10^9/L into the Milan criteria significantly increased the predictive power for HCC recurrence, over that of Milan criteria alone.

The molecular mechanisms through which the NLR and PLR are associated with HCC recurrence after LT remain unknown, but several hypotheses have been proposed. Both neutrophils and platelets are involved in vascular invasion and metastatization by increasing the production of proangiogenic factors such as

Table 2 Main selection criteria for liver transplantation including des-gamma-carboxyprothrombin

Reference	Country	*n*	Cutoff values	Criteria	Validated in
Takada *et al*[61],2007	Japan	125	DCP: 400	Kyoto criteria: up to 10 tumors ≤ 5 cm and DCP ≤ 400	Fujiki *et al*[63], 2009; Kaido *et al*[64], 2013
Soejima *et al*[62], 2007	Japan	60	DCP: 300	Kyushu criteria: any number of tumors < 5 cm and DCP < 300	Shirabe *et al*[65], 2011
Todo *et al*[66], 2007	Japan	551	AFP:200, DCP: 100	A-P level: AFP ≤ 200 and DCP ≤ 100	
Chaiteerakij *et al*[70], 2015	United States	127	AFP:250, DCP: 7.5		
Yang *et al*[67], 2016	Korea	88 (training cohort); 198 (validation cohort)	AFP: 200; DCP: 200	A-P 200: AFP ≤ 200 or DCP ≤ 200	
Kim *et al*[68], 2016	Korea	461	AFP: 150; DCP:100	--	

AFP: Alpha-fetoprotein; DCP: Des-gamma-carboxyprothrombin; MoRAL: Model of recurrence after liver transplantation.

VEGF[86,87]. Moreover, neutrophils, the common inflammatory infiltrate in tumors, have been found to be enriched predominantly in the peritumoral stroma of HCC tissue[75,88], correlating with angiogenesis and disease progression[89]. Within the circulatory system, platelets could help to establish metastatic lesions by blocking tumor cell removal[90,91]. On the other hand, low lymphocyte numbers, which also increase NLR and PLR values, could result in an impaired immunosurveillance against disease development and progression.

C-reactive protein

The C-reactive protein (CRP) is an acute-phase reactant synthesized by hepatocytes in response to systemic inflammation that has been related with the prognosis of various malignancies, including HCC[92]. Two independent groups from Korea have reported that high CRP level (with cutoff values at 1 mg/dL[93] or 0.3 mg/dL[94]) is an independent risk factor for HCC recurrence after LT, but only in patients beyond Milan criteria.

COMBINATION OF SERUM BIOLOGICAL MARKERS

In recent years, several studies have showed that the combination of several systemic inflammation biomarkers and tumor biomarkers predict the risk of HCC recurrence after LT more accurately (Table 3). In all the nine studies summarized, the relationship among tumor features and HCC recurrence was evidenced, and interestingly all studies analyzing pre-LT AFP level, except for one[95], found AFP to be an independent risk factor for HCC recurrence[80,96-98]. Also, Lai *et al*[78] found that although AFP and PLR were associated with HCC recurrence in univariate analysis, AFP > 200 ng/mL was the best prognostic factor with an area under the receiver operating characteristic curve (AUC) of 70.6 compared to 66.1 for PLR. Similarly, only two studies[80,99] out of three, found DCP to be an independent factor for HCC recurrence.

Regarding the systemic inflammation markers, NLR was found to be associated with HCC recurrence in six[82,95-99] out of nine studies and CRP in one[96] of two studies, while PLR was not shown as an independent risk factor in any of the four studies in which it was analyzed[78,79,99,100]. The two studies that analyzed inflammation marker sonly, found none of the biomarkers included to be independent risk factors for HCC recurrence[79,100]. Parisi *et al*[79] analyzed NLR, PLR and the inflammation-based index score (CRP ≥ 10 mg/dL and albumin < 35 gr/L; one point each) in 150 patients within Milan criteria before LT and found that absence of neoadjuvant therapy before LT and exceeding Milan criteria on explant pathology were the only risk factors for HCC recurrence. Fu *et al*[100] investigated the prognostic role of the systemic inflammation index (SII; absolute platelet count × absolute neutrophil count/absolute lymphocyte count) compared with PLR, NLR and monocyte-to-lymphocyte ratio in patients fulfilling the Hangzhou criteria for LDLT. At a cutoff of 226 × 10^9/mL, high SSI was associated with larger tumor size, greater total tumor volume, poorer differentiation grade and higher AFP level. Nevertheless, although SII was the best prognostic factor for overall survival, neither SSI nor the other systemic inflammatory markers

Table 3 Main studies analyzing several pre-liver transplantation systemic inflammation biomarkers and proposed scores

Reference	Country	LT type	*n*	Biomarkers	Time of biomarker test	Risk factors by multivariate analysis	Risk score
Yoshizumi *et al*[95], 2013	Japan	LDLT	104	AFP > 400, DCP > 300, NLR ≥ 4	Not specified	NLR, tumor size + number ≥ 8	No
Na *et al*[96], 2014	Korea	LDLT	224	AFP ≥ 100, NLR ≥ 6, CRP ≥ 1,	Day of LT	NLR ≥ 6 and AFP ≥ 100	Prognostic factor score: NLR ≥ 6 and CRP ≥ 1 (one point each)
Shindoh *et al*[80], 2014	Japan	LDLT	124	AFP, DCP, NLR	Day before LT, maximum and mean values within 90 d before LT	Tumor ≥ 5, MVI, mean NLR and maximum AFP and DCP before LT	Tokyo criteria, AFP > 250 and DCP > 450 (one point each)
Lai *et al*[78], 2014	Belgium	DDLT	146	AFP > 200, NLR > 5.4, PLR > 150	At inclusion on the waiting list, at LT,	AFP, NLR and PLR in univariate analysis	No
Parisi *et al*[79], 2014	UK	DDLT	150	NLR ≥ 5, PLR ≥ 150, IBI score	Day of LT	Absence of neoadjuvant therapy, beyond Milan criteria on explant	No
Harimoto *et al*[99], 2016	Japan	LDLT	190	DCP ≥ 300, NLR ≥ 2.66, PLR ≥ 70.4, CRP ≥ 0.27	Not specified	NLR, DCP, and tumor number ≥ 5	No
Wang *et al*[97], 2016	China	DDLT/LDLT	248	NLR continuous, AFP > 400	Within 1 wk before LT	NLR , AFP > 400, age, tumor number and size	Model TFS: 1.094 × tumor number (≤ 3, 0 points; > 3 (1 point) + 0.094 × maximum tumor diameter + 0.754 × AFP (≤ 400, 0 points; > 400, 1 point) + 0.085 × NLR -0.024 × age
Halazun *et al*[98], 2017	United States	DDLT/LDLT	339	NLR ≥ 5, AFP	NLR at day of LT; serial AFP at HCC diagnosis, before pre-LT treatment and at LT.	Tumor size and number, NLR ≥ 5, maximum pre-LT AFP, vascular invasion and poor differentiated tumors	MoRAL score: (1) pre-MoRAL: NLR ≥ 5 (6 points) + AFP > 200 (4 points) + largest tumor size > 3 cm by imaging (3 points); (2) post-MoRAL: grade IV tumors (6 points) + vascular invasion (2 points) + tumor size > 3 on pathology (3 points) + tumor number > 3 on pathology (2 points); and (3) combined score.
Fu *et al*[100], 2018	China	LDLT	150	NLR, PLR, MLR, SII	Within 1 wk before LT	No association	No

AFP: Alpha-fetoprotein; CRP: C-reactive protein; DCP: Des-gamma-carboxyprothrombin; DDLT: Deceased donor liver transplantation; IBI: Inflammation based index; LDLT: Living donor liver transplantation; LT: Liver transplantation; MVI: Microvascular invasion; NLR: Neutrophil-to-lymphocyte ratio; PLR: Platelet-to-lymphocyte ratio; SII: Systemic inflammation index; TFS: Tumor-free survival.

analyzed were associated with recurrence-free survival.

Prognostic scores including inflammatory markers for HCC recurrence after LDLT have been proposed by three different groups from Asia and one group from the United States. Na *et al*[96] proposed a prognostic factor score assigning 1 point for pre-LT NLR ≥ 6 and CRP ≥ 1 each, and Wang *et al*[97], who included only males receiving a LDLT, proposed the model tumor free survival, combining tumor morphological features with tumor biological information. Interestingly, both scores were

informative only in patients beyond Milan criteria, and not predictive of HCC recurrence in patients within Milan criteria.

The score proposed by Shindoh *et al*[80] incorporates pre-LT maximum AFP and DCP in the Tokyo criteria (≤ 5 tumors of ≤ 5 cm) to better stratify patients at risk of HCC recurrence after LT. After evaluating three different pre-LT values for NLR, AFP and DCP (the last value before LT, and the maximum and mean values within the 90 d before LDLT), the maximum AFP and DCP values and the mean value of NLR were independently associated with HCC recurrence. However, NLR had a limited prognostic impact (AUC: 0.62) and only maximum AFP and DCP values had sufficient discriminative power (AUC: 0.88 and 0.76 respectively). So, the authors proposed extending the Tokyo criteria by adding AFP > 250 and DCP > 450 (1 point for each variable; maximum score of 3). Patients with a score 0-1 had a 5-year disease-free survival rate of 97%, opposed to only 20% of patients with a score 2-3.

In 2017, Halazun *et al*[98] carried out a prospective study of 339 patients to identify predictors of HCC recurrence after LT. Preoperative NLR > 5 ($P < 0.0001$, HR: 6.2), AFP > 200 ($P < 0.0001$, HR: 3.8) and tumor size > 3 cm ($P < 0.001$, HR: 3.2) were found to be independently associated with a worse recurrence-free survival. The authors developed a new MoRAL score for predicting HCC recurrence after LT, mainly in individuals receiving a liver from deceased donors[98]. They constructed three scores: the pre-MoRAL, the post-MoRAL and the combined-MoRAL score, the latter including both pre-MoRAL and post-MoRAL scores. The pre-MoRAL score, included the three preoperative significant variables with a minimum of 0 points (no factors) and a maximum of 13 points (all 3 factors). The highest risk patients in the pre-MoRAL (score > 10) had a 5-year recurrence-free survival of 17.9% compared with 98.6% for the low risk group ($P < 0.0001$). The post-MoRAL score included four postoperatively available factors related to pathological features in liver explant, namely grade 4 HCCs, vascular invasion, tumor size > 3 cm and tumor number > 3. The pre-MoRAL, post-MoRAL and combo-MoRAL better predicted HCC recurrence after LT than Milan criteria with C-statistics of 0.82, 0.87 and 0.91 respectively.

LIMITATIONS OF PRETRANSPLANT SERUM BIOMARKERS

Most of the studies to date have been retrospective and include a small sample size; moreover, the included patients in the different studies are highly heterogeneous regarding indications for LT, handling of incidental tumors or inclusion of salvage LT. Also, frequent exclusion of patients who died within 1 mo or even 3 mo after LT could have restricted data about the most aggressive tumors. Besides, there is a great variation of time elapsed between the measurement of the markers and LT. Most studies considered these markers from the analytical data of the day before LT, while others considered these values within 1 wk before LT or did not specify it. Also, there is no consensus about the best cutoff value for each biomarker, and it maybe those different cutoffs should be considered in different populations or centers. In addition, comparison of results from multiple laboratories is uncertain because of different laboratory methods and processing techniques for measuring these biomarkers. Another limitation of the different studies reviewed here relies on the analyses of HCC recurrence as a time-dependent variable, such as recurrence or disease-free survival, without accounting for competing risk, such as death. So, patients who died early after LT or whose death was not related to HCC may never have had the chance to experience HCC recurrence.

Albeit the serum markers reviewed here are potential markers to be included in patient selection for LT, their utility is limited and they cannot be universally applied in all patients. Although AFP is considered the most useful pretransplant marker of HCC recurrence after LT, its utility is restricted by the existence of non-AFP secreting HCC. More restricted is the utility of systemic inflammatory markers, for different reasons. Although some meta-analyses have suggested NLR[82] and PLR[83] as useful pretransplant biomarkers for HCC recurrence, they are based on very few retrospective studies (four and five studies respectively), with most having small sample size. However, the most important limitation may be that these inflammatory serum biomarkers can be affected by other conditions, such an acute infection, hematologic disorders, hypersplenism, gastrointestinal tract bleeding or systemic inflammatory diseases, which are frequent in patients with end-stage liver diseases.

OTHER POTENTIAL SERUM BIOMARKERS

In addition to the serum biomarkers reviewed here, some other markers have been

proposed as potential risk factors for HCC recurrence after LT.

AFP-L3%, which represents a serum AFP fraction reactive with lens culinaris agglutinin, has been associated with HCC diagnosis[101,102]. In the LT context, an AFP-L3% level > 50 ng/mL combined with Milan criteria improved HCC recurrence prediction, when compared with Milan criteria alone[70]. Interestingly, AFP-L3% has been suggested as a highly specific marker of HCC in patients with low AFP level[102], which could overcome the limitation of AFP usefulness as a biomarker of HCC recurrence in patients with AFP-negative HCC. However, more studies are needed for this promising biomarker.

Liquid biopsy has attracted much attention as a feasible and noninvasive tool to identify tumoral markers in peripheral blood for diagnosis, monitoring and prognosis of cancer, overcoming tissue biopsy limitations. Circulating tumoral cells and tumoral cell free nucleic acids in peripheral blood could be advisory of micro metastasis, and their utility has been explored in HCC diagnosis and prognosis[103]. Very few data are available about the potential role of these circulating tumoral components as preoperative predictors of HCC recurrence after LT, and it is still a controversial issue. Although circulating HCC cells have been detected before LT, they have not been associated with HCC recurrence after LT[104]. Regarding circulating nucleic acids, AFP mRNA expression in peripheral blood has been suggested as a surrogate of circulating tumoral cells and has been associated with an increased risk of HCC recurrence after LT[105]. However, their utility is controversial and some authors consider AFP mRNA to be nonspecific for HCC micro metastases.

Some other circulating RNA have been explored, but none of them has been widely recognized as valuable marker of HCC recurrence, probably because none of them are specific for HCC[103]. Circulating tumor DNA has been isolated in patients with HCC, and has been associated with microvascular invasion[106]. However, much effort is still needed in order to consider these circulating tumor components as valuable markers in clinical practice since some limitations still need to be overcome. Although the complex methodology to isolate these tumoral components has improved dramatically, their extremely low frequencies in peripheral blood require more sensitive and cost effective techniques. Also, HCC-specific biomarkers should be validated and evidence of their association with HCC recurrence after LT should be proven.

Finally, different micro (mi) RNA signatures in liver tissue have been associated with HCC recurrence after LT[107,108]. However, the necessity of liver tissue samples limits their application preoperatively, and circulating miRNAs are at present being explored. Several circulating miRNAs have been suggested as potential biomarkers for HCC diagnosis[109], vascular invasion and prognosis[110,111]. To date, to the best of our knowledge, there is no data about the association of miRNAs with HCC recurrence after LT, and future studies are warranted to explore the utility of these promising biomarkers in preoperative prediction of HCC recurrence after LT.

CONCLUSION

Although the Milan criteria have improved survival of patients receiving a LT for small HCC, tumor recurrence after transplantation still develops in about 15% of patients. On the other hand, patients with less aggressive tumors and at lower risk of recurrence have proven benefit of LT. Since the Milan criteria are based on morphological tumor feature sonly, combination of these criteria with other preoperative available biomarkers related with tumor biology could better predict HCC recurrence after LT. Some serum biomarkers have been proposed but there is no consensus about their use, mainly due to the several limitations commented on in this review. In addition, considering that tumor growth patterns are highly variable among individuals, there probably is no perfect single biomarker for HCC prognosis after LT; thus, the combination of biomarkers could be more informative than any single biomarker alone.

For those reasons and taking into account the limitations highlighted here, multicenter prospective studies are demanded and an international consensus is mandatory in order to provide practical recommendations to guide the implementation of serum biomarkers combined with morphological criteria to better stratify patients at high or low risk of HCC recurrence after LT.

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MINIREVIEWS

Persistent risk for new, subsequent new and recurrent hepatocellular carcinoma despite successful anti-hepatitis B virus therapy and tumor ablation: The need for hepatitis B virus cure

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Abstract

Hepatitis B virus (HBV) is one of the most significant hepatocarcinogens. The ultimate goal of anti-HBV treatment is to prevent the development of hepatocellular carcinoma (HCC). During the last two decades, with the use of currently available anti-HBV therapies (lamivudine, entecavir and tenofovir disoproxil fumatate), there has been a decrease in the incidence of HBV-associated HCC (HBV-HCC). Furthermore, several studies have demonstrated a reduction in recurrent or new HCC development after initial HCC tumor ablation. However, during an observation period spanning 10 to 20 years, several case reports have demonstrated the development of new, subsequent new and recurrent HCC even in patients with undetectable serum HBV DNA. The persistent risk for HCC is attributed to the presence of covalently closed circular DNA (cccDNA) in the hepatocyte nucleus which continues to work as a template for HBV replication. While a functional cure (loss of hepatitis B surface antigen and undetectable viral DNA) can be attained with nucleos(t)ide analogues, these therapies do not eliminate cccDNA. Of utmost importance is successful eradication of the transcriptionally active HBV cccDNA from hepatocyte nuclei which would be considered a complete cure. The unpredictable nature of HCC development in patients with chronic HBV infection shows the need for a complete cure. Continued support and encouragement for research efforts aimed at developing curative therapies is imperative. The aims of this minireview are to highlight these observations and emphasize the need for a cure for HBV.

Key words: Hepatitis B; Hepatocellular carcinoma; Antiviral therapy; Persistent Risk for hepatocellular carcinoma; Tumor ablation

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Core tip: Despite the advances in the management of hepatitis B virus (HBV) infection and HBV-associated hepatocellular carcinoma (HCC), the risk for new, subsequent new and recurrent HCC persists even after over a decade of antiviral therapy and initial tumor ablation. This is due to the inability of the current antiviral therapy to eliminate the covalently closed circular DNA from the hepatocyte nucleus. There is a great need for an HBV cure drug.

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INTRODUCTION

Since the discovery of hepatitis B virus (HBV) by Blumberg *et al*[1] in 1965 and the development of the first HBV vaccine in 1983, much knowledge has been obtained regarding the pathogenesis, hepatocarcinogenesis, epidemiology and molecular biology of HBV as well as the antiviral treatment.

With vaccination, there has been a decrease in the number of infected people globally especially in the younger generations. There has also been a significant increase in survival of HBV infected individuals due to antiviral treatment and earlier detection of hepatocellular carcinoma (HCC) with simultaneous antiviral treatment.

However HBV is still responsible for 786000 deaths in 2010 with 341000 from HCC and 312000 attributed to cirrhosis[2].

HBV is a major public health issue with more than 257 million carriers living today, and up to one million who die annually from HBV-related liver disease including HCC[3,4]. HCC is the sixth most common cancer and the third leading cause of cancer deaths worldwide. Chronic infection with HBV is responsible for 50% of HCC cases worldwide[5,6]. Current therapies available to treat chronic hepatitis B (CHB) include interferon and the nucleos(t)ide analogues (NAs): Lamivudine, adefovir, entecavir, telbivudine, tenofovir disoproxil fumarate and the recently FDA-approved tenofovir alafenamide. Several studies have been conducted in the past to assess the impact of antiviral therapies, specifically lamivudine, entecavir and tenofovir, on disease progression and HCC development[7-10]. Multiple studies have demonstrated that for patients diagnosed with HBV-related HCC, improved survival can be attained with adjuvant antiviral therapy following curative liver resection and/or local tumor ablation[11,12].

Current antiviral therapies used to treat CHB control viral replication through inhibition of reverse transcriptase and subsequent interruption of HBV DNA formation. While treatment can slow and/or prevent the progression of liver disease, it does not eliminate the covalently closed circular DNA (cccDNA) from infected hepatocytes and is therefore unable to cure the infection. As a result, patients usually require lifelong therapy to control viral replication and help prevent hepatocarcinogenesis.

However, it has been reported that despite several years of antiviral therapy, a persistent risk for carcinogenesis still exists[13-15]. Studies have also demonstrated that even after more than a decade of successful HBV suppression, the risk for HBV-related HCC remains[16-18]. The aims of this minireview are to bring light to these observations and emphasize the need for a cure for HBV[19-21].

HBC REPLICATION CYCLE AND HEPATOCARCINOGENESIS

HBV related hepatocarcinogenesis has been extensively described in the past[22-27]. HBV is an enveloped partially double-stranded relaxed circular DNA virus of the *Hepadnaviridae* family. The viral replication cycle begins when HBV recognizes highly-sulfated heparin sulfate proteoglycans on the hepatocyte surface and gains entry by

binding the liver-specific receptor, sodium taurocholate co-transporting polypeptide (NTCP or SLC10A1)[28,29]. Once in the cell, the virus enters the hepatocyte nucleus where the relaxed circular DNA is converted to cccDNA. While little is known about the formation and regulation of cccDNA, it is thought that most of the steps needed for this conversion are provided by the host cell[19-21,30]. Viral cccDNA remains in the nucleus of the infected host cell and is used as the template for transcription of four viral mRNA intermediates. These mRNA intermediates eventually undergo translation to produce seven viral proteins including DNA polymerase and the core protein. One of these mRNA intermediates, called pregenomic RNA, is critical for the viral replication. It undergoes reverse transcription and serves as the template for new viral DNA. The newly formed viral DNA and viral proteins form viral nucleocapsids that obtain HBV envelope proteins prior to being released from the hepatocyte as mature enveloped virions[19-21,30]. These virions then go on to infect other hepatocytes.

As to HBV associated hepatocarcinogenesis, inside the hepatocyte nucleus, HBV DNA integration with the host genome takes place during the acute phase of infection[31,32]. This integration is thought to be one of several mechanisms that leads to carcinogenesis and HCC. Activation of cellular oncogenes, inactivation of tumor suppressor genes, chronic liver injury, inflammation and regeneration, activation of cellular proto-oncogenes, suppression of growth regulating genes and increased HBx protein have all been implicated in the development of HCC[33] (Figure 1).

CURRENT ANTIVIRAL THERAPIES FOR CHRONIC HBV AND THE IMPACT ON HCC INCIDENCE

Current therapies available to treat CHB include interferon and NAs: lamivudine, adefovir, entecavir, telbivudine, tenofovir disoproxil fumarate and the recently FDA-approved tenofovir alafenamide. While interferon works through immune modulation and has a weak antiviral effect, the NAs inhibit viral replication through direct inhibition of viral reverse transcriptase. The goal of these antiviral medications is to improve quality of life and survival by preventing the progression of CHB and development of cirrhosis and HCC. Currently the treatment objectives are categorized as shown in Table 1. While a "functional cure" is defined as the loss of hepatitis B surface antigen (HbsAg) and/or seroconversion to antibody to hepatitis B surface antigen with undetectable serum HBV DNA, it is important to remember that this is not a complete cure[19]. This complete cure is what is desperately needed to end the persistent risk for HCC.

Decreased incidence of HCC with antiviral therapy

While a complete cure is not yet available, several studies have assessed the impact of NAs on disease progression and HCC development. A randomized controlled trial by Liaw *et al*[7] randomized patients with advanced fibrosis or cirrhosis secondary to CHB to receive daily Lamivudine or placebo for up to 5 years. HCC occurred in 17/436 (3.9%) of patients treated with Lamivudine and 16/215 (7.4%) of patients who received placebo (P = 0.047). After a median treatment duration of 32.4 mo, the incidence of HCC was significantly reduced in the Lamivudine group and the study was stopped[7].

A retrospective study by Eun *et al*[8] conducted from March 1997 to February 2005 also showed a decreased incidence of HCC with use of lamivudine in patients with chronic HBV and compensated cirrhosis. HCC occurred in 4.9% of patients in the group treated with Lamivudine with sustained viral suppression compared to 25% of patients in the untreated group.

Similar results have also been shown with newer antivirals such as entecavir and tenofovir. Hosaka *et al*[9] assessed the risk of HCC in patients with CHB treated with entecavir compared to a control group of treatment-naïve patients. After 5 years of treatment, patients treated with entecavir had a cumulative HCC incidence of 3.7% compared to 13.7% in the treatment-naïve group (P < 0.001). Similarly, Kim *et al*[10] examined the incidence of HCC in patients treated with tenofovir disoproxil fumerate (TDF). Patients with CHB, including those with cirrhosis, were treated with TDF and assessed for incidence of HCC. The investigators found that there was a decreased incidence of HCC noted by the third year of treatment with TDF compared to the predicted incidence.

Improved survival with antiviral therapy after the development of HCC

Since the first antiviral drug for HBV became available, antiviral therapy has been used in patients following surgical resection or local ablative therapy for HBV-associated HCC. Longer survival with concomitant antiviral therapy following tumor

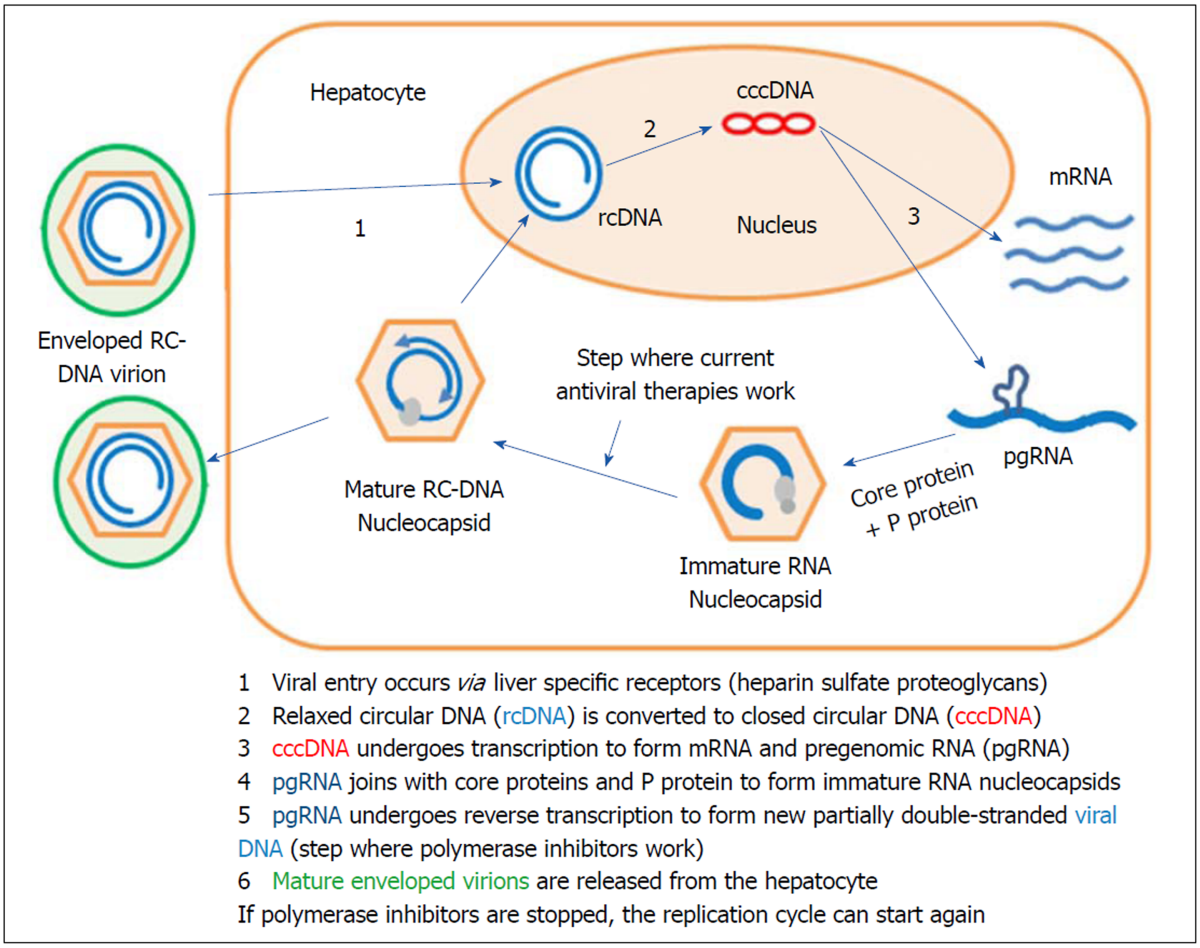

Figure 1 Hepatitis B replication life cycle.

ablation has been observed in a number of studies conducted in Japan, Hong Kong, Taiwan and China since 2007. Similar observations of improved survival were first reported in the United States in 2011[34]. A meta-analysis conducted by Yuan *et al*[11] reviewed sixteen of these studies in an effort to investigate the effect of antiviral therapy after curative therapy in patients with HBV-associated HCC. The papers in the analysis included four studies from China, three from Hong Kong, one from Taiwan, six from Japan and one from the United States. The meta-analysis looked at the impact of NAs on the one and three year recurrence rates, as well as one, three and five year overall survival and disease-free survival rates. The results revealed that NAs significantly reduced the recurrence of HBV-related HCC after curative therapy and improved both overall survival and disease-free survival[11].

Liu *et al*[12] also conducted a meta-analysis to assess the effect of adjuvant antiviral therapy following hepatectomy and/or percutaneous ablation for HBV-related HCC. They looked at twenty-one studies that compared antiviral therapy with placebo or no treatment. The primary end-points assessed were recurrence-free survival and overall survival. While this meta-analysis did have some limitations including significant between-study heterogeneity and most of the studies being observational in nature, NAs did show a significant improvement in prognosis after curative treatment in patients with HBV-associated HCC[12].

Development of new HCC while on successful antiviral therapy

While overall incidence of HCC has reduced with antiviral therapy, HCC can still develop despite successful viral suppression, most commonly in patients with cirrhosis. The most commonly reported cases of HBV-associated HCC developed within five to ten years despite antiviral therapy[13-15]. However, recent observations at the Liver Disease Prevention Center, Division of Gastroenterology and Hepatology of Thomas Jefferson University Hospital have demonstrated development of HBV-associated HCC even after a decade of successful viral suppression[16,17]. Patients from these observations were maintained on antiviral treatment anywhere from 5.2-18.4 years with undetectable serum HBV DNA for up to 12.4 years before HCC

Table 1 Definition of hepatitis B virus cure[19]

	HBsAg	Anti-HBs	Viremia	cccDNA
Functional cure	-	+	-	+
Complete cure	-	+	-	-

HbsAg: Hepatitis B surface antigen; Anti-HBs: Antibody to hepatitis B surface antigen; cccDNA: Covalently closed circular DNA.

development. Due to close monitoring and follow up, most of the tumors were discovered early and were ablated successfully (Table 2)[17].

Development of the subsequent new and recurrent HCC

After resection or ablation of the initial HCC, the patients were continued on antiviral therapy and maintained successful viral suppression. However, the persistent risk for subsequent new and recurrent HCC in this patient population has been observed. We recently described three cases of subsequent new and recurrent HCC in patients who underwent successful initial tumor ablation and had suppression of serum HBV DNA for years. Figure 2 depicts these three patients and shows the time from their HBV diagnosis to the initial HCC and eventual development of subsequent new and recurrent HCC. One of these patients described in Figure 3 was diagnosed as HbsAg (+) in 1987. She did not receive antiviral therapy as it was not available until after 1998. In 2000, thirteen years later, she was diagnosed with HCC. Following successful transarterial chemoembolization (TACE) and radiofrequency ablation she was maintained on antiviral therapy for five years at which point she was found to have a 1.1 cm × 0.8 cm arterially enhancing lesion with washout appearance near the previously treatment site on abdominal magnetic resonance imaging consistent with recurrent HCC. She underwent TACE and was maintained on antiviral therapy for another ten years at which point she developed another 0.8 cm recurrent HCC despite an undetectable serum HBV DNA[18].

Need for HBV Cure

Without eradication of cccDNA from infected hepatocytes, HBV continues to carry the risk of development of HCC despite functional cure being achieved as described above. As we become more aware of the persistent risk for new, subsequent new and recurrent HCC, we become more aware of our need for a cure for HBV infection. Antiviral therapy is able to control viral replication and slow the progression of liver disease in the majority of treated patients, but the persistence of cccDNA and continual suppression of the innate and adaptive host immune response causes a persistent risk of hepatocarcinogenesis. Several steps in the HBV replication cycle have been identified as potential targets for future therapies. These therapies can be separated into four categories based on their target of action: (1) complete inhibition of HBV replication; (2) restoration of host innate immunity and adaptive anti-HBV T and B cell responses; (3) selective sensitization of HBV infected hepatocytes to immune elimination; and (4) direct targeting of cccDNA[21].

Complete inhibition of HBV replication: The first category, complete inhibition of HBV replication, should have in theory already been achieved with existing NAs. However, complete inhibition of HBV replication is not achieved with NAs alone since inhibition of DNA polymerase is only one step of the entire HBV replication cycle. If this had been achieved, we would see elimination of HBV DNA and even cccDNA as infected hepatocytes are replaced. In addition to direct inhibition of HBV DNA polymerase, other steps in the replication cycle are being targeted. Entry inhibitors are being investigated and work through targeting the NTCP receptor preventing de novo infection of hepatocytes as well as the spread of the virus from infected hepatocytes to non-infected hepatocytes. Capsid inhibitors are also being investigated and have the potential to work at several different steps in the HBV replication cycle where the capsid is essential including genome packaging, reverse transcription, intracellular trafficking and re-importation into the nucleus. These therapies have the potential to augment the action of NAs and lead to complete inhibition of HBV DNA[19].

Restoration of host innate and adaptive immunity: In regards to restoration of host innate and adaptive immune responses, several targets have been identified. However, the challenge of stimulating an antiviral immune-mediated response without triggering detrimental anti-HBV flares is difficult. Inhibiting HBV gene

Table 2 Development of hepatocellular carcinoma in patients with cirrhosis on long-term antiviral therapy[17]

Patient	Date start Tx	Chang in child class on Tx	Date HCC Dx	Years on anti-HBV Tx at HCC DX	Years with HBV DNA (-)	Age (yr) at HCC Dx	Size (cm) and site of HCC	HBV DNA at HCC Dx	Anti-HBV Tx	Status
1	4/1998	B→A	7/2007	9.3	3.4[1]	53	1.1 Junction	UD	LAM + TDF	Alive
2	6/2002	A→A	8/2007	5.2	4.7	70	1.0 Rt	UD	LAM + TDF	Alive
3	1/1998	B→A	3/2008	10.2	8.2	68	2.8 × 2.5	UD	LAM + TDF	Dead
4	5/1998	A→A	2/2008	9.8	6.7	76	1.8 × 0.9 Lt	UD	LAM + TDF	Alive
5	7/2004	B→B	9/2009	5.2	4.7	52	3.9 Rt	UD	LAM + TDF	Alive
6	7/2001	B→B	9/2010	9.2	4.1	54	2.8 Rt	UD	LAM + TDF	Dead
7	2/2004	A→A	6/2013	9. 3	7.7	57	2.5 Lt med	UD	TDF	Dead
8	2/1996	A→A	7/2013	17.4	9.7	73	1.6 × 1.4 Rt	UD	TDF	Dead
9	8/1997	A→A	6/2014	16.8	5.9	54	2.2 × 1.9 Lt lat	UD	ETV	Alive
10	5/1996	A→A	10/2014	18.4	10.4	74	3.4 Rt	UD	LAM + TDF	Dead
11	2/2000	A→A	4/2015	15.2	12.4	62	3.4 × 3.4 Rt	UD	TDF	Alive
12	2/2000	B→A	5/2015	15.3	12.2	65	3.8 Rt	UD	TDF	Alive

[1]Patient has been hepatitis B virus (HBV) DNA (-) until 3 years before HBV DNA became detectable (22 IU/mL) when tenofovir disoproxil fumarate was added. HCC: Hepatocellular carcinoma; HBV: Hepatitis B virus; LAM: Lamivudine; TDF: Tenofovir disoproxil fumarate. UD: Undetectable.

expression has been investigated with RNA interference and nucleic acid polymers. Checkpoint inhibitors such as anti-PD-1 monoclonal antibodies currently used in oncology are also being investigated. In HBV, the HBV-specific CD8+ T cells express high levels of inhibitory molecules so inhibition of these checkpoint molecules can rescue HBV-specific CD8+ T cells. Engineering HBV-specific T cells through transfer of HBV-specific T cell receptors and therapeutic vaccines aimed at stimulating HBV-specific T cell immunity in patients chronically infected with HBV are also under investigation. Finally, toll-like receptor agonists are being developed to help recognize the virus and subsequently stimulate production of interferon-alpha and other cytokines in an effort to activate natural killer cells and other lymphocytes[21].

Immune elimination of HBV infected hepatocytes: The third category of therapies under investigation includes medications that target cellular inhibitor of apoptosis proteins (cIAPs). These proteins have been found to attenuate TNF signaling during HBV infection which leads to restricted death of infected hepatocytes. This lack of infected hepatocyte death leads to viral persistence. Birinapant, a specific therapy currently being investigated in preclinical models, antagonizes cIAP and has shown anti-HBV activity[21].

Direct Targeting of cccDNA: The final category of potentially curative HBV therapies under investigation involves direct targeting of cccDNA. There are several strategies to target cccDNA which include inhibition of cccDNA formation, destruction of cccDNA and functional silencing of cccDNA through targeting proteins involved in cccDNA transcription and/or stability. Given the knowledge we have regarding persistent cccDNA and impaired immune responses in patients with CHB, it is likely that a combination of these newly investigated therapies will need to be used in order to achieve complete cure[21].

CONCLUSION

The ultimate goal of HBV treatment is to prevent the development of HCC and death from the virus. Of utmost importance is successful eradication of the transcriptionally active HBV cccDNA from hepatocyte nuclei. While we currently aim for a functional cure with loss of HBsAg and undetectable viral DNA, a complete cure can only be attained with eradication of cccDNA. The several concerns regarding the unpredictable nature of HCC development in patients with CHB shows the need for a cure and we should continue to support and encourage research efforts aimed at developing curative therapies.

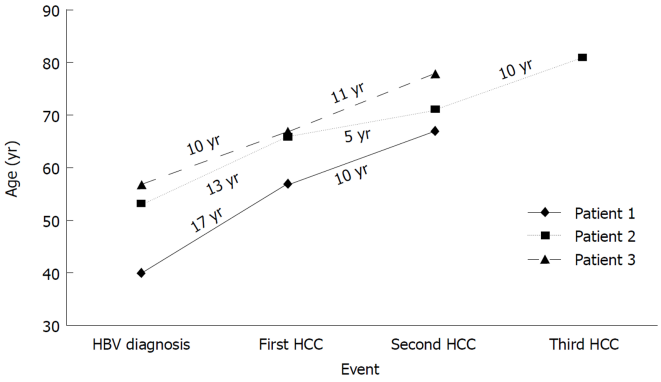

Figure 2 Hepatocellular carcinoma in three patients with chronic hepatitis B[18]**.** HCC: Hepatocellular carcinoma; HBV: Hepatitis B virus.

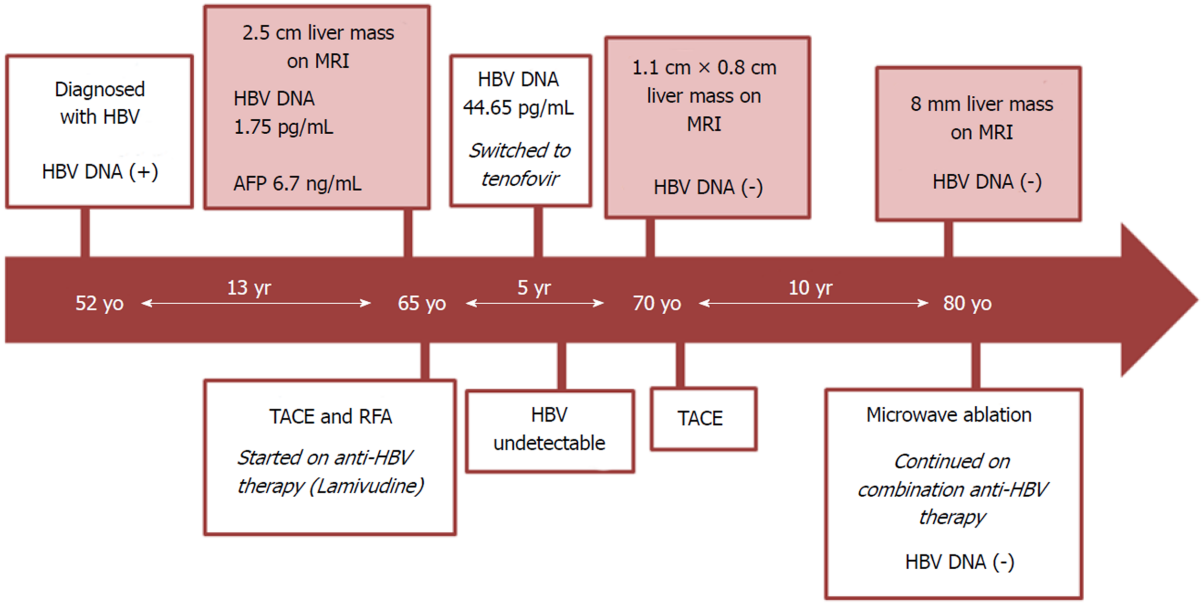

Figure 3 Persistent risk for recurrent hepatocellular carcinoma in a patient with chronic hepatitis B[18]**.** HCC: Hepatocellular carcinoma; HBV: Hepatitis B virus; MRI: Magnetic resonance imaging; TACE: Transarterial chemoembolization; RFA: Radiofrequency ablation.

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ORIGINAL ARTICLE

Basic Study

Temporal trends of cirrhosis associated conditions

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Abstract

BACKGROUND

Chronic liver disease and cirrhosis is the 12th leading cause of death in the United States. Patients with decompensated-cirrhosis, especially with hepatic encephalopathy/coma (HC), have a higher rate of early readmission and contribute to higher healthcare cost.

AIM

To evaluate the national inpatient trends of discharges, mortalities and financial impacts associated with four common conditions of cirrhosis.

METHODS

The publicly available Healthcare Cost and Utilization Project National Inpatient Sample database was utilized to examine the temporal trends of total number of discharges, mortalities and inpatient costs related to hospitalization with a primary diagnosis of HC, transjugular intrahepatic portosystemic shunt (TIPS), esophageal varices with bleeding (EV) and spontaneous bacterial peritonitis (SBP) from 2005 to 2014. The ten-year temporal trends were assessed using simple linear regressions and multiple regression analysis. Two-sided $P < 0.05$ was considered statistically significant.

RESULTS

From 2005 to 2014, the total number of discharges with cirrhosis-associated complications trended up for HC, SBP and EV (HC by 70% increase, $P < 0.0001$; SBP by 819% increase, $P = 0.0002$; EV by 9% increase, $P = 0.016$), but not for TIPS ($P = 0.90$). HC related to viral hepatitis showed faster increase by 357% ($P < 0.0001$) in comparison to HC not related to viral hepatitis by 33 % ($P = 0.0006$). Overall, in-hospital mortality rates for each condition decreased from 2005 to 2014 (HC by 29% reduction, $P = 0.0024$; SBP by 26% reduction, $P = 0.0038$; TIPS by 32% reduction, $P = 0.021$) except for EV ($P = 0.34$). After adjustment for inflation, aggregate cost of hospitalization for EV, HC, and SBP significantly increased by 20%, 86%, and 980%, respectively, from 2005 to 2014 (all $P < 0.02$), while TIPS had trend toward decreasing cost by 3% ($P = 0.95$).

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CONCLUSION
The number of hospitalizations and costs for some of the cirrhosis-associated conditions increased. However, the inpatient mortality rates for most of these conditions decreased.

Key words: Cirrhosis; Hepatic encephalopathy; Spontaneous bacterial peritonitis; Esophageal varices; Transjugular intrahepatic portosystemic shunt

Core tip: Understanding recent temporal trends of cirrhosis-associated conditions is an important aspect of developing strategies to reduce health care cost. Our study showed increasing trends of hospital discharges related to cirrhosis-associated conditions despite the decreasing trends for total hospital discharges across the nation. Importantly, hepatic coma associated with viral hepatitis showed rapid increase in discharge volume in comparison to hepatic coma not associated with viral hepatitis. After adjusting for inflation, cirrhosis associated conditions showed disproportionately greater increase in aggregate cost compare to national trends. This suggests that prevention of hospitalizations secondary to cirrhosis-associated conditions likely reduces overall health care cost.

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INTRODUCTION

Background

Chronic liver disease and cirrhosis is the 12th leading cause of death in the United States[1]. Cirrhosis is associated with multiple life-threatening complications such as intraabdominal infections, hepatic encephalopathy/coma (HC), portal hypertension, esophageal varices and hepatocellular carcinoma. These complications are suggestive of decompensated-cirrhosis and are indicative of worse prognosis among patients with cirrhosis[2,3]. In addition, patients with decompensated-cirrhosis, especially with HC, have a higher rate of early readmission and contribute to higher healthcare cost[4-6]. Other cirrhosis-associated complications such as portal hypertension, renal dysfunction and infections were also associated with higher inpatient mortality[7].

Objective

Reducing the preventable hospitalizations and readmissions are the focus of recent national initiatives with the goal of improving quality of care and reducing health care costs. Therefore, optimal management of cirrhosis to prevent decompensated-cirrhosis is emphasized. However, there is limited data on the temporal trend of hospitalizations and mortality associated with cirrhosis-associated complications. Recent retrospective studies showed a decline in the number of hospitalizations for esophageal varices with bleeding (EV)[8-10]. However, there is paucity of data on other cirrhosis-associated conditions. Therefore, we conducted a larger population-based study to evaluate the national temporal trends of hospitalizations, mortalities and financial impact associated with four common conditions of cirrhosis: HC; intraabdominal venous shunt (transjugular intrahepatic portosystemic shunt or TIPS); EV; spontaneous bacterial peritonitis (SBP).

MATERIALS AND METHODS

Data sources

National Inpatient Sample (NIS), the largest all-payer database of inpatient care in the United States, from the Healthcare Cost and Utilization Project (HCUP) published by the Agency for Healthcare Research and Quality, contains data from over 7-million

hospital discharges each year from over 1000 hospitals and is weighted to produce national estimates[11]. The HCUP validates the NIS for biases by comparing it with other population-based data sets[12] and NIS data have been utilized and published in the past[13-17]. There was a change in sampling methods in 2012, but the collection date was adjusted for this change[11,18]. Data reporting met the NIS data use agreement as established by the HCUP[11]. Since we utilized publicly-accessible, de-identified administrative level aggregate data, rather than patient-specific data, approval from the institutional review board was not required to conduct the study.

Study population

We conducted a retrospective study utilizing the NIS to assess 10 year temporal trends from 2005 to 2014 for total number of discharges, death, mortality, length of stay (LOS), mean charges, aggregate charges, aggregate costs, age, sex, insurance types (Medicare, Medicaid, Private insurance, Uninsured, Other or Missing) and bed size (Small, Medium and Large) related to hospitalization, with a primary diagnosis of HC, TIPS, EV and SBP, using International Classification of Diseases (ICD)-9 codes (HC: 572.2; HC secondary to viral hepatitis: 070.0, 070.20-23, 070.41-44, 070.49, 070.6, 070.71; TIPS: 39.1; EV: 456.0; SBP: 567.23)[11,19]. A primary diagnosis is the main reason why patients are hospitalized, rather than a secondary diagnosis which sometimes used to identify continuation of treatment for chronic conditions in the hospital. These ICD-9 codes have been validated in literature except HC secondary to viral hepatitis[19]. Codes for HC secondary to viral hepatitis include acute viral hepatitis resulted in HC. However, if providers used codes for unspecified acute liver failure which resulted in HC from viral hepatitis, our analysis was unable to include these populations due to lack of specificity of codes. As the coding behavior is becoming more specific due to insurance requirement, we expect that inclusion codes related to HC secondary to viral hepatitis will be an important part of analysis. This will allow us to evaluate if the changes related to HC is merely an artifact of coding behavior. We also obtained data for a National all-cause hospitalization (national) for a comparison. Importantly, the ICD-9 code for SBP, 567.23, was introduced in 2005[13]. We only evaluated a primary diagnosis of ICD-9 codes rather than a secondary diagnosis to avoid duplication in the data. We chose to end the study period in 2014 since the official transition from ICD-9 codes to ICD-10 codes occurred nationally in 2015.

Statistical analysis

A biomedical statistician GZ reviewed and performed statistical analysis. Both charges and costs were inflation-adjusted using the appropriate Consumer Price Index (http://www.bls.gov/cpi/) and all converted to 2014 United States dollars. We compared the 10-year temporal trends of discharges, mortalities, charges and costs data using simple linear regressions using SAS version 9.4 (SAS Institute Inc., Cary, NC). We also used multiple regression analysis to explore the adjustments of mean age and LOS for outcomes that showed significant temporal trends in simple regressions. Nonrandom temporal trends (or "special-cause variation") were also demonstrated by run charts using the statistical process control decision rule: there are 6 or more consecutive data points always going up or going down over time[20]. Two-sided $P < 0.05$ was considered statistically significant without multiple comparison adjustments.

RESULTS

Temporal trends of discharges for cirrhosis-associated conditions are shown in Figure 1A-C. From 2005 to 2014, the primary diagnosis of four cirrhosis related pathologies were all trending up except TIPS [HC, 47268 to 80470 by 70% increase, slope estimate (annual change rate) = 4205 [95% confidence interval (CI) = 3739 to 4671], $P < 0.0001$; SBP, 821 to 7545 by 819% increase, slope = 660 (428 to 893), $P = 0.0002$; EV, 3942 to 4305 by 9% increase, slope = 55 (13 to 96), $P = 0.016$; TIPS, 4079 to 4060 by < 1% decrease, slope = 9 (-151 to 168), $P = 0.90$]. Nationally, the total number of discharges decreased from 37843039 to 35358818 by 7% [slope = -316319 (-433063 to -199576), $P = 0.0002$]. Similar significance of temporal trends of these discharges remained if adjusted for mean age and LOS, and both variables did not show significant change over time (all $P > 0.11$). HC not related to viral hepatitis (ICD-9 code 572.2) increased from 41796 to 55485 by 33% from 2005 to 2014 [slope = 1688 (968 to 2408), $P = 0.0006$], while HC related to viral hepatitis (070.0, 070.20-23, 070.41-44, 070.49, 070.6, 070.71) increased faster, from 5472 to 24985 by 357% [slope = 2517 (1732 to 3302), $P < 0.0001$].

Overall as described in Figure 2, in-hospital mortality rates for each condition significantly decreased from 2005 to 2014 [HC: 8.06% to 5.73% by 29% reduction, slope

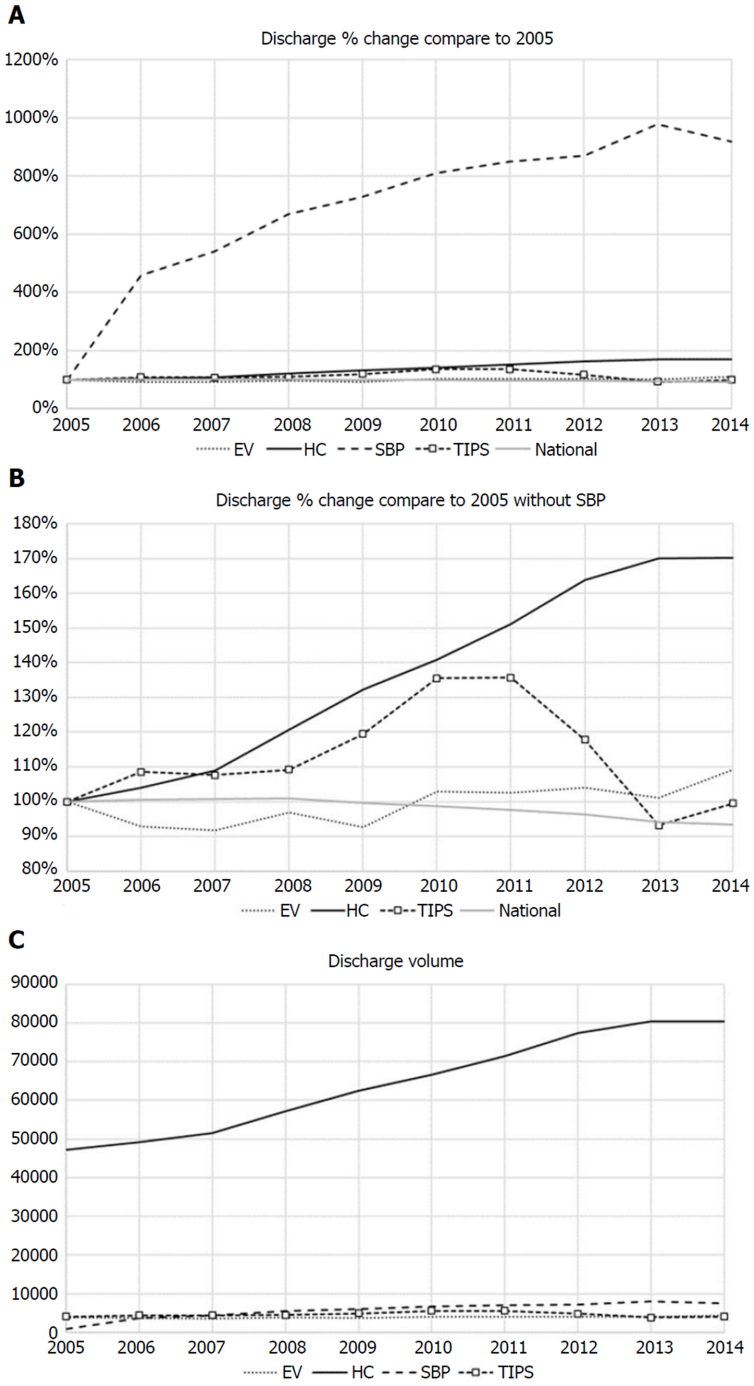

Figure 1 10-year temporal trends of discharges. A: Percent changes of discharge numbers compare to 2005; B: Percent changes of discharge numbers compare to 2005 without SBP; C: Discharge volume for cirrhosis associated conditions. From 2005 to 2014, the primary diagnosis of four cirrhosis related pathologies were all trending up except TIPS. EV: Esophageal varices with bleeding; HC: Hepatic encephalopathy/coma; TIPS: Transjugular intrahepatic portosystemic shunt; SBP: Spontaneous bacterial peritonitis.

= -0.21% (-0.33% to -0.10%), P = 0.0024; SBP: 8.34% to 6.16% by 26% reduction, slope = -0.25% (-0.39% to -0.10%), P = 0.0038; TIPS: 10.81% to 7.39% by 32% reduction, slope = -0.35% (-0.64% to -0.07%), P = 0.021] except for EV (5.84% to 6.04%; P = 0.34). If adjusted for mean age and LOS, SBP was still reduced significantly (P = 0.034), but TIPS were no longer significant (P = 0.067). The changes in in-hospital mortality rates might be associated with the variations of mean LOS but not with mean age. For example, one-day reduction in mean LOS seemed significantly associated with a reduction in TIPS mortality rates of 1.73% (0.69% to 2.76%), P = 0.007. There was also a statistically significant reduction on national mortality rate, from 2.04% to 1.90%, or a 7% reduction, slope = -0.018% (-0.031% to -0.006%), P = 0.011.

Figure 3 shows the 10-year trends for mean LOS and mean ages. Mean LOS for

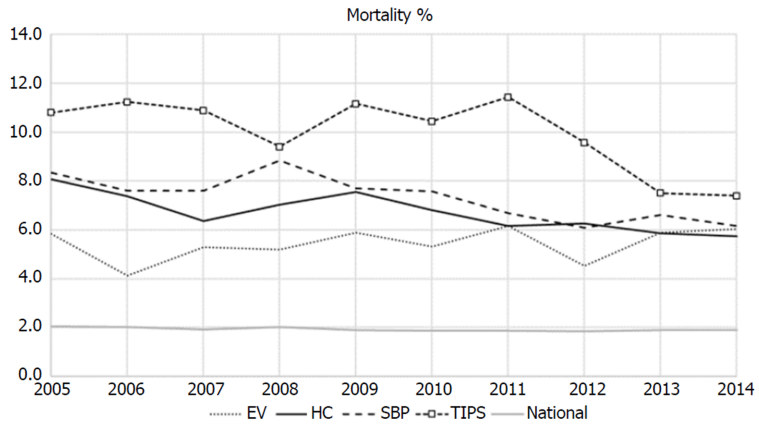

Figure 2 10-year temporal trends of mortality rates. In-hospital mortality rates for each condition significantly decreased from 2005 to 2014 except for esophageal varices with bleeding. EV: Esophageal varices with bleeding; HC: Hepatic encephalopathy/coma; TIPS: Transjugular intrahepatic portosystemic shunt; SBP: Spontaneous bacterial peritonitis.

TIPS decreased from 9.6 d in 2005 to 8.0 in 2014 with 17% decrease [slope = -0.18 (-0.34 to -0.01), P = 0.038]. However, other cirrhosis related conditions and national LOS did not show any significant changes. TIPS had persistently higher LOS as compared to other conditions. Mean ages of cirrhosis-associated conditions were consistently higher than national average, and HC persistently had the highest mean age.

Figure 4A shows mean charges of four cirrhosis-associated conditions that have been increasing more than 30% compared to 2005 data, after adjustment for inflation [HC, $32045 to $47929 by 50% up, slope = 2042$/year (1425 to 2660), P < 0.0001; EV, $33979 to $51336 by 51% up, slope = 1822$/year (1238 to 2406), P < 0.0001; TIPS, $45425 to $59130 by 30% up, P = 0.0022; SBP, $33151 to $50824 by 53% up, slope = 2019$/year (687 to 3352), P=0.008; from 2005 to 2014). Nationally, mean charge increased from $26323 to $41633, or a 58% increase [slope = 1739$/year (1646 to 1833), P < 0.0001]. Temporal trends of these mean charges were still significant (all P < 0.012) after further adjustment for mean age and LOS. Figure 4B shows mean costs associated with four cirrhosis-associated conditions. This figure demonstrates that TIPS has persistently higher cost related to hospitalizations. Compared to mean charges, mean costs had less variation over time. The mean costs of TIPS, EV and SBP did not increase significantly over time (all P > 0.15). Only HC significantly increased mean costs from $11041 in 2005 to $12282, or a 12% increase, slope = 217$/year (92 to 341), P = 0.004 (after adjusting for mean age and LOS, P = 0.002).

After adjustment for inflation, aggregate charges (so called "national bill") for hospitalizations related to EV, HC, SBP, TIPS and national increased by 65%, 150%, 1296%, 30 % and 48 %, respectively, from 2005 to 2014 (all $P \leq 0.0001$ except for EV, P = 0.30) (Figure 5a and 5b). Inflation adjusted aggregate costs of hospitalization for EV, HC, SBP and national increased by 20%, 86%, 980% and 7%, respectively, from 2005 to 2014 (all P < 0.02), while TIPS trended toward a decreased by 3% (P = 0.95) (Figure 5C and 5D).

Table 1 and Table 2 shows age and sex distributions, insurance coverage and bed size for 2005 and 2014 respectively. For four cirrhosis-associated conditions, proportions of patients are shifting from large hospitals to small-medium sized hospitals.

DISCUSSION

Discharges with a primary diagnosis of HC, SBP, or EV showed increasing trends despite the decreasing trend for total hospitalizations across the nation over a 10-year period, from 2005 to 2014. Especially for SBP, trends show an 819% increase in hospitalization for this primary diagnosis over 10 years. Hospitalization due to HC also demonstrated significant increase over the study period, mainly attributable to the increase in HC coding specifically related to viral hepatitis. Approximately 20.5% of HC is precipitated by SBP[21]. Therefore, increasing SBP with increasing HC suggests actual increase in case number along with change in coding behavior. One recent study showed that there was an increase in hepatitis C virus infection-related mortality from 2007 to 2013, and this may explain increasing trends of hepatic coma

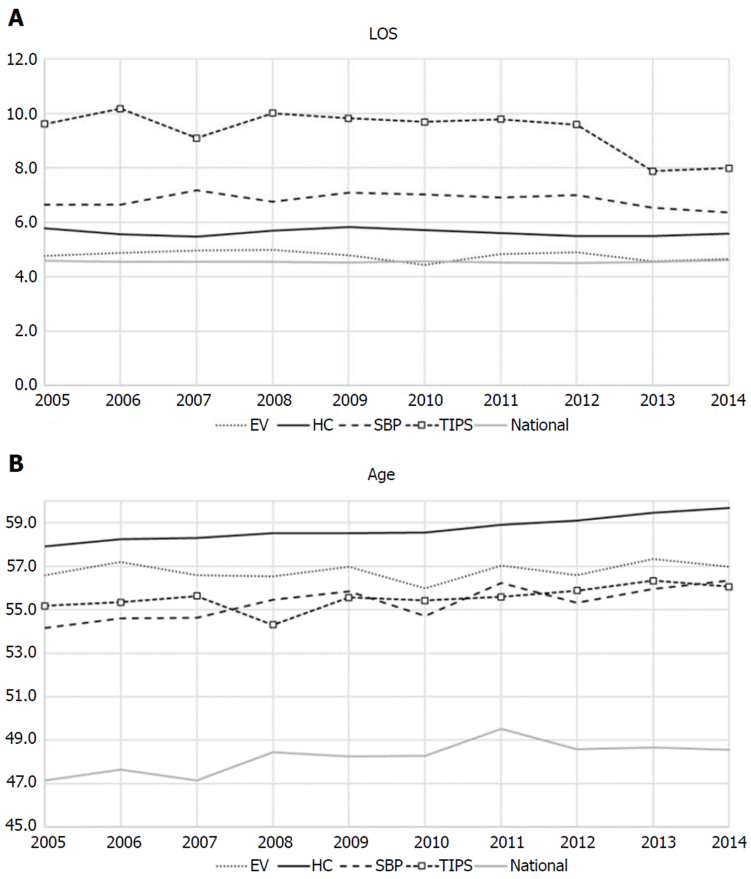

Figure 3 10-year temporal trends of mean length of stay and mean ages. A: 10-year temporal trends of mean length of stay (LOS); B: 10-year temporal trends of mean ages. Mean LOS for transjugular intrahepatic portosystemic shunt (TIPS) decreased from 9.6 d in 2005 to 8.0 in 2014 with 17%. However, other cirrhosis related conditions and national LOS did not show any significant changes. TIPS had persistently higher LOS as compared to other conditions. Mean ages of cirrhosis-associated conditions were consistently higher than national average, and HC persistently had the highest mean age. EV: Esophageal varices with bleeding; HC: Hepatic encephalopathy/coma; TIPS: Transjugular intrahepatic portosystemic shunt; SBP: Spontaneous bacterial peritonitis.

related to viral hepatitis[22]. The referenced study also noted decreasing mortality related to hepatitis C infection from 2014 to 2016 due to introduction of direct-acting antiviral agents[22]. Therefore, trends may change beyond 2014 and future study to assess this change will be necessary. Previous population-based studies[8-10] showed declining numbers of esophageal varices-related hospitalizations, which is in contrast to our findings. One possible explanation is that our study consisted of more recent data compared to the published studies. Jamal *et al*[8] used data from 1988 to 2002, Lim *et al*[9] used data from 1998 to 2009 and Pant *et al*[10] used data from 2002 to 2012. Furthermore, Pant *et al*[10] who also utilized data from NIS by HCUP, studied only the EV patients who had concurrent diagnosis of cirrhosis. Our study only looked at a primary diagnosis of EV, therefore secondary diagnosis of EV was not included which may have accounted for the differences in the study results. In addition, there was a change in sampling methods for NIS in 2012, which may have also impacted the differences in our results compared to the prior studies[11].

Overall, in-hospital mortality rates for cirrhosis-associated conditions generally decreased over a 10-year period except for EV, even though there were no significant trends in the overall all-cause inpatient national mortality rate. This may reflect the advances in pharmacotherapy such as introduction of rifaximin for HC treatment. Another possible explanation is that mortality related to hepatitis B virus infection declined from 2007 to 2016, likely due to improvement of care of hepatitis B virus infection[22]. Since patients with decompensated cirrhosis, especially from HC, are at risk for increased frequency of hospital readmission[4-6], reducing the in-patient mortality rates may be contributing to the number of readmissions following the index admission. However, our study was not designed to assess the number of readmissions in order to address this possibility. Kanwal *et al*[23] showed there is an increasing trend for post discharge mortality related to cirrhosis admission, which has likely replaced inpatient mortality. Therefore, decreasing inpatient mortality may not

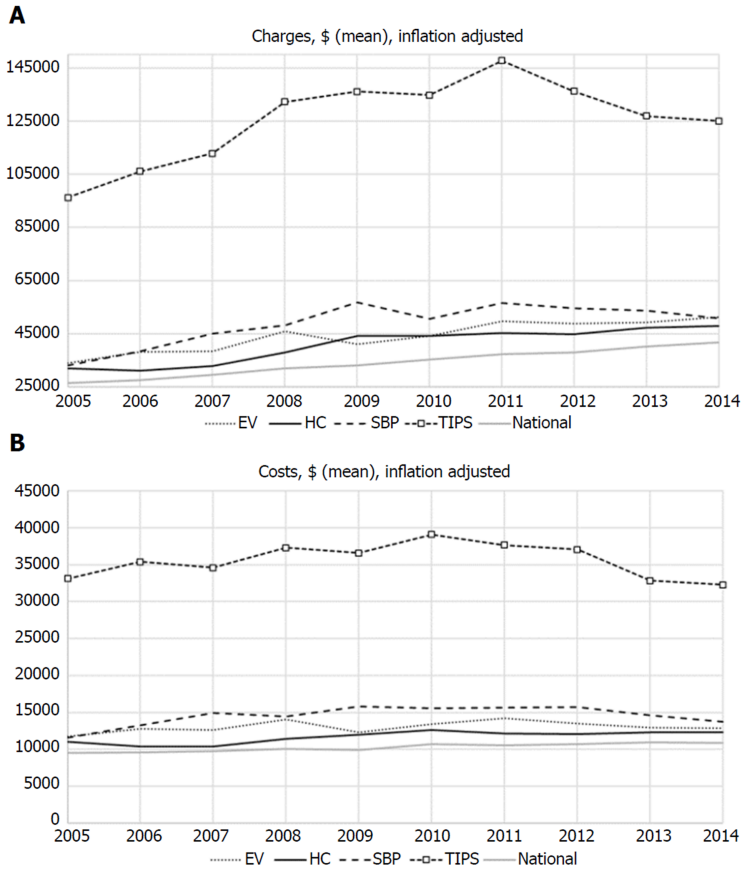

Figure 4 Inflation adjusted 10-year temporal trends of mean charges and mean costs. A: Shows mean charges of four cirrhosis-associated conditions that have been increasing more than 30% compared to 2005 data, after adjustment for inflation; B: Demonstrates that transjugular intrahepatic portosystemic shunt (TIPS) has persistently higher cost related to hospitalizations. Compared to mean charges, mean costs had less variation over time. The mean costs of TIPS, EV and SBP did not increase significantly over time (all $P > 0.15$). EV: Esophageal varices with bleeding; HC: Hepatic encephalopathy/coma; TIPS: Transjugular intrahepatic portosystemic shunt; SBP: Spontaneous bacterial peritonitis.

reflect actual improvement of care related to cirrhosis. Interestingly, TIPS showed significant decrease in the hospital LOS from 9.6 in 2005 to 8.0 in 2014 by 17% down ($P < 0.04$), but other cirrhosis-associated conditions did not play a significant role in the LOS. This finding is different from a previous study that evaluated TIPS from 1998 to 2012, which showed relatively constant LOS for TIPS-related admissions[24]. TIPS had persistently high LOS compared to other diagnosis, however this comparison may not be appropriate as the ICD-9 code for TIPS was a procedure code rather than a diagnostic code. In addition, there was a recent trend of minimizing post-procedural hospital stays[25].

Finally, our aggregate financial analysis suggests that mean charges of cirrhosis-associated conditions increased over a 10-year period after adjusting for inflation. A similar increased trend was also seen in the national all-cause hospitalization. However, increase in aggregate cost for primary diagnosis of EV, HC and SBP was disproportionately greater (45% increase) than the increase in aggregate cost for national trends (7% increase) after adjusting for inflation. This suggests that prevention of hospitalizations secondary to cirrhosis-associated conditions likely reduces overall health care cost.

There are some limitations to our study, which are mostly associated with the lack of patient-level, detailed clinical information as a result of using an aggregate claims database. Also, we cannot rule out the possibility of temporal changes in the reporting bias for these conditions, since the reporting process for the discharge diagnoses may have become more specific over recent years with more prevalent use of electronic medical records. Therefore, it is unclear if increasing numbers of cirrhosis-associated discharges based on ICD-9 codes reflects actual increases in prevalence of the conditions *vs* increase in more accurate reporting of these conditions. In addition, previous studies have not validated ICD-9 codes related to HC secondary to viral hepatitis. Therefore, use of unvalidated codes may skew results. Further prospective

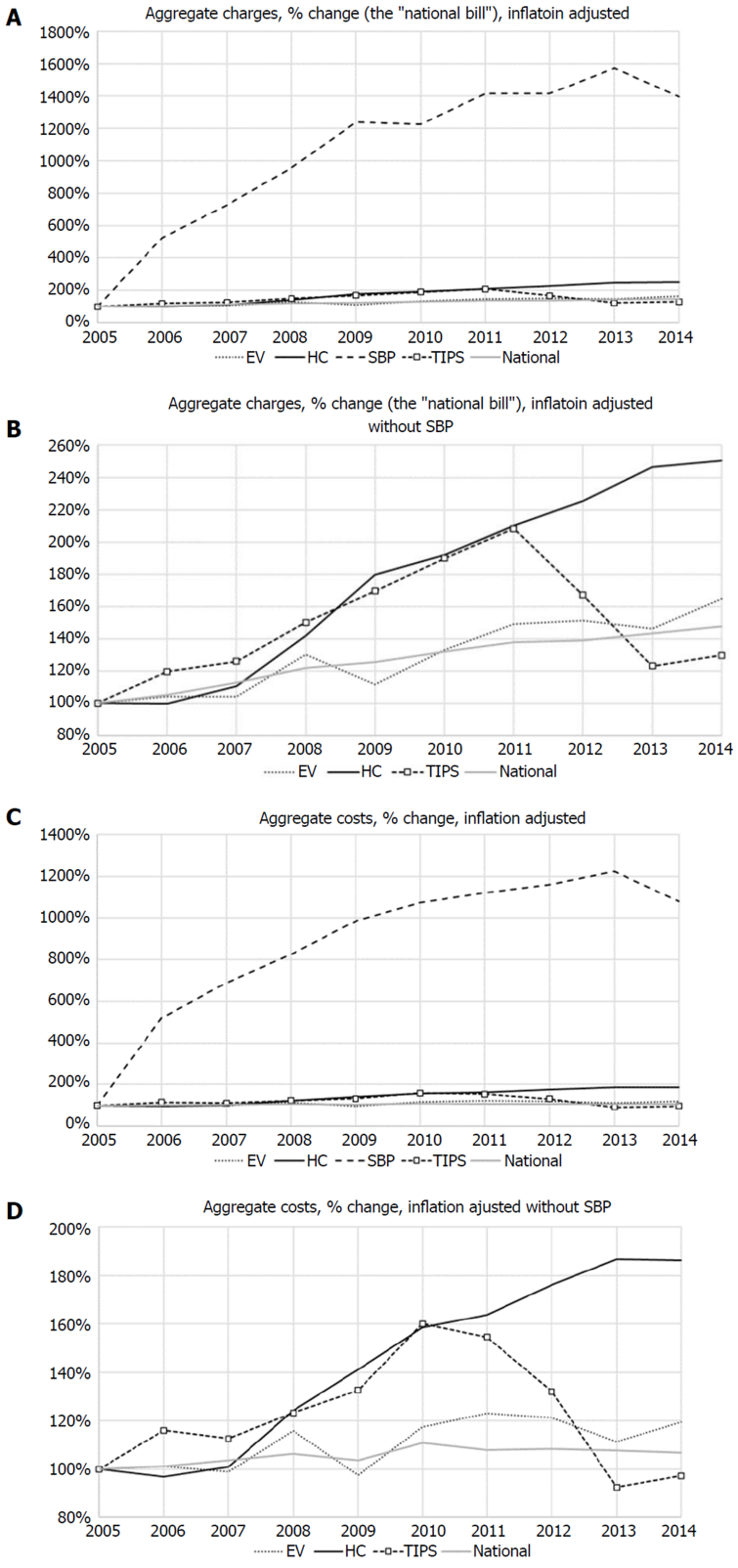

Figure 5 Inflation adjusted 10-year temporal trends of aggregate charges and aggregate costs. Same figures without SBP for charges and costs. A and B: After adjustment for inflation, aggregate charges for hospitalizations related to HC, SBP, transjugular intrahepatic portosystemic shunt (TIPS) and national increased from 2005 to 2014 except EV; C and D: Inflation adjusted aggregate costs of hospitalization for EV, HC, SBP and national increased from 2005 to 2014, while TIPS trended toward a decreased. EV: Esophageal varices with bleeding; HC: Hepatic encephalopathy/coma; TIPS: Transjugular intrahepatic portosystemic shunt; SBP: Spontaneous bacterial peritonitis.

studies are needed to find the explanations of these trends. We also utilized ICD-9 diagnostic codes for HC, EV and SBP, and an ICD-9 procedure code was utilized for TIPS. Therefore, comparison between ICD-9 diagnostic codes and procedure codes

Table 1 Number of all discharges, aggregate cost ($), percent of male (%), mean age, age distribution, payer type, bed size

Diagnosis (2005)	HC		SBP		EV		TIPS	
All discharges	47268		821		3942		4079	
Male (%)	61.4		66.7		67.7		66.5	
Age, mean	57.9		54.2		56.6		55.2	
Age	*n*	%	*n*	%	*n*	%	*n*	%
18-44	4648	9.8	136	16.6	566	14.4	479	11.8
45-64	29350	62.1	471	57.4	2196	55.7	2600	63.7
65-84	12623	26.7	189	23.0	996	25.3	888	21.8
85+	622	1.3	N/A	N/A	122	3.1	N/A	N/A
Payer								
Medicare	20002	42.3	338	41.2	1298	32.9	1243	30.5
Medicaid	11819	25.0	181	22.1	639	16.2	870	21.3
Private insurance	10833	22.9	193	23.5	1271	32.2	1555	38.1
Uninsured	2785	5.9	65	7.9	537	13.6	237	5.8
Other	1782	3.8	N/A	N/A	192	4.9	174	4.3
Missing	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Bed sizes								
Small	4869	10.3	111	13.5	520	13.2	55	1.3
Medium	12046	25.5	167	20.4	1017	25.8	861	21.1
Large	30354	64.2	543	66.1	2405	61.0	3163	77.5

n: Number; N/A: Not available; HC: Hepatic coma; SBP: Spontaneous bacterial peritonitis; EV: Esophageal varices; TIPS: Transjugular intrahepatic portosystemic shunt.

may not reflect differences in the respective trends. We understand that determining the reason for TIPS is important as prognosis may differ for different causes. However, NIS data did not allow us to separate etiologies. Additional studies to look at this difference would be appropriate.

In summary, we observed significant temporal increase in hospitalization due to cirrhosis-associated complications except for TIPS[2,3]. EV, HC and especially SBP had significant increases in aggregate hospitalization cost. Strategies to prevent readmission in the era of decreasing mortality rate are needed to effectively reduce healthcare cost.

Table 2 Number of all discharges, aggregate cost ($), percent of male (%), mean age, age distribution, payer type, bed size

Diagnosis (2014)	HC		SBP		EV		TIPS	
All discharges	80470		7545		4305		4060	
Male (%)	60.4		62.5		65.5		64.3	
Age, mean	59.7		56.3		57.0		56.1	
Age	*n*	%	*n*	%	*n*	%	*n*	%
18-44	5525	6.9	945	12.5	600	13.9	425	10.5
45-64	50085	62.2	4715	62.5	2540	59.0	2660	65.5
65-84	23770	29.5	1680	22.3	1000	23.2	885	21.8
85+	1080	1.3	105	1.4	125	2.9	N/A	N/A
Payer								
Medicare	38995	48.5	3005	39.8	1445	33.6	1455	35.8
Medicaid	19545	24.3	2005	26.6	985	22.9	990	24.4
Private insurance	15745	19.6	1940	25.7	1225	28.5	1200	29.6
Uninsured	3240	4.0	360	4.8	470	10.9	270	6.7
Other	2715	3.4	220	2.9	175	4.1	125	3.1
Missing	229.999	0.3	N/A	N/A	N/A	N/A	N/A	N/A
Bed sizes								
Small	13620	16.9	1065	14.1	830	19.3	255	6.3
Medium	23280	28.9	2060	27.3	1265	29.4	1000	24.6
Large	43570	54.1	4420	58.6	2210	51.3	2805	69.1

n: number; N/A: Not available; HC: Hepatic coma; SBP: Spontaneous bacterial peritonitis; EV: Esophageal varices; TIPS: Transjugular intrahepatic portosystemic shunt.

ARTICLE HIGHLIGHTS

Research background

There is limited data on recent temporal trends of cirrhosis associated conditions in the United States, which is critical to identify problems related to hospitalizations.

Research motivation

Healthcare cost reduction, especially in the United States, is a current focus on providing cost-effective care. Recognizing problems in temporal trends enables to create action plan to reduce unnecessary costs.

Research objectives

We aim to conduct a descriptive study to identify 10-year temporal trends of cirrhosis associated conditions which can guide future prospective studies.

Research methods

We used publicly available National Inpatient Sample to conduct 10-year trends analysis of cirrhosis associated conditions identified by ICD-9 codes from 2005 to 2014. Simple linear regression and multiple regression models were utilized for statistical analysis.

Research results

The total number of discharges of Hepatic encephalopathy/coma (HC), Spontaneous bacteria peritonitis (SBP) and esophageal varices with bleeding (EV) had significant increase. Notably, HC associated with viral hepatitis showed faster rate of increase of hospitalizations. Mortalities has decreased for HC, SBP and transjugular intrahepatic portosystemic shunt (TIPS), but no change was observed for EV. Aggregate cost of hospitalizations for HC, EV and SBP had significant increase after adjustment for inflation; however TIPS showed non-significant trends toward decreasing cost.

Research conclusions

The number of hospitalizations and costs for some of the cirrhosis-associated conditions increased. Especially, HC related to viral hepatitis showed fast rate of increase which suggest appropriate treatment of viral hepatitis maybe necessary to reduce HC in these population. In addition, the inpatient mortality rates for most of these conditions decreased.

Research perspectives

Viral hepatitis related cirrhosis maybe contributing to high cost of hospitalization especially hospitalization related to HC. These findings suggest necessity of studies beyond 2014 after

introduction of newer antiviral agents for hepatitis C as well as studies to identify trends of re-admission and post-hospitalization mortality.

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ORIGINAL ARTICLE

Retrospective Study

Clinical factors associated with hepatitis B screening and vaccination in high-risk adults

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Author contributions: Ayoola R was involved in the study concept design, acquisition of data, and drafting of the manuscript; Larion S was involved in the study concept and design, analysis and interpretation of data, and drafting the manuscript; Poppers DM was involved in the critical revision of the manuscript for important intellectual content. Williams R was involved in the study concept and design, critical revision of the manuscript for important intellectual content, and study supervision.

Institutional review board statement: This study was reviewed and approved by the Institutional Review Board at New York University/New York Langone Health.

Informed consent statement: Patients were not required to give informed consent to the study because the analysis used anonymous retrospective clinical data. The study analyzed only existing data that spanned multiple years, and included a large number of patients hindering the ability to obtain retrospective consent from all patients. The study was descriptive in nature and lacked identifying patient information.

Conflict-of-interest statement: We have no relevant financial relationships to disclose.

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Abstract

BACKGROUND

Hepatitis B virus is a viral infection that can lead to acute and/or chronic liver disease, and hepatocellular carcinoma (HCC). Hepatitis B vaccination is 95% effective in preventing infection and the development of chronic liver disease and HCC due to hepatitis B. In 2011, the Centers for Disease Control updated their guidelines recommending that adults at high-risk for hepatitis B infection be vaccinated against hepatitis B including those with diabetes mellitus (DM). We hypothesize that adults at high-risk for hepatitis B infection are not being adequately screened and/or vaccinated for hepatitis B in a large urban healthcare system.

AIM

To investigate clinical factors associated with Hepatitis B screening and vaccination in patients at high-risk for Hepatitis B infection.

METHODS

We conducted a retrospective review of 999 patients presenting at a large urban healthcare system from 2012-2017 at high-risk for hepatitis B infection. Patients were considered high-risk for hepatitis B infection based on hepatitis B practice recommendations from the Center for Disease Control. Medical history including hepatitis B serology, concomitant medical diagnoses, demographics, insurance status and social history were extracted from electronic health records. Multivariate logistic regression was used to identify clinical risk factors independently associated with hepatitis B screening and vaccination.

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RESULTS

Among the 999 patients, 556 (55.7%) patients were screened for hepatitis B. Of those who were screened, only 242 (43.5%) patients were vaccinated against hepatitis B. Multivariate regression analysis revealed end-stage renal disease [odds ratio (OR): 5.122; 2.766-9.483], alcoholic hepatitis (OR: 3.064; 1.020-9.206), and cirrhosis or end-stage liver disease (OR: 1.909; 1.095-3.329); all $P < 0.05$ were associated with hepatitis B screening, while age (OR: 0.785; 0.680-0.906), insurance status (0.690; 0.558-0.854), history of DM (OR: 0.518; 0.364-0.737), and human immunodeficiency virus (OR: 0.443; 0.273-0.718); all $P < 0.05$ were instead not associated with hepatitis B screening. Of the adults vaccinated for hepatitis B, multivariate regression analysis revealed age (OR: 0.755; 0.650-0.878) and DM were not associated with hepatitis B vaccination (OR: 0.620; 0.409-0.941) both $P < 0.05$.

CONCLUSION

Patients at high-risk for hepatitis B are not being adequately screened and/or vaccinated. Improvements in hepatitis B vaccination should be strongly encouraged by all healthcare systems.

Key words: Health prevention; Vaccination; Hepatitis B virus; Screening; Diabetes mellitus; Cirrhosis; End-stage renal disease; Human immunodeficiency virus; Intravenous drug users

Core tip: This is a retrospective study evaluating clinical factors associated with Hepatitis B virus (HBV) screening and vaccination in high-risk adults. Among the 999 high-risk adults included in this study, 556 (55.7%) adults were screened for HBV. Of those who were screened, only 242 (43.5%) adults were vaccinated against HBV. Clinical factors such as End Stage Renal Disease, and cirrhosis were associated with HBV screening, while diabetes mellitus (DM) was not. Patients with DM were less likely to undergo HBV vaccination. HBV vaccination is highly effective in preventing HBV-related liver disease and its sequelae. Increasing HBV vaccination in all high-risk adults should be strongly encouraged by all healthcare systems.

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INTRODUCTION

Hepatitis B virus (HBV) is a major cause of acute and chronic liver disease (CLD) both in the United States and worldwide. More than 350 million people worldwide are infected with HBV, of whom approximately 1.4 million reside in the United States[1,2]. HBV is one of the leading causes of cirrhosis and the most common cause for hepatocellular carcinoma (HCC), accounting for 50% of all HCC cases and virtually all childhood cases of this condition[3].

The primary approach to HBV prevention is immunization through vaccination. The vaccine is usually given as 2, 3, or 4 injections over a 6 mo period. With the advent of a highly effective HBV vaccine, HBV infection rates have decreased from an estimated 13.8 cases per 100000 in 1987 to roughly 1.5 cases per 100000 in 2007 in the United States[4-7]. In 2006, the Centers for Disease Control (CDC) recommended HBV vaccination for unvaccinated adults who are at high-risk for HBV infection[8]. These indications were expanded in 2011 to include patients with diabetes mellitus (DM), patients between 19 and 59 years of age, and those greater than 60 years of age at the discretion of the supervising clinician (Table 1)[9].

HBV vaccination is the most effective measure to prevent hepatitis B infection and its sequelae, including acute liver failure, cirrhosis, HCC, and overall liver-related death. However, HBV vaccination among high-risk patients has been limited by a

Table 1 High-risk condition for which hepatitis B vaccine is recommended for amongst unvaccinated adults

High-risk conditions for HBV infection
People whose sex partners have hepatitis B
Sexually active persons who are not in a long-term monogamous relationship
Persons seeking evaluation or treatment for a sexually transmitted disease
Men who have sexual contact with other men
People who share needles, syringes, or other drug-injection equipment
People who have household contact with someone infected with the hepatitis B virus
Health care and public safety workers at risk for exposure to blood or body fluids
Residents and staff of facilities for developmentally disabled persons
Persons in correctional facilities
Victims of sexual assault or abuse
Travelers to regions with increased rates of hepatitis B
People with chronic liver disease, kidney disease, HIV infection, or diabetes
Anyone who wants to be protected from hepatitis B

HBV: Hepatitis B virus; HIV: Human immunodeficiency virus.

number of factors including a lack of appropriate physician implementation of CDC recommendations, as well as inadequate insurance coverage to pay for patient vaccination. Most health insurance plans cover recommended vaccines for adults at little or no cost, but many people in the United States remain without health insurance coverage. A 2012 US National Health Interview Survey (NHIS) study on vaccination coverage (defined as those having received at least the three recommended vaccination doses) reported only 24.6% of adults aged ≥ 19 yr being vaccinated (16.5% among adults aged ≥ 50 yr) with rates of 42% in adults deemed at high-risk[9,10]. Despite CDC recommendations and significant public health efforts, HBV vaccination rates increased by less than 5% between 2004 and 2009, in the United States[11]. Since the addition of DM as a vaccination criterion in 2011, rates of HBV vaccination in the high-risk population is unclear. Previous epidemiological studies on HBV vaccination rates have also demonstrated that underrepresented high-risk patient populations have been noted to have the highest prevalence of HBV infection[12].

The purpose of this study was to perform a retrospective chart review of patients at high-risk for HBV infection from 2012-2017 in a large, urban safety-net hospital and tertiary care center. Our study had several aims: (1) To determine serologically evident HBV vaccination and screening rates in adults at high-risk for HBV; (2) to identify clinical factors significantly associated with screening and vaccination rates; and (3) to identify the key baseline characteristics of these individuals.

MATERIALS AND METHODS

A retrospective cohort study was performed using our center's electronic medical record (EMR) of randomly selected patients presenting to our health system between 2012 and 2017 who were considered at high-risk for HBV. Our health system is a large, academic medical center that incorporates a safety-net hospital that serves a diverse patient population in an urban setting. The most recent CDC guidelines were used to determine medical conditions considered high-risk for HBV infection (Table 1). Patients were included in the study if a high-risk condition or activity was noted in the EMR. A patient list was generated using ICD-10 identifiers, with subject demographics including clinical history obtained via individual chart review. Patients from both inpatient and outpatient settings were included. Patients were excluded if a high-risk ICD-10 designator was not documented in the medical history, or if the patient had previously contracted HBV (positive serology for hepatitis B surface antigen). Data accrual was terminated following review of 1100 individual patient records.

The final study population was stratified into two cohorts: a screening cohort and a vaccination cohort. The screening cohort consisted of all patients at high-risk for HBV as determined by the study inclusion criteria. Patients were considered to have undergone HBV screening if HBV serology [hepatitis B surface antigen (HBsAg), hepatitis B core antibody (anti-HBc) and hepatitis surface B surface antibody (anti-

HBs)] was documented in the EMR. Patients without HBsAg, anti-HBc, and anti-HBs in our EMR were not considered to have been screened. Demographic and clinical factors were compared between patients who were screened for HBV versus those who were not in order to identify factors associated with screening. The vaccination cohort consisted of the subset of patients in the screening cohort who had HBV serology on file. Patients were considered to have undergone HBV vaccination if the anti-HBs was positive, and the HBsAg and anti-HBc were negative. Patients with negative anti-HBs, HBsAg, and anti-HBc were not considered vaccinated. As with the screening cohort, demographic and clinical factors were compared between patients who were vaccinated for HBV and those who were not in order to identify factors associated with vaccination.

Statistical analysis

Data are presented as percentages for categorical variables and medians for non-parametric continuous variables. Differences in proportions were determined using the chi-square test with Yates correction factor or the Fisher exact test, as appropriate. Differences in continuous variables were determined using the Mann-Whitney U-test. Factors found significant ($P < 0.05$) on univariate analysis were inputted into a multivariate logistic regression with the dichotomous dependent variable (screening or vaccination) coded as 1. Dummy variables were used to code categorical variables. For regression purposes, health insurance coverage was coded as follows "0" for private insurance, "1" for Medicare, "2" for Medicaid, and "3" for uninsured or other types of coverage. Age was coded by increasing deciles. BMI was grouped into increasing 5 kg/m^2 units as listed and coded as integers. Missing data fields were left blank. Logistic regression results are reported as odds ratios with 95% confidence intervals. The Hosmer-Lemeshow statistic was used to test model goodness-of-fit. A biomedical statistician performed the statistical review of this study. Data analysis was completed using Sigmaplot 12.0 (Systat Software, San Jose, CA, United States). This study was approved by the Institutional Review Board of NYU Langone Health (s16-01837).

RESULTS

A total of 1,100 patients were identified during the data collection period, of which 101 were excluded due to evidence of prior HBV infection (*i.e.*, positive HBsAg). Of the remaining 999 patients, 556 (55.7%) patients had been screened for hepatitis B and 443 patients (44.3%) had not been screened ("screening cohort"; Figure 1). Demographics for the screening cohort are listed in Table 2, showing that a higher proportion of patients were male (60.6%), between 50-70 yr of age (63.1%), and obese (BMI > 25 kg/m^2; 66.4%). Almost half of the study cohort was non-white (46.5%), and 40.6% did not have private health insurance.

High-risk medical conditions as determined by the CDC are listed in Table 3, revealing that chronic kidney disease (CKD) (48.1%), DM (46.8%), and end-stage renal disease (ESRD) (41.3%) were highly enriched in the study population. Hemoglobin A_1c was available for 499 (49.9%) patients, with a median A_1c of 6.5% (25-75th percentile: 5.6%-7.7%). CLD was present in 25.7% of patients, including 10.3% with hepatitis C virus (HCV) infection (*i.e.*, a positive hepatitis C virus antibody [HCV Ab] with a detectable viral load on RNA PCR assay), and 10.1% of patients were noted to have non-alcoholic fatty liver disease (NAFLD). Cirrhosis was reported in 10.6% of patients (median MELD: 16; 25-75th percentile: 10-21), with 70 patients (7.0%) currently listed for liver transplant. A total of 75 (7.5%) expired at the end of data collection period, including 13 from liver-related deaths. Most patients (76.0%) had at least two or more high-risk conditions or activities as defined by the CDC.

Cardiovascular risk factors and other major comorbid conditions are listed in Table 4, showing that hypertension (59.6%), dyslipidemia (43.2%; median LDL: 80; 25-75th percentile: 57-107), and coronary artery disease (CAD; 21.1%) were also common in our study population. A total of 39.1% of patients had a current or former smoking history, including 6.6% who were active tobacco users. As expected in underserved patient populations, only 32.6% of patients had seen a primary care provider within the previous year, and only 25.8% had been evaluated by a gastroenterologist within the past year.

Demographic and clinical factors in the screening cohort were compared between those who had been successfully screened for hepatitis B ($n = 556$; 55.7%) and those who had not been screened ($n = 443$; 44.3%). Univariate analysis revealed that patients who had been screened for HBV were more likely to be under 50 yr of age and have a BMI of less than 25.0 kg/m^2 ($P < 0.05$; Table 2). Race was significantly associated with

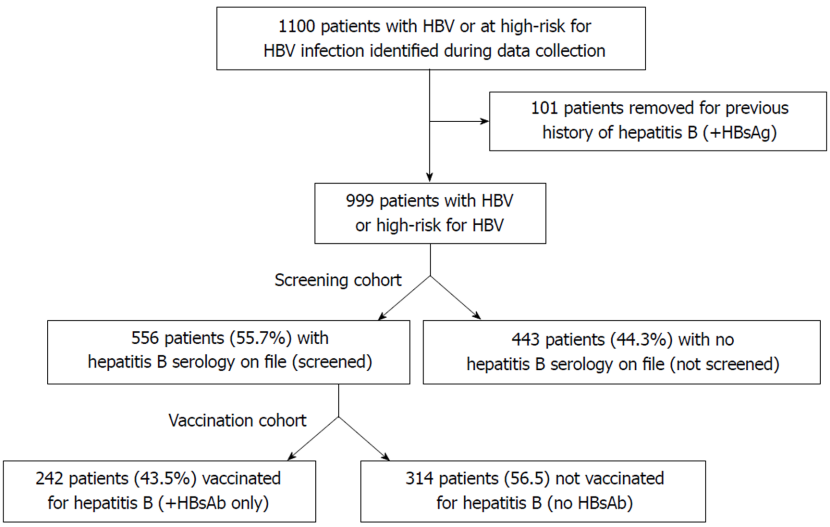

Figure 1 Study design showing screening and vaccination cohorts.

screening status (P = 0.006), ranging from 67.5% in Hispanic patients to 51.9% in white patients (Table 2). Insurance status was also significantly associated with screening (P < 0.001), peaking at 59.5% in individuals with private health insurance to less than 30% in uninsured patients (Table 2).

High-risk medical conditions are listed in Table 3, revealing that HBV screening was significantly more common in patients with CKD or ESRD (both P < 0.001). Screening was also more frequent in patients with major cardiovascular risk factors such as hypertension, CAD, or congestive heart failure (all P < 0.05; Table 4). In contrast, a history of intravenous drug use (3.2% *vs* 0.9%), human immunodeficiency virus (HIV) infection (22.3% *vs* 7.9%), or current tobacco use (8.8% *vs* 4.9%, all P < 0.05) were significantly more frequent in patients who had not been screened for HBV, suggesting a bias against HBV screening in patients with a history of high-risk activities such as polysubstance abuse.

Multivariate analysis revealed that medical conditions such as ESRD [odds ratio (OR): 5.122; 2.766-9.483], alcoholic hepatitis (OR: 3.064; 1.020-9.206), and cirrhosis (OR: 1.909; 1.095-3.329) were positively associated with HBV screening (Figure 2). Demographics including age (OR: 0.785; 0.680-0.906) or insurance status (OR: 0.690; 0.558-0.854), and chronic medical conditions such HIV (OR: 0.443; 0.273-0.718), and DM (OR: 0.518; 0.364-0.737) were inversely correlated with screening (all P < 0.05), suggesting that socioeconomic factors strongly impact likelihood of screening. The Hosmer-Lemeshow goodness-of-fit test was not significant (P = 0.116), indicating that the regression fit the data. Patients who were screened for HBV were also significantly more likely to have undergone HCV screening, hepatitis A vaccination, or have a documented hepatitis A serology on file (all P < 0.05; Table 5). These patients were also more likely to have been evaluated by primary care providers, emergency department personnel, or gastroenterologists within the past year (all, P < 0.001). MELD score was significantly higher in patients who had undergone HBV screening (median: 16 *vs* 12), and these patients tended to succumb more frequently to all-cause (11.3% *vs* 2.7%, P < 0.001) and liver-related mortality (2.0% *vs* 0.5%, P = 0.067 trend).

Of the 556 patients who had been screened for HBV, a total of 242 (43.5%) patients had been vaccinated for HBV, while 314 (56.5%) patients had not been vaccinated ("vaccination cohort"; Figure 1). Demographic information is listed in Table 2, revealing that most patients were male (59.7%), obese (BMI > 25 kg/m^2; 62.2%), and 19.4% were under 50 years of age. The vaccination cohort was socioeconomically diverse with 50.2% non-white and 36.5% without private medical insurance. Similar to the screening cohort, the vaccination cohort was characterized by a large burden of cardiovascular and other high-risk medical conditions including 64.6% with CKD, 26.4% with CLD 20.5% with high-risk sexual behavior, and 7.9% with HIV (Tables 2 and 3).

Clinical information was compared between those who had been vaccinated against HBV *vs* those who had not been vaccinated. Univariate analysis revealed that HBV vaccination was more frequently performed in non-obese patients (BMI < 25 kg/m^2; 41.7% *vs* 34.6%) and those younger than 50 years of age (26.9% *vs* 13.7%, both P < 0.05). Race significantly impacted vaccination status, with HBV vaccination ranging from 55.2% in black patients to only 36.1% of white patients (P = 0.001).

Table 2 Screening and vaccination cohort patient demographics

Demographic	Screening cohort				Vaccination cohort			
	Entire screening cohort (n = 999)	Screened for HBV (n = 556)	Not screened for HBV (n = 443)	P-value	Entire vaccination cohort (n = 556)	Vaccinated against HBV (n = 242)	Not vaccinated against HBV (n = 314)	P-value
Male	60.6%	59.7%	61.6%	0.583	59.7%	59.5%	59.9%	0.999
Age				< 0.001				< 0.001
< 40	4.9%	7.9%	1.1%		7.9%	12.8%	4.1%	
41-50	6.7%	11.5%	0.7%		11.5%	14.1%	9.6%	
51-60	26.4%	22.3%	31.6%		22.3%	24.4%	20.7%	
61-70	36.7%	28.2%	47.4%		28.2%	25.2%	30.6%	
71-80	14.6%	15.6%	13.3%		15.6%	12.4%	18.2%	
> 80	10.6%	14.4%	5.9%		14.4%	11.2%	16.9%	
BMI				0.028				0.047
< 20	7.0%	8.7%	4.8%		8.7%	11.9%	6.3%	
20-24.9	26.6%	29.0%	23.5%		29.0%	29.8%	28.3%	
25-29.9	32.7%	31.5%	34.2%		31.5%	31.2%	31.8%	
30-34.9	20.6%	19.0%	22.5%		19.0%	19.3%	18.9%	
> 35	13.1%	11.7%	14.9%		11.7%	7.8%	14.7%	
Race				0.006				0.001
White	53.5%	51.9%	48.1%		49.8%	36.1%	63.9%	
Black	17.1%	61.4%	38.6%		18.9%	55.2%	44.8%	
Hispanic	11.7%	67.5%	32.5%		14.2%	41.8%	58.2%	
Other	17.7%	53.7%	46.3%		17.1%	53.7%	46.3%	
Insurance				< 0.001				0.488
Private	59.4%	59.5%	40.5%		63.5%	41.4%	58.6%	
Medicare	30.1%	53.5%	46.5%		29.0%	46.0%	54.0%	
Medicaid	6.8%	45.6%	54.4%		5.6%	51.6%	48.4%	
Uninsured	3.7%	29.7%	70.3%		2.0%	54.5%	45.5%	

HBV: Hepatitis B virus; BMI: Body mass index.

Vaccination remained low among all insurance classes (range: 41.4%-54.5%) and was not affected by type of coverage (P > 0.05). Moreover, vaccination was significantly more common in patients with hypertension, CKD, or ESRD, while less common in patients with CLD, NAFLD, DM, or cancer (all P < 0.05; Tables 3 and 4).

Multivariate analysis revealed that hypertension (OR: 1.626; 1.019-2.594) was positively associated with HBV vaccination, while DM (OR: 0.620; 0.409-0.941) and BMI (OR: 0.799; 0.671-0.952) were inversely correlated with vaccination (all P < 0.05; Figure 3). Older age (OR: 0.755; 0.650-0.878; P < 0.05) was again inversely correlated with vaccination, further suggesting that patient demographics are an important clinical factor affecting HBV management. The Hosmer-Lemeshow test was not significant (P = 0.470), indicating that the regression fit the data.

Additional data on gastrointestinal history and healthcare utilization is listed in Table 5. MELD score (median: 16 *vs* 17), all-cause mortality (10.7% *vs* 11.8%), and liver-disease specific mortality (2.4% *vs* 1.6%, all P > 0.05) were not significantly different between patients with or without HBV vaccination.

DISCUSSION

The CDC recommends all individuals at high-risk for HBV infection undergo vaccination. Updated CDC guidelines on HBV management in 2011 greatly increased the number of eligible patients who should undergo HBV vaccination by expanding vaccination criteria to include most patients with a history of DM. Despite this, only 55.7% of high-risk patients were screened for HBV, and only 43.5% were appropriately vaccinated against infection. Socioeconomic factors such as age and insurance status significantly affected HBV management, as well as high-risk medical conditions including HIV. DM was a significant risk factor in patients who were

Table 3 High-risk medical conditions or activities

High risk condition	Screening cohort				Vaccination cohort			
	Entire screening cohort (n = 999)	Screened for HBV (n = 556)	Not screened for HBV (n = 443)	P-value	Entire vaccination cohort (n = 556)	Vaccinated against HBV (n = 242)	Not vaccinated against HBV (n = 314)	P-value
Intravenous drug use	1.9%	0.9%	3.2%	0.018	0.9%	1.2%	0.6%	0.769
Men who have sex with Men	5.0%	4.0%	6.3%	0.120	4.0%	5.8%	2.5%	0.085
Chronic kidney disease	48.1%	64.6%	27.5%	< 0.001	64.6%	73.1%	58.0%	< 0.001
End stage renal disease (dialysis)	41.3%	59.2%	19.0%	< 0.001	59.2%	69.0%	51.6%	< 0.001
Chronic liver disease	25.7%	26.4%	24.8%	0.614	26.4%	21.5%	30.3%	0.026
Alcohol hepatitis	3.2%	4.9%	1.1%	0.002	4.9%	2.9%	6.4%	0.091
Primary sclerosing cholangitis	0.5%	0.5%	0.5%	0.799	0.5%	0%	1.0%	0.347
Primary biliary cirrhosis	0.7%	0.2%	1.4%	0.067	0.2%	0%	0.3%	0.896
Cryptogenic liver	0.6%	0.7%	0.5%	0.895	0.7%	0.8%	0.6%	0.807
Hemochromatosis	0.2%	0%	0.5%	0.382	0%	0%	0%	n/a
Hepatitis C	10.3%	9.4%	11.5%	0.312	9.4%	8.9%	9.9%	0.799
Non-alcoholic fatty liver disease	10.1%	11.2%	8.8%	0.264	11.2%	7.9%	13.7%	0.042
Non-alcoholic steatohepatitis	1.6%	1.6%	1.6%	0.837	1.6%	0.8%	2.2%	0.337
Autoimmune hepatitis	0.7%	1.1	0.2%	0.221	1.1%	0.8%	1.3%	0.926
End stage liver disease (cirrhosis)	10.6%	12.8%	7.9%	0.017	12.8%	9.5%	15.3%	0.058
Human immunodeficiency virus	14.3%	7.9%	22.3%	< 0.001	7.9%	9.9%	6.4%	0.168
High risk sexual behavior	22.5%	20.5%	25.1%	0.102	20.5%	23.6%	18.2%	0.145
Diabetes mellitus	46.8%	43.0%	51.7%	0.007	43.0%	37.2%	47.5%	0.019

HBV: Hepatitis B virus.

suboptimally managed, suggesting a failure to fully implement new CDC recommendations in clinical practice. Results of a 2012 NHIS study found similar HBV vaccination rates amongst high-risk individuals, but despite additional recommendations by the CDC, as well as a 6-year time lapse, there has not been a significant increase in HBV vaccination during this period. One would expect that vaccination rates over time would increase as CDC HBV vaccination awareness had increased. This may be due to lack of awareness by physicians, and the high demand of quality patient care particularly in underserved populations. More awareness regarding HBV vaccination recommendations is needed for both primary care physicians and gastroenterology subspecialists.

As our demographics represent a diverse cohort typical of many large, urban safety-net hospitals, our study identifies a significant disparity in HBV management that disproportionately affects the most vulnerable and underserved patient population. Older patients and patients with DM were less likely to be screened and

Table 4 Other medical conditions

Comorbidity	Screening cohort				Vaccination cohort			
	Entire screening cohort (*n* = 999)	Screened for HBV (*n* = 556)	Not screened for HBV (*n* = 443)	*P*-value	Entire vaccination cohort (*n* = 556)	Vaccinated against HBV (*n* = 242)	Not vaccinated against HBV (*n* = 314)	*P*-value
Acute liver failure	0.4%	0.4%	0.5%	0.782	0.4%	0.4%	0.3%	0.597
Dyslipidemia	43.2%	41.9%	44.9%	0.373	41.9%	38.8%	44.3%	0.231
Hypertension	59.6%	63.0%	55.3%	0.017	62.9%	68.2%	58.9%	0.031
Coronary artery disease	21.1%	24.3%	17.2%	0.008	24.3%	26.0%	22.9%	0.455
Chronic heart failure	10.2%	12.2%	7.7%	0.024	12.2%	11.6%	12.7%	0.775
Chronic obstructive pulmonary disease	5.6%	5.6%	5.6%	0.927	5.6%	4.1%	6.7%	0.265
Peripheral arterial disease	5.2%	6.1%	4.1%	0.191	6.1%	4.5%	7.3%	0.239
Cerebrovascular accident	7.2%	8.6%	5.4%	0.067	8.6%	11.2%	6.7%	0.088
Psychiatric disorder	10.0%	9.2%	11.1%	0.378	9.2%	8.7%	9.6%	0.836
Current tobacco user	6.6%	4.9%	8.8%	0.018	4.9%	3.7%	5.7%	0.370
Current Alcohol use	29.0%	31.3%	26.2%	0.090	31.3%	29.8%	32.5%	0.551
Cancer (any)	18.7%	19.1%	18.3%	0.816	19.1%	12.8%	23.9%	0.001

vaccinated for HBV, while patients with cardiac or renal comorbidities such as chronic kidney disease and hypertension were more frequently evaluated for HBV. This result is likely related to the necessity of hepatitis B serology before initiation of hemodialysis, as well as the significant association between renal and cardiac disease.

The efficacy of HBV vaccination in a high-risk population has been found to be greater than 90% in adults[13,14]. Despite the CDC and Advisory Committee on Immunization Practices (ACIP) long-standing recommendations to vaccinate high-risk adult populations, national HBV vaccination coverage rates have remained low[9,11,15]. Compared with high-risk vaccination coverage in the United States, reported to be around 42% in 2012, these rates vary greatly in other industrialized countries from 14%-38% in England in 2004, 25%-45% in France in 2004, and 6%-29% in the Netherlands in 2007. Compared with previously published vaccination rates in the US, our results were similar or lower[16-18].

From 2010–2015, HBV vaccination coverage decreased overall in adults 19 years of age or older, and coverage still remains suboptimal, minimally changed from previous years, with room for improvement, particularly amongst higher-risk populations, especially based upon current recommendations[10].

In 2011, the CDC and ACIP created new recommendations for HBV vaccination for all unvaccinated adults with DM under 60 years of age. Vaccination of patients greater than 60 years of age was advised at the discretion of individual health care providers. People with both type 1 and type 2 DM have higher rates of HBV than the general population and are at additional risk because of shared blood glucose meters, fingerstick devices, and other diabetes-care equipment such as syringes or insulin pens[9,19]. HBV outbreaks in people with DM in assisted living, long-term care facilities, and nursing homes have been seen related to inadequate hand hygiene between fingerstick procedures and the maintenance of sterility of blood glucose monitoring and podiatric equipment and supplies. Consistent with these studies, we report that patients with DM had lower vaccination rates than those with similar high-risk medical conditions. Previous NHIS data from a 2014-15 collection period reports that vaccination coverage for DM patients was 24.4% for those aged 19–59 years and 12.6% for those 60 years of age and older[10]. Our data and those from other studies highlight the need to educate both patients and clinicians regarding the increased risk of HBV transmission among patients with DM and the need to increase HBV screening and

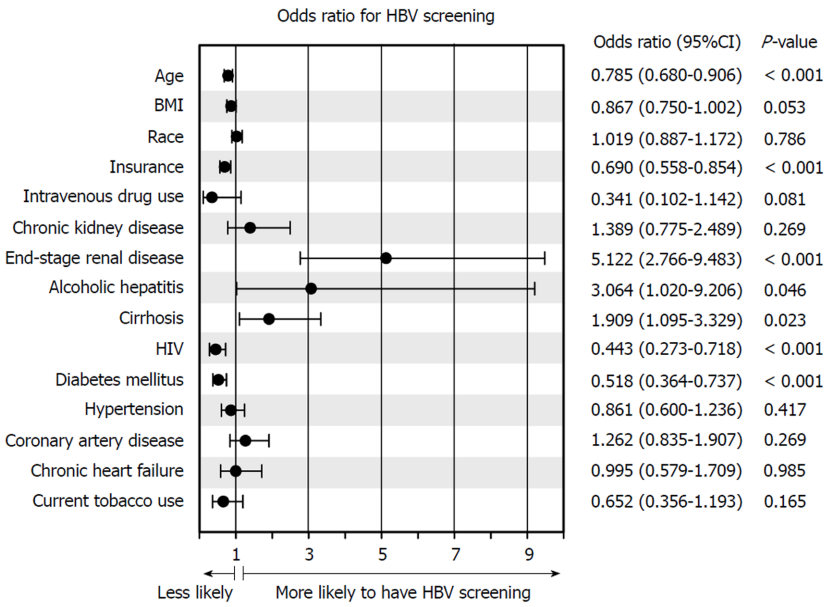

Figure 2 Multivariate logistic regression showing odds ratio for variables independently associated with hepatitis B screening.

vaccination in this higher-risk subpopulation.

Given the high prevalence of HBV infections in high-risk patients, it is reasonable to screen patients for hepatitis B prior to administering the vaccine[20]. Although screening is currently not universally recommended, it can aid in identifying patients who are already immune to HBV, and help distinguish between those who may or may not require vaccination, minimizing unnecessary vaccinations. One challenge in HBV screening is its cost effectiveness.

Post-vaccination screening can also identify those who do not seroconvert after completing the requisite vaccination series. Post-vaccination screening may be indicated in CLD as superimposed viral hepatitis B is associated with morbidity and mortality in these patients[21,22]. Patients with CLD who receive HBV vaccination have generally lower rates of seroconversion compared to otherwise healthy adults, which may be as low as 18% in patients with advanced fibrosis[23-25]. Post-vaccination screening is justified in patients with CLD and a repeat course of HBV vaccination should be considered in those who initially fail to seroconvert.

Despite the safety and well-documented benefits, rates of HBV immunization have not increased as expected. Different actions can be implemented to improve vaccination coverage. This may start with educating patients and physicians about the importance of immunization and the diseases it prevents, staying updated with current vaccination guidelines, identifying barriers to vaccination including cost, and addressing patient misconceptions. Implementing population-based immunization registries can provide access to comprehensive immunization records for patients at the community level. These registries have been shown to be effective at improving immunization rates due to their various capabilities.

Vaccination reminder-recall systems are another cost-effect method to notify adults who should undergo immunization. Many EMRs can implement standing orders, assisting healthcare professionals with identifying patient who may benefit from additional screening. These prompts may consist of electronic pop-ups in the EMR that automatically display alerts to help notify viewers that the patient is due or overdue for vaccination.

A strength of our study is an emphasis on the determination of clinical factors that may be used to identify patients in the inpatient and outpatient setting who are likely to benefit from further review of their HBV vaccination record. Previous population-based HBV vaccination and screening data have been based on surveys, and our study population is more representative of real-world data based on patient serologies in underserved urban populations. Previously reported survey-based 2012 NHIS HBV vaccination rates in high-risk adults were similar but did not include patients with DM. Our study also included anti-HBc data in the serologic panel to differentiate between immunity due to vaccination compared with prior HBV infection.

This retrospective study is subject to a number of limitations. High-risk factors in

Table 5 Gastrointestinal history and healthcare utilization

Variate	Screening cohort			Vaccination cohort		
	Screened for HBV (*n* = 556)	Not screened for HBV (*n* = 443)	*P*-value	Vaccinated against HBV (*n* = 242)	Not vaccinated against HBV (*n* = 314)	*P*-value
HCV serology	82.4%	19.2%	< 0.001	78.5%	85.4%	0.047
HCV infection	9.4%	11.5%	0.312	12.1%	10.2%	0.629
Hepatitis A vaccination	36.0%	0%	< 0.001	51.7%	23.9%	< 0.001
Hepatitis A (HAVab IgM or IgG)	26.1%	4.3%	0.002	27.9%	24.7%	0.616
1 or more primary care visit per year	43.8%	30.9%	< 0.001	38.1%	48.1%	0.030
1 or more emergency department visit per year	56.6%	26.7%	< 0.001	57.0%	56.4%	0.956
1 or more gastroenterology visit per year	35.4%	19.4%	< 0.001	31.9%	38.0%	0.166
MELD score (median; 25-75th percentile)	16 (11-23)	12 (8-20)	0.019	16 (11-22)	17 (12-24)	0.586
All-cause mortality	11.3%	2.7%	< 0.001	10.7%	11.8%	0.804
Liver-related mortality	2.0%	0.5%	0.067	2.4%	1.6%	0.662
Listed for liver-transplant	8.1%	5.6%	0.167	6.6%	9.2%	0.333

MELD: Model for end-stage liver disease.

patients are often underreported by patients (and physicians) or may not be consistently documented. Patients may frequently hesitate to report high-risk activities or history such as intravenous drug use or high-risk sexual behavior, thereby underestimating the proportion of high-risk individuals in the general study population. Patients may also not be cognizant of their complete medical histories, underscoring the need to thoroughly review and document a patient's medical history. Further, this study only used data accessible from the EMR, and not all outside records or serological information were available in the EMR. This may underestimate screening and vaccination data as many patients seek care at several inpatient and outpatient centers, along with other primary care services. Hence, the true number of unvaccinated individuals may be even lower. However, given the large proportion of underserved individuals in our study population, it is likely that our safety-net hospital (as part of our larger urban academic center) is the primary site for many of these patients' healthcare. Future studies are needed to further identify and improve ways to improve HBV vaccinations, particularly in high-risk patients.

In conclusion, Despite the most recent CDC guidelines, patients at high-risk for HBV infection are not being adequately screened and vaccinated against hepatitis B infection and show little improvement compared to historical averages, even when compared to other studies. Despite numerous studies and taskforce- or professional society-based guidelines, there has been minimal improvement in vaccination rates over the past several years. We found that comorbid conditions such as older age, and diabetes were associated with a lower likelihood of being screened or vaccinated for HBV, while the opposite was found in patients with ESRD. Vaccination rates were lower in Black and Hispanic populations.

An improvement in HBV vaccination coverage is needed. Educating patients and clinicians alike to help identify highest-risk populations is essential, in order to raise awareness that could potentially increase HBV vaccination rates, with the end goal of decreasing the burden of chronic HBV-related liver disease, including advanced fibrosis, cirrhosis, portal hypertension, and HCC. Public health programs and initiatives are essential at providing these clinical services. Greater vaccination coverage can be achieved by routinely assessing patients' vaccination status, using standing orders for vaccination, incorporating vaccination information guidelines and

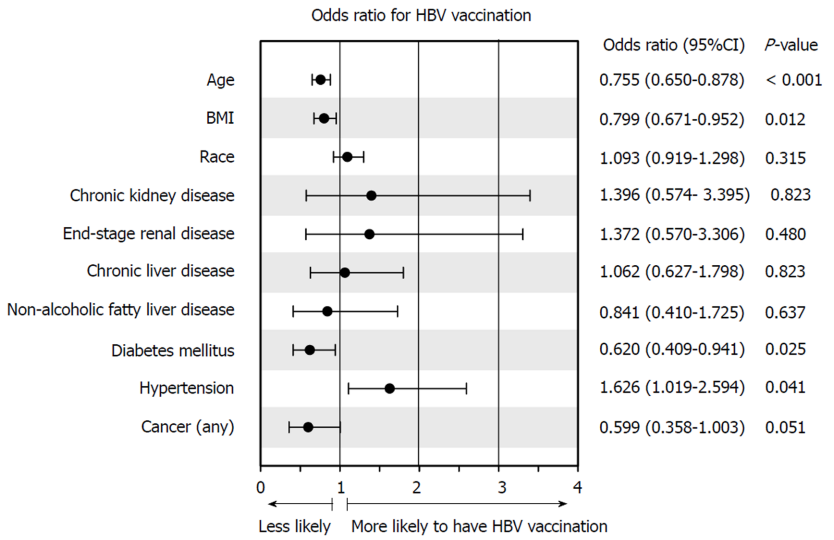

Figure 3 Multivariate logistic regression showing odds ratio for variables independently associated with hepatitis B vaccination.

prompts in electronic medical records, and using other immunization information systems[26,27].

Identifying patients who are at high-risk and implementation of the CDC HBV vaccination recommendations is important in helping decrease the incidence (and ultimately the prevalence) of HBV infections, along with the physical, emotional, and financial burden of both acute and chronic HBV and its numerous associated sequelae. Educating patients and physicians about hepatitis B vaccination, and implementation of immunization registries, reminder-recall systems and provider prompts may help increase vaccination rates.

ARTICLE HIGHLIGHTS

Research background

Hepatitis B is a liver infection caused by the hepatitis B virus (HBV), affecting 1.4 million people in the United States, and 350 people worldwide. HBV infection accounts annually for 4000 to 5500 deaths in the United States and 1 million deaths worldwide from cirrhosis, liver failure, and hepatocellular carcinoma (HCC). Hepatitis B vaccination is 95% effective in preventing infection and the development of chronic disease and liver cancer due to hepatitis B in adults vaccinated before being exposed to the virus. Hepatitis B disproportionately affects certain high-risk populations. HBV vaccination coverage in high-risk individuals in the United States was reported to be around 42% in 2012. The Centers for Disease Control (CDC) recommends all individuals at high-risk for HBV infection undergo vaccination. These guidelines expanded in 2011 to include those with diabetes mellitus (DM). The purpose of our study is to evaluate clinical factors associated with HBV screening and vaccination in high-risk individuals.

Research motivation

Hepatitis B infection is a significant cause of liver disease in the United States. With the advent of HBV vaccination, rates of hepatitis B infection have declined, but the rates of vaccination in high-risk individuals have not significantly increased over previous years. With the recommendation for expanded HBV vaccination guidelines from the CDC, current rates in high-risk individuals may be underestimated. Our research study looks to evaluate clinical factors associated with HBV screening and vaccination in high-risk individuals, which may provide better understanding to the current vaccination rates in this population. Estimating current vaccination rates in high-risk individuals is important for future research that can study different methods to improving vaccination rates.

Research objectives

The main objective of this study was to evaluate screening and vaccination rates in high-risk individuals, and clinical factors associated with screening and vaccination. We found that the vaccination rates in high-risk individuals remains low in our study population, and that these rates are similar to previous national rates despite updated CDC guidelines.

Research methods

We conducted a retrospective review of 999 patients presenting at a large urban healthcare system from 2012-2017 at high-risk for hepatitis B infection. Patients were considered high-risk for hepatitis B infection based on hepatitis B practice recommendations from the Centers for

Disease Control. Medical history including hepatitis B serology, medical diagnoses, demographics, insurance status and social history were extracted from electronic health records. Multivariate logistic regression was used to identify clinical risk factors independently associated with hepatitis B screening and vaccination.

Research results

Among the 999 patients, 556 (55.7%) patients were screened for hepatitis B. Of those who were screened, only 242 (43.5%) patients were vaccinated against hepatitis B. Multivariate regression analysis revealed end-stage renal disease (ESRD) [odds ratio (OR): 5.122; 2.766-9.483], alcoholic hepatitis (OR: 3.064; 1.020-9.206), and cirrhosis or end-stage liver disease (OR: 1.909; 1.095-3.329; all $P < 0.05$) were associated with hepatitis B screening, while increasing age (OR: 0.785; 0.680-0.906), insurance status (0.690; 0.558-0.854), history of DM (OR: 0.518; 0.364-0.737), and human immunodeficiency virus (OR: 0.443; 0.273-0.718; all $P < 0.05$) were less likely to undergo hepatitis B screening. Of adults vaccinated for hepatitis B, multivariate regression analysis revealed increasing age (OR: 0.755; 0.650-0.878), BMI (0.799; 0.671-0.952), and DM (OR: 0.620; 0.409-0.941; all $P < 0.05$) were less likely to undergo hepatitis B vaccination.

Research conclusions

Vaccination rates in high-risk individuals remain low at 43.5% in our study and ways to improve these rates need to be evaluated. The CDC recommends all individuals at high-risk for HBV infection undergo vaccination. Our study reveals that patients at high-risk for hepatitis B are not being adequately screened and/or vaccinated. With the addition of DM in the CDC HBV vaccination guidelines, we found that older age, diabetes, and decreasing insurance coverage were associated with a lower likelihood of being screened or vaccinated for HBV, while ESRD was associated with increased likelihood of screening. Vaccination rates likely remain low due to lack of knowledge by patients and physicians on appropriate implementation of CDC guidelines. Identifying patients who are at high-risk for infection is an important step in decreasing the incidence (and ultimately the prevalence) of HBV infections in the United States. Future studies are needed to further identify and improve ways to improve HBV vaccinations, particularly in high-risk patients.

Research perspectives

Identifying high-risk patients who are likely to benefit from further review of their HBV vaccination status and implementation of vaccination to those in need is of high importance in the prevention of hepatitis B infection and its sequelae including chronic liver disease, cirrhosis and HCC. Despite CDC recommendations, HBV vaccination rates in high-risk individuals are still not optimal. The direction of future research should be aimed at obtaining national rates to better gauge vaccination in the United States. Also, with the knowledge of current vaccinations rates, future studies can evaluate different modalities including patient and physician education, immunization registries, reminder-recall systems and provider prompts that can help improve HBV management.

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ORIGINAL ARTICLE

Retrospective Study

Low platelet count: Predictor of death and graft loss after liver transplantation

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Author contributions: Beltrame P conceptualized and designed the data, collected the data, analyzed and interpreted the data, drafted the article, and critically revised the article; Rodriguez S collected the data, and critically revised the article; Brandão ABM conceptualized and designed the data, analyzed and interpreted the data, drafted the article, and critically revised the article; all authors have read and approved of the final version of the article.

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Abstract

BACKGROUND

The impact of platelets on liver transplantation (LT) is well recognized, but not completely understood. Platelets exert dichotomous effects on the graft and on the patient. On the one hand, they are essential for primary hemostasis and tissue repair and regeneration. On the other hand, they support ischemia/reperfusion injury and inflammatory processes. Recent evidence has shown a new role for platelet count (PC) in predicting outcomes after LT.

AIM

To evaluate if low PC is a predictor of short- and long-term outcomes after LT.

METHODS

Four hundred and eighty consecutive LT patients were retrospectively assessed. PC from the preoperative to the seventh postoperative day (POD) were considered. C-statistic analysis defined the ideal cutoff point for PC. Cox regression was performed to check whether low PC was a predictor of death, retransplantation or primary changes in graft function within one year after LT.

RESULTS

The highest median PC was 86 × 10^9/L [interquartile range (IQR) = 65–100 × 10^9/L] on seventh POD, and the lowest was 51 × 10^9/L (IQR = 38–71 × 10^9/L) on third POD. The C-statistic defined a PC < 70 × 10^9/L on fifth POD as the ideal cutoff point for predicting death and retransplantation. In the multivariate analysis, platelets < 70 × 10^9/L on 5POD was an independent risk factor for death at 12 mo after LT [hazard ratio (HR) = 2.01; 95% confidence interval (CI) 1.06-3.79;

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P = 0.031]. In the Cox regression, patients with PC < 70 × 10^9/L on 5POD had worse graft survival rates up to one year after LT (HR = 2.76; 95%CI 1.52-4.99; P = 0.001).

CONCLUSION
PC < 70 × 10^9/L on 5POD is an independent predictor of death in the first year after LT. These results are in agreement with other studies that indicate that low PC after LT is associated with negative outcomes.

Key words: Predictive factors; Prognosis; Platelet count; Liver transplantation; Graft survival; Mortality

Core tip: Recent evidence shows that low platelet count (PC) can predict outcomes after liver transplantation (LT). We evaluated if a low PC in the immediate postoperative period of LT, defined as a PC < 70 × 10^9/L on the fifth postoperative day (5POD), is a predictor of death or retransplantation. We retrospectively assessed 480 consecutive LT patients. This study showed that a PC < 70 × 10^9/L on the 5POD was independently associated with shorter patient and graft survival within one year after LT. These results are in agreement with other studies indicating that thrombocytopenia in the immediate postoperative period of LT is associated with negative outcomes.

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INTRODUCTION

Low platelet count (PC) is common in candidates for liver transplantation (LT). Its etiology is multifactorial, including increased spleen destruction, inability to produce bone marrow and reduced production of thrombopoietin[1-3]. Reduction in PC is also commonly observed in the postoperative period of LT. In this period, there are other possible contributing factors for its occurrence, such as graft and splenic sequestration, hemodilution, use of some medications and immunological reactions[4-8]. After LT, PC reach their lowest level around the third or fifth postoperative day (POD), returning to preoperative values in around two weeks[9]. Low PC after LT is associated with shorter graft and patient survival and higher rates of negative outcomes[10], possibly due to the fact that platelets play an important role in the promotion of angiogenesis[11-14] and hepatic regeneration[15,16].

Lesurtel *et al*[17] observed that a PC < 60 × 10^9/L on the fifth POD was an independent risk factor for complications and shorter graft and patient survival within the first 90 d posttransplantation. They then proposed the "60-5 criterion", where a PC of < 60 × 10^9/L on 5POD of LT could be used to predict severe complications[17]. Subsequently, Takahashi *et al*[18] reported that a PC of < 72 × 10^9/L on 5POD was associated with graft loss and shorter patient survival. The present study aimed to confirm the hypothesis that a low PC in the immediate postoperative period of LT is a predictor of death or retransplantation.

MATERIALS AND METHODS

All adult patients consecutively submitted to LT with a deceased donor between June 2006 and June 2016 were eligible at a referral center in southern Brazil. Excluded from the study were patients who underwent double organ transplantation (liver and kidney), late liver retransplantation (more than one year between LT and retransplantation) or LT due to acute liver failure and who had incomplete medical records. The study followed the recommended guidelines for observational studies[19] and was approved by the Institutional Review Board of Santa Casa de Misericórdia de Porto Alegre (No. 1.183.375).

Variables analyzed

The following variables were analyzed: (1) Demographic characteristics of the recipient and donor (sex, age and body mass index); (2) variables related to the procedure (times of cold and warm ischemia, volume of bleeding and transfusions during transplantation); (3) blood components transfused up to the first week after LT; (4) biochemical tests performed immediately before LT and up to the seventh POD: PC, international normalized ratio (INR), aspartate aminotransferase, alanine aminotransferase (ALT), total bilirubin, factor V, alkaline phosphatase and gamma-GT; and (5) relevant preoperative characteristics of the recipient: Need for ventilation or hemodynamic support, renal replacement therapy, hepatorenal syndrome, cirrhosis etiology, model for end-stage liver disease (MELD) score, and Child-Turcotte-Pugh (CTP) score[20-22].

Surgical procedure

All patients underwent LT by the same team, where the technique of choice was hepatectomy with preservation of the inferior vena cava (piggy-back technique). The preservation solutions used during the period were: First, the University of Wisconsin solution, then histidine-tryptophan-ketoglutarate and, since 2013, the Institute Georges Lopez-1 solution. The decision to transfuse blood components was based on clinical, laboratory and hemodynamic parameters. Blood loss was restored by transfusion of packed red blood cells, with the goal of maintaining hemoglobin levels between 8.0 and 10.0 g/dL. Platelet concentrate was administered only in patients with platelet dysfunction and persistent bleeding, even after correction of other coagulation factors.

Outcomes

The primary outcome was death, by any cause, within 30, 90 and 365 d after LT. The secondary outcome was the need for liver retransplantation in the same period. We considered any cause of death and retransplantation after LT, which could be primary or secondary. Secondary death/retransplantation was due to technical, immunological, infectious or cardiovascular causes. Primary death/retransplantation was due to delayed graft function or primary graft dysfunction. Delayed graft function was defined as the presence of at least 1 of the following parameters 7 d after LT: A serum bilirubin level ≥ 10 mg/dL and an INR ≥ 1.6 or an ALT level > 2000 IU/L[17]. Primary graft dysfunction was defined as the presence of one or more of the following postoperative laboratory parameters: A serum bilirubin level ≥ 10mg/dL on day 7, INR ≥ 1.6 on day 7, and an ALT level > 2000 IU/L within the first 7 d[23].

Statistical analysis

Statistical analysis was performed with IBM SPSS software version 22.0 for Windows (IBM, Armonk, NY, United States). Quantitative variables were described by mean and SD, and significance was determined by Student *t*-test. Categorical variables were described by counts and percentages, and significance was assessed using the chi-square test or Fisher exact, when needed. The C-statistic, equivalent to the area under the receiver operating characteristics (ROC) curve (AUC) assessed on each POD of LT, was adopted to establish the day on which the PC showed the best performance. Recursive analyses of ROC curves, within the day previously detected, allowed us to identify the cutoff point. Univariate and multivariate Cox regression analyses were performed to adjust the PC findings with the potential effect of other factors. The pre-operative variables chosen, with potential confounding effect over PC in the postoperative period, as previously shown in similar studies[17,18,23], were: MELD score before LT > 20, pre-LT PC < 70 × 10^9/L, age of the recipient > 60 yr, donor age > 40 yr and diagnosis of hepatorenal syndrome at the time of transplantation. In addition, the need for intraoperative transfusion of platelet concentrates, perioperative bleeding > 2500 mL and cold ischemia time > 8 h were considered. Survival curves were obtained using the Kaplan-Meier method. $P < 0.05$ was considered statistically significant.

RESULTS

Population studied

Of the 617 eligible patients for study, 137 (22.2%) were excluded from the analysis for the following reasons: Underwent combined liver and kidney transplantation (n = 32; 5.2%); late liver retransplantation (performed one year after the initial LT) (n = 21; 3.4%); transplantation for acute liver failure (n = 21; 3.4%); or incomplete information in medical records up to seventh POD, due to either death, retransplantation or inadequate completion of medical records (n = 63; 10.2%). Therefore, 480 patients

were included in the study. Approximately 20% of them were older than 60 yr, with the majority being male, and hepatitis C virus infection was the most frequent etiology of cirrhosis. At the time of LT, the mean MELD score was 16; approximately 49% of patients were in the B classification of the CTP score and 3.5% underwent hemodialysis immediately before transplantation. About 48% of the donors were older than 40, and most times the cold ischemia time was < 8 h (Table 1).

Characteristics of patients in relation to platelet count

The highest median number of PC was observed on seventh POD: 86 × 10^9/L [interquartile range (IQR) = 65-100 × 10^9/L], exceeding the preoperative median, *i.e.*, 77 × 10^9/L (IQR = 57-97 × 10^9/L). The lowest median was found on the third POD: 51 × 10^9/L (IQR = 38-71 × 10^9/L).

C-statistical analysis showed that a PC < 70 × 10^9/L on 5POD was the ideal cutoff point for predicting death and retransplantation at 365 d after LT [AUC-ROC = 0.632; 95% confidence interval (CI) 0.558-0.705; P = 0.001]. Patients were then stratified into two groups according to PC on 5POD: Patients with ≥ 70 × 10^9/L platelets and those with < 70 × 10^9/L platelets (Table 1). In the group with < 70 × 10^9/L PC, the recipients were transplanted at later stages of their disease according to the CTP score (P = 0.014) and, although without statistical significance, they received organs with a longer time of cold ischemia and from donors over 40 yr old (Table 1).

Outcomes and platelet counts on 5POD

Univariate analysis (Table 2) showed that patients with a PC of < 70 × 10^9/L on 5POD had higher all-cause mortality rates at 90 and 365 d after LT, compared to patients with a higher PC. The 90-day mortality rate was 7.5%, higher in patients with a PC < 70 × 10^9/L on 5POD (9.7% *vs* 4.2%, P = 0.037). Overall mortality at 365 d post-LT was 13.3%. Likewise, the highest mortality rates occurred in the group with PC < 70 × 10^9/L on 5POD (17.4% *vs* 7.3%; P = 0.002). Retransplantation rates at 30, 90 and 365 d were higher in patients with lower PC (< 70 × 10^9/L) on 5POD. In fact, the need for retransplantation up to 90 d after LT was only observed in the group of patients with a lower PC, and only one patient with a PC of ≥ 70 × 10^9/L on 5POD required retransplantation within 365 d post-LT (5.2% *vs* 0.5%; P = 0.004) (Table 2).

Multivariate analysis

Multivariate analysis (Figure 1) showed that, in the first year after LT, a PC < 70 × 10^9/L on 5POD was a risk factor for death, independently of age of the recipient > 60 yr, pre-LT MELD score > 20, bleeding volume > 2500 mL intraoperatively and need for transfusion of platelet concentrates during the procedure. Pre-LT MELD > 20 and age of the recipient > 60 yr also appeared to be independent risk factors for mortality up to one year after LT (P = 0.031 and P = 0.012, respectively).

Survival of grafts and recipients

Eighty grafts were lost in up to one year of follow-up: 64 (80.0%) due to death of the recipient and 16 (20.0%) due to the need for liver retransplantation. Of the patients who underwent liver retransplantation, six were due to primary causes: three for primary graft nonfunction (18.75%), two for primary graft dysfunction (12.5%) and one for delayed graft function (6.25%). The most common causes of death were infection (28.1%), cardiovascular events (21.8%) and primary causes related to the graft (7.8%). Graft survival in the first year after LT was significantly lower in the group with PC < 70 × 10^9/L on 5POD compared to ≥ 70 × 10^9/L on 5POD group, even when adjusted for the factors used in multivariate analysis (77.5% *vs* 92.2%, P = 0.001) (Figure 2). Overall survival at 12 mo for patients with a PC ≥ 70 × 10^9/L on 5POD was higher than for those with a lower PC (92.8% *vs* 82.7%, P = 0.002) (Figure 3).

DISCUSSION

This study showed that PC < 70 × 10^9/L on 5POD of LT were independently associated with shorter patient and graft survival within one year after LT. These results are in agreement with other studies indicating that thrombocytopenia in the immediate postoperative period of LT is associated with negative outcomes[17,18,23].

Platelets contain a marked number of secretory granules, filled with proteins essential for hemostasis and different tissue growth factors, such as platelet-derived growth factor (PDGF), hepatocyte growth factor (HGF), insulin-like growth factor type 1 (IGF-1), vascular endothelial growth factor (VEGF), serotonin, ADP and ATP[14]. Platelets are activated by various types of stimuli and release these substances depending on the situation. In addition to its known role in primary hemostasis and thrombosis, platelets exert other functions, such as promoting liver regeneration and

Table 1 Comparison of patients according to platelet count on fifth postoperative day of liver transplantation, *n* (%)

Variables prior to LT	All patients (*n* = 480)	Platelets < 70 × 10⁹/L - 5POD (*n* = 288)	Platelets ≥ 70 × 10⁹/L - 5POD (*n* = 192)	*P*-value
Related to recipients				
Age > 60 yr	97 (20.2)	65 (22.6)	32 (16.7)	0.071
Sex: Male	311 (64,8)	189 (65.6)	122 (63.5)	0.355
BMI (kg/m^2)	25.7 (± 3.8)	25.7 (± 3.5)	25.4 (± 3.4)	0.410
Hepatitis C	141 (29.4)	85 (29.5)	56 (29.2)	> 0.99
Hepatitis B	20 (4.2)	12 (4.2)	8 (4.2)	> 0.99
MELD score (± SD)	16.3 (± 5.3)	16.8 (± 5.4)	15.7 (± 5.0)	0.033
CTP score				
A	117 (24.5)	59 (20.6)	58 (30.4)	0.014
B	228 (47.8)	140 (49)	88 (46.1)	
C	132 (27.7)	87 (30.4)	45 (23.6)	
Life support[1]	3 (0.6)	3 (1)	0 (0.0)	0.215
HRS	45 (9.4)	27 (9.4)	18 (9.4)	0.560
Hemodialysis[2]	17 (3.5)	10 (3.5)	7 (3.6)	0.554
Related to donors				
Age > 40 yr	223 (47.2)	139 (49.3)	84 (44.2)	0.161
CIT > 8 h	192 (40.6)	116 (41.1)	76 (39.8)	0.422
Variables during LT				
Bleeding > 2500 mL	276 (57.5)	172 (59.7)	104 (54.2)	0.133
Transfusion of platelets	180 (37.5)	109 (37.8)	71 (36.9)	0.890
Transfusion of fresh frozen plasma	288 (60.0)	171 (59.4)	117 (60.9)	0.805

[1]Patients needing vasoactive drugs or mechanical ventilation before liver transplantation.
[2]Patients needing renal replacement therapy before liver transplantation. 5POD: Fifth postoperative day; BMI: Body mass index; CIT: Cold ischemia time; CTP: Child-Turcotte-Pugh; HRS: Hepatorenal syndrome; LT: Liver transplantation; MELD: Model for End-stage Liver Disease.

liver protection[10-14]. In liver recipients, it is common to have thrombocytopenia[1,24], a situation that worsens during transplantation, for reasons not completely understood[10]. However, many factors have been identified and associated with thrombocytopenia, such as their consumption in the process of hemostasis and sequestration by reperfusion graft or spleen[4,10,25,26].

The relationship between low PC and events after LT was suggested in the late 1990s, when thrombocytopenia (defined arbitrarily) in the immediate postoperative period of LT was associated with shorter patient and graft survival[10,25,27,28]. However, it was the Lesurtel *et al*[17] who established a cutoff point in the number of platelets that could be used as a predictor of outcomes after LT. In a retrospective study, Lesurtel *et al*[17] evaluated a cohort of 257 patients who underwent LT with a deceased donor. They observed that a PC < 60 × 10^9/L on 5POD was an independent predictor of Clavien-Dindo IIIb/V complications (OR = 1.96; 95%CI 1.07-3.56), graft loss [hazard ratio (HR) = 2.0; 95%CI 1.1-3.6], and lower survival of patients (HR = 2.2; 95%CI 1.4-4.6, *P* = 0.03) within 90 d after LT. They then proposed the "60-5" criterion, in which the determination of the number of platelets on 5POD could be used to predict outcomes, anticipate complications, and thus be used prophylactically. Later, Takahashi *et al*[18], also retrospectively analyzing a cohort of transplanted patients with a deceased donor, reported that a PC < 72 × 10^9/L on 5POD was associated with graft loss and shorter patient survival within one year after LT. The authors postulated that in patients with a greater number of platelets, more thrombocytes accumulate in the graft, which would allow the release of a greater amount of growth factors (PDGF, HGF, IGF-1 and VEGF), improving graft survival and survival in general[18]. In studying 234 adult patients submitted to LT with living donors, a Chinese study[23] concluded that a PC of ≤ 68 × 10^9/L at any time in the immediate postoperative period was an independent risk factor for early graft dysfunction (OR = 2.88; 95%CI 1.22-6.82, *P* = 0.016).

The present study validated the "60-5 criterion", where we found that a PC < 70 × 10^9/L on 5 POD was independently associated with mortality at 365 d after LT. Overall survival of patients with < 70 × 10^9/L on 5POD was 2.5-fold lower than for

Table 2 Univariate analyses of postoperative outcomes according to patients' platelet count after liver transplantation, *n* (%)

Outcome	Platelet count < 70 × 10^9/L - 5POD (*n* = 288)	Platelet count ≥ 70 × 10^9/L - 5POD (*n* = 192)	HR (95%CI)	*P*-value
Mortality in 30 d	17 (5.9)	7 (3.6)	1.62 (0.68-3.83)	0.37
Mortality in 90 d	28 (9.7)	8 (4.2)	2.33 (1.09-5.00)	0.037
Mortality in 1 yr	50 (17.4)	14 (7.3)	2.38 (1.36-4.18)	0.002
Retransplantation within 30 d	10 (3.5)	0 (0.0)	-	0.007
Retransplantation within 90 d	12 (4.2)	0 (0.0)	-	0.002
Retransplantation within 1 yr	15 (5.2)	1 (0.5)	10 (1.33-76.92)	0.004
Delayed graft, function	28 (9.7)	15 (7.8)	1.24 (0.68-2.27)	0.58

5POD: Fifth postoperative day; HR: Hazard ratio.

patients with a PC ≥ 70 × 10^9/L on 5POD. In addition, lower liver graft survival was observed in the first year after surgery: patients with PC < 70 × 10^9/L on 5POD had a 2.76-fold greater risk of graft loss than patients with a higher PC. Therefore, our results are in line with the "60-5 criterion", although the cutoff point was different. In fact, another study[29] validating the "60-5 criterion" also identified a cutoff point different from the one originally proposed. At the time of transplantation, patients with a lower PC on 5POD had a more severe disease: The mean MELD was higher (16.8 ± 5.4 *vs* 15.7 ± 5.0; $P = 0.033$), and most of them were in the C category of the CTP score (30.4% *vs* 23.6%, $P = 0.014$), which could predict the onset of intra- and postoperative complications. However, the intraoperative variables analyzed, such as the requirement for transfusion of blood components (including platelets), were similar in the two groups, and it is unlikely that the severity of the disease at the time of LT showed any clinical significance. Another issue to be pondered is how a decreased PC on 5POD can have an influence on late outcomes. Considering that patients with a reduced number of platelets on 5POD have a higher rate of biliary tract complications[29], and that these are associated with infectious complications, hospital readmissions and endoscopic or surgical interventions, it is possible that such complications may have an impact on the survival of grafts and patients in the long run[18,29].

Theoretically, for patients with post-transplant thrombocytopenia, preventive measures should be taken to avoid or minimize adverse effects: Platelet transfusion, suspension of potentially myelosuppressive drugs, or administration of serotonin or thrombopoietin. However, further studies are needed before these interventions are considered in clinical practice, although transfusion of platelets in transplant recipients seems to be positively associated with graft regeneration[30,31]. In addition, this study pointed out that a pre-LT MELD > 20 and age of the recipient > 60 yr was also independent risk factors for mortality up to one year after LT.

This study had some limitations. One of them is inherent to retrospective studies; this study was carried out in a single center. However, the same team of surgeons and anesthesiologists performed the procedures, and in the immediate postoperative period, patients were treated in an intensive care unit exclusively for patients undergoing organ transplantation. The study design also did not allow us to demonstrate that platelet transfusion could effectively exert a protective effect for the graft and patient. In addition, with this study, it was not possible to clarify, in fact, if low PC in the postoperative period is the cause of negative outcomes after LT or if it is a surrogate marker of another clinical condition, which could be the real problem.

In summary, patients with PC of < 70 × 10^9/L on 5POD of LT showed higher mortality within 365 d after transplantation and lower graft survival. This study reinforces the need to evaluate the role of interventions to maintain a minimum PC after LT to potentially improve outcomes after LT.

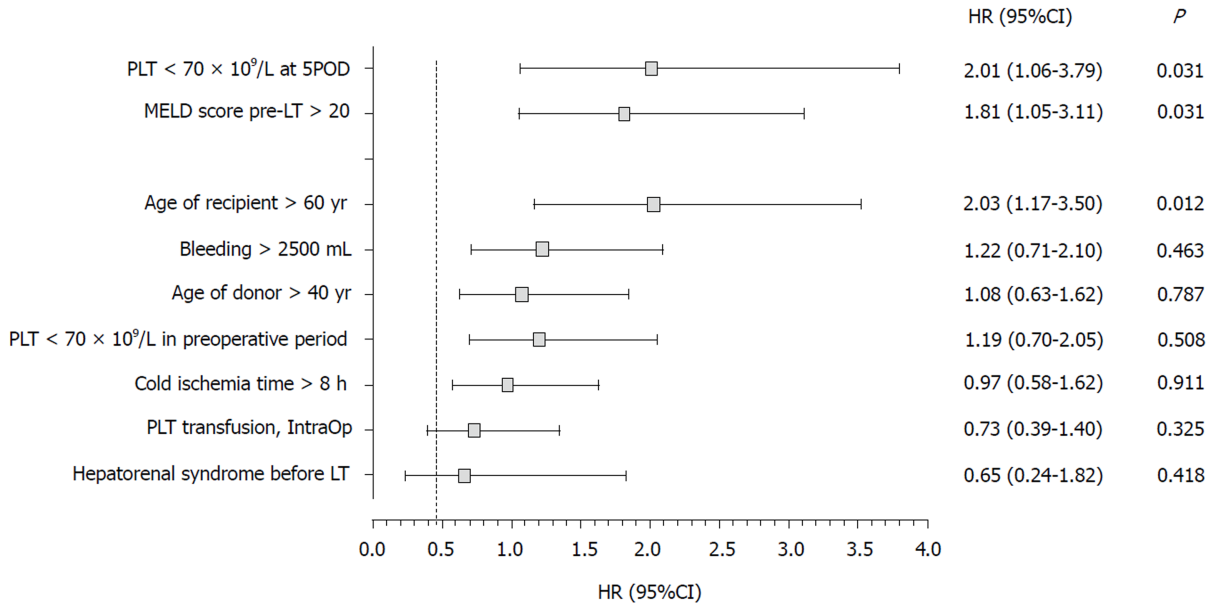

Figure 1 Independent risk factors of death within one year after liver transplantation, in multivariate analysis. 5POD: Fifth postoperative day; 95%CI: 95% confidence interval; HR: hazard ratio; IntraOp: Intraoperative; PLT: Platelets; MELD: Model for end-stage liver disease; LT: Liver transplantation.

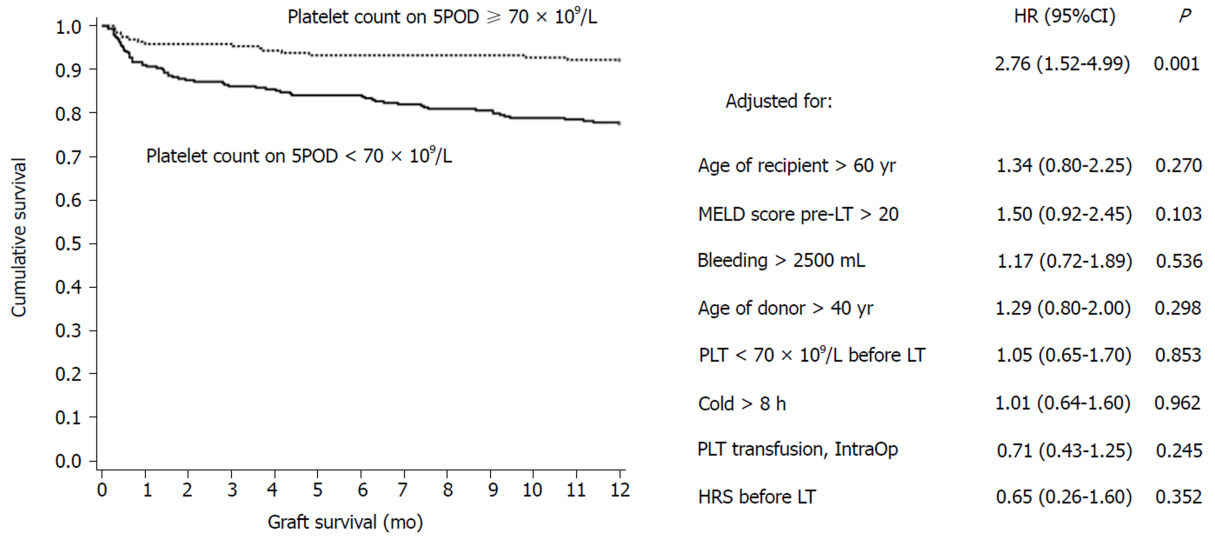

Figure 2 Cumulative survival of liver graft of patients with platelets < 70 × 10⁹/L on fifth postoperative day, adjusted for different variables, during first year of follow-up after liver transplantation. 5POD: Fifth postoperative day; CI: confidence interval; HR: hazard ratio; HRS: Hepatorenal syndrome; IntraOp: Intraoperative; PLT: platelets; MELD: Model for end-stage liver disease; LT: Liver transplantation.

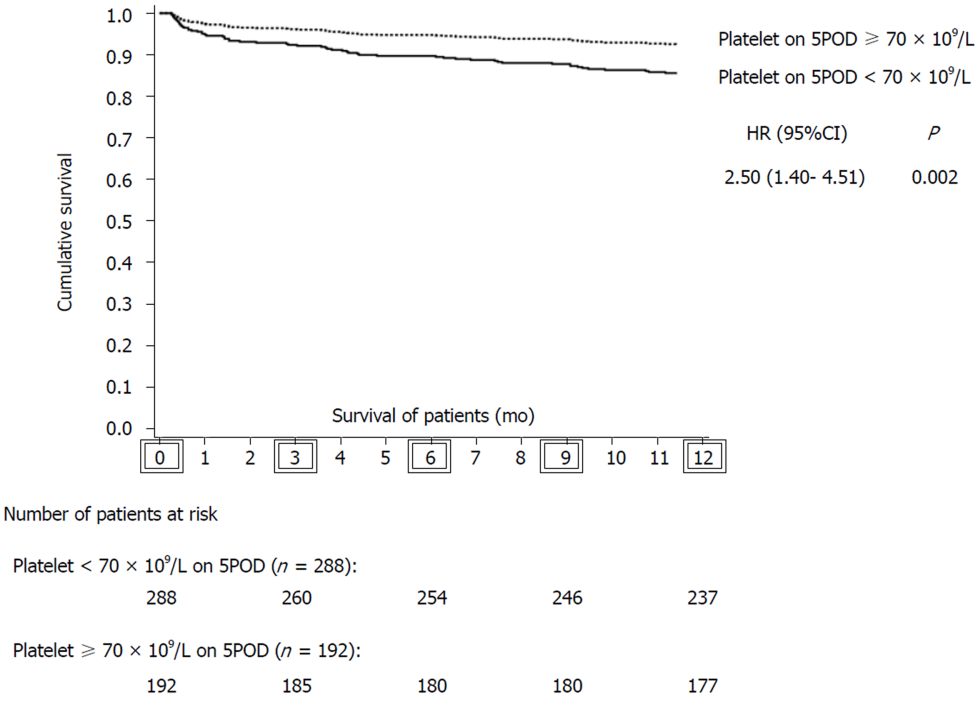

Figure 3 General survival within first year after liver transplantation (LT) of patients with platelet count < 70 × 10^9/L on fifth postoperative day *vs* patients with platelet count ≥ 70 × 10^9/L on 5POD. Together, the description of the number of patients at risk of death at, 0, 3, 6, 9 and 12 mo after liver transplantation, respectively. 5POD: Fifth postoperative day; CI: confidence interval; HR: hazard ratio; LT: liver transplantation.

ARTICLE HIGHLIGHTS

Research background

Platelets have several functions and exert dichotomous effects on the graft and on the patient in the context of liver transplantation (LT). Low platelet count (PC) after LT is associated with higher rates of complications. However, it is not clear whether low PC in the postoperative period is the cause or a surrogate marker of negative outcomes.

Research motivation

The accurate prediction of which LT recipients will do well and which ones will have serious complications remains somewhat elusive. Some authors suggest that low PC after LT can predict early posttransplant survival or graft loss. Confirmation of these findings can provide the clinician with the opportunity to intervene early and theoretically change the postoperative course of the patient.

Research objectives

To confirm the hypothesis that a low PC after LT is a predictor of death or graft loss.

Research methods

We performed a retrospective database analysis. PC from the preoperative to the seventh postoperative day (POD) were considered. C-statistic analysis was adopted to establish the day on which the PC showed the best performance. Recursive analyses of receiver operating characteristics curves allowed us to identify the cutoff point. Cox regression was performed to check whether low PC was a predictor of death, retransplantation or primary changes in graft function within one year after LT.

Research results

PC < 70 × 10^9/L on 5POD was defined as the ideal cutoff point for predicting death and retransplantation. PC < 70 × 10^9/L on 5POD was an independent risk factor for death at 12 mo after LT. In the Cox regression, patients with PC < 70 × 10^9/L on 5POD had worse graft survival rates up to one year after LT.

Research conclusions

A low PC on 5POD was associated with graft loss and mortality one year after LT. This result is in agreement with previous studies indicating that low PC in the immediate postoperative period of after LT is associated with negative outcomes.

Research perspectives

Our results reinforce the need to evaluate the role of interventions to maintain a minimum PC

after LT. Preventive measures, such as platelet transfusion, suspension of potentially myelosuppressive drugs, and administration of serotonin or thrombopoietin, could be used in the future in the LT setting. However, further studies are still required before these interventions can be considered in clinical practice.

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ORIGINAL ARTICLE

Observational Study

High prevalence of occult hepatitis C infection in predialysis patients

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Author contributions: Sette LHBC conceived of the presented idea, designed the report, collected and analyzed the clinical data, and wrote the paper; Lopes EPA conceived of the presented idea, designed the report, analyzed the data and wrote the paper; Guedes dos Anjos NC designed the report and collected the clinical data; Valente LM conceived of the presented idea, and analyzed the data; Vieira de Oliveira SA performed the biochemical and PCR testing; Lucena-Silva N conceived of the presented idea, performed the biochemical and PCR testing, analyzed the data, and wrote the paper.

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Abstract

BACKGROUND

Occult hepatitis C virus (HCV) infection (OCI) may be associated with extrahepatic diseases and it is known that the patients with chronic kidney disease (CKD) who are on hemodialysis (HD) present a higher prevalence of this type of infection than the general population, with a worse clinical outcome. However, there are no data in the literature to assess the presence of OCI in patients prior to the initiation of renal replacement therapy (RRT). Therefore, this study aimed to evaluate the occurrence and epidemiological aspects of OCI in patients with Predialysis CKD. We hypothesize that this infection could occur before RRT initiation.

AIM

To research the status in predialysis patients when HD patients have high prevalence of OCI.

METHODS

A cross-sectional study was conducted between 2015 and 2017. Adults with creatinine clearance < 60 mL/min·1.73 m^2 (predialysis patients) were recruited to the study. Pregnant and postpartum women, patients with glomerulopathies, and patients showing positivity for serological markers of hepatitis B virus (HBV), HCV or human immunodeficiency virus infection were excluded. Patients were diagnosed with OCI according to test results of anti-HCV antibody negativity and HCV RNA positivity in either ultracentrifuged serum or, if serum-

Conflict-of-interest statement: All of the authors declare no conflicts of interest.

Data sharing statement: The dataset is available upon request made to the main investigator, Luis Henrique Bezerra Cavalcanti Sette (luis.sette@ufpe.br).

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negative, in peripheral blood mononuclear cells.

RESULTS

Among the 91 total patients included in the study, the prevalence of OCI was 16.5%. Among these 15 total OCI patients, 1 was diagnosed by 14 ultracentrifuged serum results and 14 were diagnosed by peripheral blood mononuclear cell results. Compared to the non-OCI group, the OCI patients presented higher frequency of older age (P = 0.002), patients with CKD of mixed etiology (P = 0.019), and patients with markers of previous HBV infection (*i.e.*, combined positivity for anti-hepatitis B core protein antibody and anti-hepatitis B surface protein antibody) (P = 0.001).

CONCLUSION

Among predialysis patients, OCI involved the elderly, patients with CKD of mixed etiology, and patients with previous HBV infection.

Key words: Occult hepatitis infection; Chronic hepatitis C; Chronic kidney disease; Hemodialysis; Hepatitis C virus-RNA; Peripheral blood mononuclear cells

Core tip: Evaluation of patients with chronic renal disease and glomerular filtration rate lower than 30 mL/min·1.73 m^2 showed high occurrence of occult hepatitis C virus infection (OCI). In addition, the study population showed higher occurrence of OCI among patients who were older, had chronic kidney disease (CKD) of multifactorial etiology, and had prior contact with the hepatitis B virus. Further studies will be needed to clarify the pathophysiology of renal injury caused by OCI, the influence of this type of infection on the transmissibility of hepatitis C virus, and the role of treatment for patients with OCI and CKD.

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INTRODUCTION

Occult hepatitis C virus (HCV) infection (OCI) is characterized by presence of HCV genetic material (HCV RNA) in a patient's peripheral blood mononuclear cells (PBMCs), ultracentrifuged serum, or hepatic tissue, along with the absence of HCV antibodies (anti-HCV) and HCV RNA in the non-ultracentrifuged serum[1]. This form of viral hepatitis may be associated with tissue damage in the liver (*i.e.*, chronic cryptogenic liver disease and nonalcoholic fatty liver disease)[2-4] and extrahepatic diseases (*i.e.*, glomerulopathies, lymphoproliferative diseases, and end-stage renal disease)[5-7].

It has been suggested that a specific cellular immune response underlies OCI, one that is less effective than the response in individuals without infection but which is more effective than that in individuals with the classic form of the disease (HCV RNA-positive serum)[8]. It is known that the patients with chronic kidney disease (CKD) who are on hemodialysis (HD) present a higher prevalence of HCV infection than the general population, both in its classical form[9] and the occult form, reaching up to 45% prevalence among patients with OCI[7,10].

Nowadays, due to universal precautions in HD units and the reduced need for blood transfusion, some authors suggest that HCV infection may occur in the predialytic period[11]. Indeed, patients with predialysis (PD)CKD have a higher prevalence of infection with HCV and hepatitis B virus (HBV), as compared to the general population[12-14]. However, there are no data in the literature to assess the presence of OCI in patients prior to the initiation of renal replacement therapy.

The study of the epidemiology of OCI in patients with PDCKD is relevant according to: (1) the higher prevalence of classic HCV infection in HD patients[9,15]; (2)

the outlook for the use of diagnostic OCI techniques in these patients before the onset of HD[1]; (3) the greater difficulty in identifying hepatic aggression in patients with CKD due to reduced serum aminotransferase levels resulting from hemodilution[16]; and, finally; and (4) the possibility of HCV treatment with the new direct-acting antiviral agents that have few side effects and high efficacy[17].

Therefore, this study aimed to evaluate the occurrence and epidemiological aspects of OCI in patients with PDCKD.

MATERIALS AND METHODS

Study design

A cross-sectional study was carried out from October 2015 to April 2017, in which patients with PDCKD were evaluated in the CKD Outpatient Clinic of the Nephrology Service of the Federal University of Pernambuco (Brazil). Adult patients with a follow-up period of more than 3 mo and with an estimated creatinine clearance of less than 60 mL/min·1.73m^2 were included. Pregnant and postpartum women, patients with glomerulopathies, and patients with serological markers of infection with either HBV [hepatitis B surface antigen (HBsAg)-positive], HCV (anti-HCV-positive) or human immunodeficiency virus (commonly known as HIV) (anti-HIV-positive) were excluded.

Each study participant provided written informed consent following the explanation that study participation would: (1) not disrupt the patient's normal attendance at the outpatient clinic; (2) include a copy of all exam results being placed in their medical record, so that the doctor accompanying the patient in clinic will know the results; and (3) provide information for research for publication, with any exam results being published without the patient name. The risks and benefits of study participation were also explained. Samples were only taken from patients after agreement to study participation and signing of the informed consent form.

Each study participant enrolled in the study filled out a questionnaire on demographic characteristics (sex, age, ethnicity), clinical diagnoses of CKD (stage, etiology) and risk factors for viral hepatitis acquisition (transfusion, tattooing, piercing, intravenous drug use). A blood sample (20 mL in vacutainer tube) was drawn from each participant's peripheral vein, for use in biochemical, serological and virological laboratory tests.

Serum levels of aminotransferases (aspartate aminotransferase, alanine aminotransferase) and creatinine were determined by automated method (UV Diasys-Architect c8000; Abbott Diagnostics, Lake Forest, IL, United States). The creatinine clearance rate was evaluated by the Chronic Kidney Disease Epidemiology Collaboration formula (commonly known as the CKD-EPI equation)[18].

Evaluation of viral infections included third-generation ELISA serum screening for anti-HCV, rapid immunochromatographic assay for the qualitative determination of HBsAg, and immunoassays for anti-HBs detection by direct chemiluminescence (ADVIA Centaur® automated system; Siemens Healthcare Diagnostics S.A., Sao Paulo, Brazil) and for total qualitative anti-hepatitis B core protein (HBc) (IMMUNITE 2000® Imunoassay; Siemens Healthcare Diagnostics S.A.). Patients with negative results on anti-HCV and HBsAg screening were included in the study and evaluated for OCI.

Patients were diagnosed with OCI if they were positive for HCV RNA by PCR screening of ultracentrifuged serum or PBMCs. If the screening was positive for HCV RNA in the ultracentrifuged serum, the patient's sample of non-ultracentrifuged serum was screened using the same procedure to confirm the diagnosis of OCI.

Detection of HCV RNA

Each peripheral blood sample was initially centrifuged at 3000 x *g* for 7 min at room temperature, to obtain serum. Then, a 2 mL aliquot of the serum was overlaid by a 10% sucrose buffer, in a ratio of 1:1, and ultracentrifuged at 100000 x *g* for 17 h at 4 °C. The precipitate obtained by ultracentrifugation was eluted in 200 µL of diethylpyrocarbonate (DEPC; Invitrogen, Carlsbad, CA, United States), to generate an RNase-free sample. A separate aliquot of the peripheral blood with anticoagulant) was subjected to the density gradient centrifugation with Ficoll-Paque (GE Healthcare, Little Chalfont, United Kingdom), to isolate PBMCs. The cDNA synthesis was performed with random primers having as template RNA strands extracted from peripheral blood mononuclear cells and/or ultracentrifuged serum, using the enzyme M-MVL reverse transcriptase (Invitrogen™), following the manufacturer's specifications.

Detection of HCV RNA in the ultracentrifuged serum and PBMCs was performed by PCR prepared with 100 ng of cDNA, 5 µM of the primers specific for amplification

of the HCV genome (UTRLC1: 5'-CAAGCACCCTATCAGGCAGT-3'; UTRLC2: 5'-CTTCACGCAGAAAGCGTCTA-3'), 1 x PCR Rxn buffer (Invitrogen), 5 mmol/L $MgCl_2$, and 10 pmol dNTPs. The reaction conditions consisted of an initial cycle of 10 min at 95 °C, followed by 30 cycles of 95 °C for 30 s, 55 °C for 30 s and 72 °C for 30 s, and with a final 5-min extension at 72 °C, performed in the SimpliAmp Thermal Cycler (Applied Biosystems Inc., Foster City, CA, United States). Positive and negative controls consisted of a sample of patients known to be positive for classic hepatitis C and the PCR mix without DNA addition, respectively. The amplified product was subjected to 2% agarose gel electrophoresis and visualized on the SYBR-safe gel through the L-PIX Transilluminator (Loccus, São Paulo, Brazil). The presence of a fragment of approxiately 230 base pairs in the absence of nonspecific bands was considered a positive result. The positive result was confirmed by a new PCR from another aliquot of the patient's sample, using the same procedure.

Statistical analysis

Numerical variables were represented by measures of central tendency and dispersion measures. The categorical variables were submitted to χ^2 test and Fisher's exact test to evaluate the occurrence of association between them. The Kolmogorov-Smirnov normality test was applied to the quantitative variables. When the normal distribution was evidenced, the Student t test was used. When the variable was non-normal the comparison was performed using Mann-Whitney (Non-Normal). STATA/SE 12.0 and Excel 2010 were used for data storage and processing. In all tests, 95%CIs were applied.

RESULTS

We initially recruited 154 patients with creatinine clearance < 60 mL/min·1.73 m^2 as potential participants for the study; of those, 130 were enrolled, including 24 patients who had follow-up time of < 3 mo. Ultimately, 39 of the enrolled patients were excluded from study participation, for presence of glomerulopathies (n = 23), refusal to participate (n = 7), positivity in HIV serology (n = 3), positivity for anti-HCV (n = 3), positivity for HBsAg (n = 2), and impossibility of venipuncture (n = 1).

The demographic, clinical and laboratory characteristics of the 91 study participants are described in Table 1. The prevalence of OCI among them was 16.5% (15/91), including 14 cases for who the HCV RNA positivity was identified in the PBMCs and 1 with positivity in the ultracentrifuged serum. Figure 1 shows an electrophoresis of a patients positive for OCI.

OCI showed an age relationship, since older patients presented a higher risk of infection compared to younger patients (69.4 ± 7.9 *vs* 60.9 ± 13.5, P = 0.002). CKD of mixed etiology was observed in 11 of the 15 cases (73.3%) of OCI and was much more frequent than in cases without OCI (P = 0.019). No relationship was found between the occurrence of OCI and sex, skin color or stage of CKD. There was no difference between serum levels of aminotransferases between the groups with and without OCI neither with albumin, bilirubin or gamma glutamyl transferase serum levels.

Risk factors associated with viral hepatitis transmission were also tested (previous HD, use of injectable drugs, occupational exposure, contaminated family member, history of hemotransfusion, piercing, tattoo, acupuncture, sexually transmitted infection, and condom use). None showed an association to the occurrence of OCI.

Of the 91 total patients evaluated, 2 provided an insufficient sample for assessment of HBV antibodies. Thus, of the 89 patients analyzed, 75 (84.2%) were anti-HBc negative, 9 (9.8%) were anti-HBc positive, and 5 (5.5%) were undetermined. A total of 4 out of the 5 patients with undetermined anti-HBc were positive for anti-HBs and were considered as having had previous contact with HBV. A total of 31 patients were anti-HBs positive only and this antibody was not found more frequently in OCI patients (P = 0.59); however, the presence of total anti-HBc and anti-HBs in combination (indicating previous HBV infection) was more frequent in patients with OCI, when compared to uninfected patients (P = 0.001), as shown in Table 2.

DISCUSSION

This was the first study to describe the occurrence of OCI in patients with PDCKD. It was previously known that patients with PDCKD present a higher occurrence of HCV infection in the classic form than the general population, with reported prevalence rates range from 3.9% to 7.3% and being up to seven times higher than in the control populations studied[12-14].

Table 1 Distribution of clinical parameters according to the occurrence of occult hepatitis C virus infection in 91 patients with chronic kidney disease: 2015 to 2017

	Total (%), *n* = 91	Occult hepatitis C infection		*P*-value
		Yes (%), *n* = 15	No (%), *n* = 76	
Age	62.4 ± 13.1	69.4 ± 7.9	60.9 ± 13.5	0.002[1]
Age ≥ 60 yr				0.001[1]
Yes	59 (64.8)	15 (100)	44 (57.9)	
No	32 (35.2)	0 (0)	32-42.1	
Sex				0.323[2]
Male	44 (48.4)	9 (60)	35 (46.1)	
Female	47 (51.6)	6 (40)	41 (53.9)	
Ethnicity				0.719[1]
Mixed	50 (54.9)	7 (46.7)	43 (56.6)	
Caucasian	30 (33.0)	6 (40.0)	24 (31.6)	
African Americans	11 (12.1)	2 (13.3)	9 (11.8)	
BMI	27.1 ± 4.4	27.4 ± 4.7	27.0 ± 4.3	0.750[1]
GFR	21.6 ± 10.3	24.6 ± 10.6	21.0 ± 10.2	0.231[1]
CKD stage				0.580[2]
IIIa	2 (2.2)	0 (0)	2 (2.7)	
IIIb	22 (24.4)	6 (40.0)	16 (21.3)	
IV	38 (42.2)	5 (33.3)	33 (44.0)	
V	28 (31.1)	4 (26.7)	24 (32.0)	
Etiology of CKD				0.019[2]
Mixed	31 (34.1)	11 (73.3)	20 (26.3)	
DM	26 (28.6)	1 (6.70)	25 (32.9)	
HA	19 (20.9)	3 (20.0)	16 (21.1)	
ADPKD	7 (7.70)	0 (0)	7 (9.20)	
Others	1 (1.1)	0 (0)	1 (1.3)	
ALT[e]	18.1 (14.2; 23.2)	17.9 (15.2; 20.9)	18.1 (14.2; 24.7)	0.859
AST[e]	19.5 (16.6; 24.8)	18.1 (17; 22.6)	19.5 (16.6; 25.3)	0.713

[1]Student's *t* test;
[2]Fisher's exact test;. ADPKD: Autosomal dominant polycystic kidney disease; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; BMI: Body mass index; CKD: Chronic kidney disease; DM: Diabetes mellitus; GFR: Glomerular filtration rate; HA: Hypertension.

One of the possible reasons underlying the high prevalence of OCI (16.5%) in our study could be the low sensitivity of the ELISA tests used, that is, false negative anti-HCV results. However, in this study, patients underwent at least two anti-HCV screenings, with the first one in the diagnosis of CKD and the second as an inclusion criterion for this study. Along with both having negative results, a third-generation immunoenzymatic assay was employed, which shows close to 100% accuracy[19]. The other possibility would be the immunological deficiency of patients with PDCKD who would not be able to produce anti-HCV antibodies in detectable titers.

We could still consider the possibility of a false positive result in the PCR reaction to detect the HCV RNA in the PBMCs or ultracentrifuged serum; however, this is unlikely since the different steps in the procedure were performed in different environments under laminar flow with quality reagents. In addition, positive results were confirmed by a new PCR from another aliquot of the patient's blood sample, using the same technique for detection of HCV RNA.

In this study, we did not perform HCV RNA screening by PCR in the serum (non-ultracentrifuged) of all patients since our objective was not to identify patients with serum anti-HCV negativity and HCV RNA positivity. However, HCV RNA screening was performed in all patients' non-ultracentrifuged serum when HCV RNA was positive in PBMCs or in ultracentrifuged serum. Moreover, it would be unlikely to find patients with anti-HCV negativity and HCV RNA negativity in both PBMCs and in ultracentrifuged serum, but with positivity in non–ultracentrifuged serum. According to these parameters, then, we question the need for performing the HCV RNA screening by PCR of serum before RNA screening of PBMCs and

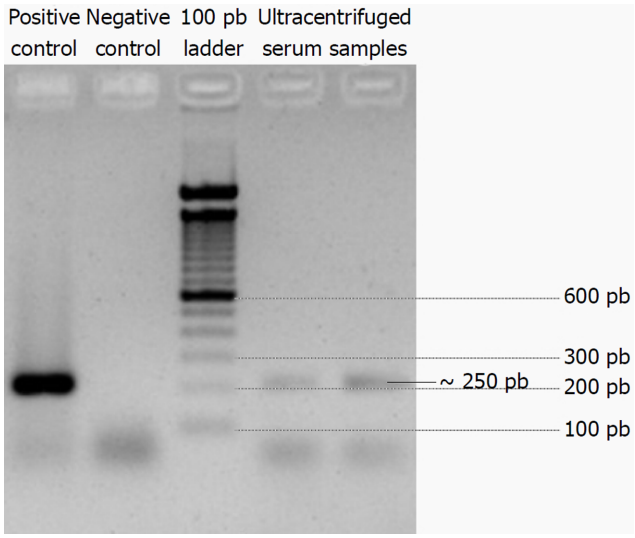

Figure 1 Electrophoresis showing a positive sample visualized on the SYBR-safe gel through the L-PIX Transilluminator (Loccus, São Paulo, Brazil).

ultracentrifuged plasma for diagnostic confirmation of OCI.

The practical relevance of the findings from our study is uncertain. However, the high frequency of OCI found in our study population may play a significant role in the transmissibility of the disease within HD units once the patients do not have detectable antibodies and circulating virions, when they could potentially be infectious[20]. Moreover, the vast majority of patients (14/15) with OCI had viral genome components at detectable levels in PBMCs, making them potential transmitters of the infection[21]. These are intriguing findings since, in general, about 50% of HCV-infected patients do not have identifiable risk factors for HCV infection[22].

Epidemiologically, a phylogenetic study conducted by Castillo *et al*[23] found that patients with OCI have high intrafamilial transmissibility. Moreover, Roque-Cuellar *et al*[24] evaluated the immunological response in the presence of OCI in heterosexual partners of patients with the classic form of infection; they found a specific CD4+ and CD8+ immune response and a higher prevalence of OCI in the partners compared to those with uninfected heterosexual partners. These findings were corroborated by Shazly *et al*[25], who evaluated 50 partners of patients with positive anti-HCV in serum and found a about 4% prevalence rate for OCI, higher than expected in the general population.

In addition to the exclusion of patients with glomerulopathies (since this group may present a prevalence of OCI of up to 39%), our results are strengthened by the fact that two techniques were used to identify OCIboth the serum ultracentrifugation and the identification of the viral genome in PBMCs, which increases the chance of detection of OCI. In fact, Castillo *et al*[5] demonstrated that positivity for HCV in PBMCs by PCR corresponded to 82% of positive cases for OCI; in addition, when using ultracentrifugation, the number of positive patients increased. In none of the cases of that study was the examination of OCI positive by both techniques.

In our study, OCI was associated with age, mixed etiology CKD, and positivity for HBV antibodies (anti-HBc and anti-HBs), suggesting previous infection.

Certainly, older patients are more exposed to contamination during their longer lifetimes. These patients also have more comorbidities, as described in a meta-analysis published in 2016 by Alvarez *et al*[26]. Age has been characterized as an independent risk factor for acquisition of HCV infection[27]. In addition, a North American study found that about 75% of HCV-infected patients were born between the years of 1945 and 1965, representing the so-called Baby Boomer generation[28].

Patients with CKD of mixed etiology are reported to have more comorbidities and, therefore, have greater chance of exposures to other diseased patients, such as those infected with hepatitis, in hospital settings and to diagnostic and therapeutic procedures, all known risk factors for HCV infection[29]. In fact, a case-control study conducted by Perez *et al*[30] in the United States, involving acute and symptomatic HCV infections in individuals over 55 years of age, found that exposure in a healthcare environment with intravenous drug infusion was related to a higher risk of HCV infection; namely, 2.7-fold higher than in the controls, with risk attributable to exposure of 37% (OR = 2.7, 95%CI: 1.3-5.8).

Table 2 Distribution of antibodies against hepatitis B virus according to the occurrence of hepatitis C virus occult infection in 89 patients with chronic kidney disease: 2015 to 2017

	Total (%), *n* = 89	Occult HCV infection		*P*-value
		Yes (%), *n* = 14	No (%), *n* = 75	
Anti-HBs + anti-HBc total				
Positive	13 (14.6)	7 - 50	6 - 8	0.001[1]
Anti-HBs (anti-HBc negative)				
Positive	31 (34.8)	4 (28.6)	27 - 36	0.590[1]
Anti-HBs + anti-HBc total				
Negative	45 (50.6)	3 (21.4)	42 - 56	0.01[1]

[1]Fisher's exact test. HBc: Hepatitis B core protein; HBs: Hepatitis B surface protein; HBV: Hepatitis B virus; HCV: Hepatitis C virus.

In our study, patients with OCI showed a higher frequency of positivity for anti-HBc and anti-HBs, when compared to those not infected. It is also known that patients with HCV infection present a higher chance of HBV contamination, since the risk factors for transmission of these infections are similar[31].

Our study had some limitations that must be considered when interpreting our findings. Specifically, by not quantifying the viral load and not genotyping the patients, the epidemiological and transmissibility factors could not be determined for our study population of patients with OCI.

The reasons underlying the emergence of the occult form of HCV infection are not known. We can speculate that virus-related factors may be involved in this form of infection, as described in genomic studies where genetic variations between different viral strains of HCV constitute a mechanism of viral survival, contributing to their pathogenicity and resistance to the host's immune system[32,33]. We can also speculate that host-related factors may be involved in the occult form of HCV infection. Of course, the specific cellular immune response (CD4+ and CD8+) in patients with OCI would be less effective than in individuals without infection but more intense than in those with the classic form of disease, presenting with HCV RNA positivity in the serum[8].

In addition, although most infected patients show a specific cellular immune response to HCV, only 20% of infected individuals are able to achieve viral clearance in the acute phase of the disease[34,35]. Indeed, Burke *et al*[36] observed that patients who were unable to achieve viral clearance had lower cell responses and decreased numbers of CD4+ and CD8+ T lymphocytes. In addition, existing differences between the human leukocyte antigen (commonly known as HLA) molecules can target poorly effective immune responses, as some HLA super types affect the recognition and binding affinity of T lymphocytes to antigenic epitopes[37].

Treatment of OCI can be performed with antiviral therapy, as reported by Pardo *et al*[38]. Those authors administrated pegylated-interferon and ribavirin for 6 mo to 10 patients who presented with OCI and abnormalities in serum levels of aminotransferases that were associated with chronic hepatitis (diagnosed by biopsy). At the end of treatment, 6 patients presented normalization of the serum alanine aminotransferase levels and in 7 cases the HCV RNA was no longer detectable in the PBMCs, suggesting viral behavior similar to that of the classic HCV infection[38].

In our study, presented herein, occurrence of OCI was high among patients with PDCKD. OCI in this patient population was more common in the elderly, in patients with CKD of mixed etiology and in patients with serological markers of HBV (indicating previous infection). Further studies will be needed to evaluate the influence of this form of infection on the transmissibility of HCV in the HD setting and the role of treatment for HD patients with OCI.

ARTICLE HIGHLIGHTS

Research background

Patients with chronic kidney disease (CKD) and who are on hemodialysis (HD) have a higher prevalence of hepatitis C virus (HCV) infection, both in its classical form and the occult (OCI) form. However, no studies in the literature have evaluated the occurrence of OCI in predialysis patients.

Research motivation
The motivation of this study was borne of the new lines of evidence showing OCI having implications on transmissibility, prognosis and progression of CKD in patients on dialysis, and knowledge that this infection could be diagnosticated and treated before end-stage kidney disease is reached.

Research objectives
The main objective of this study was to evaluate the prevalence of OCI in predialysis CKD patients. The findings of this study could help in future efforts to trace the transmissibility of OCI and even to develop a treatment to improve these patients' prognosis.

Research methods
This was a cross-sectional study, wherein patients were allocated if they had glomerular filtration rate < 60 mL/min·1.73 m^2 and were anti-HCV negative. PCR was performed on patient samples of ultracentrifuged plasma and peripheral blood mononuclear cells.

Research results
OCI was found in 16.5% of the study population of CKD patients. It was more common in the elderly, in patients with CKD of mixed etiology, and in patients with previous hepatitis B virus infection.

Research conclusions
This study found a high prevalence of OCI among CKD patients, suggesting that patients with low kidney function may be reservoirs for HCV. This high prevalence appears to be associated with infection occurring before the beginning of dialysis. Moreover, OCI could be an important transmission route of HCV infection in dialysis centers. The collective findings from this study could help future studies evaluating the transmissibility of hepatitis infection and the progression of CKD to need for dialysis.

Research perspectives
This is the first study to evaluate the prevalence of OCI in this specific group of patients, who are more vulnerable to viral infections. Future research should evaluate the influence of this form of infection on the transmissibility of HCV in the HD setting and the role of treatment for these OCI patients. A prospective study evaluating the clinical behavior of patients with OCI will be insightful.

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CASE REPORT

Multidisciplinary approach for multifocal, bilobar hepatocellular carcinoma: A case report and literature review

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Abstract

BACKGROUND

Hepatocellular carcinoma (HCC) is the second most lethal malignancy worldwide. There has been virtually no change in the survivability of HCC in spite of improvement in therapies. Surgery is considered the ideal first, curative intervention, however most patients present in advanced stages with unresectable disease. Therefore, systemic and liver-directed non-operative therapies are initially offered to downstage the disease. To ensure optimal management, a multidisciplinary team approach is often warranted. Our case highlights the benefits of a multidisciplinary approach in a young woman with multifocal, bilobar HCC.

CASE SUMMARY

A 36-year-old Chinese woman with untreated hepatitis B was found to have large bilobar HCC during work up for abdominal pain. Her initial serum alpha-fetoprotein was significantly elevated to 311136 ng/mL. Computed tomography scan demonstrated bulky bilobar liver masses, consistent with intermediate stage HCC, Barcelona Clinic Liver Cancer Stage B. Her case was discussed and a personalized care plan was developed at the Multidisciplinary Center for Advanced Minimally Invasive Liver Oncologic Therapies at the University of Washington. She initially underwent bilobar transarterial chemoembolization with partial response of the left lobar tumor. Salvage hypofractionated proton beam radiation therapy was delivered to the right lobe followed by two additional transarterial chemoembolizations to the left lobe with good response. Finally, to remove left lobar residual disease, she was taken to the operating room

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for a left hepatectomy eleven months after her initial presentation. She continues to be without evidence of disease.

CONCLUSION
Coordinating the multiple HCC treatment modalities is complex and our case highlights the benefits of a multidisciplinary approach.

Key words: Hepatocellular carcinoma; Liver cancer; Multidisciplinary care; Case report

Core tip: Hepatocellular carcinoma is a challenging disease that requires a personalized and multidisciplinary approach for treatment. Our case highlights a favorable patient outcome in a young woman with multifocal bilobar hepatocellular carcinoma as a result of a coordinated multimodal treatment approach utilizing catheter-based ablative techniques, external beam radiation therapy, and surgical resection.

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INTRODUCTION

Hepatocellular carcinoma (HCC) is the second most lethal malignancy worldwide, claiming over 745000 lives in 2012[1]. This figure is projected to rise despite new and effective antiviral drugs to eradicate hepatitis C infection and is due to increasing rates of fatty liver disease from diabetes and the obesity epidemic[2]. There has been virtually no change in the survivability of HCC over the last three decades[3]. This is especially true for intermediate and advanced HCC where the standard of care therapy with sorafenib provides only a 2% response rate and 3-mo survival advantage[4]. While checkpoint inhibition immunotherapy (*e.g.*, nivolumab and pembrolizumab) have demonstrated significant response rates, a large proportion of HCC patients do not respond to this immunotherapy[5]. Therefore, more effective treatments for advanced cases of HCC are greatly needed.

Several modalities exist for treatment of intermediate and advanced stage HCC. Potential therapies include ablative techniques using ethanol (percutaneous ethanol injection), microwave or radiofrequency; catheter-directed transarterial chemoembolization (TACE) or radioembolization (TARE); external beam radiation therapy in the form of stereotactic body radiation therapy or proton beam therapy (PBT); systemic targeted small molecules; and immunotherapy and investigational agents. In certain cases, these therapies can be utilized simultaneously or in sequence to achieve optimal tumor control. Furthermore, this approach can be used to effectively downstage advanced HCC and make it amenable for curative liver resection (LR) or transplantation[6].

Coordinated management of the HCC patient with these different modalities has become increasingly complex. To navigate this complexity, discuss suitable therapeutic options, and generate personalized treatment plans, many centers have adopted a multidisciplinary team (MDT) approach with established "tumor boards." These teams include members from several complementary specialties including surgical, medical and radiation oncology, transplant surgery and hepatology, diagnostic and interventional radiology, pathology, physical and occupational therapy, nursing, nutrition, genetic counseling, spiritual, and palliative care. The MDT approach has been adopted for the treatment of a wide variety of malignancies, and it is in fact required for accreditation as a comprehensive cancer care center by the American College of Surgeons Commission on Cancer. Although the MDT approach has several putative benefits for patient care, there is a concern over the paucity of high quality evidence demonstrating an improvement in patient outcomes[7,8]. Furthermore, it has been associated with high operating costs.

Our patient presented with advanced, multifocal, and bilobar HCC; an extent of

disease associated with a dismal prognosis. Herein, we highlight a year-long MDT approach that demonstrates how it can downstage advanced disease and lead to improved patient outcomes. We report a young patient with advanced, multifocal, bilobar, and initially unresectable HCC that benefited from a year-long MDT approach.

CASE PRESENTATION

A 36-year-old woman originally from China was evaluated in September 2016 for management of large bilobar liver masses. Her pertinent past medical history was significant for untreated hepatitis B virus infection diagnosed in 1999 while pregnant with her first child. The rest of her past medical and surgical history was remarkable only for a previously excised benign breast mass. Her family history was positive for lymphoma in her father. She had never smoked tobacco, consumed alcohol, or used illicit drugs. Her physical examination was unremarkable with no appreciable liver masses.

Her oncologic history began in 2015 when she first experienced right shoulder and chest pain with work-up revealing mildly elevated transaminases. No further evaluation was performed until 2016 when the pain worsened with associated weight loss and fatigue. She underwent right upper quadrant ultrasound, which demonstrated large bilobar liver masses. The serum alpha-fetoprotein (AFP) was significantly elevated to 311136 ng/mL (reference 0-8.6). A multiphase liver protocol computed tomography (CT) scan demonstrated bulky bilobar liver masses (Figure 1A), including a left lobe lesion measuring 8.1 cm x 14.4 cm x 10.7 cm with central necrosis, in addition to a multilobular right lobe lesion measuring 10.5 cm x 9.5 cm x 14.9 cm. No IVC, hepatic, or portal venous invasion or thrombosis was identified, and there was no evidence of extrahepatic disease. Her serum was positive for HBsAg with a HBV quantitative PCR of 12000 IU/mL for which she initiated entecavir therapy. Her liver function was well-preserved with a child-turcotte-pugh score of A5 and model for end-stage liver disease score of 6.

MULTIDISCIPLINARY EXPERT CONSULTATION

Her case was discussed at the University of Washington Multidisciplinary Center for Advanced Minimally Invasive Liver Oncologic Therapies composed of members from the surgical, medical and radiation oncology, transplant surgery and hepatology, pathology, and diagnostic and interventional radiology teams. Given her history of hepatitis B, elevated AFP, and imaging characteristics consistent with HCC, a diagnosis was made.

FINAL DIAGNOSIS

Intermediate stage HCC, Barcelona Clinic Liver Cancer Stage B.

TREATMENT

She was not a candidate for orthotopic liver transplantation, LR, or any ablative therapies given the large size of the masses and their intimate relationship to the vasculature. She underwent bilobar TACE in October 2016, resulting in a decrease of the serum AFP to 132150 ng/mL, and post-treatment imaging demonstrated a partial response (Figure 1B). However, during chemoembolization of the right lobar mass, the right inferior phrenic artery was inaccessible, leaving part of the right tumor untreated.

Given this and the large size of the right lobar mass, she underwent salvage hypofractionated PBT to the right lobe tumor using a simultaneously integrated boost intensity modulated proton therapy technique with pencil beam scanning in December 2016. A total dose of 60 GyE to the tumor periphery and 67.5 GyE to internal sub-volumes within the tumor were delivered in 15 fractions (Figure 2A and B). Because optimally preserving the future normal liver remnant from radiation was critically important, a pre-treatment functional liver imaging scan with technetium-99m sulfur colloid single-photon emission CT was employed to assist in functional liver avoidance radiation planning (Figure 2C and D). She tolerated radiotherapy well with maintenance of normal liver function.

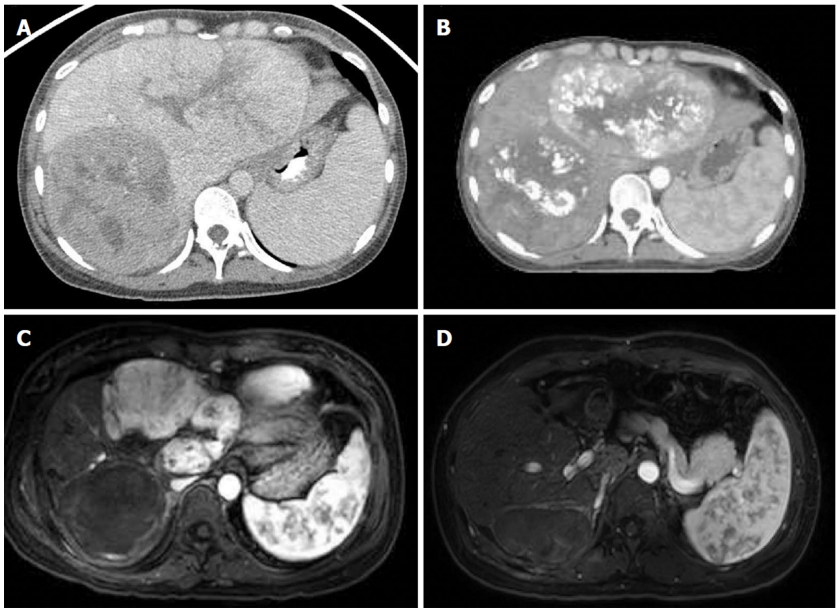

Figure 1 Initial portal venous phase contrast-enhanced computerized tomography showing bilobar lesions consistent with hepatocellular carcinoma. A: Arterial phase contrast-enhanced low-dose radiation therapy planning computerized tomography showing post-transarterial chemoembolization treatment changes to bilateral lobes 3 mo after diagnosis; B: Arterial phase T1-weighted fat-saturated post-contrast magnetic resonance imaging showing complete right lobar mass response following proton radiation therapy 6 mo after diagnosis; C: Axial arterial phase T1-weighted fat-saturated post-contrast magnetic resonance imaging showing no evidence of disease following left hepatectomy; D: Axial arterial phase T1-weighted fat-saturated post-contrast magnetic resonance imaging showing no evidence of disease 14 mo after diagnosis.

Three weeks after completion of PBT, the serum AFP declined to 62728 ng/mL, with a liver protocol magnetic resonance imaging (MRI) scan demonstrating complete response of her right lobar mass but significant residual tumor in the left lobe (Figure 1C). In February 2017 and April 2017, she underwent two additional TACE sessions to the left lobe. The serum AFP continued to decrease with each treatment to a nadir of 22700 ng/mL. However, post-treatment MRI demonstrated residual disease in the left lobe. She was referred for consideration of hepatic resection.

Pre-operative MRI demonstrated an adequate future liver remnant but with an obscured middle hepatic venous outflow with drainage of the non-tumor involved anterior right lobe *via* a large collateral vessel connecting to the middle hepatic vein and circumscribing the previously irradiated right lobe tumor. Doppler ultrasound was performed and confirmed appropriate venous caval outflow through this collateral vessel, which drained through the right hepatic vein into the IVC. Therefore, the middle hepatic vein, the anomalous venous collateral, and the right hepatic vein all required preservation. The patient underwent formal left hepatectomy in August 2017, eleven months after initial presentation. The case was quite challenging due to the tumor's long segment abutment of the middle hepatic vein, but LR with salvage of all aforementioned vasculature was successful. Pathology demonstrated a 11.5 cm x 10.5 cm x 7.5 cm HCC involving segments 2, 4a, and 8 with 25% tumor necrosis. The tumor was confined to the liver without vascular invasion and negative surgical margins was achieved with a final pathologic stage of ypT1bN0M0.

OUTCOME AND FOLLOW-UP

The serum AFP level at 16, 29, and 42 wk postoperatively was 13.3, < 5.0 and < 5.0 ng/mL, respectively (Figure 3). MRI scans performed 16, 29, and 42 wk postoperatively have been negative for any evidence of residual or recurrent disease (Figure 1D). The patient continues to do well greater than 12 mo postoperatively, with normal liver function (Na 139 mEq/L, Cr 0.63 mg/dL, bilirubin 0.7 mg/dL, albumin 4.1 g/dL, and platelet count 117000/μL) and performance status of ECOG 0. There have been no adverse or unanticipated events.

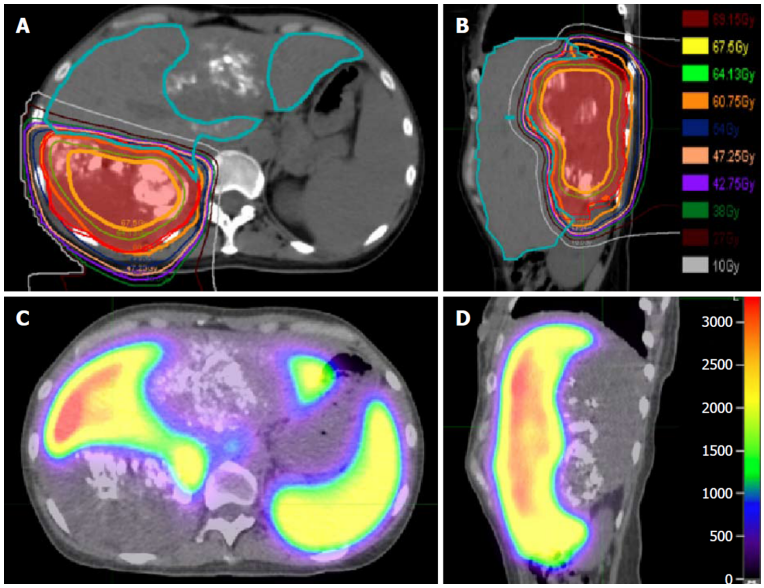

Figure 2 Radiation therapy approach. A, B: Proton radiation therapy treatment using a 2-beam approach for the treatment of the right-sided hepatocellular carcinoma highlighting low dose to the contralateral lobe of the liver; C, D: Demonstrated functional liver imaging using technetium-99m sulfur colloid single-photon emission computerized tomography scan co-registered to the radiation therapy planning computed tomography to aid in target delineation of the gross tumor volume and sparing of the highly functional liver (max sulfur colloid single-photon emission counts shown in red). Key: Liver minus gross tumor volume is demonstrated by the teal line (panels A, B), internal target volume (gross tumor volume + tumor motion on 4D-computed tomography scan) is shaded in red (panels A, B).

DISCUSSION

The MDT approach has been increasingly utilized for the management of a variety of diseases from diabetes to cancer. In oncology, some of the first successes were demonstrated in the management of breast cancer in the National Health Service[9]. It has been widely adopted internationally as the standard of care for newly diagnosed malignancies. Published evidence demonstrates improved accuracy of pre-operative staging[10,11], increased access to care, adherence to clinical guidelines[12,13], and satisfaction for both patients and health care providers[14-16]. In spite of this widespread adoption, there has been concern over the paucity of evidence demonstrating a significant improvement in clinical outcomes with the MDT approach[7,15]. Two recent systematic reviews published by Croke *et al*[8] and Pillay *et al*[11] demonstrated MDT approaches resulted in a significant impact on patient management, but there was little evidence to support an improvement in clinical outcomes. On an individual level, our case supports the significant patient impact of the MDT approach.

The management of HCC is increasingly complex as advances in more effective liver-directed therapies and systemic therapies become available. Optimal management of intermediate or advanced stage HCC requires thoughtful sequencing and timing of the various therapeutic modalities, which is best achieved through a tightly knit multidisciplinary team. We have found in our experience at the University of Washington Center for Advanced Minimally Invasive Liver Oncologic Therapies that multidisciplinary management of these patients is a beneficial endeavor, as seen in our presented case. With complementary multidisciplinary therapy, a combination of local, loco-regional, and systemic therapies can be successful in downstaging an advanced-stage HCC to one amenable to curative intervention with LR or orthotopic liver transplantation[6,17-19]. This has been demonstrated in prior series with multiple studies indicating the impact of downstaging prior to LR or transplantation. A prospective study by Yao *et al*[17] demonstrated that with careful patient selection, loco-regional therapies was successful in downstaging > 50% of patients to liver transplantation. This has similarly been examined in LR for HCC where liver-directed non-operative therapies have been successful in downstaging tumors for resection. Our present case is novel in that it highlights a patient with extensive multifocal disease involving both lobes requiring several rounds of sequential TACE and proton beam radiation therapy followed by a complex partial hepatectomy. A limitation of this approach is that it is resource intensive with complex scheduling issues that arise in routine clinical practice.

An important technique in tumor downstaging includes catheter based therapies

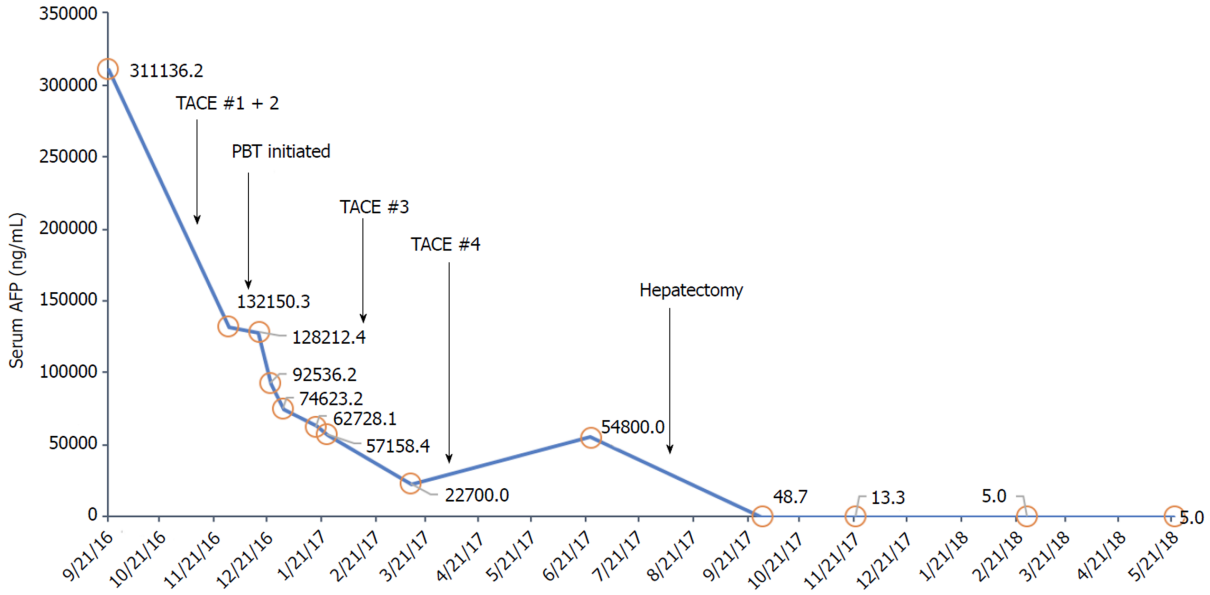

Figure 3 Serum alpha-fetoprotein level (ng/mL) during treatment course.

such as TACE and TARE. These have been demonstrated to both improving overall survival[20] in addition to serving as both bridging and downstaging treatment modalities. A study published in 2009 by Lewandowski *et al*[21] reported successful downstaging from UNOS T3 to T2 in 61% and 37% for TARE and TACE, respectively[13]. Six of twenty-one patients in a 2012 case series who underwent TARE for palliative intent for UNOS T3 HCC were sufficiently downstaged, enabling definitive LR, definitive ablation, or orthotopic liver transplantation[22]. TACE was chosen over TARE for our patient due to the potential of inadvertent lung dose of yttrium-90 due to significant shunting.

Technological advancements have resulted in the increasing use of external beam radiation therapy in the treatment of HCC given high rates of durable local control ranging from 87%-100% at 1 to 3 years[23-26]. For our patient, radiotherapy offered a promising local treatment option for the right-sided lesion due to the large tumor size and vascular anatomy prohibitive of further catheter-based therapy or surgical resection. PBT was the preferred radiation modality due to the tumor size, and dosimetric advantage with improved normal liver parenchyma sparing from low-to-moderate doses as compared to photon-based radiation, *e.g.*, stereotactic body radiation therapy. In radiation therapy akin to surgery, a "critical volume model" is applied to describe the minimum volume of normal liver that should be uninjured by radiation as to limit the incidence of radiation-induced liver disease. Dose and dosimetric constraint guidelines were derived from Hong *et al*[26] multicenter phase II clinical trial of dose-escalated proton beam radiation for unresectable primary hepatic cancers, where they demonstrated a low incidence of radiation-induced liver disease as measured by worsening Child-Turcotte-Pugh-score in 3.6% and no grade 4 or 5 radiation-related toxicities. Given the complexity of this case, we used technetium-99m sulfur colloid single-photon emission CT imaging co-registered to our radiation planning CT to assist in delineating the borders of the residual gross tumor volume, as well as a tool for more accurate global and spatial liver function to guide placement of radiation beams through less functional normal liver parenchyma[27-29].

LR continues to be a potentially curative treatment option in 15% to 20% of HCC patients with an operative mortality of less than 5% even in cirrhotic patients or those undergoing major LR, resulting in a 5-year overall survival of over 50%. The safety and outcomes of LR for HCC have improved substantially over the last three decades, which is attributed to refinements in patient evaluation and selection, the ability to manipulate the future liver remnant volume, advances in surgical and anesthetic techniques, and the enhanced peri-operative management. In this case, the patient was young and medically fit, with an ECOG performance status 0, adequate hepatic reserve in the future liver remnant, and absence of portal hypertension.

Biologic markers to predict HCC tumor biology are under development. Current surrogate markers predicting prognosis are based on tumor size, number, and vascular invasion. For HCCs, some researchers reported that larger tumor sizes exceeding the University of California at San Francisco criteria and serum AFP levels

over 400 ng/mL were associated with post-resection recurrence. Other markers such as retinoic acid-induced protein 3 as well as miRNA expression profiles have been used to predict poor prognosis and assess the risk of disease recurrence after orthotopic liver transplantation. Large tumor size has traditionally been a relative contraindication to LR given the elevated risk of vascular invasion. However, HCCs over 10 cm in size without invasion amenable to LR can result in good outcomes, and therefore identification of such tumors is important[30,31]. In this case, the lack of macrovascular invasion, and good response to TACE and PBT were considered in the surgical decision making.

CONCLUSION

The primary aim of MDT approach in cancer care is to improve patient outcomes by bringing together key specialists with complementary expertise to review cases, ensure accurate diagnosis and develop optimal, individualized plans of care. They often result in significant changes in patient diagnosis and management and are generally considered beneficial for patient care. A multidisciplinary approach to our presented case clearly resulted in an improved patient outcome on an individualized level; however, recent systematic reviews have failed to demonstrate improvement in patient outcomes on a population level. Further research is needed to understand the true clinical impact of a MDT approach to cancer care.

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CASE REPORT

Non-uremic calciphylaxis associated with alcoholic hepatitis: A case report

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Author contributions: Sammour YM designed the research and collected the patient's clinical data; Piliang M provided the patient's histopathological information; Sammour YM, Saleh HM, Gad MM, Healey B, Piliang M analyzed the data and wrote the paper.

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Abstract

BACKGROUND

Calciphylaxis is a form of vascular calcification more commonly associated with renal disease. While the exact mechanism of calciphylaxis is poorly understood, most cases are due to end stage kidney disease. However, it can also be found in patients without kidney disease and in such cases is termed non-uremic calciphylaxis for which have multiple proposed etiologies.

CASE SUMMARY

We describe a case of a thirty-year-old morbidly obese Caucasian female who had a positive history of alcoholic hepatitis and presented with painful calciphylaxis wounds of the abdomen, hips, and thighs. The hypercoagulability panel showed low levels of Protein C and normal Protein S, low Antithrombin III and positive lupus anticoagulant and negative anticardiolipin. Wound biopsy confirmed the diagnosis of non-uremic calciphylaxis in the setting of alcoholic liver disease. The calciphylaxis wounds did not improve when Sodium Thiosulfate was used alone. The patient underwent a series of bedside and surgical debridement. Broad spectrum antibiotics were also used for secondary wound bacterial infections. The patient passed away shortly after due to sepsis and multiorgan failure.

CONCLUSION

Non-uremic Calciphylaxis can occur in the setting of alcoholic liver disease. The treatment of choice is still unknown.

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Key words: Calciphylaxis; Alcoholic hepatitis; Vascular calcification; Sodium thiosulfate; Debridement; Case report

Core tip: In this case report, we present a patient with alcoholic liver disease and low levels of Protein C who developed calciphylaxis and died shortly after due to complications. The pathogenesis is not completely understood but the disruption of calcium-phosphate-byproduct has been implicated to play a role in the disease process. Liver dysfunction can lead to low levels of coagulation inhibitors specifically Protein C and Protein S. The aim of the medical treatment is to lower the calcium-phosphate-byproduct and decrease the vascular calcification. The use of surgical wound debridement is less established.

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INTRODUCTION

Calciphylaxis is a form of vascular calcification commonly associated with renal disease. Patients diagnosed with calciphylaxis usually face an unfavorable prognosis with most patients dying within 12 mo of diagnosis[1]. Although rare, calciphylaxis has an incidence rate of 35 per 10000 patients in the United States with around 70% of the patients being females and the average age of diagnosis being at 50-70 years[1]. The vascular calcification in calciphylaxis results in ischemic skin lesions that are very painful, treatment resistant, and predisposed to bacterial infections. Most patients with calciphylaxis have a diagnosis of end stage renal disease or other forms of kidney dysfunction including chronic kidney disease, kidney transplantation, or acute kidney injury. Some studies have reported calciphylaxis in patients with normal kidney function, termed non-uremic calciphylaxis[2,3]. Other non-uremic causes of calciphylaxis reported in the literature included primary hyperparathyroidism, malignancy, connective tissue disease, and liver disease[1]. As the main clinical manifestation associated with calciphylaxis, the cutaneous lesions can range from minor painful induration to skin necrosis[3].

CASE PRESENTATION

A thirty-year-old Caucasian female was transferred to our medical center for further management of painful wounds of the abdomen, hips, and thighs. She had a past medical history of alcoholic hepatitis, diagnosed four months earlier with a liver biopsy that showed steatohepatitis and stage 3 fibrosis rather than cirrhosis, and morbid obesity (BMI = 56) status post Roux-en-Y gastric bypass done twelve years ago (unknown pre-surgical BMI). History was negative for diabetes mellitus, kidney dysfunction, autoimmune diseases, hyperparathyroidism or Warfarin intake.

Eight weeks after the onset of alcoholic hepatitis, the patient developed tender erythema on her abdomen, hips, and thighs, which evolved into painful firm subcutaneous nodules.

On admission to our hospital, vital signs were notable for temperature 36.7 °C (98.1 °F), blood pressure 98/41 mmHg, pulse 119 bpm, and respiratory rate 20/min. Physical examination showed woody, indurated, exquisitely tender erythematous plaques on the abdomen, hips, and thighs, with central stellate necrotic eschar and purpura (Figure 1A). She also had anterior abdominal wall edema and bilateral lower extremity pitting edema. Laboratory workup was significant for leukocytosis 24 × 10^9/L , with absolute neutrophil count 21.5 × 10^9/L , hemoglobin 7.2 g/dL, MCV 87.5 fL, total protein 6.1 g/dL, albumin 1.7 g/dL, AST 56 U/L, ALT 19 U/L, alkaline phosphatase 172 U/L, total bilirubin 1.8 mg/dL, PT 17.7 s, INR 1.6, APTT 37.3, BUN 15 mg/dL, creatinine 1.32 mg/dL, calcium 8.2 mg/dL, phosphorus 3.9 mg/dL, PTH 22 (normal), and 1,25 OH Vit D3 5.8 (low). The hypercoagulability panel showed low

levels of Protein C 33 IU/dL (normal: 76-147), low normal levels of protein S 67 IU/dL (normal: 65-135), low antithrombin III levels, positive lupus anticoagulant and negative anticardiolipin. Wound biopsy showed dermal hemorrhage, dermal vascular occlusion, calcium deposition within the walls of large veins and the surrounding adipose tissue (Figure 2). These pathologic findings, correlated clinically, were most consistent with non-uremic calciphylaxis in the setting of alcoholic liver disease.

FINAL DIAGNOSIS

These pathologic findings, correlated clinically, were most consistent with non-uremic calciphylaxis in the setting of alcoholic liver disease.

TREATMENT

Management consisted of sodium thiosulfate infusions, a series of bedside non-excisional and surgical excisional debridement (Figure 1B); in addition to broad spectrum antibiotic treatment for secondary *pseudomonas aeruginosa* and *morganella morganii* wound bacterial infections.

OUTCOME AND FOLLOW-UP

The patient was eventually transferred to a regional burn unit for specialized management of the extensive calciphylaxis wounds. Shortly after, the patient passed away due to sepsis and multiorgan failure.

DISCUSSION

We present a patient with alcoholic liver disease and low normal levels of protein C who developed calciphylaxis and died shortly thereafter from related complications.

The pathogenesis of non-uremic calciphylaxis is not completely understood, but disruption in the calcium-phosphate-byproduct has been implicated to play a role in the disease process[4]. Abnormalities of the Receptor Activator of Nuclear Factor-B (RANK, NF-κB), RANK ligand, and osteoprotegerin may be involved. Factors such as liver disease, hyperparathyroidism and corticosteroid use are known to stimulate the expression of RANK ligand and decrease osteoprotegerin, thus activating NF-κB and ultimately leading to osseous mineral loss and extraosseous mineral deposits[5].

Liver dysfunction can lead to low levels of coagulation inhibitors, specifically protein C and S, which can lead to vascular injury[6] as well as thromboembolic manifestations such as deep venous thrombosis and pulmonary embolism. Another theory behind the link between liver dysfunction and calciphylaxis could be related to Fetuin-A which is a protein synthesized in the liver that acts as a circulating inhibitor of vascular ossification-calcification. Its effects are mediated by ''calciprotein particles'', which clear the circulating calcium and phosphorus, and therefore selectively inhibit vascular ossification–calcification without affecting the bone mineralization. Another inhibitor of that pathway is the Matrix-GLA-Protein (MGP). Activated MGP, through Vitamin K dependent carboxylation, forms a complex with fetuin-A which inhibits the Bone-Morphogenetic-Protein-2 induced osteogenic differentiation. Thus, liver dysfunction induced vitamin K deficiency can lead to decreased MGP activity and increased vascular ossification-calcification. This mechanism may also explain the association between calciphylaxis and Warfarin-a Vitamin K antagonist[7]. Total uncarboxylated MGP (t-ucMGP) could reflect arterial calcification, with lower values being associated with more widespread calcium deposits[8]. However, its level was not assessed in our patient; its measurement in future studies may be required.

Gastric bypass surgery can also predispose to Vitamin D and Calcium deficiency with secondary hyperparathyroidism due to alterations in the digestive anatomy which could set up a suitable environment for calciphylaxis[9].

The abdomen and thighs are the commonest predilection sites for calciphylaxis lesions due to higher adipose tissue density. The lesions present as indurated plaques or nodules that may have ulcerations and eschar and can be associated with livedo reticularis[10]. A tissue biopsy is essential to confirm the diagnosis[11,12]. Histopathologic changes are similar in both uremic and non-uremic calciphylaxis. Microscopic findings include calcification of dermal vessels and diffuse dermal thrombi. Dermal

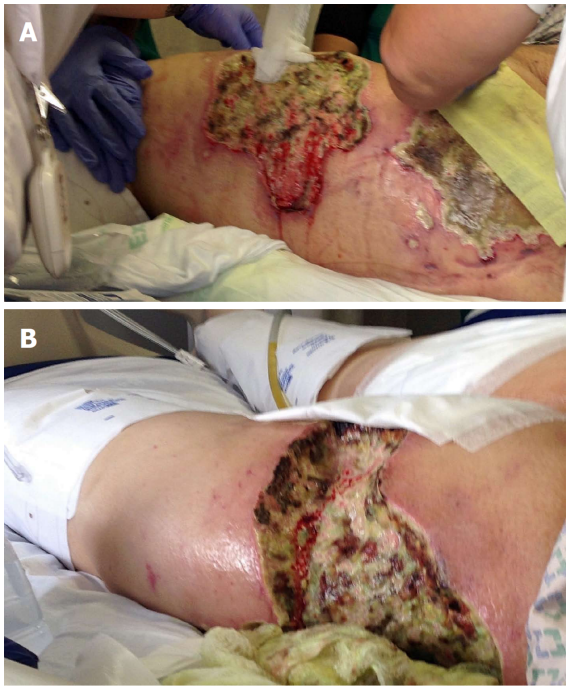

Figure 1 Calciphylaxis wounds in the thigh. A: Before debridement; B: After debridement.

angioplasia was frequently reported[13]. Pseudoxanthoma elasticum-like changes were also reported and described as thickened, fragmented and curled elastic fibers[14].

Non-uremic calciphylaxis usually has a poor prognosis with mortality that can reach 50%, most commonly due to sepsis[4]. When calciphylaxis affects proximal areas of the body, such as the abdomen, thighs and buttocks, the mortality rates can reach up to 63%. Distal calciphylaxis, however, is associated with lower mortality, being 23% as reported in one series. The presence of associated ulceration carries a mortality rate of greater than 80%[1,5].

The aim of medical treatment is to reduce the serum calcium-phosphate-byproduct, which can decrease the vascular calcification. Sodium thiosulfate increases the solubility of the calcium deposits and is considered a successful therapy for uremic calciphylaxis[1,2] but our non-uremic patient did not improve when sodium thiosulfate was used alone. Cases of calciphylaxis are usually treated with analgesics, wound care, and proper nutrition. Treatments that have been studied specifically for such cases include sodium thiosulfate, bisphosphonate, and hyperbaric oxygen therapy. The use of surgical wound debridement is less established and the decision is typically individualized based on the patient characteristics and presentation[1]. No effective treatment is available for non-uremic etiologies of calciphylaxis as the pathology remains unclear[6]. Few cases of non-uremic calciphylaxis were reported with alcoholic liver disease and were treated mainly by serial debridement procedures with wound care and sodium thiosulfate infusions[6,15].

Corticosteroid use was believed to be a predisposing factor for non-uremic calciphylaxis[3], however, Biswas *et al*[16] reported a case with acute non-uremic calciphylaxis that improved on systemic corticosteroids. Similarly, Elamin *et al*[17] described another case of calcifying panniculitis that was treated with a 10 d course of oral prednisone resulting in complete healing.

An increasing number of cases of calciphylaxis have been reported in the setting of alcoholic liver disease. The treatment of choice for those patients is still unknown. There is a gap in literature about the role of extensive debridement of the calciphylaxis wounds and whether it can lead to improvement of the outcomes or cause more complications such as sepsis. At this time, further research and interventional studies need to be done to better understand the mechanism of calciphylaxis in those patients, which can help us develop a more effective treatment regimen.

CONCLUSION

An increasing number of cases of calciphylaxis in the setting of liver failure have

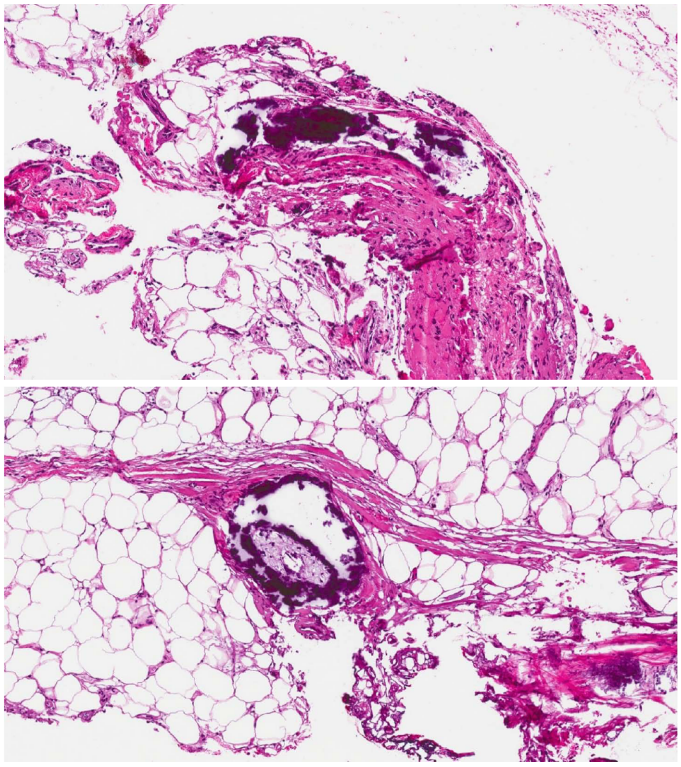

Figure 2 Dermal vascular occlusion and calcium deposition within the walls of large veins and the surrounding adipose tissue.

recently been reported, but no primary treatment option has been discovered. Surgical debridement and sodium thiosulfate were utilized in this patient, but success was unable to be evaluated as the patient passed from complications before healing could occur. Future studies should expand upon and investigate other therapeutic options for management of non-uremic calciphylaxis in the setting of liver failure.

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CASE REPORT

Caval replacement with parietal peritoneum tube graft for septic thrombophlebitis after hepatectomy: A case report

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Abstract

BACKGROUND

Caval vein thrombosis after hepatectomy is rare, although it increases mortality and morbidity. The evolution of this thrombosis into a septic thrombophlebitis responsible for persistent septicaemia after a hepatectomy has not been reported to date in the literature. We here report the management of a 54-year-old woman operated for a peripheral cholangiocarcinoma who developed a suppurated thrombophlebitis of the vena cava following a hepatectomy.

CASE SUMMARY

This patient was operated by left lobectomy extended to segment V with bile duct resection and Roux-en-Y hepaticojejunostomy. After the surgery, she developed *Streptococcus anginosus, Escherichia coli*, and *Enterococcus faecium* bacteraemias, as well as *Candida albicans* fungemia. A computed tomography scan revealed a bilioma which was percutaneously drained. Despite adequate antibiotic therapy, the patient's condition remained septic. A diagnosis of septic thrombophlebitis of the vena cava was made on post-operative day 25. The patient was then operated again for a surgical thrombectomy and complete caval reconstruction with a parietal peritoneum tube graft. Use of the peritoneum as a vascular graft is an inexpensive technique, it is readily and rapidly available, and it allows caval replacement in a septic area. Septic thrombophlebitis of the vena cava after hepatectomy has not been described previously and it warrants being added to the spectrum of potential complications of this procedure.

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CONCLUSION
Septic thrombophlebitis of the vena cava was successfully treated with antibiotic and anticoagulation treatments, prompt surgical thrombectomy and caval reconstruction.

Key words: Bilioma; Septic thrombophlebitis; Septicaemia; Parietal peritoneum tube graft; Complete caval reconstruction; Case report

Core tip: Caval vein thrombosis after hepatectomy is rare, although it increases mortality and morbidity. Its evolution into a septic thrombophlebitis responsible for persistent septicaemia after a hepatectomy has not been reported to date in the literature. This study reports the management of a 54-year-old woman with peripheral cholangiocarcinoma who developed a suppurated thrombophlebitis of the vena cava following a hepatectomy. A combination of antibiotic and anticoagulation treatments, prompt surgical thrombectomy and complete caval reconstruction was associated with a favorable outcome. Use of the peritoneum as a vascular graft is an inexpensive technique, and it allows caval replacement in a septic area.

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INTRODUCTION

Caval vein thrombosis after hepatectomy is rare, although it increases mortality and morbidity[1]. The evolution of this thrombosis into a septic thrombophlebitis has not been reported to date in the literature. We here report the management of a 54-year-old woman operated for a peripheral cholangiocarcinoma who developed a suppurated thrombophlebitis of the vena cava following a hepatectomy.

CASE PRESENTATION

This patient was operated by left lobectomy extended to segment V with bile duct resection and Roux-en-Y hepaticojejunostomy. Besides its cancer, the patient had no medical history, including thromboembolic or cardiovascular diseases. On post-operative day 3 (POD3), she developed *Streptococcus anginosus*, *Escherichia coli*, and *Enterococcus faecium* (*E. faecium*) bacteraemias, as well as *Candida albicans* (*C. albicans*) fungemia. A computed tomography (CT) scan revealed a 5 cm bilioma. Antibiotic treatment based on ceftriaxon, teicoplanin, and caspofungin was started while the bilioma was percutaneously drained on POD10, and cultures were positive for *C. albicans*. However, both the *E. faecium* bacteraemia and the *C. albicans* fungemia persisted, with sepsis (high-grade fever, hyperleukocytosis and tachycardia). The choice and the administration of these antibiotics were deemed to be appropriate, as were the teicoplanin serum levels.

The clinical condition of the patient continued to deteriorate, despite appropriate antibiotic treatment. Another CT scan on POD25 revealed thrombophlebitis of the inferior vena cava closed to the bilioma (Figure 1), and new subpleural nodular lesions suggestive of an embolic mechanism.

FINAL DIAGNOSIS

A diagnosis of septic thrombophlebitis of the inferior vena cava was made.

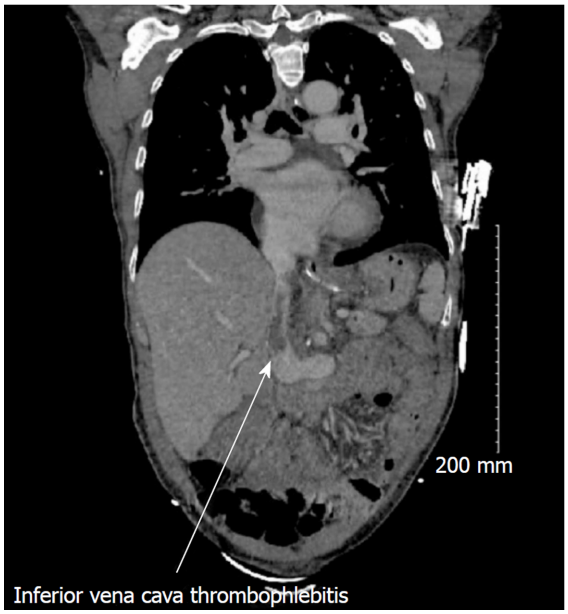

Figure 1 Coronal computed tomography scan of thrombophlebitis of the vena cava after hepatectomy.

TREATMENT

The patient was operated again on POD27 for a thrombectomy and replacement of the inferior vena cava. First, the vena cava was controlled above and below the hepatic vein and the upper the renal veins after performing the Kocher manoeuvre. The bilioma was found along with an infectious necrosis with suppuration and thrombosis of the front side of the vena cava at the rear of the hepatic pedicle (Figure 2). A vascular resection was performed with vena cava clamping without exclusion of the liver. A reconstruction of the vena cava with a parietal peritoneum patch harvested from the posterior aponeurosis of the left rectus abdominal muscle was carried out. The segment of peritoneum was sutured into a 20 mm tubular shape with a longitudinal Prolene 5-0 running suture. The segment of peritoneal tube was sutured to the vena cava with a longitudinal Prolene 5-0 running suture (Figure 3). The thrombus was positive for both *E. faecium* and *C. albicans*.

OUTCOME AND FOLLOW-UP

In the days that followed, the fever abated progressively, while the sepsis disappeared and all of the repeated blood cultures remained negative. The patient was discharged on POD67 with anticoagulation treatment. She remained afebrile, and the antibiotic treatment has been terminated.

DISCUSSION

Septic thrombophlebitis related to vascular invasion from adjacent non-vascular infections is best illustrated by thrombophlebitis of the internal jugular vein[2]. This complication has also been reported for limb veins, portal (pylephlebitis) and mesenteric veins after abdominal infections, pelvic veins following postpartum infections, and dural sinuses[3].

Isolated septic thrombophlebitis of the vena cava has been reported in a small number of cases, mainly caused by prolonged central venous catheterization, but never after hepatectomy[4-6]. Septic thrombophlebitis of the vena cava is a rare disease that induce the increase of the morbidity and the mortality, as a result of sepsis and septic emboli that can cause septic pulmonary emboli and infective endocarditis[7]. The rarity of these complication results in diagnostic delay and a higher level of severity.

Optimal management of septic thrombophlebitis remains unclear due to the limited data available from comparative trials[8]. Key components comprise appropriate antibiotic treatment along with surgical or radiological drainage of the primary or secondary embolic infectious foci. Anticoagulation is an option, although it should be considered early in the management of septic thrombophlebitis in the absence of non-

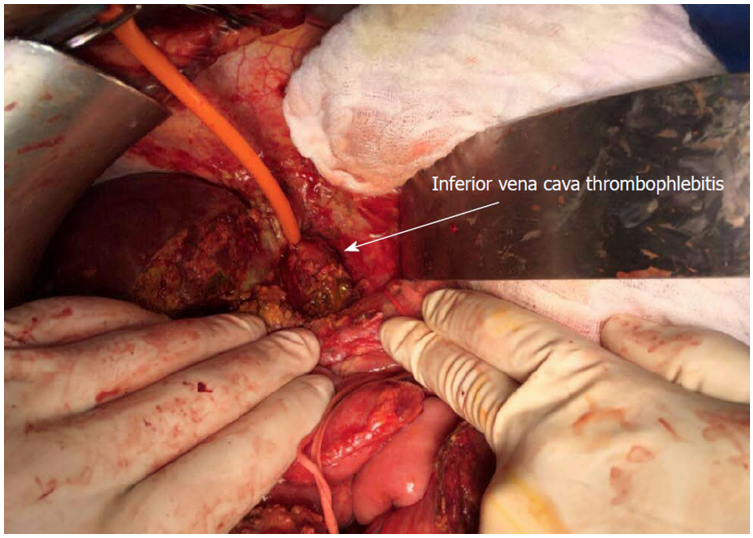

Figure 2 Image of the thrombus during the second surgery.

acceptable haemorrhagic risk[9]. Finally, endovascular or surgical thrombectomy can be an option in cases of deep vein septic thrombophlebitis that are refractory to conservative treatment[3,9,10]. In this unstable septic patient, management relied on removal of the foci of infection by surgical thrombectomy of the inferior vena cava, thereby allowing prompt and definitive control of the sepsis. In this septic environment, autologous parietal peritoneum for complete caval replacement was used to avoid reconstruction of the vena cava with synthetic materials. The parietal peritoneum was harvested from the posterior aponeurosis of the rectus abdominal muscle, thereby allowing a particularly stiff graft for support of the vena cava flow to be obtained[11]. Use of the peritoneum as a vascular graft has been reported in animal models[12] and has been described as a safe and good option for circumferential replacement of the vena cava[11,13-16]. This technique is inexpensive, it is readily and rapidly available, it uses the same surgical incision, and it allows caval replacement in a septic area.

Septic thrombophlebitis of the vena cava after hepatectomy has not been described previously, and it warrants being added to the spectrum of potential complications of this procedure. A combination of antibiotic and anticoagulation treatments, prompt surgical thrombectomy, and complete caval reconstruction with a parietal peritoneum tube graft was associated with a favorable outcome in this severe case.

EXPERIENCES AND LESSONS

Septic thrombophlebitis of the vena cava after hepatectomy is a rare complication of this procedure. Septic thrombophlebitis of the vena cava was successfully treated with antibiotic and anticoagulation treatments, prompt surgical thrombectomy and caval reconstruction. Use of the peritoneum as a vascular graft is an inexpensive technique, it is readily and rapidly available, and it allows caval replacement in a septic area.

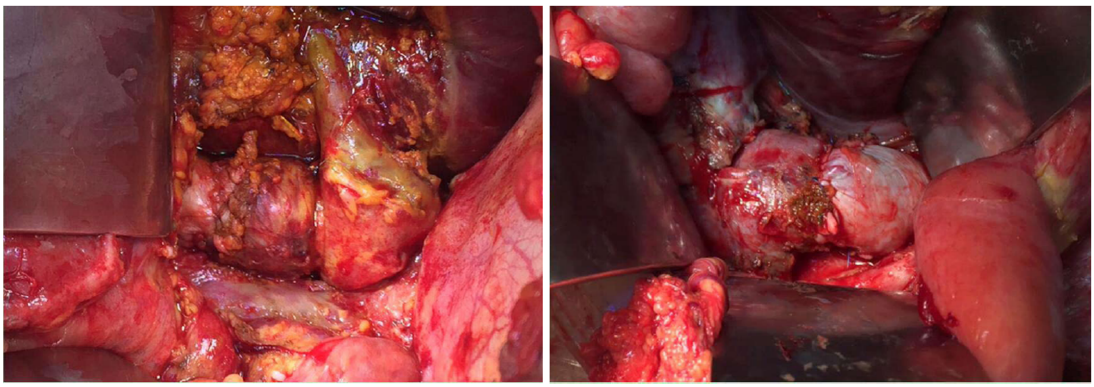

Figure 3 Vena cava reconstruction with a parietal peritoneum tube graft.

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