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REVIEW

Peroxisome proliferator-activated receptor gamma as a therapeutic target for hepatocellular carcinoma: Experimental and clinical scenarios

Swati Katoch, Vinesh Sharma, Vikram Patial

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Abstract

Hepatocellular carcinoma (HCC) is the most common type of liver cancer worldwide. Viral hepatitis is a significant risk factor for HCC, although metabolic syndrome and diabetes are more frequently associated with the HCC. With increasing prevalence, there is expected to be > 1 million cases annually by 2025. Therefore, there is an urgent need to establish potential therapeutic targets to cure this disease. Peroxisome-proliferator-activated receptor gamma (PPARγ) is a ligand-activated transcription factor that plays a crucial role in the patho-physiology of HCC. Many synthetic agonists of PPARγ suppress HCC in experimental studies and clinical trials. These synthetic agonists have shown promising results by inducing cell cycle arrest and apoptosis in HCC cells and preventing the invasion and metastasis of HCC. However, some synthetic agonists also pose severe side effects in addition to their therapeutic efficacy. Thus natural PPARγ agonists can be an alternative to exploit this potential target for HCC treatment. In this review, the regulatory role of PPARγ in the pathogenesis of HCC is elucidated. Furthermore, the experimental and clinical scenario of both synthetic and natural PPARγ agonists against HCC is discussed. Most of the available literature advocates PPARγ as a potential therapeutic target for the treatment of HCC.

Key Words: Anticancer; Hepatocellular carcinoma; Natural agonists; Peroxisome proliferator-activated receptor-γ; Thiazolidinediones

Core Tip: Hepatocellular carcinoma (HCC) is the most common type of liver cancer worldwide. Viral infections and metabolic syndrome are the major risk factors for HCC, and its incidence is expected to increase to > 1 million cases annually by 2025. The crucial role of peroxisome-proliferator-activated receptor gamma (PPARγ) in HCC pathophysiology makes it a potential therapeutic target. Along with synthetic agonists, natural PPARγ agonists provide alternative and safer options for HCC treatment; however, they need to be validated clinically. This review discusses the regulatory role of PPARγ in HCC pathogenesis and experimental and clinical scenarios of PPARγ agonists in HCC treatment.

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INTRODUCTION

Liver cancer is the sixth most common cause of cancer-related death worldwide, with a higher prevalence in men than women. Hepatocellular carcinoma (HCC) incidence was expected to increase to > 1 million individuals annually by 2025[1]. HCC, a primary subtype of liver cancer, primarily occurs in Asia and Africa due to the high prevalence of hepatitis B virus (HBV), hepatitis C virus, and diabetes [2]. These conditions are linked to the inflammatory response in the liver, leading to the development of HCC. Furthermore, other conditions such as obesity, dietary mycotoxin exposure, and excessive alcohol consumption are also among the risk factors for the development of HCC. These factors lead to the development of cirrhosis in 70%-80% of HCC patients. Liver transplantation is currently the best option for curing HCC, but there is a limitation to the availability of donors[3]. During the last two decades, the understanding and management of HCC have changed dramatically due to the extensive basic and clinical research, which may further help to reveal potential targets for the treatment of HCC. Sorafenib is the first-line defense therapy approved by the United States food and Drug Administration (FDA) for the advanced stages of HCC. It is a type of multikinase inhibitor that shows tumor-suppressing activity *via* targeting vascular endothelial growth factor receptor, adenosine monophosphate-activated protein kinase (AMPK), and platelet-derived growth factor receptor[4]. Apart from their therapeutic potential, sorafenib shows acquired resistance in HCC cells. The low response rate indicates that patients sensitive to sorafenib during the treatment will develop resistance within 6 mo. These negative impacts of approved drugs prompted many researchers to find novel drugs or targets to cure HCC[5].

Peroxisome proliferator-activated receptor gamma (PPARγ) is a ligand-activated nuclear receptor activated by synthetic and natural agonists[6]. It is highly expressed in adipose tissue, where it plays a central role in regulating adipose tissue function. Many studies have established the role of PPARγ in the pathophysiology of HCC. *In vitro* and *in vivo* data have shown the inhibitory role of PPARγ activation in tumor cell growth, migration, and invasion suggesting its therapeutic role in the growth regulation of HCC[7,8]. The antitumor effects of PPARγ are fulfilled by various mechanisms, including the induction of cell cycle arrest and activation of genes/proteins involved in immune and inflammatory responses[9]. Previous reports have revealed the mechanism underlying the development of HCC and suggested the presence of PPARγ in human HCC tissues, which shows a dose-dependent decrease in the growth of HCC cell lines[10]. Thus, molecules modulating PPARγ signaling pathways will provide a novel solution for the effective treatment of HCC. This review focuses on the role of PPARγ in the HCC pathophysiology and the experimental and clinical status of PPARγ agonists in the treatment of HCC.

MOLECULAR ARRANGEMENT OF PPARγ

The PPARs protein belong to the superfamily of nuclear hormone factors containing 48 members. PPARs were mainly recognized for their proficiency in promoting peroxisome proliferation in the liver, and their expression is mainly regulated in response to ligand binding[11]. Three isoforms of PPARs, namely PPARα, PPARγ, and PPARδ, have been studied to a large extent. Of these isoforms, PPARγ is highly expressed in adipose tissue, where it plays a vital role in regulating lipid homeostasis, energy balance, adipogenesis, and inflammation. Due to the presence of different promotor regions and 5′ exons, PPARγ has three distinct mRNAs (PPARγ1, PPARγ2, and PPARγ3). The translation products of PPARγ1 and PPARγ3 yield identical proteins; however, PPARγ2 results in a product with an additional N-terminal region[12]. PPARγ1 and PPARγ3 are biologically expressed in different tissues (hepatocytes, muscles, and endothelial cells), whereas PPARγ2 is only widely expressed in adipose tissue[9,13]. PPARγ plays a significant role in maintaining metabolic alterations, inflammation, glucose homeostasis,

cell cycle regulation, differentiation, and migration, making it a potential therapeutic target for treating metabolic disorders and cancers[14]. The structural arrangement of PPARs is similar to steroid and thyroid hormone receptors. Its ligand-binding cavity is 3- to 4 -times higher than that of the other nuclear receptors. They can be activated by various natural and synthetic agonists, such as essential fatty acids[15,16]. The three-dimensional structure of PPARγ consists of a canonical domain shared with other nuclear receptors, named A-E from N to C terminus (Figure 1). These domains include the amino-terminal AF-1 domain, a DNA-binding domain with two zinc finger motifs, and a ligand-binding domain (LBD or E/F domain) at the C-terminus responsible for specific ligand binding at the peroxisome proliferator response element (PPRE)[16,17]. After interaction with specific ligands, the LBD facilitates the heterodimerization of PPARs with retinoid X receptor (RXR), which subsequently binds to the PPRE of the target gene. RXR is activated by the natural ligand 9-cis-retinoic acid receptor and synthetic retinoids receptors. However, in the absence of specific ligands, heterodimers bind with co-repressors, ultimately inhibiting the gene[12]. This complex subsequently recruits coactivation or co-repressors to regulate the expression of targets genes related to lipid glucose metabolisms and inflammation (Figure 1)[6].

ROLE OF PPARγ IN HCC

PPARγ plays a multifunctional role in many tissues and cell types such as adipocytes, pancreas, macrophages, liver, kidney, and skeletal muscle. It plays a regulatory role in adipocyte differentiation, lipid metabolism, and insulin sensitivity *via* downregulating leptin concentration[7]. Despite the low expression in the healthy liver, PPARγ plays a significant role in several hepatic conditions such as fatty liver, fibrosis, and HCC. Many *in vitro* and *in vivo* studies have reported that natural and synthetic PPARγ agonists inhibit tumor growth and cell migration in HCC[18]. The activation of PPARγ inhibits cell growth by inducing G0/G1 cell cycle arrest in HCC cells, which is suggested to be associated with p21, p27, and p18 upregulation (Figure 2). Furthermore, p27 upregulation downregulates S-phase kinase-associated protein-2 (Skp2) in HCC, an F-box protein component of the Skp, Cullin, F-box ubiquitin-ligase complex. p27 plays a vital role in G0/G1 arrest instead of p21[10,19]. The direct overexpression of PPARγ in hepatic cancerous cells also inhibits cell growth; however, the cells are arrested in the G2/M phase instead of the G0/G1 phase after PPARγ agonist treatment. G2/M phase arrest in PPARγ overexpression is attributed to activating cell division cycle 25C phosphatase by Ser216 phosphorylation and preventing premature mitosis[20]. Compared to wild-type mice, another study on PPARγ-deficient mice showed increased hepatocarcinogenesis after treatment of diethylnitrosamine (DENA). Growth differentiation factor 15 (GDF 15) is a target gene of PPARγ and is induced by its activation. GDF 15 overexpression in many cancers is associated with an antitumorigenic response, as it was suggested to reduce cancer cell viability and induce cell apoptosis. PPARγ activation by agonist or direct overexpression induces apoptosis by intrinsic and extrinsic pathways[21]. Activation of the extrinsic apoptosis pathway by PPARγ overexpression is attributed to the induction of tumor necrosis factor α (TNFα) and Fas, leading to the activation of downstream caspases (Figure 2). In the intrinsic pathway, PPARγ overexpression stimulates B-cell lymphoma 2 (Bcl-2)-associated X protein transcription and release into the cytosol, activating apoptotic protease activating factor 1 and caspase-9 complex, which further triggers caspase 3 and 7 to induce apoptosis[6,21]. The antitumorigenic effect of PPARγ in HCC is also suggested *via* modulation of the phosphoinositide 3-kinase (PI3K)/Akt pathway [22]. PPARγ activation attenuates p85 activation, which is essential for Akt induction, thus inhibiting PI3K/Akt signaling and inducing apoptosis[23].

Hepatic inflammation is crucial in the progression of HCC, and PPARγ plays a central role in regulating inflammation. PPARγ inhibits inflammation by interfering with nuclear factor-kappa B (NF-κB) and suppressing the production of proinflammatory cytokines (TNFα and interleukin 1 beta [IL-1β]). Activation of PPARγ by specific ligands in T-cell differentiation promotes an inflammatory response, thereby playing a significant role in the adaptive immune response. Thus, PPARγ act as an important therapeutic target for regulating inflammatory markers (TNFα, IL-2, IL-1β, and IL-6) against the progression of several diseases[7,16]. Hepatic stellate cell (HSC) activation and fibrogenic factor significantly contribute to the development of HCC (Figure 2). PPARγ is highly expressed in quiescent HSCs and has a role in their transdifferentiation. HSC activation and PPARγ are inversely related as increased expression of PPARγ inhibits HSC proliferation and induces apoptosis in activated HSCs[24]. It also reduces the expression of alpha-smooth muscle actin (αSMA) and hydroxyproline to inhibit hepatic fibrosis. Hepatic injury induces microvascular complications in the liver, stimulating various sinusoidal cells such as HSCs, liver sinusoidal endothelial cells, and Kupffer cells. PPARγ regulates the role of these cells in liver inflammation and fibrosis. The deactivation of HSCs by PPARγ agonists further reduces extracellular matrix deposition and expression patterns of matrix metalloproteinase (MMP)/tissue inhibitors of MMPs (TIMP). The expression of MMP9 and MMP13, TIMP, heparinase, and E-cadherin is associated with cancer cell migration and metastasis[25]. The expression patterns of these markers are directly linked to PPARγ activation. Reports also link PPARγ activation with autophagy in HCC. Autophagy is thought to be inhibited after autophagosome formation in the absence

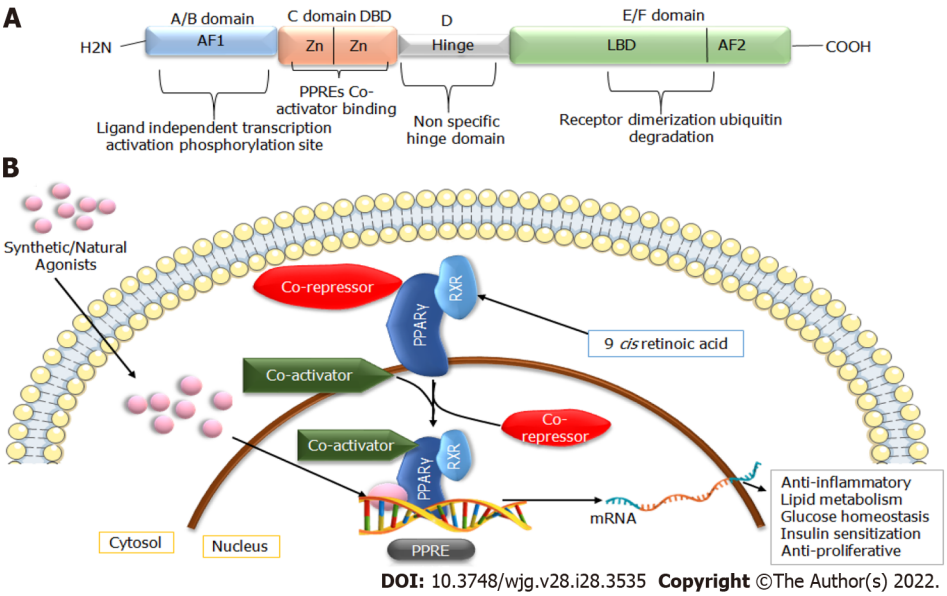

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Figure 1 General structure and ligand-activated transcription of peroxisome proliferator-activated receptor-gamma. A: Peroxisome proliferator-activated receptor (PPAR) structure includes four distinct structural domains A/B, C, D, and E/F; B: Ligand-activated transcription of PPARγ, which includes heterodimerization with nuclear receptor retinoid X receptor (RXR) and binding with peroxisome proliferator response elements located in the target genes through the DNA-binding domain (DBD). In the absence of ligand, PPAR is linked with the corepressor complex, whereas, in the presence of ligand, it is associated with the coactivator complex. LBD: Ligand-binding domain; PPRE: Peroxisome proliferator response element.

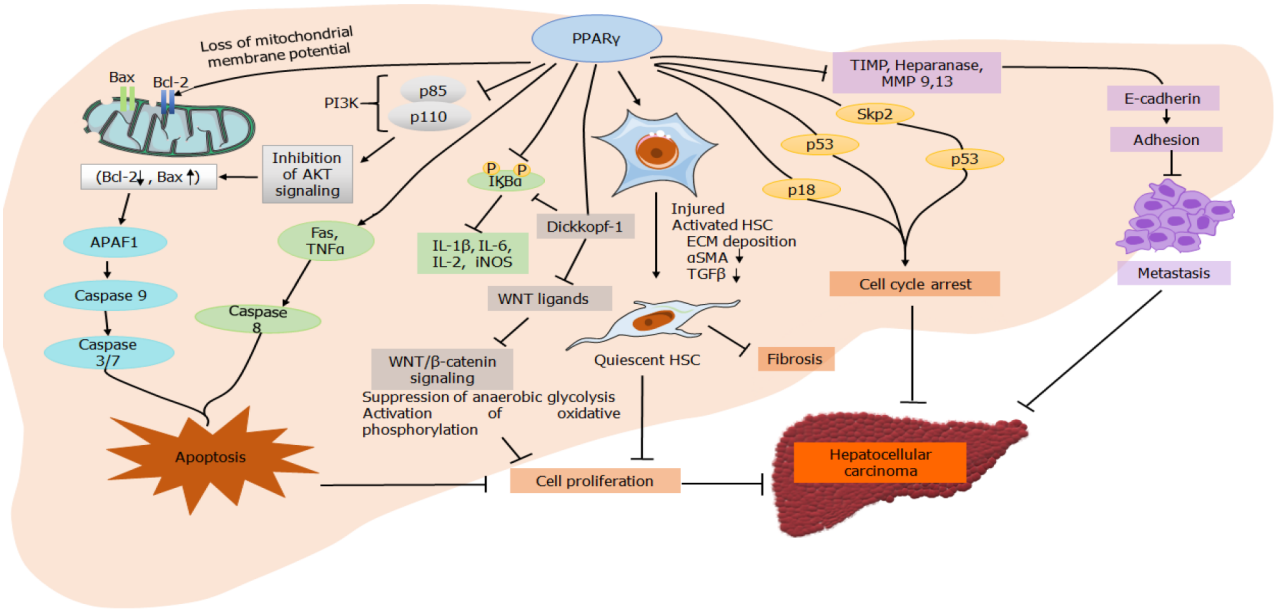

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Figure 2 Schematic diagram showing the protective effect of peroxisome proliferator-activated receptor γ against the progression of hepatocellular carcinoma. Activated peroxisome proliferator-activated receptor γ (PPARγ) interacts with multiple pathways, leading to cell cycle arrest, apoptosis, inhibition of cell proliferation, and cell metastasis in hepatocellular carcinoma. BAX: B-cell lymphoma 2 (Bcl-2)-associated X protein; IκBα: Nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibition alpha; IL: Interleukin; TIMP: Tissue inhibitor of metalloproteinases; MMP: Matrix metalloproteinase; ECM: Extracellular matrix; αSMA: Alpha-smooth muscle actin; TGFβ: Transforming growth factor beta; iNOS: Inducible nitric oxide synthase; TNFα: Tumor necrosis factor alpha; APF1: Apoptotic protease activating factor 1.

of PPARγ, resulting in increased light chain 3 protein expression and accumulation of p62 in the autophagosome[26,27]. Therefore, induction of autophagy in HCC is linked to the activation of PPARγ in HCC. A recent study elucidated the role of PPARγ coactivator-1α (PGC1α) in suppressing HCC metastasis. The levels of PGC1α were downregulated in human HCC and associated with a poor prognosis, large tumor size, and vascular invasion[28]. However, PGC1α overexpression in the HCC cells inhibited tumor cell migration and invasion. The suppression of metastasis by PGC1α overex-

pression was suggested due to PPARγ-dependent downregulation of pyruvate dehydrogenase kinase isozyme 1 and inhibition of aerobic glycolysis through Wnt/β-catenin/pyruvate dehydrogenase kinase-1 (PDK1) axis regulation[29].

Zinc finger protein 746 (ZNF746) is a Parkin-interacting substrate (PARIS), acting as a transcriptional regulator of PPARγ co-activator 1 alpha (PGC1α) which further regulates the activity of PPARγ and is involved in the onset of HCC. The elevated levels of insoluble parkin with PARIS accretion in the hepatic cells of diethylnitrosamine (DEN)-injected mice were observed with the downregulation of PGC1α and NRF1. Moreover, Chang liver cells treated with hydrogen peroxide showed PARIS accretion and alleviation of PGC1α. As the co-activator, PGC1α is directly linked to PPARγ regulation, further monitoring the oncogenic stress promoting cancer development. Thus, the modulation of PPARγ and its co-activators can be a promising therapeutic target for HCC[30]. In a clinical study, it was subsequently observed that the expression of PGC1α is negatively associated with tumor size and vascular influx. The increased expression of PGC1α could elevate the degree of oxidative phosphorylation, further slowing down the rate of metastasis and the Warburg effect of HCC cells[31]. Rapid proliferation is the prime feature of cancerous cells for which cells need to meet the high energy demand through the aerobic glycolysis pathway rather than the pyruvate oxidation pathway. The canonical Wnt/β-catenin signaling was also targeted to observe the expression of PDK1 in the PGC1α knockdown model by employing two popular inhibitors of this signaling pathway (XAV-939 and ICG-001). Gene Set Enrichment Analysis indicated that these inhibitors alleviate the overexpression of extracellular lactate, suggesting the possible role of PGC1α in the inhibition of aerobic glycolysis *via* Wnt/β-catenin signaling. Dual-luciferase reporter assays showed that the transcriptional actions of PPARγ are significantly increased in HCCLM3 and MHCC97H cells with PGC1α augmentation. These results show that the tumor-suppressive activity of PGC1α depends on PPARγ, which makes PPARγ a key regulator of HCC[29,32]. An earlier report revealed the role of PPARγ in HCC by analyzing the mRNA and protein expression in 20 patients with cirrhosis and chronic hepatitis. The results indicated a statistically pronounced drop in levels of PPARγ in HCC compared to the non-tumorous liver tissue[33]. A report confirmed that miR-130b aids cell aggressiveness by suppressing PPARγ in human HCC[34]. Similarly, evidence on the oncogenic role of miR-1468 in HCC *via* activating the PPARγ/Akt pathway was also recently confirmed. The increased levels of miR-1468 elevated the malignant prognostic features and improved survival. Carboxy-terminal domain 2 and UPF1 RNA Helicase And ATPase were identified as the downstream targets for miR-1468, which regulate PPARγ/Akt pathway activation. Restoration of the expression of these targets partially abolished the effects of miR-1468, explaining the regulation *via* PPARγ/Akt signaling[35].

EXPERIMENTAL AND CLINICAL SCENARIOS

Many studies have explored the therapeutic effects of synthetic and natural PPARγ agonists against HCC in preclinical and clinical trials. The activation of PPARγ significantly suppresses HCC progression and invasion. Several findings have identified PPARγ as a target for tumor suppression, a mediator of apoptosis, and a suppressor of carcinogenesis and metastasis by triggering intrinsic pathways and mainly inhibiting the PI3K/Akt survival pathway[8,21,36]. The various synthetic and natural PPARγ agonists used for HCC are listed in Table 1.

SYNTHETIC PPARγ AGONISTS IN HCC

PPARγ itself and its agonists have anticancer activities, such as growth inhibition, induction of apoptosis, and cell differentiation. Thiazolidinediones (TZDs) are a class of synthetic PPARγ agonists, and many compounds of this class have been studied for their efficacy in experimental models and clinical trials. These compounds were used as a bioregulatory remedial approach to target the communicative framework of HCC in patients with non-curative HCC[37]. TZDs are also effective for glycemic control and the likelihood of HCC and hepatic manifestation in diabetic patients with chronic hepatitis B (CHB). Of the 28999 patients with CHB, 3963 patients developed HCC at a median follow-up of 7.1 years, whereas 1153 patients were administered TZD during the follow-ups. The findings showed the co-relation of TZD use with lowering the risk of poor hepatic manifestations in diabetic patients with CHB[38]. A population-based case-control study performed in 23580 diabetic patients demonstrated the negative relationship between the risk of HCC and use of TZDs. There is a time-dependent effect of TZD use on the risk of HCC. The longer the duration of TZD use, the lower the risk of HCC[39-41]. Many other reports have also suggested that the administration of PPARγ agonists ameliorates several types of cancers, *i.e.* colorectal, bladder, lung, and liver cancers. The effects are more substantial at higher cumulative dosages with longer durations[42].

Table 1 Various synthetic and natural peroxisome-proliferator-activated receptor gamma agonist used in experimental and clinical trials for hepatocellular carcinoma

Agonist name	Drug bank/ PubChem ID	Model	Concentration/dose of agonist	Effects	Ref.
Synthetic agonists					
Pioglitazone	DB01132	*In vivo* (Rats, and Mice)	3 mg/kg; 10 mg/kg	Reduced HCC progression and decreased tumor size and volume	[44]
Rosiglitazone	DB00412	*In vivo* (Orthotopic Mice) *In vitro* (MHCC97L, and BEL-7404)	50 μmol/L	Decreased HCC migration, and invasiveness	[25]
		In vitro (HepG2 and PC3)	0.1, 1, 10, 100 μmol/L	Reduced cancer growth, Increased apoptosis	[49]
		In vitro (HepG2 and Hep3B)	80 μmol/L	Restricted the oncogenic activity of SEPT2	[50]
Telmisartan	DB00966	*In vitro* (HLF, HLE, HuH-7, PLC/PRF/5, and HepG2)	10, 50 or 100 μmol/L	Inhibit proliferation, induce cell cycle arrest	[53]
		In vivo (Mice)	15 mg/kg	Reversed malignant anomalies, antioxidant, anti-inflammatory	[54]
Troglitazone	DB00197	*In vitro* (Hep G2, HuH-7, KYN-1, and KYN-2)	5, 10, 25 μmol/L	Reduced cell proliferation and increased apoptosis	[56]
		In vitro (HepG2)	5, 10, 20, 40, 80, and 100 μmol/L	Apoptosis and growth inhibition	[57]
		In vitro (Hep G2, HuH-7, KYN-1, and KYN-2)	5, 10, and 25 μmol/L	Inhibited DNA synthesis, cell cycle growth, and α-fetoprotein levels	[58]
		In vitro (PLC/PRF/5, and HuH-7)	5, 10, 20, 40, 60, 80, and 100 μmol/L	Reduced cell proliferation and increased apoptosis	[59]
		In vitro (HLF, HAK-1A, HAK-1B, and HAK-5)	10, 20, 30, 40, and 50 μmol/L	Reduced cell proliferation and increased apoptosis	[19]
Saroglitazar	DB13115	*In vivo* (Mice)	4 mg/kg	Reduced inflammation in hepatic lobules, hepatocellular ballooning, and steatosis	[61]
		In vivo (Rats)	4 mg/kg	Improved lipid profile, and histopathological changes	[62]
Natural agonists					
Cannabinol, Cannabinoids	DB14737	*In vitro* (HepG2 and HUH-7); *In vivo* (Mice)	8 μmol/L; 15 mg/kg	Increased apoptosis, autophagy, anti-proliferative	[66]
		In vitro (HEK-293T and Neuro-2a); *In vivo* (Mice)	1, 5, 10, 25 μmol/L; 20 mg/kg	Antitumor, antioxidant, anti-inflammatory	[68]
Capsaicin	DB06774	*In vivo* (Rats)	0.5 and 1 mg/kg	Inhibit hepatic injury, and collagen deposition, anti-inflammatory	[71]
Curcumin	DB11672	*In vivo* (Rats)	20 mg/kg	Attenuated histopathological, serological, proliferative, and apoptotic parameters	[77]
		In vitro (H22); *In vivo* (Mice)	5, 10, 20, 40, and 80 μmol/L; 50, 100 mg/kg	Antiproliferative, decrease tumor growth, induce apoptosis	[78]
		In vivo (Mice)	150 mg/kg	Reduced inflammation, and tumor size	[79]
		In vivo (Rats)	0.5, 1, 2, 5, 10, 15, and 20 ng/mL	Interrupted TGFβ signaling, activated hepatic stellate cells	[80]
		In vitro (SMMC7721 and Huh-7)	10, 20, 40, 80, and 160 μmol/L	Suppressed cellular proliferation	[82]
Hesperidin	DB04703	*In vivo* (Rats)	50 and 100 mg/kg	Suppressed TGFβ signaling and hepatocarcinogenesis	[85]
		In vivo (Rats)	200 mg/kg	Inhibited PI3K/Akt pathway, Antioxidant	[86]

		In vitro (HepG2); *In vivo* (Rats)	100 μmol/L; 150 mg/kg	Inhibited Wnt3a/5a signaling pathway, anti-inflammatory	[87]
Hispidulin	DB14008	*In vitro* (SMMC7721 and Bel7402); *In vivo* (mouse tumor xenograft)	10 and 20 μmol/L; 20 and 40 mg /kg	Anticancerous, inhibited cell migration	[89]
		In vitro (NCI-H460 and A549)	4, 8, 15, 30, and 60 μmol/L	Induced ROS-mediated apoptosis, anti-cancerous	[90]
Isoflavone	DB12007	*In vivo* (Bel-7402 and SK-Hep-1)*In vivo* (Mice)	75 and 12 μmol/L resp.; 25 and 7.5 mg/kg resp.	Anti-inflammatory, anti-tumorigenic, reduced the size and volume of tumor	[94]
		In vitro (Hepa 1-6 cells)	1, 5, 10, 15, 20, 25, 50, 75, and 100 μmol/L	Antitumorigenic and antiproliferative	[95]
		In vitro (HCC-LM3, SMMC-7721, Hep3B, Bel-7402, and Huh-7)*In vivo* (Mice)	40, 60, and 80 μmol/L; 20, 40, and 80 mg/kg	Suppressed aerobic glycolysis and increased apoptotic rate	[96]
Oroxyloside	14655551	*In vitro* (HepG2) and SMMC-7721); *In vivo* (Mice)	100, 200, and 300 μmol/L; 90 mg/kg	Cell cycle arrest and growth repression	[100]
Resveratrol	DB02709	*In vivo* (Rats)	100 mg/kg	Antioxidant, anti-inflammatory, anticancer	[101]
		In vitro (HepG2); *In vivo* (Rats)	7.81, 15.63, 31.25, 62.5, 125, and 250 μg/mL; 20 mg/kg	Attenuated histopathological, serological, proliferative, and apoptotic parameters	[102]
Miscellaneous					
Avicularin	5490064	*In vitro* (HuH-7)	25, 50, and 100 μg/mL	Decreased the cell migration and invasiveness	[107]
Honokiol	72303	*In vitro* (HEK-293 and 3T3-L1); *In vivo* (Mice)	1, 3, and 10 μmol/L; 100 mg/kg	Activated PPARγ/RXR heterodimers; Reduced hyperglycemia	[108]
Chrysin	DB15581	*In vitro* (MDA-MB-231 and HepG2)*In vivo* (Mice)	10 μmol/L; 10 mg/kg	Increased apoptosis	[112]
Quercetin	DB04216	*In vitro* (HepG2 and SMCC-7721); *In vivo* (Mice)	0.05, 0.1, and 0.15 mmol/L; 40 mg/kg	Promoted the autophagy	[114]
		In vitro (PATU-8988 and PANC-1)	20, 40, 80, and 160 μmol/L	Suppressed HCC *via* STAT3 pathway	[117]
		In vitro (LM3); *In vivo* (Mice)	40, 80, and 120 μmol/L; 100 mg/kg	Reduced invasiveness, Cell cycle regulation	[118]
Clinical trials					
		Population type	No. of patients		
Thiazolidinediones	NA	Hongkong	1153	Reduce the synergistic effect of diabetes with liver disorders; Reduced risk of HCC	[38],[39], [40],[41]
		Taiwanese	77396		
			32891		
			76349		
Pioglitazone	DB01132	Chinese	75	Blocked RAGE signaling; Reduced HCC	[45]
		Japanese	85	Reduced growth and invasion of HCC cells	[46]
		Thai	10000	Reduced risk of HCC	[47]
Rosiglitazone	DB00412	French	44	Reduced NASH activity and ballooning score, Ameliorated histopathological aberrations	[51]
Saroglitazar	DB13115	Indian	30	Improved glycemic index and liver stiffness	[63]
			90	Improved fibrosis score	[64]

Isoflavone	DB12007	Japanese	302	Antioxidant, reduced risk of HCC	[97]
			191	Antioxidant, reduced risk of HCC	[98]

Akt/PKB: Protein kinase B; HCC: Hepatocellular carcinoma; NASH: Non-alcoholic steatohepatitis; PI3K: Phosphoinositide 3-kinase; PPARγ: Peroxisome proliferator-activated receptor gamma; RAGE: Receptor for advanced glycation end products; ROS: Reactive oxygen species; RXR: Retinoid X receptor; SEPT2: Septin 2; STAT3: Signal transducer and activator of transcription 3; TGFβ: Transforming growth factor beta; Wnt: Wingless-related integration site.

Pioglitazone

Pioglitazone (PGZ), a PPARγ ligand, works by improving the insulin sensitivity of tissues and exhibits anticancer activity. It selectively stimulates PPARγ *via* modulating the transcriptional alterations of genes involved in glucose metabolism and insulin resistance and further decreasing the gluconeogenesis and levels of glycated hemoglobin in the bloodstream[43]. PGZ treatment inhibits fibrosis progression and HCC development and reduces tumor size in DENA-induced rats at 3 mg/kg and mice at 10 mg/kg. PGZ is suggested to exhibit protective effects by reducing mitogen-activated protein kinase (MAPK) and upregulating adiponectin levels, resulting in activation of the hepatoprotective AMPK pathway[44].

The anticancer activity of PGZ is attributed to the pathological receptors for advanced glycation end products (RAGE). HCC tissues from 75 patients showed high expression of RAGE in HCC tissues, which was closely linked to pathological staging and lymph-vascular space influx. However, PGZ treatment suppressed cellular proliferation, ameliorated apoptosis, and cell cycle arrest, which further elevated PPARγ expression and decreased the expression of RAGE, NF-κB, high mobility group box 1, p38MAPK Ki-67, MMP2, and cyclin D1. The results demonstrated that PGZ as a PPARγ agonist possibly slows down the growth and invasion of HCC cells by blocking RAGE signaling[45]. Another prospective study confirmed the effect of PGZ on HCC by investigating 85 patients with HCC and hepatitis C virus infection to investigate recurrence-free survival. The spline-model analysis showed that the lessened risk of HCC recurrence is associated with increased body weight and body mass index ≥ 23. PGZ was also observed to alleviate insulin resistance and serum adiponectin levels[46]. A lifetime Markov model was employed among the population of Thailand to study the life expectancy, quality-adjusted life years, lifetime costs, and the incremental cost-effectiveness ratios in HCC patients. The weight reduction program with the administration of PGZ demonstrated that PGZ can reduce the number of HCC cases[47]. These therapeutic potentials also have limitations. PGZ has adverse effects such as body weight gain, peripheral edema, bone loss, and heart failure. Additionally, the risk of bladder cancer significantly limits the use of this agonist in the medical field[48].

Rosiglitazone

Rosiglitazone is a member of the TZD class of insulin-sensitizing PPARγ agonists. An inhibitory effect of PPARγ was reported on the invasive and metastatic potential of HCC *in vitro* (MHCC97L and BEL-7404 cell lines) and *in vivo* (orthotopic HCC mouse model). A pronounced expression of PPARγ was demonstrated in HCC cell lines treated with adenovirus-expressing mouse PPARγ1 (Ad-PPARγ), rosiglitazone (50 μmol/L), or Ad-PPARγ plus rosiglitazone. The induction of PPARγ markedly repressed HCC cell migration, invasiveness, levels of pro-metastatic genes (MMP9, MMP13, heparanase [HPSE]), and hepatocyte growth factor. However, the levels of cell adhesion genes (E-cadherin and SYP), extracellular matrix regulator TIMP3, and tumor suppressor gene retinoblastoma 1 were elevated. Additionally, direct transcriptional regulation of the genes TIMP3, MMP9, MMP13, and HPSE regulating PPARγ levels was also validated by chromatin immunoprecipitation-PCR[25]. Bcl-2 is a well-known family of anti-apoptotic proteins regulating endogenous apoptotic pathways and are highly expressed in carcinomas. (-)-gossypol ((-)-G) is the (-) enantiomer of gossypol that acts as a small molecule to induce apoptosis in several types of cancers by inhibiting Bcl-2 proteins. In a study, rosiglitazone was employed to sensitize (-)-G to induce apoptosis at different concentrations (0.1, 1, 10, 100 μmol/L). The (-)-G induced Mcl-1 (myeloid cell leukemia-1) stability was the prime concern for its apoptotic activity. However, rosiglitazone attenuated this stability *via* Janus kinase phosphorylation, further repressing cancer growth. These results suggest that rosiglitazone can reduce cancer growth and sensitize the other apoptotic factors for performing a similar activity. The study also provides insights into the novel cancer therapeutic activity of BH3 mimetics in the case of carcinomas based on the combination of PPARγ agonists and BH3 mimetics[49]. Rosiglitazone (80 μmol/L) also inhibits HCC cell growth by restricting the oncogenic activity of septin 2[50].

A long-term clinical trial was conducted in which 53 patients underwent liver biopsies and were further treated with rosiglitazone (8 mg/d) for the next 2 years. Forty-four patients fulfilled the criteria of the extension period and underwent another biopsy. During the extension phase, serum insulin and alanine aminotransferase (ALT) levels were decreased by 26% and 24%, respectively. Non-alcoholic steatohepatitis activity, ballooning, and fibrotic stage were decreased but not on a significant scale. The treatment was continued for another 2 years, but no significant results were obtained, showing that rosiglitazone does attenuate insulin sensitivity and transaminase levels but might not significantly

improve other histopathological parameters. However, additional targets were suggested to be explored [51]. However, there is increasing evidence of bone fractures in females medicated with rosiglitazone after menopause, limiting its use. In September 2010, the FDA restricted the use of rosiglitazone based on meta-analyses of mostly short-term randomized controlled trials, which showed evidence of myocardial infection risk. However, these restrictions were removed in 2013 based on other large clinical trials by Duke Clinical Research Institute, which showed no complications regarding heart failure[52].

Telmisartan

Telmisartan (TEL) is an angiotensin II receptor blocker with a high affinity for the angiotensin II receptor type 1, whose impromptu link with HCC has been discovered; however, the underlying mechanism is not clear. TEL shows basal resemblance with a well-known PPARγ agonist, PGZ. TEL (at concentrations of 10, 50, or 100 μmol/L) inhibits the proliferation and G0 to G1 cell cycle transition leading to G0/G1 cell cycle arrest in hepatic cancer cells (HLF, HLE, HuH-7, PLC/PRF/5, and HepG2) in a dose-dependent manner. The cell cycle arrest was accompanied by reduced cell cycle-related proteins, including cyclin D1 and cyclin E. Further TEL was suggested to increase the activity of AMPK and inhibit the mammalian target of rapamycin (mTOR) pathway[53]. Another study used a DENA-induced HCC mouse model to evaluate the effects of TEL (15 mg/kg), sorafenib (SRF) (30 mg/kg), and a combination of these two agonists. The treatment downregulated the mRNA expression of NF-κBp65, AFP, TNFα, and transforming growth factor beta 1 (TGFβ1) resulting in the reversion of malignant anomalies and suppression of extracellular signalregulated protein kinase 1/2 (ERK1/2) activation. SRF and TEL showed antiproliferative, antimetastatic, and anti-angiogenic effects by improving the expression of hepatic cyclin D1, MMP2, and vascular endothelial growth factor (VEGF). However, only TEL has exhibited agonistic activity for PPARγ receptors, as indicated by the elevated PPARγ DNA-binding activity, mRNA expression of cluster of differentiation 36, heme oxygenase 1, and enhanced hepatic antioxidant capacity. Moreover, TEL and SRF both ameliorate phosphorylation-induced activation of TGFβ-activated kinase 1 (TAK1), suggesting that TAK1 might act as the core mediator for the interaction between ERK1/2 and NF-κB. TEL exerts its anticancer effects by modulating the ERK1/2, TAK1, and NF-κB signaling axis from the perspective of its PPARγ agonistic activity. Thus, TEL may be a useful PPARγ agonist for further clinical studies in the context of HCC treatment[54]. Despite its potential, it has adverse effects including headaches, dizziness, fatigue, upper respiratory tract or stomach-related infections, sinusitis, nonspecific pain, and diarrhea[55].

Troglitazone

Troglitazone (TGZ) is a member of the TZD class of drugs and acts as a PPARγ agonist. The antiproliferative and antitumorigenic effects of TGZ were studied in the BEL-7402 HCC cell line at 5, 10, and 25 μmol/L concentrations. TGZ induced cell death in a concentration-dependent manner resulting in the increased presence of fragmented DNA and terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL)-positive cells. TGZ enhanced cell cycle arrest in the G0/G1 phase and increased caspase activities (caspase 3, 6, 7, and 9), indicating increased cell apoptosis[56]. In another study, the HepG2 cell line treated with TGZ showed significant growth inhibition in a dose-dependent manner. The TUNEL assay and immunohistochemistry showed apoptosis induction and elevated expression of apoptotic proteins such as caspase 3 and survivin[57]. PPARγ was functionally expressed in hepatic cancer cell lines (HepG2, HuH-7, KYN-1, and KYN-2) with TGZ treatment. This was followed by the profound inhibition of cellular proliferation, DNA synthesis, cell cycle growth, and α-fetoprotein levels [58]. Similar results have also been shown by other groups that used other HCC cell lines such as PLC/PRF/5, HuH-7[59], HLF, HAK-1A, HAK-1B, and HAK-5 with TGZ[10,19]. The reduction in cell proliferation and increased apoptosis in most of these studies demonstrated the usefulness of TGZ for chemoprevention in HCC. Some recent studies showed the hepatotoxic effect of TGZ on diabetic patients. There is a significant elevation in liver enzymes level (ALT and aspartate aminotransferase [AST]) in 1.9% of patients with diabetes treated with TGZ for 24 to 48 wk. Furthermore, the cost of TGZ is much higher than that of other oral antihyperglycemic agents or insulin, which also limits the use of TGZ[60].

Saroglitazar

Saroglitazar is a first-class drug that acts as a dual PPARα/γ agonist. It is indicated for enhanced diabetic dyslipidemia, inflammation, steatosis, ballooning, and fibrosis progression. The agonistic effects of this drug have a favorable impact on insulin resistance and lipid profile. Saroglitazar treatment is thought to ameliorate high-fat diet-induced aberrations. The improvements were observed in hepatic lobular inflammation, hepatocellular ballooning, steatosis, and fibrosis. The effects of saroglitazar were more pronounced compared to PGZ. Transcriptomic analyses revealed the elevated expression of PPARγ in hepatic tissue with the anti-inflammatory effects of saroglitazar treatment[61]. Similarly, saroglitazar improved liver function parameters, degenerative changes, glucose and insulin levels, and lipid profile in high-fat emulsion plus lipopolysaccharide (LPS)-treated rats. The positive effects on serum leptin, TNFα, and adiponectin levels were also observed. The multiple protective roles of PPARα

/γ agonists in liver disorders suggest the usefulness of saroglitazar in managing liver cancer[62].

In a prospective observational study, 30 diabetic patients with liver fibrosis were enrolled and treated with 4 mg saroglitazar daily for 6 mo. A profound improvement in glycemic index, liver stiffness, and serum triglyceride levels of the patients was observed with no significant adverse side effects[63]. Another study conducted in 90 NAFLD patients who underwent liver biopsies, fibrosis scores, and other non-invasive parameters showed that saroglitazar treatment significantly improved the serum biomarker levels and fibrosis score. The study concluded the reversal effect of saroglitazar on fibrosis and advocated its use in treating HCC[64]. The most common adverse events associated with saroglitazar included asthenia, gastritis, chest discomfort, peripheral edema, dizziness, and tremors[65].

NATURAL PPARγ AGONISTS IN HCC

Natural PPARγ agonists have many beneficial properties including antioxidant, anti-inflammatory, antifibrotic, and antitumor effects. In addition to therapeutic effects, synthetic drugs have many adverse effects due to full PPARγ activation. Therefore, researchers are exploring potential natural PPARγ modulators with high specificity in terms of their binding at the active site and improving drug safety. The PPARγ-activating effect of natural products is recognized as having great potential in developing anticancer therapy. There are many reports on the natural PPARγ agonist against HCC in various experimental models.

Cannabinoids

The hemp plant *Cannabis sativa L.* produces approximately 60 unique compounds known as cannabinoids, of which ∆9-tetrahydrocannabinol (THC) is the most important due to its high potency and abundance in cannabis. Various studies have reported the fair safety profile of cannabinoids, in accordance with its probable antiproliferative activity on cancerous cells, may set the basis for future trials to evaluate the potential antitumor activity of cannabinoids. Vara *et al*[66] reported that cannabinoids THC and JWH-015 increased the intracellular mRNA and protein levels of PPARγ in HCC cells, and inhibition of PPARγ decreased cannabinoid-induced cell death and apoptosis. Further, increased PPARγ levels were correlated with endoplasmic reticulum stress and autophagy in HCC cells, suggesting the antiproliferative effects of cannabinoids through PPARγ-dependent pathways. The antitumor activity of THC was evaluated in patients who had failed standard therapy norms. *In vitro* studies have shown the suppression of tumor cell proliferation, and Ki67 immunostaining exhibits a reduced number of tumor cells[67]. THC is suggested to induce transcriptional modulation of the PPARγ pathway, and the activation is much more potent by cannabinoid acids than its decarboxylated products, indicating that cannabinoids act as a PPARγ agonist[68]. *Cannabis* contains some psychoactive agents that increase sociability and exert euphoric effects. Repeated use of *cannabis* has been linked to short- and long-term side effects, including respiratory and cardiovascular disorders, cognitive alterations, psychosis, schizophrenia, and mood disorders[69]. A recent study highlighted the side effects of a common preparation from *C. sativa* named *marijuana*. This study gave the putative association of the use of *cannabis* with a higher risk of gingival and periodontal diseases, oral infection, and cancer of the oral cavity[70]. Given the growing popularity of cannabinoid-based drugs for recreational and medical purposes and their potentially harmful effects, there is a need for further investigation in this field.

Capsaicin

Capsaicin (8-methyl-N-vanillyl-6-nonenamide) is a vital constituent of chili peppers belonging to the family of Capsicum. These phytoconstituents possess anti-inflammatory and chemopreventive properties. They counter various compounds' mutagenic properties and exert anticancer effects on breast, colon, prostate, and hepatic cancers. The DENA-induced models of HCC in rats and hepatic stellate cell lines were used to study the effects of capsaicin. Capsaicin was observed to inhibit hepatic injury, NF-κB activation, and collagen deposition. It has also ameliorated the levels of α-SMA, collagen type I, MMP2, TGFβ1, and TNFα. Furthermore, TGFβ1 expression and the phosphorylation of Smad2/3 were also inhibited through induction of PPARγ expression. The findings showed that capsaicin attenuates hepatic fibrosis by upregulating PPARγ expression[71]. The limitations of this natural PPARγ agonist should be mentioned. Capsaicin is a well-known irritant responsible for producing a painful, burning sensation when applied to the skin. Exposure to the eyes is painful and causes tearing, conjunctivitis, and blepharospasm[72]. Capsaicin is also a tussive agent, and inhaled capsaicin can be used to induce cough under experimental conditions. In humans, inhaled capsaicin induces a cough response immediately upon administration[73,74]. Interestingly, there is evidence that topical capsaicin can exacerbate angiotensin-converting enzyme (ACE) inhibitor-induced cough. A patient taking an ACE inhibitor for several years with no complaint of coughing reported coughing associated with applying a 0.075% capsaicin cream[75]. Additionally, oral administration of the ACE inhibitor captopril was found to cause a shift in the dose-response curve of inhaled capsaicin-induced cough in a trial with healthy adults[76].

Curcumin

Curcumin is a polyphenol compound present in *Curcuma longa* and is well known for its multiple therapeutic effects. Our previous study reported the effect of curcumin and piperine on DENA-induced HCC in rats. Curcumin prevented HCC progression by improving hepatic pathology, apoptosis induction, and inhibiting cell proliferation. However, the synergistic effect on HCC suppression was observed with the combination of curcumin and piperine[77]. Similarly, another study also reported the inhibition of cell proliferation, tumor growth, and apoptosis induction by curcumin treatment in HCC. The effect was suggested to decrease VEGF expression and PI3K/Akt signaling[78]. A study in a transgenic mouse model (expressing double HBV oncoproteins, HBx and pre-S2 in the liver) of HBV-related HCC reported the protective effects of phytosomal curcumin *via* targeting PPARγ as a key regulator. Curcumin decreased HCC formation and reduced the tumor size. Moreover, considerably more potent effects were observed on activation of PPARγ and inhibition of NF-κB. The report suggested that curcumin is an agonist for PPARγ, upregulating the genes involved in lipid metabolism, antiproliferation, and anti-inflammation. Furthermore, PPARγ activation regulates the suppression of NF-κB and subsequent pro-inflammatory cytokines. In addition, curcumin also is suggested to repress mTOR[79]. Recently, the antitumor effect of curcumin on HCC was suggested due to the involvement of miR-21 targeting TIMP3 and inhibition of the TGFβ1/Smad3 signaling pathway. The inhibition of TGFβ 1/Smad3 signaling by curcumin is reportedly linked to activation of the PPARγ gene[80,81]. It was further suggested to suppress cell proliferation through long non-coding RNA downregulation and inhibition of Wnt/β-catenin signaling[82]. The major disadvantage of this medication is the usage of high doses, which ultimately leads to liver injury in humans and experimental animals. A study showed that curcumin supplementation with paracetamol at doses of 50 and 100 mg/kg per day in experimental rabbits showed elevation of liver injury markers (ALT, AST, ALP, total protein, and albumin level) in plasma. Furthermore, levels of red blood cells and platelets were raised[83]. Also, the poor bioavailability of curcumin leads to its combined usage with other drugs such as piperine, which reportedly causes adverse drug reactions[84].

Hesperidin

Hesperidin is a flavanone glycoside found in the rind of citrus fruits including oranges and lemon. It possesses several pharmacological activities including antioxidant, anti-inflammatory, and anticancer effects. The chemopreventive efficacy of hesperidin was evaluated in DENA-induced HCC in rats. The hesperidin significantly reduced hepatic serological and tumor biomarkers along with TNFα. Furthermore, it also reduced the hepatic degenerative changes, oxidative stress, collagen deposition, TGFβ1, and NF-κB expression. However, the upregulated expression of nuclear factor erythroid 2–related factor 2, HO-1, and PPARγ suggested the effect of hesperidin *via* suppressing TGFβ signaling and subsequently activating PPARγ[85]. Another study investigated the efficacy of hesperidin *via* the PI3K/Akt pathway as a probable mechanism for curing HCC. Treatment with hesperidin elevated the protein levels of PI3K, Akt, and cyclin-dependent kinase 2 and ameliorated HCC progression[86]. In addition, hesperidin reportedly alters Wnt3a/β-catenin signaling in preventing HCC[87]. There are few reports on the bioavailability and solubility of hesperidin. Ameer *et al*[88] reported that hesperidin is absorbed across the gastrointestinal tract on oral administration, but cumulative recovery indicates low bioavailability. The factors limiting the bioavailability of hesperidin are poor water solubility and its precipitation in an acidic environment.

Hispidulin

Hispidulin, a phenolic flavonoid, exhibits anticancer activity against several types of cancers. The effect of hispidulin on HCC was studied in tumor cell lines (SMMC7721 and Bel7402) and mouse tumor xenograft models. Hispidulin activates caspase 3, triggers apoptosis, and inhibits cell migration *via* PPARγ activation, which is further linked to escalated phosphorylation of AMPK, ERK, and JNK *in vitro*. Specifically, GW9662 (a PPARγ inhibitor), compound C (an AMPK inhibitor), and PD98059 (a MEK inhibitor) negated the protective effects of hispidulin on PPARγ signaling. However, no pronounced changes in PPARγ levels were noted with pre-treatment of SP6000125 (a JNK inhibitor) *in vitro,* whereas it attenuated the anticancer activity of hispidulin. The suppression of Bel7402 xenograft tumor growth was successfully achieved by hispidulin through PPARγ activation, indicating the cardinal role of PPARγ signaling in HCC cell growth[89]. Recently, Lv *et al*[90] suggested that induction of reactive oxygen speciesmediated apoptosis through activation of the endoplasmic reticulum stress pathway is also responsible for the anticancer effect of hispidulin. Some evidence links hispidulin to its limited large-scale preparation. Studies have shown the lack of a single-dose design of hispidulin, which further limits the bioavailability[91,92].

Isoflavones

Isoflavones are a group of phytochemicals, a type of naturally occurring isoflavonoids. Studies have shown the anticancer effects of different isoflavones in the case of HCC[93]. A combination of two well-known isoflavones, Biochanin A and SB590885, was evaluated for their anticancer activities in HCC. The combination showed synergistic inhibition of cell growth and induced cell cycle arrest and apoptosis *in*

vitro. The inhibition of cellular proliferation and tumor suppression were attributed to the aberration of ERK MAPK and PI3K/Akt pathways. *In vivo*, a profound reduction in the size and volume of HCC tumors was noted, indicating the combination therapy of isoflavones as a potential lead for the management and treatment of advanced HCC[94]. The antitumorigenic and antiproliferative role of genistein was also studied in HCC *in vitro*. The isoflavone suppressed the proliferation of Hepa 1-6 cells and caused apoptosis in time- and dose-dependent manners[95]. In another study, genistein treatment suppressed aerobic glycolysis and increased the apoptotic rate in HCC cell lines. Additionally, genistein exhibited inhibitory effects on tumor progression and aerobic glycolysis. This may be identified as an effective treatment for advanced HCC[96]. Studies have reported the PPARγ-modulating effect of isoflavones and inhibition of HCC through inhibition of the PI3K/Akt pathway, and aerobic glycolysis further validates the involvement of PPARγ signaling. Clinical studies have also suggested that the more the dietary intake of flavonoids, the lesser the risk of developing HCC. In the Japanese population, a correlation between the isoflavone-rich diet and risk of HCC was observed[97,98]. Despite the therapeutic potential, some contentious health issues are associated with their intake. Soy proteins rich in isoflavones showed unfavorable effects at a higher dose, including gastrointestinal upset, constipation, nausea, allergic reactions, and loss of appetite. In animals, the intake of isoflavone (genistein) reportedly impacts the fertility and morphogenesis of ovaries. In addition, long-term use of soy extract may result in abnormal tissue growth in the uterus[99].

Oroxyloside

Oroxyloside (OAG), a flavonoid, was explored as a new dual agonist of PPARγ/α, which acts as a potent cell proliferation inhibitor in HCC-based metabolic transition. It regulates the glycolipid metabolic enzymes (PPAR-dependent or PPAR-independent), inhibits the breakdown of glucose, and promotes fatty acid oxidation, which generates acetyl-CoA for the tricarboxylic acid cycle and oxidative phosphorylation. The metabolic transition produced by OAG exhibits a profound generation of reactive oxygen species, leading to G1 cell cycle arrest and growth repression of HCC cells. OAG requires pyruvate dehydrogenase kinase 4 and β-oxidation to inhibit cell proliferation, explaining its PPARγ agonistic behavior. OAG is a new PPARγ/α agonist drug candidate and an effective therapeutic approach for HCC based on metabolic reprogramming[100]. Although many bioactive flavones' sources are very well known, information on their bioavailability and their active forms *in vivo* is limited. In particular, most flavonoid agents' absorption, metabolism, and blood delivery are poorly understood. Due to limited literature, it is difficult to elucidate the whole molecular mechanism. Hence, further studies are required to uncover their therapeutic potential against liver diseases.

Resveratrol

Resveratrol (RS) is a popular natural polyphenolic PPARγ agonist, well known for its anticancer properties, and has been recognized as the alternate mode in cancer treatment. A study revealed the effect of RS against alcohol-aflatoxin B1-induced HCC. During the progression of HCC, a decline in the antioxidant markers was effectively restored by resveratrol treatment. RS modulated the activity of the sirtuin 1 (SIRT1) enzyme in HCC by negatively regulating the levels of NF-κB, and cross-talk between this PPARγ agonist and SIRT1 signaling was observed[101]. A nano-formulation of RS using liposomes was developed to establish a specific drug delivery system for managing HCC. *In vitro* studies have revealed the increased internalization and enhanced anticancer activity of liposomal formulation (RL5) compared to naïve RS. A profound reduction in liver injury markers, hepatocyte nodules, and degenerative changes in the liver was observed in an *in vivo* HCC model. The results indicated the promising action of nano-formulation of RS and its substantial activity in controlling the severity of HCC[102]. Earlier, similar approaches were briefly reviewed by Santos *et al*[103] to study the pharmacokinetics of RS-loaded nanoparticles (RS-NPs) and study their effects on cancer tissue. A comprehensive analysis was carried out in various *in vivo* models, which revealed the markedly enhanced anticancer activity of RS-NPs. However, the poor bioavailability and rapid metabolism restricted the successful translation of resveratrol to clinical form. The *in vivo* efficacy of RS is affected due to its low solubility and low bioavailability. Oral intake of 25 mg of RS showed extremely low bioavailability; only a trace amount of unmetabolized RS was detected in plasma. The gastrointestinal tract absorbs approximately 70% of RS, but it is further metabolized by three distinct metabolic pathways leading to low bioavailability[104].

Miscellaneous

Avicularin (quercetin-3-α L arabinofuranoside), a glycoside related to quercetin, reportedly reduces obesity, inflammation, and drug resistance[105,106]. It also induces cytotoxicity in cancer cells by promoting intrinsic apoptosis pathways. One study investigated the activity of avicularin in HCC by employing HuH-7 cell lines. Avicularin inhibited cell proliferation in a dose-dependent manner and markedly decreased the cell migration and invasiveness of the cancer cells. Gene and protein expression studies revealed reduced levels of NF-κB, cyclooxygenase 2, and PPARγ. Avicularin may have the potential to modulate PPARγ to induce antineoplastic activity in HCC[107].

Honokiol (C18H18O2) is a bioactive, biphenolic phytoconstituent derived from the bark and leaves of *Magnolia Officinalis*. Honokiol exhibits various protective activities such as anticarcinogenic, anti-inflammatory, anti-angiogenic, antioxidative, and repressive potency towards the malignant conversion of papillomas to carcinomas without any noticeable toxicity effects. A group of researchers employed a great blend of *in silico, in vitro*, and *in vivo* techniques to pinpoint and validate honokiol as a potent lead for being a PPARγ agonist. The binding of honokiol into the ligand-binding pocket of PPARγ was anticipated *via* various *in silico* techniques. The luciferase reporter assay confirmed this binding and advocated that honokiol could act as a partial PPARγ agonist. Further, using 3T3-L1 and mouse embryonic cell lines, it was observed that honokiol stimulated basal glucose uptake but did not induce adipogenesis. However, the oral administration of honokiol resulted in reduced hyperglycemia and weight gain[108]. Various studies have suggested that honokiol acts as an RXR agonist forming RXR dimers and activating PPARγ/RXR heterodimers. Additionally, it also potentiates the activation of PPARγ/RXR heterodimers induced by rosiglitazone[109-111]. Also, no peer-reviewed papers proving the abuse, misuse, or dependence on or addiction to avicularin and honokiol have been retrieved yet.

Chrysin is a dihydroxyflavone belonging to the family of flavonoids. A study revealed that chrysin reduced cell viability and promoted apoptosis in all cell lines *via* inhibiting the Skp2 and low-density lipoprotein receptor-related protein 6 expression. However, reduced MMP2, MMP9, and fibronectin levels were observed[112]. Despite these interesting bioactivities, the clinical applications of chrysin have been constrained by its hydrophobicity, poor bioavailability, and degradation at alkaline pH[113]. Similarly, quercetin (QE) is a classic flavonoid and a yellow crystalline pigment present in plants, used as a food supplement to reduce allergic responses or boost immunity. It has been known to inhibit the development of various types of cancer hepatic conditions[114,115]. QE was suggested to effectively suppress HCC due to its close interaction with the signal transducer and activator of transcription 3 (STAT3) pathway[116,117]. It inhibits cell proliferation, cell cycle regulation, and invasiveness of the cancer cells by promoting the autophagy of HCC[118]. However, the bioavailability of QE is very low due to its poor aqueous solubility and instability, challenging its therapeutic application in the pharma sector[119].

CONTRADICTORY ROLE OF PPARγ

Cancer tissues display metabolic and thermodynamic aberrations with dysregulated cellular growth. Although the role of PPARγ and its agonists in HCC and other cancers have been extensively studied, as discussed above, several conflicting reports exist concerning the PPARγ expression in cancers. It is unclear whether PPARγ induction promotes or suppresses tumor growth and viability. In the case of several cancers, PPARγ mainly exhibits the down-regulated expressions while activating several other pathways like the canonical Wnt/beta-catenin pathway, PI3K/Akt pathway, STAT3 pathway, *etc*[82,87,118]. The activation of Wnt/β-catenin signaling leads to the upregulated PDK1, which leads to aerobic glycolysis and mitochondrial stress[29]. A recent report by Galbraith *et al*[120] revealed that the activation of PPARγ, in turn, induced Akt serine/threonine kinase 3 (AKT3), which eventually led to the more aggressive form of cancer. AKT3 enhances PGC1α localization to the nuclear space by repressing chromosome maintenance region 1, while the latter served as the downstream target for PGC1α. All these led to mitochondrial biogenesis, which fueled the progression of the tumor. Previous studies have also reported such inconsistent findings for PPARγ in HCC. Koga *et al*[10] tested five patients with cirrhotic livers and found no significant change in the PPARγ expressions compared to the surrounding non-cancerous tissue. Another study reported the consistently overexpressed PPARγ in HCC tissue having null expression in the surrounding tissues, even though all the patients were infected with viral hepatitis (B or C)[121]. Although the well-known inhibitory effects of PPARγ agonists are reported, they are also suggested to have PPARγ-independent effects on cancers. Troglitazone, as discussed above, has a prominent antitumorigenic role in HCC. However, there are reports of it exhibiting PPARγ-independent activity. Palakurthi *et al*[122] studied troglitazone and ciglitazone on both PPARγ-/- and PPARγ+/+ mouse embryonic stem cells considering various concentrations. Both the agonists could inhibit cellular proliferation in a dose-dependent manner by suppressing the G1-S transition. This evidence demonstrated that the antiproliferative effect was induced by suppressing the translation initiation. More similar reports back up the PPARγ-independent antitumorigenic property of PPARγ agonists[123,124]. One of the studies focused on the HCC progression in HBV-transgenic mice demonstrated that the anticancerous, antiproliferative, and apoptotic effects of TZD were more significant in PPARγ-deficient mice in comparison with the control mice, exhibiting normal PPARγ levels[125]. It is well-understood that PPARγ could potentially affect various pathways, so it is vital to understand the underlying mechanisms critically. This understanding is an absolute requirement as PPARγ may be inconsistent. However, it highlights its crucial role in tumor development, suggesting that targeted biomedical research against PPARγ could provide a highly efficacious avenue for treating and managing of HCC and various other cancers.

CONCLUSION

The majority of current studies support the fact that PPARγ may be a potential target against the progression of HCC. They have extensively explored the various signaling cascades through which PPARγ exerted inhibitory against HCC using synthetic and natural agonists in preclinical and clinical trials. PPARγ was suggested as a potential target as it suppresses cell proliferation, migration, and invasion in HCC cells through different signaling pathways. TZD, a class of synthetic PPARγ agonists, were extensively studied for their efficacy against HCC. TZD showed significant results against the progression of HCC; however, due to their adverse effect on different organs, these drugs are not approved for any cancer treatment. Therefore, increased focus was employed to identify natural and endogenous PPARγ agonists having high bioavailability and specificity in terms of their binding at the active site. Several studies reported the safety profiles and therapeutic role of natural agonists against HCC in various experiment modals. Natural agonists are also effectively reported to mediate apoptosis and inhibit cell proliferation, tumor growth, and metastasis in HCC. Few reports also highlighted the contradictory role of PPARγ in HCC. These contradictions might be due to some unidentified link between PPARγ and cancer. With the well-established role of PPARγ in the progression of HCC, better efficacy of its agonists may be achieved by a complete understanding of underlying mechanisms through which PPARγ showed therapeutic effects. Future studies should be focused on developing novel PPARγ targeting therapy for the treatment of HCC.

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REFERENCES

1 **El-Serag HB**. Epidemiology of viral hepatitis and hepatocellular carcinoma. *Gastroenterology* 2012; **142**: 1264-1273.e1 [PMID: 22537432 DOI: 10.1053/j.gastro.2011.12.061]
2 **Sayiner M**, Golabi P, Younossi ZM. Disease Burden of Hepatocellular Carcinoma: A Global Perspective. *Dig Dis Sci* 2019; **64**: 910-917 [PMID: 30835028 DOI: 10.1007/s10620-019-05537-2]
3 **Santopaolo F**, Lenci I, Milana M, Manzia TM, Baiocchi L. Liver transplantation for hepatocellular carcinoma: Where do we stand? *World J Gastroenterol* 2019; **25**: 2591-2602 [PMID: 31210712 DOI: 10.3748/wjg.v25.i21.2591]
4 **Llovet JM**, Ricci S, Mazzaferro V, Hilgard P, Gane E, Blanc JF, de Oliveira AC, Santoro A, Raoul JL, Forner A, Schwartz M, Porta C, Zeuzem S, Bolondi L, Greten TF, Galle PR, Seitz JF, Borbath I, Häussinger D, Giannaris T, Shan M, Moscovici M, Voliotis D, Bruix J; SHARP Investigators Study Group. Sorafenib in advanced hepatocellular carcinoma. *N Engl J Med* 2008; **359**: 378-390 [PMID: 18650514 DOI: 10.1056/NEJMoa0708857]

5 **Fan G**, Wei X, Xu X. Is the era of sorafenib over? *Ther Adv Med Oncol* 2020; **12**: 1758835920927602 [PMID: 32518599 DOI: 10.1177/1758835920927602]
6 **Hsu HT**, Chi CW. Emerging role of the peroxisome proliferator-activated receptor-gamma in hepatocellular carcinoma. *J Hepatocell Carcinoma* 2014; **1**: 127-135 [PMID: 27508182 DOI: 10.2147/JHC.S48512]
7 **Willson TM**, Lambert MH, Kliewer SA. Peroxisome proliferator-activated receptor gamma and metabolic disease. *Annu Rev Biochem* 2001; **70**: 341-367 [PMID: 11395411 DOI: 10.1146/annurev.biochem.70.1.341]
8 **Wu CW**, Farrell GC, Yu J. Functional role of peroxisome-proliferator-activated receptor γ in hepatocellular carcinoma. *J Gastroenterol Hepatol* 2012; **27**: 1665-1669 [PMID: 22742931 DOI: 10.1111/j.1440-1746.2012.]
9 **Tan Y**, Wang M, Yang K, Chi T, Liao Z, Wei P. PPAR-α Modulators as Current and Potential Cancer Treatments. *Front Oncol* 2021; **11**: 599995 [PMID: 33833983 DOI: 10.3389/fonc.2021.599995]
10 **Koga H**, Sakisaka S, Harada M, Takagi T, Hanada S, Taniguchi E, Kawaguchi T, Sasatomi K, Kimura R, Hashimoto O, Ueno T, Yano H, Kojiro M, Sata M. Involvement of p21(WAF1/Cip1), p27(Kip1), and p18(INK4c) in troglitazone-induced cell-cycle arrest in human hepatoma cell lines. *Hepatology* 2001; **33**: 1087-1097 [PMID: 11343236 DOI: 10.1053/jhep.2001.24024]
11 **Lee WS**, Kim J. Peroxisome Proliferator-Activated Receptors and the Heart: Lessons from the Past and Future Directions. *PPAR Res* 2015; **2015**: 271983 [PMID: 26587015 DOI: 10.1155/2015/271983]
12 **Mirza AZ**, Althagafi II, Shamshad H. Role of PPAR receptor in different diseases and their ligands: Physiological importance and clinical implications. *Eur J Med Chem* 2019; **166**: 502-513 [PMID: 30739829 DOI: 10.1016/j.ejmech.2019.01.067]
13 **Aouali N**, Broukou A, Bosseler M, Keunen O, Schlesser V, Janji B, Palissot V, Stordeur P, Berchem G. Epigenetic Activity of Peroxisome Proliferator-Activated Receptor Gamma Agonists Increases the Anticancer Effect of Histone Deacetylase Inhibitors on Multiple Myeloma Cells. *PLoS One* 2015; **10**: e0130339 [PMID: 26091518 DOI: 10.1371/journal.pone.0130339]
14 **Peters JM**, Shah YM, Gonzalez FJ. The role of peroxisome proliferator-activated receptors in carcinogenesis and chemoprevention. *Nat Rev Cancer* 2012; **12**: 181-195 [PMID: 22318237 DOI: 10.1038/nrc3214]
15 **Tyagi S**, Gupta P, Saini AS, Kaushal C, Sharma S. The peroxisome proliferator-activated receptor: A family of nuclear receptors role in various diseases. *J Adv Pharm Technol Res* 2011; **2**: 236-240 [PMID: 22247890 DOI: 10.4103/2231-4040.90879]
16 **Grygiel-Górniak B**. Peroxisome proliferator-activated receptors and their ligands: nutritional and clinical implications--a review. *Nutr J* 2014; **13**: 17 [PMID: 24524207 DOI: 10.1186/1475-2891-13-17]
17 **Guan Y**. Peroxisome proliferator-activated receptor family and its relationship to renal complications of the metabolic syndrome. *J Am Soc Nephrol* 2004; **15**: 2801-2815 [PMID: 15504933 DOI: 10.1097/01.ASN.0000139067.83419.46]
18 **Wu L**, Guo C, Wu J. Therapeutic potential of PPARγ natural agonists in liver diseases. *J Cell Mol Med* 2020; **24**: 2736-2748 [PMID: 32031298 DOI: 10.1111/jcmm.15028]
19 **Koga H**, Harada M, Ohtsubo M, Shishido S, Kumemura H, Hanada S, Taniguchi E, Yamashita K, Kumashiro R, Ueno T, Sata M. Troglitazone induces p27Kip1-associated cell-cycle arrest through down-regulating Skp2 in human hepatoma cells. *Hepatology* 2003; **37**: 1086-1096 [PMID: 12717389 DOI: 10.1053/jhep.2003.50186]
20 **Cheung KF**, Zhao J, Hao Y, Li X, Lowe AW, Cheng AS, Sung JJ, Yu J. CITED2 is a novel direct effector of peroxisome proliferator-activated receptor γ in suppressing hepatocellular carcinoma cell growth. *Cancer* 2013; **119**: 1217-1226 [PMID: 23212831 DOI: 10.1002/cncr.27865]
21 **Yu J**, Shen B, Chu ES, Teoh N, Cheung KF, Wu CW, Wang S, Lam CN, Feng H, Zhao J, Cheng AS, To KF, Chan HL, Sung JJ. Inhibitory role of peroxisome proliferator-activated receptor gamma in hepatocarcinogenesis in mice and in vitro. *Hepatology* 2010; **51**: 2008-2019 [PMID: 20512989 DOI: 10.1002/hep.23550]
22 **Yousefnia S**, Momenzadeh S, Seyed Forootan F, Ghaedi K, Nasr Esfahani MH. The influence of peroxisome proliferator-activated receptor γ (PPARγ) ligands on cancer cell tumorigenicity. *Gene* 2018; **649**: 14-22 [PMID: 29369787 DOI: 10.1016/j.gene.2018.01.018]
23 **Bo QF**, Sun XM, Liu J, Sui XM, Li GX. Antitumor action of the peroxisome proliferator-activated receptor-γ agonist rosiglitazone in hepatocellular carcinoma. *Oncol Lett* 2015; **10**: 1979-1984 [PMID: 26622783 DOI: 10.3892/ol.2015.3554]
24 **Zhang Q**, Xiang S, Liu Q, Gu T, Yao Y, Lu X. PPARγ Antagonizes Hypoxia-Induced Activation of Hepatic Stellate Cell through Cross Mediating PI3K/AKT and cGMP/PKG Signaling. *PPAR Res* 2018; **2018**: 6970407 [PMID: 29686697 DOI: 10.1155/2018/6970407]
25 **Shen B**, Chu ES, Zhao G, Man K, Wu CW, Cheng JT, Li G, Nie Y, Lo CM, Teoh N, Farrell GC, Sung JJ, Yu J. PPAR gamma inhibits hepatocellular carcinoma metastases *in vitro* and in mice. *Br J Cancer* 2012; **106**: 1486-1494 [PMID: 22472882 DOI: 10.1038/bjc.2012.130]
26 **Mahmood DFD**, Jguirim-Souissi I, Khadija EH, Blondeau N, Diderot V, Amrani S, Slimane MN, Syrovets T, Simmet T, Rouis M. Peroxisome proliferator-activated receptor gamma induces apoptosis and inhibits autophagy of human monocyte-derived macrophages *via* induction of cathepsin L: potential role in atherosclerosis. *J Biol Chem* 2011; **286**: 28858-28866 [PMID: 21700710 DOI: 10.1074/jbc.M111.273292]
27 **Sun M**, Tan L, Hu M. The role of autophagy in hepatic fibrosis. *Am J Transl Res* 2021; **13**: 5747-5757 [PMID: 34306323]
28 **Mastropasqua F**, Girolimetti G, Shoshan M. PGC1α: Friend or Foe in Cancer? *Genes (Basel)* 2018; **9** [PMID: 29361779 DOI: 10.3390/genes9010048]
29 **Zuo Q**, He J, Zhang S, Wang H, Jin G, Jin H, Cheng Z, Tao X, Yu C, Li B, Yang C, Wang S, Lv Y, Zhao F, Yao M, Cong W, Wang C, Qin W. PPARγ Coactivator-1α Suppresses Metastasis of Hepatocellular Carcinoma by Inhibiting Warburg Effect by PPARγ-Dependent WNT/β-Catenin/Pyruvate Dehydrogenase Kinase Isozyme 1 Axis. *Hepatology* 2021; **73**: 644-660 [PMID: 32298475 DOI: 10.1002/hep.31280]
30 **Kim H**, Lee JY, Park SJ, Kwag E, Koo O, Shin JH. ZNF746/PARIS promotes the occurrence of hepatocellular carcinoma. *Biochem Biophys Res Commun* 2021; **563**: 98-104 [PMID: 34062393 DOI: 10.1016/j.bbrc.2021.05.051]
31 **Bost F**, Kaminski L. The metabolic modulator PGC-1α in cancer. *Am J Cancer Res* 2019; **9**: 198-211 [PMID: 30906622]
32 **Gerhold DL**, Liu F, Jiang G, Li Z, Xu J, Lu M, Sachs JR, Bagchi A, Fridman A, Holder DJ, Doebber TW, Berger J,

Elbrecht A, Moller DE, Zhang BB. Gene expression profile of adipocyte differentiation and its regulation by peroxisome proliferator-activated receptor-gamma agonists. *Endocrinology* 2002; **143**: 2106-2118 [PMID: 12021175 DOI: 10.1210/endo.143.6.8842]

33 **Yu J**, Qiao L, Zimmermann L, Ebert MP, Zhang H, Lin W, Röcken C, Malfertheiner P, Farrell GC. Troglitazone inhibits tumor growth in hepatocellular carcinoma in vitro and in vivo. *Hepatology* 2006; **43**: 134-143 [PMID: 16374840 DOI: 10.1002/hep.20994]

34 **Tu K**, Zheng X, Dou C, Li C, Yang W, Yao Y, Liu Q. MicroRNA-130b promotes cell aggressiveness by inhibiting peroxisome proliferator-activated receptor gamma in human hepatocellular carcinoma. *Int J Mol Sci* 2014; **15**: 20486-20499 [PMID: 25387077 DOI: 10.3390/ijms151120486]

35 **Liu Z**, Wang Y, Dou C, Sun L, Li Q, Wang L, Xu Q, Yang W, Liu Q, Tu K. MicroRNA-1468 promotes tumor progression by activating PPAR-γ-mediated AKT signaling in human hepatocellular carcinoma. *J Exp Clin Cancer Res* 2018; **37**: 49 [PMID: 29510736 DOI: 10.1186/s13046-018-0717-3]

36 **Hyun S**, Kim MS, Song YS, Bak Y, Ham SY, Lee DH, Hong J, Yoon DY. Peroxisome proliferator-activated receptor-gamma agonist 4-O-methylhonokiol induces apoptosis by triggering the intrinsic apoptosis pathway and inhibiting the PI3K/Akt survival pathway in SiHa human cervical cancer cells. *J Microbiol Biotechnol* 2015; **25**: 334-342 [PMID: 25563418 DOI: 10.4014/jmb.1411.11073]

37 **Walter I**, Schulz U, Vogelhuber M, Wiedmann K, Endlicher E, Klebl F, Andreesen R, Herr W, Ghibelli L, Hackl C, Wiest R, Reichle A. Communicative reprogramming non-curative hepatocellular carcinoma with low-dose metronomic chemotherapy, COX-2 inhibitor and PPAR-gamma agonist: a phase II trial. *Med Oncol* 2017; **34**: 192 [PMID: 29098441 DOI: 10.1007/s12032-017-1040-0]

38 **Yip TC**, Wong VW, Chan HL, Tse YK, Hui VW, Liang LY, Lee HW, Lui GC, Kong AP, Wong GL. Thiazolidinediones reduce the risk of hepatocellular carcinoma and hepatic events in diabetic patients with chronic hepatitis B. *J Viral Hepat* 2020; **27**: 904-914 [PMID: 32340077 DOI: 10.1111/jvh.13307]

39 **Lai SW**, Chen PC, Liao KF, Muo CH, Lin CC, Sung FC. Risk of hepatocellular carcinoma in diabetic patients and risk reduction associated with anti-diabetic therapy: a population-based cohort study. *Am J Gastroenterol* 2012; **107**: 46-52 [PMID: 22085817 DOI: 10.1038/ajg.2011.384]

40 **Lin HC**, Hsu YT, Kachingwe BH, Hsu CY, Uang YS, Wang LH. Dose effect of thiazolidinedione on cancer risk in type 2 diabetes mellitus patients: a six-year population-based cohort study. *J Clin Pharm Ther* 2014; **39**: 354-360 [PMID: 24661226 DOI: 10.1111/jcpt.12151]

41 **Huang MY**, Chung CH, Chang WK, Lin CS, Chen KW, Hsieh TY, Chien WC, Lin HH. The role of thiazolidinediones in hepatocellular carcinoma risk reduction: a population-based cohort study in Taiwan. *Am J Cancer Res* 2017; **7**: 1606-1616 [PMID: 28744408]

42 **Chang CH**, Lin JW, Wu LC, Lai MS, Chuang LM, Chan KA. Association of thiazolidinediones with liver cancer and colorectal cancer in type 2 diabetes mellitus. *Hepatology* 2012; **55**: 1462-1472 [PMID: 22135104 DOI: 10.1002/hep.25509]

43 **Yan H**, Wu W, Chang X, Xia M, Ma S, Wang L, Gao J. Gender differences in the efficacy of pioglitazone treatment in nonalcoholic fatty liver disease patients with abnormal glucose metabolism. *Biol Sex Differ* 2021; **12**: 1 [PMID: 33397443 DOI: 10.1186/s13293-020-00344-1]

44 **Li S**, Ghoshal S, Sojoodi M, Arora G, Masia R, Erstad DJ, Lanuti M, Hoshida Y, Baumert TF, Tanabe KK, Fuchs BC. Pioglitazone Reduces Hepatocellular Carcinoma Development in Two Rodent Models of Cirrhosis. *J Gastrointest Surg* 2019; **23**: 101-111 [PMID: 30367397 DOI: 10.1007/s11605-018-4004-6]

45 **Yang Y**, Zhao LH, Huang B, Wang RY, Yuan SX, Tao QF, Xu Y, Sun HY, Lin C, Zhou WP. Pioglitazone, a PPARγ agonist, inhibits growth and invasion of human hepatocellular carcinoma *via* blockade of the rage signaling. *Mol Carcinog* 2015; **54**: 1584-1595 [PMID: 25307746 DOI: 10.1002/mc.22231]

46 **Sumie S**, Kawaguchi T, Kawaguchi A, Kuromatsu R, Nakano M, Satani M, Yamada S, Okamura S, Yonezawa Y, Kakuma T, Torimura T, Sata M. Effect of pioglitazone on outcome following curative treatment for hepatocellular carcinoma in patients with hepatitis C virus infection: A prospective study. *Mol Clin Oncol* 2015; **3**: 115-120 [PMID: 25469280 DOI: 10.3892/mco.2014.435]

47 **Chongmelaxme B**, Phisalprapa P, Sawangjit R, Dilokthornsakul P, Chaiyakunapruk N. Weight Reduction and Pioglitazone are Cost-Effective for the Treatment of Non-Alcoholic Fatty Liver Disease in Thailand. *Pharmacoeconomics* 2019; **37**: 267-278 [PMID: 30430467 DOI: 10.1007/s40273-018-0736-0]

48 **Shah P**, Mudaliar S. Pioglitazone: side effect and safety profile. *Expert Opin Drug Saf* 2010; **9**: 347-354 [PMID: 20175701 DOI: 10.1517/14740331003623218]

49 **Li X**, He J, Li B, Gao M, Zeng Y, Lian J, Shi C, Huang Y, He F. The PPARγ agonist rosiglitazone sensitizes the BH3 mimetic (-)-gossypol to induce apoptosis in cancer cells with high level of Bcl-2. *Mol Carcinog* 2018; **57**: 1213-1222 [PMID: 29856104 DOI: 10.1002/mc.22837]

50 **Cao LQ**, Shao ZL, Liang HH, Zhang DW, Yang XW, Jiang XF, Xue P. Activation of peroxisome proliferator-activated receptor-γ (PPARγ) inhibits hepatoma cell growth *via* downregulation of SEPT2 expression. *Cancer Lett* 2015; **359**: 127-135 [PMID: 25592041 DOI: 10.1016/j.canlet.2015.01.004]

51 **Ratziu V**, Charlotte F, Bernhardt C, Giral P, Halbron M, Lenaour G, Hartmann-Heurtier A, Bruckert E, Poynard T; LIDO Study Group. Long-term efficacy of rosiglitazone in nonalcoholic steatohepatitis: results of the fatty liver improvement by rosiglitazone therapy (FLIRT 2) extension trial. *Hepatology* 2010; **51**: 445-453 [PMID: 19877169 DOI: 10.1002/hep.23270]

52 **Mitka M**. Panel recommends easing restrictions on rosiglitazone despite concerns about cardiovascular safety. *JAMA* 2013; **310**: 246-247 [PMID: 23860970 DOI: 10.1001/jama.2013.8141]

53 **Oura K**, Tadokoro T, Fujihara S, Morishita A, Chiyo T, Samukawa E, Yamana Y, Fujita K, Sakamoto T, Nomura T, Yoneyama H, Kobara H, Mori H, Iwama H, Okano K, Suzuki Y, Masaki T. Telmisartan inhibits hepatocellular carcinoma cell proliferation in vitro by inducing cell cycle arrest. *Oncol Rep* 2017; **38**: 2825-2835 [PMID: 29048654 DOI: 10.3892/or.2017.5977]

54 **Saber S**, Khodir AE, Soliman WE, Salama MM, Abdo WS, Elsaeed B, Nader K, Abdelnasser A, Megahed N, Basuony M, Shawky A, Mahmoud M, Medhat R, Eldin AS. Telmisartan attenuates N-nitrosodiethylamine-induced hepatocellular carcinoma in mice by modulating the NF-κB-TAK1-ERK1/2 axis in the context of PPARγ agonistic activity. *Naunyn Schmiedebergs Arch Pharmacol* 2019; **392**: 1591-1604 [PMID: 31367864 DOI: 10.1007/s00210-019-01706-2]

55 **Unger T**, Schupp M. Telmisartan: from lowering blood pressure to end-organ protection. *Future Cardiol* 2005; **1**: 7-15 [PMID: 19804057 DOI: 10.1517/14796678.1.1.7]

56 **Li MY**, Deng H, Zhao JM, Dai D, Tan XY. Peroxisome proliferator-activated receptor gamma ligands inhibit cell growth and induce apoptosis in human liver cancer BEL-7402 cells. *World J Gastroenterol* 2003; **9**: 1683-1688 [PMID: 12918101 DOI: 10.3748/wjg.v9.i8.1683]

57 **Zhou YM**, Wen YH, Kang XY, Qian HH, Yang JM, Yin ZF. Troglitazone, a peroxisome proliferator-activated receptor gamma ligand, induces growth inhibition and apoptosis of HepG2 human liver cancer cells. *World J Gastroenterol* 2008; **14**: 2168-2173 [PMID: 18407589 DOI: 10.3748/wjg.14.2168]

58 **Rumi MA**, Sato H, Ishihara S, Kawashima K, Hamamoto S, Kazumori H, Okuyama T, Fukuda R, Nagasue N, Kinoshita Y. Peroxisome proliferator-activated receptor gamma ligand-induced growth inhibition of human hepatocellular carcinoma. *Br J Cancer* 2001; **84**: 1640-1647 [PMID: 11401318 DOI: 10.1054/bjoc.2001.1821]

59 **Toyoda M**, Takagi H, Horiguchi N, Kakizaki S, Sato K, Takayama H, Mori M. A ligand for peroxisome proliferator activated receptor gamma inhibits cell growth and induces apoptosis in human liver cancer cells. *Gut* 2002; **50**: 563-567 [PMID: 11889080 DOI: 10.1136/gut.50.4.563]

60 **Kores K**, Konc J, Bren U. Mechanistic Insights into Side Effects of Troglitazone and Rosiglitazone Using a Novel Inverse Molecular Docking Protocol. *Pharmaceutics* 2021; **13** [PMID: 33670968 DOI: 10.3390/pharmaceutics13030315]

61 **Kumar DP**, Caffrey R, Marioneaux J, Santhekadur PK, Bhat M, Alonso C, Koduru SV, Philip B, Jain MR, Giri SR, Bedossa P, Sanyal AJ. The PPAR α/γ Agonist Saroglitazar Improves Insulin Resistance and Steatohepatitis in a Diet Induced Animal Model of Nonalcoholic Fatty Liver Disease. *Sci Rep* 2020; **10**: 9330 [PMID: 32518275 DOI: 10.1038/s41598-020-66458-z]

62 **Hassan NF**, Nada SA, Hassan A, El-Ansary MR, Al-Shorbagy MY, Abdelsalam RM. Saroglitazar Deactivates the Hepatic LPS/TLR4 Signaling Pathway and Ameliorates Adipocyte Dysfunction in Rats with High-Fat Emulsion/LPS Model-Induced Non-alcoholic Steatohepatitis. *Inflammation* 2019; **42**: 1056-1070 [PMID: 30737662 DOI: 10.1007/s10753-019-00967-6]

63 **Mitra A**. An Observational Study of Reduction in Glycemic Parameters and Liver Stiffness by Saroglitazar 4 mg in Patients With Type 2 Diabetes Mellitus and Nonalcoholic Fatty Liver Disease. *Cureus* 2020; **12**: e9065 [PMID: 32782883 DOI: 10.7759/cureus.9065]

64 **Shafi SM**, Yattoo GN. Spectrum, Clinico-pathological Profile of Non-Alcoholic Fatty Liver Disease and its Treatment Response with 48 wk Therapy of Saroglitazar. *JMS SKIMS* 2021; 24

65 **Sosale A**, Saboo B, Sosale B. Saroglitazar for the treatment of hypertrig-lyceridemia in patients with type 2 diabetes: current evidence. *Diabetes Metab Syndr Obes* 2015; **8**: 189-196 [PMID: 25926748 DOI: 10.2147/DMSO.S49592]

66 **Vara D**, Morell C, Rodríguez-Henche N, Diaz-Laviada I. Involvement of PPARγ in the antitumoral action of cannabinoids on hepatocellular carcinoma. *Cell Death Dis* 2013; **4**: e618 [PMID: 23640460 DOI: 10.1038/cddis.2013.141]

67 **Guzmán M**, Duarte MJ, Blázquez C, Ravina J, Rosa MC, Galve-Roperh I, Sánchez C, Velasco G, González-Feria L. A pilot clinical study of Delta9-tetrahydrocannabinol in patients with recurrent glioblastoma multiforme. *Br J Cancer* 2006; **95**: 197-203 [PMID: 16804518 DOI: 10.1038/sj.bjc.6603236]

68 **Nadal X**, Del Río C, Casano S, Palomares B, Ferreiro-Vera C, Navarrete C, Sánchez-Carnerero C, Cantarero I, Bellido ML, Meyer S, Morello G, Appendino G, Muñoz E. Tetrahydrocannabinolic acid is a potent PPARγ agonist with neuroprotective activity. *Br J Pharmacol* 2017; **174**: 4263-4276 [PMID: 28853159 DOI: 10.1111/bph.14019]

69 **Cohen K**, Weizman A, Weinstein A. Positive and Negative Effects of Cannabis and Cannabinoids on Health. *Clin Pharmacol Ther* 2019; **105**: 1139-1147 [PMID: 30703255 DOI: 10.1002/cpt.1381]

70 **Bellocchio L**, Inchingolo AD, Inchingolo AM, Lorusso F, Malcangi G, Santacroce L, Scarano A, Bordea IR, Hazballa D, D'Oria MT, Isacco CG, Nucci L, Serpico R, Tartaglia GM, Giovanniello D, Contaldo M, Farronato M, Dipalma G, Inchingolo F. Cannabinoids Drugs and Oral Health-From Recreational Side-Effects to Medicinal Purposes: A Systematic Review. *Int J Mol Sci* 2021; **22** [PMID: 34361095 DOI: 10.3390/ijms22158329]

71 **Choi JH**, Jin SW, Choi CY, Kim HG, Lee GH, Kim YA, Chung YC, Jeong HG. Capsaicin Inhibits Dimethylnitrosamine-Induced Hepatic Fibrosis by Inhibiting the TGF-β1/Smad Pathway *via* Peroxisome Proliferator-Activated Receptor Gamma Activation. *J Agric Food Chem* 2017; **65**: 317-326 [PMID: 27991776 DOI: 10.1021/acs.jafc.6b04805]

72 **Clark R**, Lee SH. Anticancer Properties of Capsaicin Against Human Cancer. *Anticancer Res* 2016; **36**: 837-843 [PMID: 26976969]

73 **Collier JG**, Fuller RW. Capsaicin inhalation in man and the effects of sodium cromoglycate. *Br J Pharmacol* 1984; **81**: 113-117 [PMID: 6423016 DOI: 10.1111/j.1476-5381.1984.tb10750.x]

74 **Midgren B**, Hansson L, Karlsson JA, Simonsson BG, Persson CG. Capsaicin-induced cough in humans. *Am Rev Respir Dis* 1992; **146**: 347-351 [PMID: 1489123 DOI: 10.1164/ajrccm/146.2.347]

75 **Hakas JF Jr**. Topical capsaicin induces cough in patient receiving ACE inhibitor. *Ann Allergy* 1990; **65**: 322-323 [PMID: 2221491]

76 **Morice AH**, Brown MJ, Higenbottam T. Cough associated with angiotensin converting enzyme inhibition. *J Cardiovasc Pharmacol* 1989; **13** Suppl 3: S59-S62 [PMID: 2474106 DOI: 10.1097/00005344-198900133-00015]

77 **Patial V**, S M, Sharma S, Pratap K, Singh D, Padwad YS. Synergistic effect of curcumin and piperine in suppression of DENA-induced hepatocellular carcinoma in rats. *Environ Toxicol Pharmacol* 2015; **40**: 445-452 [PMID: 26278679 DOI: 10.1016/j.etap.2015.07.012]

78 **Pan Z**, Zhuang J, Ji C, Cai Z, Liao W, Huang Z. Curcumin inhibits hepatocellular carcinoma growth by targeting VEGF expression. *Oncol Lett* 2018; **15**: 4821-4826 [PMID: 29552121 DOI: 10.3892/ol.2018.7988]

79 **Teng CF**, Yu CH, Chang HY, Hsieh WC, Wu TH, Lin JH, Wu HC, Jeng LB, Su IJ. Chemopreventive Effect of

Phytosomal Curcumin on Hepatitis B Virus-Related Hepatocellular Carcinoma in A Transgenic Mouse Model. *Sci Rep* 2019; **9**: 10338 [PMID: 31316146 DOI: 10.1038/s41598-019-46891-5]

80 **Zheng S**, Chen A. Disruption of transforming growth factor-beta signaling by curcumin induces gene expression of peroxisome proliferator-activated receptor-gamma in rat hepatic stellate cells. *Am J Physiol Gastrointest Liver Physiol* 2007; **292**: G113-G123 [PMID: 16959952 DOI: 10.1152/ajpgi.00200.2006]

81 **Li J**, Wei H, Liu Y, Li Q, Guo H, Guo Y, Chang Z. Curcumin Inhibits Hepatocellular Carcinoma *via* Regulating miR-21/TIMP3 Axis. *Evid Based Complement Alternat Med* 2020; **2020**: 2892917 [PMID: 32724322 DOI: 10.1155/2020/2892917]

82 **Shao J**, Shi CJ, Li Y, Zhang FW, Pan FF, Fu WM, Zhang JF. LincROR Mediates the Suppressive Effects of Curcumin on Hepatocellular Carcinoma Through Inactivating Wnt/β-Catenin Signaling. *Front Pharmacol* 2020; **11**: 847 [PMID: 32714183 DOI: 10.3389/fphar.2020.00847]

83 **Sayed MM**, El-Kordy EA. The protective effect of curcumin on paracetamol-induced liver damage in adult male rabbits: biochemical and histological studies. *Egyptian J Histol* 2014; **37**: 629-39

84 **Anand P**, Kunnumakkara AB, Newman RA, Aggarwal BB. Bioavailability of curcumin: problems and promises. *Mol Pharm* 2007; **4**: 807-818 [PMID: 17999464 DOI: 10.1021/mp700113r]

85 **Mahmoud AM**, Mohammed HM, Khadrawy SM, Galaly SR. Hesperidin protects against chemically induced hepatocarcinogenesis *via* modulation of Nrf2/ARE/HO-1, PPARγ and TGF-β1/Smad3 signaling, and amelioration of oxidative stress and inflammation. *Chem Biol Interact* 2017; **277**: 146-158 [PMID: 28935427 DOI: 10.1016/j.cbi.2017.09.015]

86 **Mo'men YS**, Hussein RM, Kandeil MA. Involvement of PI3K/Akt pathway in the protective effect of hesperidin against a chemically induced liver cancer in rats. *J Biochem Mol Toxicol* 2019; **33**: e22305 [PMID: 30779474 DOI: 10.1002/jbt.22305]

87 **Zaghloul RA**, Elsherbiny NM, Kenawy HI, El-Karef A, Eissa LA, El-Shishtawy MM. Hepatoprotective effect of hesperidin in hepatocellular carcinoma: Involvement of Wnt signaling pathways. *Life Sci* 2017; **185**: 114-125 [PMID: 28754618 DOI: 10.1016/j.lfs.2017.07.026]

88 **Ameer B**, Weintraub RA, Johnson JV, Yost RA, Rouseff RL. Flavanone absorption after naringin, hesperidin, and citrus administration. *Clin Pharmacol Ther* 1996; **60**: 34-40 [PMID: 8689809 DOI: 10.1016/S0009-9236(96)90164-2]

89 **Han M**, Gao H, Ju P, Gao MQ, Yuan YP, Chen XH, Liu KL, Han YT, Han ZW. Hispidulin inhibits hepatocellular carcinoma growth and metastasis through AMPK and ERK signaling mediated activation of PPARγ. *Biomed Pharmacother* 2018; **103**: 272-283 [PMID: 29656183 DOI: 10.1016/j.biopha.2018.04.014]

90 **Lv L**, Zhang W, Li T, Jiang L, Lu X, Lin J. Hispidulin exhibits potent anticancer activity in vitro and in vivo through activating ER stress in nonsmallcell lung cancer cells. *Oncol Rep* 2020; **43**: 1995-2003 [PMID: 32236602 DOI: 10.3892/or.2020.7568]

91 **Kavvadias D**, Sand P, Youdim KA, Qaiser MZ, Rice-Evans C, Baur R, Sigel E, Rausch WD, Riederer P, Schreier P. The flavone hispidulin, a benzodiazepine receptor ligand with positive allosteric properties, traverses the blood-brain barrier and exhibits anticonvulsive effects. *Br J Pharmacol* 2004; **142**: 811-820 [PMID: 15231642 DOI: 10.1038/sj.bjp.0705828]

92 **Chen LC**, Hsu KC, Chiou LC, Tseng HJ, Huang WJ. Total Synthesis and Metabolic Stability of Hispidulin and Its d-Labelled Derivative. *Molecules* 2017; **22** [PMID: 29113055 DOI: 10.3390/molecules22111897]

93 **Moselhy J**, Srinivasan S, Ankem MK, Damodaran C. Natural Products That Target Cancer Stem Cells. *Anticancer Res* 2015; **35**: 5773-5788 [PMID: 26503998]

94 **Xiao Y**, Gong Q, Wang W, Liu F, Kong Q, Pan F, Zhang X, Yu C, Hu S, Fan F, Li S, Liu Y. The combination of Biochanin A and SB590885 potentiates the inhibition of tumour progression in hepatocellular carcinoma. *Cancer Cell Int* 2020; **20**: 371 [PMID: 32774165 DOI: 10.1186/s12935-020-01463-w]

95 **Sanaei M**, Kavoosi F, Valiani A, Ghobadifar MA. Effect of Genistein on Apoptosis and Proliferation of Hepatocellular Carcinoma Hepa1-6 Cell Line. *Int J Prev Med* 2018; **9**: 12 [PMID: 29541427 DOI: 10.4103/ijpvm.IJPVM_249_16]

96 **Li S**, Li J, Dai W, Zhang Q, Feng J, Wu L, Liu T, Yu Q, Xu S, Wang W, Lu X, Chen K, Xia Y, Lu J, Zhou Y, Fan X, Mo W, Xu L, Guo C. Genistein suppresses aerobic glycolysis and induces hepatocellular carcinoma cell death. *Br J Cancer* 2017; **117**: 1518-1528 [PMID: 28926527 DOI: 10.1038/bjc.2017.323]

97 **Sharp GB**, Lagarde F, Mizuno T, Sauvaget C, Fukuhara T, Allen N, Suzuki G, Tokuoka S. Relationship of hepatocellular carcinoma to soya food consumption: a cohort-based, case-control study in Japan. *Int J Cancer* 2005; **115**: 290-295 [PMID: 15688396 DOI: 10.1002/ijc.20897]

98 **Zamora-Ros R**, Fedirko V, Trichopoulou A, González CA, Bamia C, Trepo E, Nöthlings U, Duarte-Salles T, Serafini M, Bredsdorff L, Overvad K, Tjønneland A, Halkjaer J, Fagherazzi G, Perquier F, Boutron-Ruault MC, Katzke V, Lukanova A, Floegel A, Boeing H, Lagiou P, Trichopoulos D, Saieva C, Agnoli C, Mattiello A, Tumino R, Sacerdote C, Bueno-de-Mesquita HB, Peeters PH, Weiderpass E, Engeset D, Skeie G, Argüelles MV, Molina-Montes E, Dorronsoro M, Tormo MJ, Ardanaz E, Ericson U, Sonestedt E, Sund M, Landberg R, Khaw KT, Wareham NJ, Crowe FL, Riboli E, Jenab M. Dietary flavonoid, lignan and antioxidant capacity and risk of hepatocellular carcinoma in the European prospective investigation into cancer and nutrition study. *Int J Cancer* 2013; **133**: 2429-2443 [PMID: 23649669 DOI: 10.1002/ijc.28257]

99 **Yu J**, Bi X, Yu B, Chen D. Isoflavones: Anti-Inflammatory Benefit and Possible Caveats. *Nutrients* 2016; **8** [PMID: 27294954 DOI: 10.3390/nu8060361]

100 **Zhou Y**, Guo Y, Zhu Y, Sun Y, Li W, Li Z, Wei L. Dual PPARγ/α agonist oroxyloside suppresses cell cycle progression by glycolipid metabolism switch-mediated increase of reactive oxygen species levels. *Free Radic Biol Med* 2021; **167**: 205-217 [PMID: 33713839 DOI: 10.1016/j.freeradbiomed.2021.02.032]

101 **Rawat D**, Chhonker SK, Naik RA, Koiri RK. Modulation of antioxidant enzymes, SIRT1 and NF-κB by resveratrol and nicotinamide in alcohol-aflatoxin B1-induced hepatocellular carcinoma. *J Biochem Mol Toxicol* 2021; **35**: e22625 [PMID: 32894639 DOI: 10.1002/jbt.22625]

102 **Jagwani S**, Jalalpure S, Dhamecha D, Jadhav K, Bohara R. Pharmacokinetic and Pharmacodynamic Evaluation of Resveratrol Loaded Cationic Liposomes for Targeting Hepatocellular Carcinoma. *ACS Biomater Sci Eng* 2020; **6**: 4969-

4984 [PMID: 33455290 DOI: 10.1021/acsbiomaterials.0c00429]
103 **Santos AC**, Pereira I, Magalhães M, Pereira-Silva M, Caldas M, Ferreira L, Figueiras A, Ribeiro AJ, Veiga F. Targeting Cancer Via Resveratrol-Loaded Nanoparticles Administration: Focusing on In Vivo Evidence. *AAPS J* 2019; **21**: 57 [PMID: 31016543 DOI: 10.1208/s12248-019-0325-y]
104 **Shaito A**, Posadino AM, Younes N, Hasan H, Halabi S, Alhababi D, Al-Mohannadi A, Abdel-Rahman WM, Eid AH, Nasrallah GK, Pintus G. Potential Adverse Effects of Resveratrol: A Literature Review. *Int J Mol Sci* 2020; **21** [PMID: 32197410 DOI: 10.3390/ijms21062084]
105 **Fujimori K**, Shibano M. Avicularin, a plant flavonoid, suppresses lipid accumulation through repression of C/EBPα-activated GLUT4-mediated glucose uptake in 3T3-L1 cells. *J Agric Food Chem* 2013; **61**: 5139-5147 [PMID: 23647459 DOI: 10.1021/jf401154c]
106 **Guo XF**, Liu JP, Ma SQ, Zhang P, Sun WD. Avicularin reversed multidrug-resistance in human gastric cancer through enhancing Bax and BOK expressions. *Biomed Pharmacother* 2018; **103**: 67-74 [PMID: 29635130 DOI: 10.1016/j.biopha.2018.03.110]
107 **Wang Z**, Li F, Quan Y, Shen J. Avicularin ameliorates human hepatocellular carcinoma *via* the regulation of NFκB/COX2/PPARγ activities. *Mol Med Rep* 2019; **19**: 5417-5423 [PMID: 31059053 DOI: 10.3892/mmr.2019.10198]
108 **Atanasov AG**, Wang JN, Gu SP, Bu J, Kramer MP, Baumgartner L, Fakhrudin N, Ladurner A, Malainer C, Vuorinen A, Noha SM, Schwaiger S, Rollinger JM, Schuster D, Stuppner H, Dirsch VM, Heiss EH. Honokiol: a non-adipogenic PPARγ agonist from nature. *Biochim Biophys Acta* 2013; **1830**: 4813-4819 [PMID: 23811337 DOI: 10.1016/j.bbagen.2013.06.021]
109 **Kotani H**, Tanabe H, Mizukami H, Amagaya S, Inoue M. A naturally occurring rexinoid, honokiol, can serve as a regulator of various retinoid x receptor heterodimers. *Biol Pharm Bull* 2012; **35**: 1-9 [PMID: 22223330 DOI: 10.1248/bpb.35.1]
110 **Pérez E**, Bourguet W, Gronemeyer H, de Lera AR. Modulation of RXR function through ligand design. *Biochim Biophys Acta* 2012; **1821**: 57-69 [PMID: 21515403 DOI: 10.1016/j.bbalip.2011.04.003]
111 **Kotani H**, Tanabe H, Mizukami H, Makishima M, Inoue M. Identification of a naturally occurring rexinoid, honokiol, that activates the retinoid X receptor. *J Nat Prod* 2010; **73**: 1332-1336 [PMID: 20695472 DOI: 10.1021/np100120c]
112 **Huang C**, Wei YX, Shen MC, Tu YH, Wang CC, Huang HC. Chrysin, Abundant in Morinda citrifolia Fruit Water-EtOAc Extracts, Combined with Apigenin Synergistically Induced Apoptosis and Inhibited Migration in Human Breast and Liver Cancer Cells. *J Agric Food Chem* 2016; **64**: 4235-4245 [PMID: 27137679 DOI: 10.1021/acs.jafc.6b00766]
113 **Zhang Y**, Zhao J, Afzal O, Kazmi I, Al-Abbasi FA, Altamimi ASA, Yang Z. Neuroprotective role of chrysin-loaded poly(lactic-co-glycolic acid) nanoparticle against kindling-induced epilepsy through Nrf2/ARE/HO-1 pathway. *J Biochem Mol Toxicol* 2021; **35**: e22634 [PMID: 32991785 DOI: 10.1002/jbt.22634]
114 **Dai W**, Gao Q, Qiu J, Yuan J, Wu G, Shen G. Quercetin induces apoptosis and enhances 5-FU therapeutic efficacy in hepatocellular carcinoma. *Tumour Biol* 2016; **37**: 6307-6313 [PMID: 26628295 DOI: 10.1007/s13277-015-4501-0]
115 **Srisa-Nga K**, Mankhetkorn S, Okonogi S, Khonkarn R. Delivery of Superparamagnetic Polymeric Micelles Loaded With Quercetin to Hepatocellular Carcinoma Cells. *J Pharm Sci* 2019; **108**: 996-1006 [PMID: 30121312 DOI: 10.1016/j.xphs.2018.08.008]
116 **Casella ML**, Parody JP, Ceballos MP, Quiroga AD, Ronco MT, Francés DE, Monti JA, Pisani GB, Carnovale CE, Carrillo MC, de Luján Alvarez M. Quercetin prevents liver carcinogenesis by inducing cell cycle arrest, decreasing cell proliferation and enhancing apoptosis. *Mol Nutr Food Res* 2014; **58**: 289-300 [PMID: 24124108 DOI: 10.1002/mnfr.201300362]
117 **Yu D**, Ye T, Xiang Y, Shi Z, Zhang J, Lou B, Zhang F, Chen B, Zhou M. Quercetin inhibits epithelial-mesenchymal transition, decreases invasiveness and metastasis, and reverses IL-6 induced epithelial-mesenchymal transition, expression of MMP by inhibiting STAT3 signaling in pancreatic cancer cells. *Onco Targets Ther* 2017; **10**: 4719-4729 [PMID: 29026320 DOI: 10.2147/OTT.S136840]
118 **Wu L**, Li J, Liu T, Li S, Feng J, Yu Q, Zhang J, Chen J, Zhou Y, Ji J, Chen K, Mao Y, Wang F, Dai W, Fan X, Wu J, Guo C. Quercetin shows anti-tumor effect in hepatocellular carcinoma LM3 cells by abrogating JAK2/STAT3 signaling pathway. *Cancer Med* 2019; **8**: 4806-4820 [PMID: 31273958 DOI: 10.1002/cam4.2388]
119 **Cai X**, Fang Z, Dou J, Yu A, Zhai G. Bioavailability of quercetin: problems and promises. *Curr Med Chem* 2013; **20**: 2572-2582 [PMID: 23514412 DOI: 10.2174/09298673113209990120]
120 **Galbraith LCA**, Mui E, Nixon C, Hedley A, Strachan D, MacKay G, Sumpton D, Sansom OJ, Leung HY, Ahmad I. PPAR-gamma induced AKT3 expression increases levels of mitochondrial biogenesis driving prostate cancer. *Oncogene* 2021; **40**: 2355-2366 [PMID: 33654198 DOI: 10.1038/s41388-021-01707-7]
121 **Schaefer KL**, Wada K, Takahashi H, Matsuhashi N, Ohnishi S, Wolfe MM, Turner JR, Nakajima A, Borkan SC, Saubermann LJ. Peroxisome proliferator-activated receptor gamma inhibition prevents adhesion to the extracellular matrix and induces anoikis in hepatocellular carcinoma cells. *Cancer Res* 2005; **65**: 2251-2259 [PMID: 15781638 DOI: 10.1158/0008-5472.CAN-04-3037]
122 **Palakurthi SS**, Aktas H, Grubissich LM, Mortensen RM, Halperin JA. Anticancer effects of thiazolidinediones are independent of peroxisome proliferator-activated receptor gamma and mediated by inhibition of translation initiation. *Cancer Res* 2001; **61**: 6213-6218 [PMID: 11507074]
123 **Baek SJ**, Wilson LC, Hsi LC, Eling TE. Troglitazone, a peroxisome proliferator-activated receptor gamma (PPAR gamma) ligand, selectively induces the early growth response-1 gene independently of PPAR gamma. A novel mechanism for its anti-tumorigenic activity. *J Biol Chem* 2003; **278**: 5845-5853 [PMID: 12475986]
124 **Gardner OS**, Shiau CW, Chen CS, Graves LM. Peroxisome proliferator-activated receptor gamma-independent activation of p38 MAPK by thiazolidinediones involves calcium/calmodulin-dependent protein kinase II and protein kinase R: correlation with endoplasmic reticulum stress. *J Biol Chem* 2005; **280**: 10109-10118 [PMID: 15649892 DOI: 10.1074/jbc.M410445200]
125 **Galli A**, Ceni E, Mello T, Polvani S, Tarocchi M, Buccoliero F, Lisi F, Cioni L, Ottanelli B, Foresta V, Mastrobuoni G, Moneti G, Pieraccini G, Surrenti C, Milani S. Thiazolidinediones inhibit hepatocarcinogenesis in hepatitis B virus-

transgenic mice by peroxisome proliferator-activated receptor gamma-independent regulation of nucleophosmin. *Hepatology* 2010; **52**: 493-505 [PMID: 20683949 DOI: 10.1002/hep.23669]

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REVIEW

Gut microbiota alteration and modulation in hepatitis B virus-related fibrosis and complications: Molecular mechanisms and therapeutic inventions

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Abstract

Hepatitis B virus (HBV) has posed a threat to public health, mainly resulting in liver damage. With long-term accumulation of extracellular matrix, patients with chronic hepatitis B are at high risk of developing into liver fibrosis and cirrhosis and even life-threatening hepatic carcinoma. The occurrence of complications such as spontaneous bacterial peritonitis and hepatic encephalopathy greatly increases disability and mortality. With deeper understanding of the bidirectional interaction between the liver and the gut (gut-liver axis), there is a growing consensus that the human health closely relates to the gut microbiota. Supported by animal and human studies, the gut microbiota alters as the HBV-related liver fibrosis initials and progresses, characterized as the decrease of the ratio between "good" and "potentially pathogenic" microbes. When the primary disease is controlled *via* antiviral treatment, the gut microbiota dysfunction tends to be improved. Conversely, the recovery of gut microbiota can promote the regression of liver fibrosis. Therapeutic strategies targeted on gut microbiota (rifaximin, probiotics, engineered probiotics and fecal microbiota transplantation) have been applied to animal models and patients, obtaining satisfactory results.

Key Words: Hepatitis B virus; Gut microbiota; Liver fibrosis; Liver cirrhosis; Hepatic encephalopathy; Fecal microbiota transplantation

Core Tip: Intimate connection between the gut microbiota alteration and hepatitis B virus (HBV)-related fibrosis and complications has been supported by animal and human studies. Researchers and clinicians are making effort to control and reverse fibrosis by rebuilding a healthy gut microbiota. We herein discuss the gut microbiota alteration in HBV-related fibrosis and therapies targeted on reconstruction of gut microbiota homeostasis.

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INTRODUCTION

Hepatitis B virus (HBV) has brought about substantial global health problems, giving rise to approximately 1.5 million new infections in 2019[1]. Balancing the pathogenic ability and immunity defense, some patients may experience chronic HBV infection, and even chronic hepatitis B (CHB). The different phrases are designed by the presence of hepatitis B e antigen (HBeAg), HBV DNA levels, alanine aminotransferase (ALT) values and liver inflammation, and CHB is mainly characterized by elevated ALT levels and moderate/severe liver diseases[2]. Chronic HBV infection tends to be asymptomatic initially, however, tissue repair against chronic inflammation may result in an immoderate accumulation of extracellular matrix (ECM), so CHB patients are at high risk of developing progressive fibrosis and life-threatening cirrhosis. Complications, such as portal hypertension, spontaneous bacterial peritonitis (SBP)[3] and hepatic encephalopathy (HE)[4], are difficult to prevent and address. With hepatocellular carcinoma (HCC) coming along stealthily[5], approximately 820000 deaths were caused by HBV infection–related causes in 2019[1].

The human intestine, as an organ directly connected with the outside world, is colonized by microbes progressively after birth[6]. The human gut microbiota is now considered to be composed of approximately 10^{14} bacteria[7], 200-300 fungal species[8] and abundant bacteriophages[9], and is increasingly seen as a significant superorganism[10]. Predominant strains in the adult intestine belong to *Bacteroidetes*, *Firmicutes*, *Actinobacteria* and *Proteobacteria*: *Bacteroidetes* and *Firmicutes* are the most dominant phyla and are mainly composed of gram-negative bacteria and gram-positive clostridia respectively[11]. The composition of the gut microbiota is influenced by age, race, nutrition, diet, immunity, disease and medication use, and has a strong interaction with the host[12-14]. The intimate association between gut microbiota homeostasis and multiple organ disease progression has been confirmed in the past decade, especially in some metabolic disorders[15], and intestinal and liver diseases[16].

The liver is closely connected with the gut *via* the gut-liver axis, defined as the bidirectional interaction between the liver and the gut *via* transport of bile acids, immunoreactive substances, nutrients, *etc.*[17]. When impairment of intestinal barriers and disturbances of the gut microbiota occur, gut-derived microbe/antigen translocation may lead to invasion of the liver. The association between gut microbiota alterations and chronic liver diseases (CLDs) has received great attention.

This review will concentrate on gut microbiota alterations in HBV-related liver fibrosis and summarize the cutting edge of new therapeutic strategies. We will summarize and discuss: (1) Gut microbiota alteration in HBV-related liver fibrosis; (2) Gut microbiota-related mechanisms of liver fibrosis; (3) Gut microbiota dysfunction in liver fibrosis complications; and (4) Gut microbiota-related treatment toward HBV-related fibrosis and complications.

GUT MICROBIOTA ALTERATION IN HBV-RELATED LIVER FIBROSIS

HBV-infected populations tend to obtain a gut microbiota that differs from that of healthy people (Table 1). Depending on host and viral factors, patients with HBV infection may experience different phrases[2]. In this part, gut microbiota alteration in the HBV persistence and different stages of HBV infection will be discussed.

HBV persistence

After the infection, HBV may be spontaneously cleared or cause chronic infection in different individuals: 95% of adult-acquired infections result in spontaneous clearance, while over 90% of newborn infections lead to chronic infections[18]. The same phenomenon has been observed in animal

Table 1 Gut microbiota alteration and additional findings in hepatitis B virus-related fibrosis

Ref.	Population (*n*)	Detection method	Gut microbiota alteration	Additional findings
Lu *et al* [30]	Healthy volunteers (*n* = 32); HBV carriers (*n* = 30); CHB (*n* = 31); Decompensated HBV-LC (*n* = 31)	qPCR	Phylum *Bacteroidetes* ↓ *Firmicutes* ↓ Family *Bifidobacteria*/*Enterobacteriaceae* ↓	Copies of operons that code for virulence factors markedly increased. Fecal sIgA and TNF-α in decompensated HBV-LC patients were higher than other groups
Xu *et al*[142]	Healthy volunteers (*n* = 15); CHB (*n* = 16); HBV-LC (*n* = 16)	qPCR	Species (*Bifidobacterium* specific) *B. catenulatum* ↓ *B. longum* ↓ *B. dentinum* ↑	*B. dentium*, which was considered to be an opportunistic pathogen, increased in HBV-LC patients. Species composition of *Bifidobacterium* shifted from beneficial to pathogenic
Wu *et al*[143]	Healthy volunteers (*n* = 38); Decompensated HBV-LC (*n* = 61); HBV-LT (after LC) (n =74)	qPCR	Species (*Lactobacilli* specific) *L. rhamnosus* ↓ *L. fermentus* ↓	Less complex fecal *lactobacilli* composition was found especially in decompensated HBV-LC patients
Wei *et al*[38]	Healthy volunteers (*n* = 120); HBV-LC (*n* = 120): CTP-A (*n* = 40); CTP-B (*n* = 40); CTP-C (*n* = 40)	Solexa sequencing	Phylum *Bacteroidetes* ↓ *Proteobacteria* ↑ Family *Enterobacteriaceae* ↑ Genera *Veillonella* ↑	A negative correlation was observed between the Child-Turcotte-Pugh scores and *Bacteroidetes* ($P < 0.01$)
Wang *et al* [23]	Healthy volunteers (*n* = 22); CHB (*n* = 85): CP-A (*n* = 76); CP-B (*n* = 9)	16S rRNA sequencing	Family *Lachnospiraceae* ↓ *Rikenellaceae*, ↓ *Porphyromonadaceae* ↓ *Ruminococcaceae* ↓ *Veillonellaceae* ↑	*Streptococcus*, *Veillonella*, *Streptococcus* and *Haemophilus* had strong correlations with liver function indices and serum metabolites. They were significantly higher in patients with higher Child-Pugh scores. The gut microbiota may be partially involved in the abnormal accumulation of serum metabolites
Deng *et al* [29]	Healthy volunteers (*n* = 20); HBV-LC (*n* = 80): CP-A (*n* = 30); CP-B (*n* = 31); CP-C (*n* = 19)	16S rRNA sequencing	Phylum *Firmicutes/Bacteroidetes* ↑ Genera *Megamonas* ↓ *Veillonella* ↓	Gut microbiota alteration mentioned on the left were all independent risk or protective factors for HBV-LC. Serum endotoxin increased in patients with higher CP classes ($P = 0.000$)
Zeng *et al* [140]	Healthy volunteers (*n* = 15); CHB (*n* = 21); HBV-LC (*n* = 25); HBV-HCC (*n* = 21)	16S rRNA sequencing	Phylum *Proteobacteria* ↑ *Bacteroidetes* ↑ *Firmicutes* ↓ Family *Bifidobacteria/Enterobacteriaceae* ↓	Higher *Bacteroidetes/firmicutes* ratio represented for higher LPS exposure
Wang *et al*	Healthy volunteers (*n* = 21); CHB (*n* = 69); F0-1 (*n* = 25);	16S rRNA sequencing	Genera *Prevotella* ↑	Genera responsible for bile acid metabolism decreased in CHB fibrosis patients

[59]	F2-4 (n = 44)		*Bacteroides* ↓ *Ruminococcus* ↓	
Chen *et al* [28]	Healthy volunteers (n = 21); HBV carriers (n = 23); CHB (n = 28); HBV-LC (n = 25)	16S rRNA sequencing	Phylum *Actinobacteria* ↑ *Bacteroidetes* ↓ *Firmicutes* ↓ *Proteobacteria* ↑	HBV-LC patients had higher bacterial network complexity with lower abundance of potential beneficial bacterial taxa
Yang *et al* [27]	Healthy volunteers (n = 31); HBV carriers (n = 24); CHB (n = 56); HBV-LC (n = 54); HBV-ACLF (n = 52)	16S rRNA sequencing	There are fluctuations in the changes	HBV carriers might be the most suitable donors for FMT for higher α diversity and abundance of potential beneficial bacteria
Wang *et al* [37]	Healthy volunteers (n = 877); CHB (n = 252); HBV-LC (n = 162); HBV-ACLF (n = 212)	16S rRNA sequencing; metagenomic sequencing	Species *Enterococcus faecium* ↑	High abundance of *Enterococcus* is associated with progression while that of *Faecalibacterium* is associated with regression of HBV-ACLF

HBV: Hepatitis B virus; CHB: Chronic hepatitis B; ACLF: Acute-on-chronic liver failure; CP: Child-Pugh scores; CTP, Child-Turcotte-Pugh scores; FMT: Faecal microbiota transplantation; LC: Liver cirrhosis; LT: Liver transplant; qPCR: Quantitative polymerase chain reaction.

experiments, in which hepatitis B surface antigen (HBsAg) of immature mice remained positive[19]. The age-related difference in immune clearance of HBV is consistent with the stabilization time of the gut microbiota, and maturation appears to facilitate HBV clearance by diminishing the tolerance phenotype and stimulating the immunoreactive pathway[19,20]. Similarly, if the gut microbiota was greatly imbalanced by antibiotics, the depletion can impair intestinal barrier function and weaken the ability of humoral and cellular immunity to clear HBV: adult mice with a mature gut microbiota managed to clear HBV after 6 wk of infection, while they failed to do so after antibiotic use[19,21].

Acute HBV infection

Due to the difficulty of studying acute HBV infection in humans, animal studies have been used: the ratio of *Firmicutes*/*Bacteroides* increased in the early stages of infection (Day 14) and decreased significantly over time (Day 49) in two mouse groups that were constructed with different plasmids[22].

Chronic HBV infection and non-end-stage CHB

Compositional changes have already occurred in the gut microbiota in early-stage CHB patients: in the Child-Pugh A and B groups, the abundance of 5 operational taxonomic units (OTUs) belonging to *Actinomyces*, *Clostridium sensu stricto*, unclassified *Lachnospiraceae* and *Megamonas* increased, while 27 OTUs decreased, which belong to *Alistipes*, *Asaccharobacter*, *Bacteroides*, *Butyricimonas*, *Clostridium IV*, *etc.* [23].

To further understand the gut microbiota dynamics in chronic HBV infection and CHB, there are also studies concentrating on the association with clinical indicators reflecting liver function and infection state. The gut microbiota of subjects from the chronic HBV infection group with normal ALT (NALT) levels was rather similar to those from the healthy volunteers, while significantly different from those from the high ALT level group[24]; however, in a recent study, the authors presented a slightly different perspective that the microbial diversity and abundance of *Lactobacillus*, *Clostridium*, and *Bifidobacterium* were lower in CHB-NALT patients than in healthy volunteers[25]. *Streptococcus*, *Veillonella*, *Streptococcus* and *Haemophilus* showed high correlations with some serum metabolites, including aromatic amino acids (phenylalanine and tyrosine), which are assumed to play pathogenic roles the progression of CHB [23]. The gut microbiota also varies according to viral load: HBV-infected individuals with a low viral load showed high diversity and carry a predominance of taxa associated with fatty acid and lipid metabolism[26].

As the disease progresses, the gut microbiota changes dynamically: the α diversity of asymptomatic HBV carriers slightly increased compared with that of healthy donators, while that of patients in the other three groups (CHB, liver cirrhosis, and acute-on-chronic liver failure (ACLF)) decreased with the severity of the disease[27]. The gut microbiota of patients with liver cirrhosis showed lower diversity and higher network complexity[28]. *Veillonellaceae* and *Lachnospiraceae* families were depleted in patients with liver cirrhosis compared with those in healthy volunteers, while *Megamonas* and *Veillonella* genera were depleted and enriched in patients, respectively[29]. Additionally, copy numbers of *Enterobacteriaceae* increased and lactic acid bacteria were depleted, with marked variation in the intestinal community of CHB patients[30]. The *Bifidobacteria*/*Enterobacteriaceae* ratio can be used for tracing the progression of liver disease[30]. With the magnitude of severity of liver disease (estimated as increasing liver Child-Pugh score), partial functional genes were correlated, such as those encoding aspartate-

ammonia ligase, transaldolase, adenylosuccinate synthetase and IMP dehydrogenase[31]. According to the combined results of multiple studies, there is a well-acknowledged decrease in *Firmicutes* abundance and increase in *Proteobacteria* during the progression of HBV-related fibrosis.

HCC and end-stage CHB

Liver cirrhosis is a dangerous premalignant condition with an increasing incidence of genetic aberrations and an elevated risk of HCC[32,33]. HCC patients tend to present a distempered gut microbiota and abnormal metabolites[34]. The butyrate-producing genera were depleted, while lipopolysaccharide (LPS)-producing genera were enriched in liver cirrhosis and HCC patients, and *Clostridioides* abundance was generally observed to be positively related to the tumor size of HCC[35]. In another study, *Bacteroides*, *Lachnospiracea incertae sedis*, and *Clostridium XIVa* were enriched in HCC patients, and there was a consistency of positive correlation with the tumor burden[36]. By integrating the clinical characteristics and database analysis, serum bile acids may be the communication mediators between these three genera and the host transcriptome[36]. HCC can be secondary to a number of causes, including HBV, *Hepatitis C virus* (HCV) and so on. Compared with non-HBV non-HCV HCC, the abundance of *Prevotella* was much greater in HBV-related HCC group[34]. HBV-related HCC group had higher abundance of pathways related to DNA formation and function (including chaperones and folding catalysts, DNA replication proteins and chromosome), which supported that HBV can impair the normal function of DNA[34].

Additionally, dynamic alteration of gut microbiota is a valuable indicator to predict the prognosis of end-stage liver disease. The richness of *Enterococcus* was significantly higher in the HBV-related ACLF progression group, while a high abundance of *Faecalibacterium* was associated with regression (groups were divided according to the model for end-stage liver disease at discharge)[37]; a higher abundance of *E. coli* is consistent with an increasing level of LPS ligand in the circulation of patients with end-stage liver disease[38-40].

GUT MICROBIOTA-RELATED MECHANISMS OF LIVER FIBROSIS

Liver fibrosis is fibrous scar caused by excess accumulation of ECM[41]. It is driven by the chronic and persistent occurrence of parenchymal injury and the activation of the inflammatory response, followed by a continuous repair reaction and liver fibrogenesis[42]. For HBV infection, liver infringement comes from not only HBV but also gut-derived microbe/antigen translocation and abnormal metabolites.

There is a close connection between the gut and liver through known organic pipelines (bile duct and portal vein)[43], and whether there are detours needs further study. The liver produces and sends primary bile acids (BAs) and immunologic active materials (some antimicrobial peptides) through the biliary tract to assist in intestinal digestion and immunity. Conversely, the portal vein carries secondary BAs, nutrients, gastrointestinal metabolites from the gut to the liver, to provide nutrients and get detoxification and biotransformation[17,44] (Figure 1A).

In a non-disease state, intestinal physical and chemical barriers effectively block pathogens or toxic substances and decrease bacterial colonization. The barriers mainly include mucin proteins secreted by goblet cells, secretory IgA (sIgA) secreted by plasma cells in lymphoid follicles of the lamina propria and tight junctions between intestinal epithelial cells (IECs)[45] (Figure 1B). Disorders of these barriers can lead to increased intestinal permeability and translocation of microbial components or metabolites (LPS, microbial DNA) in CLD patients, allowing microbes and antigens to translocate into the portal vein[45], and subsequently induce chronic or acute inflammatory responses of different tissues and organs[46] (Figure 1C).

Intestinal barrier impairment

The gastrointestinal mucus layer is the first line of defense against microbes, and the mobility enables the layer to carry pathogens distally and reduce microbial colonization[47]. The experimental mouse models with liver cirrhosis [induced by bile-duct ligation (BDL) or tetrachloromethane (CCl_4)] show a reduced thickness of the mucus layer, with loss of goblet cells[48]. These cirrhotic mice show pathological bacterial translocation, which has not been found in healthy or pre-hepatic portal-hypertensive mice[48]. sIgA is the predominant contributor to mucosal immunity, recognizing and eliminating bacterial protein antigens, and it also participates in barrier layer limitation of microbe/antigen translocation[49]. Patients with HBV-induced decompensated cirrhosis have increased sIgA content in blood and stool[30], consistent with the increased bacterial migration. Simultaneously, intestinal tight junctions are weakened in patients with liver cirrhosis, and the expression of tight junction proteins is decreased[50,51]. Zonulin, an effective physiological regulator of tight junctions, is one of the markers of intestinal permeability[52]. Serum zonulin content is significantly increased in HBV-related liver cirrhosis and HCC patients and the levels are correlated with the stages[53].

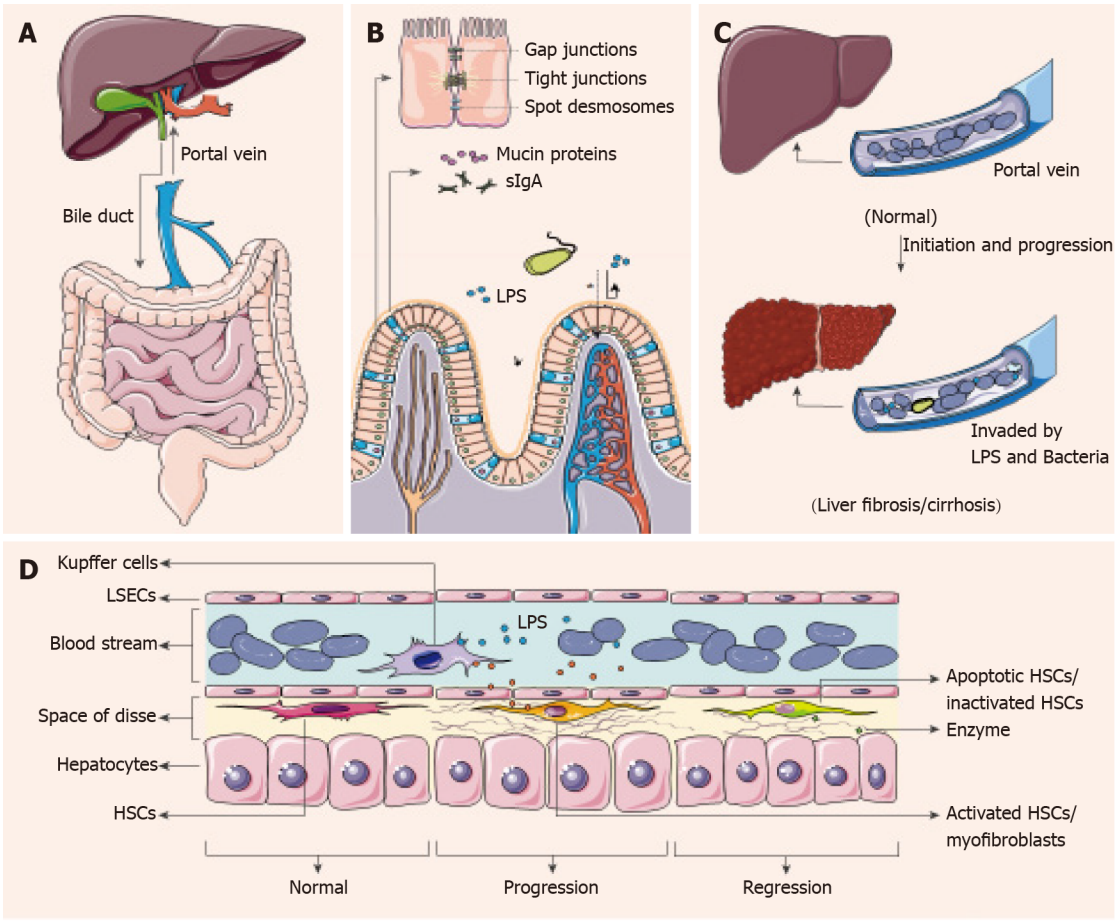

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Figure 1 Mechanism of gut microbiota-related liver fibrosis/cirrhosis. A: Gut-liver axis. The close bidirectional connection between gut and liver is mainly through the portal vein and bile duct; B: Intestinal barriers. From the intestine lumen, intestinal barriers are mainly formed by mucin proteins, sIgA and intercellular junctions, especially tight junctions between intestinal epithelial cells. The asterisk means when the intestinal barriers are weakened or broken, microbe/antigen translocation ensues; C: Liver fibrosis/cirrhosis and gut microbe/antigen translocation. Compared with normal state, gut microbe/antigen translocation and liver fibrosis/cirrhosis may drive each other in chronic hepatitis B patients; D: Mechanisms of liver fibrosis/cirrhosis process and regression. Receiving the activation signal, hepatic stellate cells (HSCs) are activated into fibroblasts to form the fiber. As the activation signal ceases, the activated HSCs are inactivated or apoptotic. When fiber degradation predominated, fibrosis is reversed. HSCs: Hepatic stellate cells; LPS: Lipopolysaccharide; LSECs: Liver sinusoidal endothelial cells.

Gut-derived microbe/antigen translocation and metabolic dysbiosis

The impairment of the intestinal barrier greatly reduces the efficiency of blocking microbe/antigen translocation. Gut-derived microbes or fragments and metabolites enter the venous system, travel through the portal vein to invade the liver. Diversity of circulating bacteria in cirrhosis patients is consistent with the presence of dysbiosis[54]. Recent studies have also supported that the occurrence of intestinal bacterial overgrowth and bacterial translocation in cirrhosis using methods such as bacterial DNA sequencing[55] and fluorescence microscopy[21] and suggested that the mechanism is associated with antimicrobial host defense[56]. Simultaneously, LPS is one of the component of the outer membrane of Gram-negative bacteria, mainly from *Enterobacteriaceae*[57]. The dysbiosis of the gut microbiota in mice leads to endotoxemia, which may bring about Kupffer Cell (KC) IL-10 production and KC-mediated T cell suppression[57]. And endotoxemia is highly related to the severity in liver diseases and complications[58].

Additionally, abnormal composition of the gut microbiota results in metabolic disorders, among which the metabolism of BAs has aroused great concern[25]. The level of fecal total BAs decreased and the ratio of conjugated and primary BAs increased in CHB patients without liver cirrhosis, which may be the prelude of following changes[25]. And there is a trend that abundance of the bacteria genera responsible for BA metabolism is decreased in CHB patients with moderate/advanced fibrosis[59,60]. There is also a link between gut bacteria-controlled BA metabolism and liver antitumor immunosurveillance *via* natural killer T (NKT) cells[61].

Immune-mediated fibrosis and regression

Pattern recognition receptors (PRRs) are highly conserved host sensors that are able to recognize

exogenous and endogenous antigens, including pathogen-associated molecular patterns (PAMPs) and host-derived damage-associated molecular patterns (DAMPs)[62]. PRRs are expressed by a plethora of immune cells, especially macrophages[63]. Macrophages could be at the center of innate immune regulation, linking microbe/antigen translocation and liver inflammation or fibrosis[64]. Recognition of PRRs sends the initial signal to active downstream adaptor proteins to undergo maturation and assemble transcription factors, such as nuclear factor (NF)-κB[65,66]. The produced cytokines then recruit inflammatory cells, drive antimicrobial activities and promote myofibroblast formation[67].

Myofibroblasts, the collagen-producing cells, are not present in healthy livers[68]. In response to toxic liver injury, myofibroblasts are mainly transformed from activated hepatic stellate cells (HSCs)[69]. There are four different stages of HSCs, namely, quiescent, activated (equivalent to collagen type I-producing myofibroblasts), inactivated and senescent[41]. Under physiological conditions, quiescent HSCs stay in the space of Disse and function as the major vitamin A storage site[70]. Simulated by several cytokines (especially transforming growth factor (TGF)-β)[71], quiescent HSCs modulate phenotypes and transform into activated HSCs, and the activated HSCs migrate and secrete ECM to produce a fibrous scar[41]. After removing the initial driver, there is a decrease in the levels of pro-inflammatory cytokines (interleukin-6, interleukin-1β and tumor necrosis factor) and TGF-β, and a rapid decline of the counts of activated HSCs[41]. Activated HSCs can be transformed into inactivated or senescent cells, and stop producing type-I collagen fibers[72]. Later, when fiber degradation by matrix metalloproteinases overwhelms fiber formation, liver fibrosis can be controlled, regressed and even reversed[73].

In conclusion, increased microbe and endotoxin loads in the portal vein cause PRR activation on immune cells, especially on macrophages, which leads to the activation of quiescent HSCs into activated HSCs[44,66]. Later, activated HSCs proliferate in response to various cytokines, secrete type-I collagen fiber and make liver fibrotic[41]. Upon cessation of underlying injury, myofibroblasts undergo inactivation or apoptosis, and fibrosis can be discontinued or reversed[41] (Figure 1D). This is the mechanism of effective treatment to control and regress liver fibrosis.

GUT MICROBIOTA DYSFUNCTION IN LIVER FIBROSIS COMPLICATIONS

As mentioned above, gut microbiota alterations may drive immune-related inflammation and fibrosis in the liver. Due to the accumulation of collagen fiber, liver stiffness is increased, bloodstream transport is blocked, healthy liver parenchyma is replaced and liver biotransformation and detoxification abilities are weakened[74]. As the disease progresses into the decompensation stage, patients may experience deadly complications, such as portal hypertension, spontaneous bacterial peritonitis (SBP) and HE. The relationship among gut microbiota alteration, liver fibrosis and portal hypertension is similar to the question of the chicken and the egg, as they drive and affect each other[75]. Compared with compensated cirrhosis, gut microbiota composition is characterized by an increase in the abundance of potentially pathogenic bacteria in the decompensation stage, especially *Alcaligenaceae*, *Porphyromonadaceae*, *Veillonellaceae* and *Enterobacteriaceae*[76].

SBP

SBP refers to the infection of ascites without an apparent intra-abdominal focus[77]. It is a severe infection and is often fatal in patients with cirrhosis and ascites[78]. The pathogen of SBP in liver cirrhosis patients is mainly from the intestinal tract.

More than two decades ago, DNA fragments of 30 bacterial isolated from ascites, mesenteric lymph nodes, portal blood, and ileal flora were compared[79]. The same bacterial strain was simultaneously isolated in ascites and in mesenteric lymph nodes and/or the ileum in 7/8 (87%) instances[79]. Intraperitoneal LPS increased TLR4 (Toll-like receptor 4, the canonical PRR for LPS) expression and amplified portal hypertension in rat liver fibrosis[80].

HE

HE is a fatal central nervous system complication caused by acute and chronic hepatitis or decompensated cirrhosis[81], which is considered consciousness disturbance after ammonia-related cerebral edema[82]. HE patients tend to have a poor prognosis and high mortality and recurrence rates, with greatly increasing economic and nursing burdens[83].

Currently, there is an increasing consensus that the gut microbiota and gastrointestinal metabolites play an important role in the initiation and progress of HE. On the basis of the gut-liver axis mentioned above, researchers proposed the concept of the gut-brain-liver axis to describe the role of the gut microbiota[84]. Cognitive dysfunction in cirrhosis is related to a decrease in the abundance of autochthonous families and an increase in *Alcaligenaceae* and *Porphyromonadaceae*[85,86].

On the one hand, gut microbiota alteration in the decompensation stage is consistent with the accumulation of microbe-derived products, including ammonia, mercaptans, benzodiazepine-like substances, and indoles[76]. These products can pass the blood-brain barrier and alter astrocyte function, resulting in osmotic or oxidative stress, mitochondrial dysfunction, neurotransmission

disorder, *etc.*[81]. On the other hand, neurotransmitters produced by the microbiota, including serotonin, dopamine, and aminobutyric acid, can act on specific receptors of exogenous primary afferent neuron cells, or cross the blood-brain barrier to act as active neurotransmitters[87]. The complex network among the enteric nervous system, the autonomic nervous system and the neuroendocrine and neuroimmunity systems of the central nervous system has a mutual impact on the gut microbiota, and the up-down or down-up regulation mechanisms need further exploration[84].

GUT MICROBIOTA-RELATED TREATMENT TOWARD HBV-RELATED FIBROSIS AND COMPLICATIONS

Based on the fibrosis regression theory mentioned above, removing the cause is the key to controlling and reversing liver fibrosis (Tables 2 and 3). For more than a decade, antiviral therapy has been recognized as an effective method to prevent, control and even reverse fibrosis and cirrhosis[88]. Rifaximin reduces the virulence of the overgrown gut microbiota[89]. With further understanding of the connection between the gut microbiota and HBV-related fibrosis, scientists have suggested that host health depends on the balance of the composition of the entire microbial community rather than one or a few dominant organisms[90]. New therapeutic strategies for HBV-related fibrosis, cirrhosis and complications have been broadened to regulate the gut microbiota through probiotic supplementation and microbiota transplantation from healthy donors.

Gut microbiota stabilization with antiviral treatment

At present, the main endpoint of all current treatment strategies is to maintain long-term suppression of HBV replication[2]. Two main options are nucleoside analogs (NAs) and interferon alpha[91]. NAs with a high barrier to HBV resistance, including entecavir (ETV), tenofovir disoproxil fumarate (TDF) and tenofovir alafenamide (TAF), are believed to be favorably safe and long-acting[92]. Antiviral treatment (AVT) exerts a positive influence on survival rate and quality of life by preventing disease progression, reversing and degrading fibrosis and cirrhosis[93,94], and even reducing HCC incidence and mortality in CHB patients[95].

ETV therapy reverses gut microbiota dysbiosis induced by HBV infection in a mouse model[96]. And in a controlled cross-sectional and longitudinal real-world study, the species abundance of the gut microbiota increased markedly after ETV treatment[97]. After 8 wk of ETV treatment, the abundance of *Clostridium sensu stricto 1*, *Erysipelotrichaceae UCG-007* and *Intestinibacter* increased significantly, and that of *Streptococcus*, *Atopobium* and *Murdochiella* was markedly reduced[97]. Although the addition of *Clostridium butyricum* (CB) to ETV failed to improve the serum biochemical, immunologic and virologic variables, addition of CB affected the gut microbiota in CHB patients treated with ETV[98]. While there is a lack of dynamic and synergetic studies on liver fibrosis outcomes and gut microbiota alterations during AVT, collaborative microbes contributing the most to antiviral-intervened HBV-related fibrosis cannot be pinpointed definitively.

Rifaximin

Rifaximin is a rifamycin-based nonsystemic antibiotic with low gastrointestinal absorption and good antibacterial activity[89,99]. The gastrointestinal tract is the main therapeutic target of rifaximin, and it has been widely used in controlling HE with infrequent side effects and a favorable long-term safety profile[100,101].

Current ideas suggest that rifaximin may have positive implications for liver cirrhosis and complications by acting on the gut microbiota. However, according to a randomized trial, there seems to be a minor impact on the composition of the gut microbiota[102]. Enrolled patients with cirrhosis and ascites were divided into two groups to receive rifaximin or placebo for 4 wk. Rifaximin decreased gut bacterial abundance, while no effect on particular species was observed; blood bacterial richness was decreased and the difference in *Pseudomonadales* abundance was relatively obvious[102]. And there was no difference in circulating markers of inflammation between the two groups[102]. Two additional studies also supported that rifaximin has little influence on gut microbiota abundance[103], but the metabolite levels altered: after rifaximin application, endotoxemia was relieved, and serum saturated and unsaturated fatty acid levels were increased significantly[104]. The former conclusion agreed with a study on experimental mice[105]. Therefore, rather than having a bactericidal effect, rifaximin seems to have direct effects on bacterial function and virulence[89].

Probiotics and synthetic probiotics

Probiotics are living nonpathogenic microorganisms, and treatment doses (at least 10^6 viable CFU/g) may help temper the gut microbiota[106]. *Lactobacillus* and *Bifidobacterium* genera are widely reported as clinically available probiotics[107]. In recent studies, probiotics have been broadly used to regulate the gut microbiota for further positive influences on primary diseases, such as gastrointestinal dysfunctions [108,109], metabolic diseases[110,111] and psychoneurotic disorders[112,113].

Table 2 Gut microbiota-related treatment toward hepatitis B virus-related fibrosis and complications (studies in animal models)

Ref.	Study populations (*n*)	Treatment and grouping (*n*)	Conclusions
Antiviral therapy			
Li *et al*[96]	AAV-mediated persistent HBV infection (AAV-HBV) mice (*n* = 10)	35 d after HBV infection, 4 wk of daily ETV treatment. ETV (*n* = 5)	Gut microbiota dysbiosis of the AAV-HBV mice was reversed by ETV treatment with restored α diversity and changed proportion of *Akkermansia*, *Lacnospiracea* and *Marvinbryantia*
Rifaximin			
Kang *et al* [105]	Germ-free mice (*n* = 16)	15 d of rifaximin 100 mg/(kg·d), or humanized with stools from a HCV-induced cirrhotic patient with MHE. Rifaximin (*n* = 4); Humanized (*n* = 4); Rifaximin + humanized (*n* = 4)	Rifaximin beneficially altered intestinal ammonia generation by regulating intestinal glutaminase expression independent of gut microbiota. MHE-associated fecal colonization resulted in intestinal and systemic inflammation. It was ameliorated with rifaximin
Engineered probiotics			
Nicaise *et al*[120]	Ornithine transcarbamoylase-deficient Sparse-fur mice; Carbon tetrachloride rats; Thioacetamide-induced acute liver failure mice	NCIMB8826 (wild-type strain *Lactobacillus plantarum*), or EV101 (engineered *Lactobacillus plantarum*, $LDH^-/AlaD^+$) oral and intrarectal administration	EV101 administration was effective in controlling hyperammonemia in constitutive animal models with a significant effect on survival, which might be involved with direct ammonia consumption in the gut
Kurtz *et al* [121]	Ornithine transcarbamylase-deficient *spfash* mice; Thioacetamide-induced acute liver failure mice; Healthy volunteers (*n* = 52)	Non-modified *Escherichia coli* Nissle 1917 (EcN), SYNB1020 (engineered EcN, Δ argR, Δ thyA, malEK:PfnrS-$argA^{fbr}$) administration	SYNB1020 converted NH3 to l-arginine in vitro, and reduced systemic hyperammonemia, improved survival in mouse models. SYNB1020 was well tolerated in healthy volunteers
Ochoa-Sanchez *et al*[122]	Bile-duct ligated rats	Non-modified EcN, S-ARG, or S-ARG + BUT administration	S-ARG converted ammonia to arginine, it was further modified to additionally synthesize butyrate, which had the potential to prevent HE
FMT			
Liu *et al* [134]	Germ-free mice	Sterile supernatant or entire stool from pre-FMT and post-FMT cirrhotic patients with HE was transplanted to Germ-free mice	Transferred microbiota mediated neuroinflammation. Cirrhosis-associated dysregulation of gut microbiota was related with frontal cortical inflammation

AAV: Adeno-associated virus; HBV: Hepatitis B virus; ETV: Entecavir; HCV: Hepatitis C virus; MHE: Minimal hepatic encephalopathy; HE: Hepatic encephalopathy; FMT: Fecal microbiota transplantation.

The role of probiotics in complications of HBV-related fibrosis and cirrhosis has been validated, especially for HE. Probiotics can drive the gut microbiota, triggering emotional brain signatures[114]. For minimal HE, probiotic therapy (*Lactobacillus acidophilus*) can improve blood ammonia and psychometric tests and reduce the risk of overt encephalopathy deterioration[115]. Further studies confirmed that patients' cognition, venous ammonia level and intestinal mucosal barrier function were significantly improved after 3 mo of probiotic use (*Clostridium butyricum* combined with *Bifidobacterium infantis*), and the predominant bacteria (*Clostridium cluster I* and *Bifidobacterium*) were obviously enriched in the probiotic-treated group, while *Enterococcus* and *Enterobacteriaceae* were depleted[116]. The combination of probiotics and lactulose is effective for the secondary prophylaxis of HE patients with cirrhosis[117]. Simultaneously, probiotics may work by promoting the growth of beneficial microbes and preventing PAMP-mediated liver inflammation and the anti-proliferative, anti-angiogenic, and anti-metastatic effects of the antioxidant can block the progress of HCC[118].

Additionally, rapid progress in synthetic biology has brought more options, which makes engineered live biotherapeutics an available and promising strategy[119]. More than one decade ago, the genetically engineered ammonia-hyperconsuming strain NCIMB8826 was verified to exhibit a beneficial effect at a lower dose than its wild-type counterpart[120]. In recent years, more engineered bacteria have been constructed to accelerate ammonia metabolism, reduce blood ammonia concentration and reduce HE incidence[121,122]. One team from Synlogic Inc. engineered oral probiotic *Escherichia coli Nissle 1917* (Ecn) to create strain SYNB1020[121]. SYNB1020 is able to convert NH_3 to L-arginine *in vivo* and reduce hyperammonemia in two mouse models (ornithine transcarbamylase-deficient spfash mice and thioacetamide-induced liver injury mice). Satisfyingly, it showed metabolic activity and good tolerance in a phase 1 clinical study of 52 healthy adult volunteers. Later, another group modified Ecn to consume and convert ammonia to arginine, which was further modified to additionally synthesize butyrate[122]. Both of these studies showed that engineered probiotics have positive therapeutic significance for hyperammonemia and underlying potential for HE prevention. However, these strains have not progressed to clinical studies in hyperammonemia patients, and the clinical effects need further study.

Table 3 Gut microbiota-related treatment toward hepatitis B virus-related fibrosis and complications (studies in human)

Ref.	Study populations (*n*)	Treatment and grouping (*n*)	Conclusions
Antiviral therapy			
Lu *et al* [97]	Healthy volunteers (*n* = 30); CHB (*n* = 30)	8 wk of daily ETV treatment. ETV (*n* = 30)	After ETV treatment, gut microbiota abundance increased markedly, blood biochemical, immunological and virological responses improved significantly
Lu *et al* [98]	Healthy volunteers (*n* = 30); CHB patients (*n* = 60)	8 wk of daily ETV treatment, or with additional CB. ETV (*n* = 30); ETV + CB (*n* = 30)	Additional CB fail to improve blood biochemical, immunological and virological responses, but affects the gut microbiota in the CHB patients treated with ETV
Rifaximin			
Bajaj *et al* [104]	Decompensated LC patients with MHE (*n* = 20):CHB (NM)	8 wk of rifaximin 550-mg BD. Rifaximin (*n* = 20)	Rifaximin affected little on gut microbiota, there was just a modest decrease in *Veillonellaceae* and increase in *Eubacteriaceae*. Rifaximin significantly improved cognition and endotoxemia, it increased increase in serum saturated and unsaturated fatty acids post-rifaximin
Lutz *et al* [144]	Decompensated LC patients with ascites (*n* = 152): Viral hepatitis (*n* = 35)	Prophylactic antibiotic treatment before the time of paracentesis. Rifaximin (*n* = 27); Other systemic antibiotics (*n* = 17)	Prophylactic rifaximin did not reduce SBP occurrence. Prophylactic rifaximin was associated with the dominant bacteria in ascites: *Escherichia coli* and *enterococci* were dominant of patients without prophylaxis, klebsiella species were mostly recovered from the rifaximin group
Kimer *et al* [102]	Decompensated LC patients (*n* = 54): CHB (NM)	4 wk of rifaximin 550-mg BD or placebo BD. Rifaximin (*n* = 36); Placebo (*n* = 18)	Rifaximin had minor effects on bacteria translocation and gut microbiota. Rifaximin showed little impact on the inflammatory state (reflected as the level of TNF-α, IL-6, IL-10, IL-18, SDF-1α, TGF-1β, CRP)
Kaji *et al* [103]	Decompensated LC patients (*n* = 30): CHB (*n* = 4)	4 wk of rifaximin 1200 mg/d. Rifaximin (*n* = 30)	Rifaximin alleviated HE and endotoxemia with improved intestinal hyperpermeability, and it is involved in a gut microbial change. Rifaximin didn't affect serum proinflammatory cytokine levels (TNF-α, IL-6, IFN-γ, IL-10)
Probiotics			
Agrawal *et al*[117]	LC patients with recovered HE (*n* = 235): CHB (*n* = 49)	3 mo of lactulose 30–60 mL/d, or 3 capsules of probiotics per day, which contained 4 strains of *Lactobacillus*. Lactulose (*n* = 80); Probiotics (*n* = 77)	Lactulose and probiotics were effective for secondary prophylaxis of HE in cirrhotic patients
Ziada *et al* [115]	Decompensated LC patients with MHE (*n* = 90): CHB (NM)	4 wk of lactulose 30–60 mL/d, or 3 capsules of probiotics per day, which contained *Lactobacillus acidophilus*. Lactulose (*n* = 30); Probiotics (*n* = 30)	Probiotic was better tolerated than lactulose. Both of them can improve blood ammonia and psychometric tests and reduce the risk of developing overt HE. Magnetic resonance spectroscopy showed more improvement in the levels of brain neurometabolites in the probiotic group
Xia *et al* [116]	Decompensated HBV-LC patients with MHE (*n* = 67)	3 mo of probiotics 1500-mg TD, which contained *Clostridium butyricum* combined with *Bifidobacterium infantis*. Probiotics (*n* = 30)	After probiotics treatment, the therapeutic bacteria were significantly enriched, while *Enterococcus* and *Enterobacteriaceae* were decreased. Probiotics contributed to the improved cognition and the decreased ammonia levels
FMT			
Ren *et al* [132]	CHB with positive HBeAg, received over 3 yr of antiviral treatment (*n* = 18)	FMT was performed by gastroscope every 4 wk until HBeAg clearance. FMT (*n* = 5)	FMT was effective on HBeAg-positive CHB, especially in patients who could not cease the oral antiviral treatment even after long-term treatment
Bajaj *et al* [135]	Decompensated LC patients with recurrent HE (*n* = 20). CHB (NM)	After 5 d of antibiotics, FMT was performed by enema, or standard of care (SOC, rifaximin/lactulose) was applied. FMT (*n* = 10); SOC (*n* = 10)	FMT increased diversity and beneficial taxa of gut microbiota, improved cognition and showed good tolerance, other than SOC
Bajaj *et al* [136]	Decompensated LC patients with recurrent HE (*n* = 20). CHB (NM)	FMT was performed by enema, or standard of care (SOC, rifaximin/lactulose) was applied. FMT (*n* = 10); SOC (*n* = 10)	Oral FMT capsules are safe and well tolerated. Post-FMT, duodenal mucosal diversity increased with higher *Ruminococcaceae* and *Bifidobacteriaceae* and lower *Streptococcaceae* and *Veillonellaceae*. Reduction in *Veillonellaceae* were noted post-FMT in sigmoid and stool
Chauhan *et al*[133]	CHB with positive HBeAg, received over 1 years of antiviral treatment (*n* = 18)	6 FMTs were performed by gastroscope every 4 wk FMT (*n* = 12)	FMT appeared to be safe and effective on HBeAg-positive CHB in viral suppression and HBeAg clearance

CHB: Chronic hepatitis B; CB: *Clostridium butyricum*; CRP: C-reactive protein; EcN: *Escherichia coli* Nissle 1917; ETV: Entecavir; HBeAg: Hepatitis B e antigen; HE: Hepatic encephalopathy; IFN: Interferon; IL: Interleukin; LC: Liver cirrhosis; MHE: Minimal hepatic encephalopathy; NM: Not mentioned; SBP: Spontaneous bacterial peritonitis; SDF-1α: Stromal cell-derived factor 1-α; TDF: Tenofovir disoproxil fumarate; TGF-1β: Transforming growth factor β-1; TNF: Tumor necrosis factor; FMT: Faecal microbiota transplantation.

Fecal microbiota transplantation

Fecal microbiota transplantation (FMT) is an emerging treatment method that transfers the gut microbiota from a healthy donor to a patient[123]. Due to its ability to directly reshape or rebuild the recipient's gut microbial communities, FMT is one of the most promising therapies balancing and stabilizing the gut microbiota[76], and it has been applied to research-based treatment in animal models of a variety of diseases[124,125] and to study the mechanisms[126,127]. In recent years, FMT has been expanded to clinical treatment for human disease as a noninvasive strategy for conditions including recurrent *Clostridium difficile* infection[128], inflammatory bowel disease[129], severe obesity and metabolic syndrome[130]. Regarding the mechanism, the gut microbiota structure can be improved by FMT, and a clinical trial employing autologous FMT supported this point[131].

Clinical trials have also aimed to determine whether CHB patients can benefit from FMT therapy. In a pilot study carried out in China, FMT showed the potential to induce HBeAg clearance in HBeAg-positive CHB patients after long-term AVT: There was a significant HBeAg level decline in the FMT group (FMT combined with AVT), while no decline in the control group (AVT only) was found[132]. The results were consistent with a nonrandomized controlled clinical trial carried out in India: after 1 year of FMT therapy for 6 terms, the FMT group (FMT + AVT) seemed to show potential effectiveness and safety compared with those of the AVT group (AVT only)[133]. Some researchers have also hypothesized that FMT of some potential beneficial bacteria can change the occurrence of disease, and HBV carriers might be the most suitable donors for slightly higher microbiota abundance[27]. However, due to the limitations of a small number of participants and a lack of randomized clinical trials, further well-designed clinical trials are needed to confirm the initial assumptions and promote clinical practicability.

Studies on FMT for HE animal models or patients show satisfactory results. In animal experiments, neuroinflammation alleviation was found in cirrhosis model mice receiving FMT[134]. In a randomized clinical trial, FMT from rationally selected donors helped reduce and improve hospitalizations and improve cognition and dysbiosis for cirrhosis with recurrent HE[135]. Later, the same team verified the safety of FMT capsules through a phase 1, randomized and placebo-controlled clinical trial[136]. In addition to integral inoculation, selective inoculation of specific strains also plays an ameliorating role. Transplanting low-urease altered Schaedler flora to mice prepared with a depleted microbiota leaded to durable reduction in fecal urease activity and ammonia production[137]. The symbiotic pair of *Lactobacillus reuteri* JBD400 and *Streptococcus rubneri* JBD420 cooperatively improved transplantation efficiency 2.3×10^3 times more than that of sole transplantation and significantly lowered blood ammonia levels [138].

CONCLUSION

Consequently, gut microbiota alteration has been observed to be related to HBV-related fibrosis initiation and progression, and it is a promising therapeutic target. According to current studies, HBV persistence and clearance show consistency with the maturity and health of the gut microbiota[19,21]. With an increase of Child-Pugh scores and the model for end-stage liver disease, the gut microbiota is characterized by a decrease in the ratio of "good" to "potentially pathogenic" bacteria, and species diversity tends to decrease[139,140]. However, it is difficult to clarify which is the initiating factor between gut microbiota alteration and HBV-related fibrosis progression. Existing studies tend to be descriptive and lack HBV-specific exploration. Gut microbiota-related mechanisms are based on the gut-liver axis and immune-mediated response, briefly including intestinal barrier impairment, PRR activation, cytokine production, HSC activation and transformation, and fiber secretion and formation [41]. When the driver is removed, activated HSCs are inhibited or become apoptotic, and fiber scars are degraded, resulting in fibrosis regression[41].

Beyond theory, quite a few studies have begun examining therapeutic inventions. AVT can effectively control or even reverse HBV-related liver fibrosis, during which the gut microbiota gradually returns to homeostasis[96,97]. Rifaximin may decrease the virulence of the overgrown gut microbiota[89]. Probiotics and FMT are the most popular gut microbiota targeted therapies, and they are moving from the laboratory to the clinic. In addition, synthetic probiotics and selective microbiota transplantation may make these therapies more precise, and bring fewer side effects.

However, current studies do have limitations. There is a lack of in-depth research on the specific molecular mechanisms of the gut microbiota. Further clinical studies are needed to determine its effectiveness in patients with HBV-induced liver cirrhosis in the real world[141]. We must also admit that age, host location, dietary habits have a great impact on the gut microbiota, which leads to the lack of consistency and comparability of the alterations in gut microbiota in different studies. Therefore, diagnosis potential of microbial markers should be considered the factors mentioned above. We are looking forward to more powerful studies to strengthen the theoretical foundation and promote clinical application.

FOOTNOTES

Author contributions: Ren ZG and Li A designed the study; Li YG, Yu ZJ, Li A and Ren ZG collected data and summary viewpoints; Li YG wrote the manuscript; Ren ZG and Li A revised the manuscript; and All authors reviewed and approved the manuscript.

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REFERENCES

1 **World Health Organization**. Global progress report on HIV, viral hepatitis and sexually transmitted infections, 2021. 2021. Available from: https://www.who.int/publications/i/item/9789240027077
2 **European Association for the Study of the Liver**. EASL 2017 Clinical Practice Guidelines on the management of hepatitis B virus infection. *J Hepatol* 2017; **67**: 370-398 [PMID: 28427875 DOI: 10.1016/j.jhep.2017.03.021]
3 **Merli M**, Lucidi C, Giannelli V, Giusto M, Riggio O, Falcone M, Ridola L, Attili AF, Venditti M. Cirrhotic patients are at risk for health care-associated bacterial infections. *Clin Gastroenterol Hepatol* 2010; **8**: 979-985 [PMID: 20621200 DOI: 10.1016/j.cgh.2010.06.024]
4 **D'Amico G**, Morabito A, D'Amico M, Pasta L, Malizia G, Rebora P, Valsecchi MG. Clinical states of cirrhosis and competing risks. *J Hepatol* 2018; **68**: 563-576 [PMID: 29111320 DOI: 10.1016/j.jhep.2017.10.020]
5 **Yang R**, Xu Y, Dai Z, Lin X, Wang H. The Immunologic Role of Gut Microbiota in Patients with Chronic HBV Infection. *J Immunol Res* 2018; **2018**: 2361963 [PMID: 30148173 DOI: 10.1155/2018/2361963]
6 **Milani C**, Duranti S, Bottacini F, Casey E, Turroni F, Mahony J, Belzer C, Delgado Palacio S, Arboleya Montes S, Mancabelli L, Lugli GA, Rodriguez JM, Bode L, de Vos W, Gueimonde M, Margolles A, van Sinderen D, Ventura M. The First Microbial Colonizers of the Human Gut: Composition, Activities, and Health Implications of the Infant Gut Microbiota. *Microbiol Mol Biol Rev* 2017; **81** [PMID: 29118049 DOI: 10.1128/MMBR.00036-17]
7 **Doré J**, Simrén M, Buttle L, Guarner F. Hot topics in gut microbiota. *United European Gastroenterol J* 2013; **1**: 311-318 [PMID: 24917977 DOI: 10.1177/2050640613502477]
8 **Hillman ET**, Lu H, Yao T, Nakatsu CH. Microbial Ecology along the Gastrointestinal Tract. *Microbes Environ* 2017; **32**: 300-313 [PMID: 29129876 DOI: 10.1264/jsme2.ME17017]
9 **Reyes A**, Semenkovich NP, Whiteson K, Rohwer F, Gordon JI. Going viral: next-generation sequencing applied to phage populations in the human gut. *Nat Rev Microbiol* 2012; **10**: 607-617 [PMID: 22864264 DOI: 10.1038/nrmicro2853]
10 **Biedermann L**, Rogler G. The intestinal microbiota: its role in health and disease. *Eur J Pediatr* 2015; **174**: 151-167 [PMID: 25563215 DOI: 10.1007/s00431-014-2476-2]
11 **Marchesi JR**, Adams DH, Fava F, Hermes GD, Hirschfield GM, Hold G, Quraishi MN, Kinross J, Smidt H, Tuohy KM, Thomas LV, Zoetendal EG, Hart A. The gut microbiota and host health: a new clinical frontier. *Gut* 2016; **65**: 330-339 [PMID: 26338727 DOI: 10.1136/gutjnl-2015-309990]
12 **Odamaki T**, Kato K, Sugahara H, Hashikura N, Takahashi S, Xiao JZ, Abe F, Osawa R. Age-related changes in gut microbiota composition from newborn to centenarian: a cross-sectional study. *BMC Microbiol* 2016; **16**: 90 [PMID: 27220822 DOI: 10.1186/s12866-016-0708-5]
13 **Chen L**, Zhang YH, Huang T, Cai YD. Gene expression profiling gut microbiota in different races of humans. *Sci Rep* 2016; **6**: 23075 [PMID: 26975620 DOI: 10.1038/srep23075]
14 **Wu GD**, Compher C, Chen EZ, Smith SA, Shah RD, Bittinger K, Chehoud C, Albenberg LG, Nessel L, Gilroy E, Star J, Weljie AM, Flint HJ, Metz DC, Bennett MJ, Li H, Bushman FD, Lewis JD. Comparative metabolomics in vegans and omnivores reveal constraints on diet-dependent gut microbiota metabolite production. *Gut* 2016; **65**: 63-72 [PMID: 25431456 DOI: 10.1136/gutjnl-2014-308209]
15 **Agus A**, Clément K, Sokol H. Gut microbiota-derived metabolites as central regulators in metabolic disorders. *Gut* 2021; **70**: 1174-1182 [PMID: 33272977 DOI: 10.1136/gutjnl-2020-323071]

16 **Jones RM**, Neish AS. Gut Microbiota in Intestinal and Liver Disease. *Annu Rev Pathol* 2021; **16**: 251-275 [PMID: 33234022 DOI: 10.1146/annurev-pathol-030320-095722]

17 **Tripathi A**, Debelius J, Brenner DA, Karin M, Loomba R, Schnabl B, Knight R. The gut-liver axis and the intersection with the microbiome. *Nat Rev Gastroenterol Hepatol* 2018; **15**: 397-411 [PMID: 29748586 DOI: 10.1038/s41575-018-0011-z]

18 **Wu LL**, Huang TS, Shyu YC, Wang CL, Wang HY, Chen PJ. Gut microbiota in the innate immunity against hepatitis B virus - implication in age-dependent HBV clearance. *Curr Opin Virol* 2021; **49**: 194-202 [PMID: 34242953 DOI: 10.1016/j.coviro.2021.06.006]

19 **Chou HH**, Chien WH, Wu LL, Cheng CH, Chung CH, Horng JH, Ni YH, Tseng HT, Wu D, Lu X, Wang HY, Chen PJ, Chen DS. Age-related immune clearance of hepatitis B virus infection requires the establishment of gut microbiota. *Proc Natl Acad Sci U S A* 2015; **112**: 2175-2180 [PMID: 25646429 DOI: 10.1073/pnas.1424775112]

20 **Xu D**, Huang Y, Wang J. Gut microbiota modulate the immune effect against hepatitis B virus infection. *Eur J Clin Microbiol Infect Dis* 2015; **34**: 2139-2147 [PMID: 26272175 DOI: 10.1007/s10096-015-2464-0]

21 **Guo W**, Zhou X, Li X, Zhu Q, Peng J, Zhu B, Zheng X, Lu Y, Yang D, Wang B, Wang J. Depletion of Gut Microbiota Impairs Gut Barrier Function and Antiviral Immune Defense in the Liver. *Front Immunol* 2021; **12**: 636803 [PMID: 33841420 DOI: 10.3389/fimmu.2021.636803]

22 **Zhu Q**, Xia P, Zhou X, Li X, Guo W, Zhu B, Zheng X, Wang B, Yang D, Wang J. Hepatitis B Virus Infection Alters Gut Microbiota Composition in Mice. *Front Cell Infect Microbiol* 2019; **9**: 377 [PMID: 31750262 DOI: 10.3389/fcimb.2019.00377]

23 **Wang J**, Wang Y, Zhang X, Liu J, Zhang Q, Zhao Y, Peng J, Feng Q, Dai J, Sun S, Zhao L, Zhang Y, Hu Y, Zhang M. Gut Microbial Dysbiosis Is Associated with Altered Hepatic Functions and Serum Metabolites in Chronic Hepatitis B Patients. *Front Microbiol* 2017; **8**: 2222 [PMID: 29180991 DOI: 10.3389/fmicb.2017.02222]

24 **Yun Y**, Chang Y, Kim HN, Ryu S, Kwon MJ, Cho YK, Kim HL, Cheong HS, Joo EJ. Alterations of the Gut Microbiome in Chronic Hepatitis B Virus Infection Associated with Alanine Aminotransferase Level. *J Clin Med* 2019; **8** [PMID: 30717396 DOI: 10.3390/jcm8020173]

25 **Sun Z**, Huang C, Shi Y, Wang R, Fan J, Yu Y, Zhang Z, Zhu K, Li M, Ni Q, Chen Z, Zheng M, Yang Z. Distinct Bile Acid Profiles in Patients With Chronic Hepatitis B Virus Infection Reveal Metabolic Interplay Between Host, Virus and Gut Microbiome. *Front Med (Lausanne)* 2021; **8**: 708495 [PMID: 34671614 DOI: 10.3389/fmed.2021.708495]

26 **Joo EJ**, Cheong HS, Kwon MJ, Sohn W, Kim HN, Cho YK. Relationship between gut microbiome diversity and hepatitis B viral load in patients with chronic hepatitis B. *Gut Pathog* 2021; **13**: 65 [PMID: 34717727 DOI: 10.1186/s13099-021-00461-1]

27 **Yang XA**, Lv F, Wang R, Chang Y, Zhao Y, Cui X, Li H, Yang S, Li S, Zhao X, Mo Z, Yang F. Potential role of intestinal microflora in disease progression among patients with different stages of Hepatitis B. *Gut Pathog* 2020; **12**: 50 [PMID: 33117435 DOI: 10.1186/s13099-020-00391-4]

28 **Chen Z**, Xie Y, Zhou F, Zhang B, Wu J, Yang L, Xu S, Stedtfeld R, Chen Q, Liu J, Zhang X, Xu H, Ren J. Featured Gut Microbiomes Associated With the Progression of Chronic Hepatitis B Disease. *Front Microbiol* 2020; **11**: 383 [PMID: 32265857 DOI: 10.3389/fmicb.2020.00383]

29 **Deng YD**, Peng XB, Zhao RR, Ma CQ, Li JN, Yao LQ. The intestinal microbial community dissimilarity in hepatitis B virus-related liver cirrhosis patients with and without at alcohol consumption. *Gut Pathog* 2019; **11**: 58 [PMID: 31788031 DOI: 10.1186/s13099-019-0337-2]

30 **Lu H**, Wu Z, Xu W, Yang J, Chen Y, Li L. Intestinal microbiota was assessed in cirrhotic patients with hepatitis B virus infection. Intestinal microbiota of HBV cirrhotic patients. *Microb Ecol* 2011; **61**: 693-703 [PMID: 21286703 DOI: 10.1007/s00248-010-9801-8]

31 **Chen Y**, Qin N, Guo J, Qian G, Fang D, Shi D, Xu M, Yang F, He Z, Van Nostrand JD, Yuan T, Deng Y, Zhou J, Li L. Functional gene arrays-based analysis of fecal microbiomes in patients with liver cirrhosis. *BMC Genomics* 2014; **15**: 753 [PMID: 25179593 DOI: 10.1186/1471-2164-15-753]

32 **Ganesan M**, Eikenberry A, Poluektova LY, Kharbanda KK, Osna NA. Role of alcohol in pathogenesis of hepatitis B virus infection. *World J Gastroenterol* 2020; **26**: 883-903 [PMID: 32206001 DOI: 10.3748/wjg.v26.i9.883]

33 **Tang Y**, Zhou H, Xiang Y, Cui F. The diagnostic potential of gut microbiome for early hepatitis B virus-related hepatocellular carcinoma. *Eur J Gastroenterol Hepatol* 2021; **33**: e167-e175 [PMID: 33208683 DOI: 10.1097/MEG.0000000000001978]

34 **Liu Q**, Li F, Zhuang Y, Xu J, Wang J, Mao X, Zhang Y, Liu X. Alteration in gut microbiota associated with hepatitis B and non-hepatitis virus related hepatocellular carcinoma. *Gut Pathog* 2019; **11**: 1 [PMID: 30675188 DOI: 10.1186/s13099-018-0281-6]

35 **Zheng R**, Wang G, Pang Z, Ran N, Gu Y, Guan X, Yuan Y, Zuo X, Pan H, Zheng J, Wang F. Liver cirrhosis contributes to the disorder of gut microbiota in patients with hepatocellular carcinoma. *Cancer Med* 2020; **9**: 4232-4250 [PMID: 32281295 DOI: 10.1002/cam4.3045]

36 **Huang H**, Ren Z, Gao X, Hu X, Zhou Y, Jiang J, Lu H, Yin S, Ji J, Zhou L, Zheng S. Integrated analysis of microbiome and host transcriptome reveals correlations between gut microbiota and clinical outcomes in HBV-related hepatocellular carcinoma. *Genome Med* 2020; **12**: 102 [PMID: 33225985 DOI: 10.1186/s13073-020-00796-5]

37 **Wang K**, Zhang Z, Mo ZS, Yang XH, Lin BL, Peng L, Xu Y, Lei CY, Zhuang XD, Lu L, Yang RF, Chen T, Gao ZL. Gut microbiota as prognosis markers for patients with HBV-related acute-on-chronic liver failure. *Gut Microbes* 2021; **13**: 1-15 [PMID: 34006193 DOI: 10.1080/19490976.2021.1921925]

38 **Wei X**, Yan X, Zou D, Yang Z, Wang X, Liu W, Wang S, Li X, Han J, Huang L, Yuan J. Abnormal fecal microbiota community and functions in patients with hepatitis B liver cirrhosis as revealed by a metagenomic approach. *BMC Gastroenterol* 2013; **13**: 175 [PMID: 24369878 DOI: 10.1186/1471-230X-13-175]

39 **Roderburg C**, Luedde T. The role of the gut microbiome in the development and progression of liver cirrhosis and hepatocellular carcinoma. *Gut Microbes* 2014; **5**: 441-445 [PMID: 25006881 DOI: 10.4161/gmic.29599]

40 **Mohamadkhani A**. On the potential role of intestinal microbial community in hepatocarcinogenesis in chronic hepatitis

B. *Cancer Med* 2018 [PMID: 29761927 DOI: 10.1002/cam4.1550]
41 **Kisseleva T**, Brenner D. Molecular and cellular mechanisms of liver fibrosis and its regression. *Nat Rev Gastroenterol Hepatol* 2021; **18**: 151-166 [PMID: 33128017 DOI: 10.1038/s41575-020-00372-7]
42 **Parola M**, Pinzani M. Liver fibrosis: Pathophysiology, pathogenetic targets and clinical issues. *Mol Aspects Med* 2019; **65**: 37-55 [PMID: 30213667 DOI: 10.1016/j.mam.2018.09.002]
43 **Milosevic I**, Vujovic A, Barac A, Djelic M, Korac M, Radovanovic Spurnic A, Gmizic I, Stevanovic O, Djordjevic V, Lekic N, Russo E, Amedei A. Gut-Liver Axis, Gut Microbiota, and Its Modulation in the Management of Liver Diseases: A Review of the Literature. *Int J Mol Sci* 2019; **20** [PMID: 30658519 DOI: 10.3390/ijms20020395]
44 **Hrncir T**, Hrncirova L, Kverka M, Hromadka R, Machova V, Trckova E, Kostovcikova K, Kralickova P, Krejsek J, Tlaskalova-Hogenova H. Gut Microbiota and NAFLD: Pathogenetic Mechanisms, Microbiota Signatures, and Therapeutic Interventions. *Microorganisms* 2021; **9** [PMID: 33946843 DOI: 10.3390/microorganisms9050957]
45 **Chen D**, Le TH, Shahidipour H, Read SA, Ahlenstiel G. The Role of Gut-Derived Microbial Antigens on Liver Fibrosis Initiation and Progression. *Cells* 2019; **8** [PMID: 31717860 DOI: 10.3390/cells8111324]
46 **Nagpal R**, Yadav H. Bacterial Translocation from the Gut to the Distant Organs: An Overview. *Ann Nutr Metab* 2017; **71** Suppl 1: 11-16 [PMID: 28950279 DOI: 10.1159/000479918]
47 **Johansson ME**, Sjövall H, Hansson GC. The gastrointestinal mucus system in health and disease. *Nat Rev Gastroenterol Hepatol* 2013; **10**: 352-361 [PMID: 23478383 DOI: 10.1038/nrgastro.2013.35]
48 **Sorribas M**, Jakob MO, Yilmaz B, Li H, Stutz D, Noser Y, de Gottardi A, Moghadamrad S, Hassan M, Albillos A, Francés R, Juanola O, Spadoni I, Rescigno M, Wiest R. FXR modulates the gut-vascular barrier by regulating the entry sites for bacterial translocation in experimental cirrhosis. *J Hepatol* 2019; **71**: 1126-1140 [PMID: 31295531 DOI: 10.1016/j.jhep.2019.06.017]
49 **Macpherson AJ**, Uhr T. Induction of protective IgA by intestinal dendritic cells carrying commensal bacteria. *Science* 2004; **303**: 1662-1665 [PMID: 15016999 DOI: 10.1126/science.1091334]
50 **Assimakopoulos SF**, Tsamandas AC, Tsiaoussis GI, Karatza E, Triantos C, Vagianos CE, Spiliopoulou I, Kaltezioti V, Charonis A, Nikolopoulou VN, Scopa CD, Thomopoulos KC. Altered intestinal tight junctions' expression in patients with liver cirrhosis: a pathogenetic mechanism of intestinal hyperpermeability. *Eur J Clin Invest* 2012; **42**: 439-446 [PMID: 22023490 DOI: 10.1111/j.1365-2362.2011.02609.x]
51 **Pijls KE**, Koek GH, Elamin EE, de Vries H, Masclee AA, Jonkers DM. Large intestine permeability is increased in patients with compensated liver cirrhosis. *Am J Physiol Gastrointest Liver Physiol* 2014; **306**: G147-G153 [PMID: 24264047 DOI: 10.1152/ajpgi.00330.2013]
52 **Fasano A**, Not T, Wang W, Uzzau S, Berti I, Tommasini A, Goldblum SE. Zonulin, a newly discovered modulator of intestinal permeability, and its expression in coeliac disease. *Lancet* 2000; **355**: 1518-1519 [PMID: 10801176]
53 **Wang X**, Li MM, Niu Y, Zhang X, Yin JB, Zhao CJ, Wang RT. Serum Zonulin in HBV-Associated Chronic Hepatitis, Liver Cirrhosis, and Hepatocellular Carcinoma. *Dis Markers* 2019; **2019**: 5945721 [PMID: 31485278 DOI: 10.1155/2019/5945721]
54 **Kajihara M**, Koido S, Kanai T, Ito Z, Matsumoto Y, Takakura K, Saruta M, Kato K, Odamaki T, Xiao JZ, Sato N, Ohkusa T. Characterisation of blood microbiota in patients with liver cirrhosis. *Eur J Gastroenterol Hepatol* 2019; **31**: 1577-1583 [PMID: 31441799 DOI: 10.1097/MEG.0000000000001494]
55 **Guarner C**, Runyon BA, Young S, Heck M, Sheikh MY. Intestinal bacterial overgrowth and bacterial translocation in cirrhotic rats with ascites. *J Hepatol* 1997; **26**: 1372-1378 [PMID: 9210626 DOI: 10.1016/s0168-8278(97)80474-6]
56 **Teltschik Z**, Wiest R, Beisner J, Nuding S, Hofmann C, Schoelmerich J, Bevins CL, Stange EF, Wehkamp J. Intestinal bacterial translocation in rats with cirrhosis is related to compromised Paneth cell antimicrobial host defense. *Hepatology* 2012; **55**: 1154-1163 [PMID: 22095436 DOI: 10.1002/hep.24789]
57 **Zhou W**, Luo J, Xie X, Yang S, Zhu D, Huang H, Yang D, Liu J. Gut Microbiota Dysbiosis Strengthens Kupffer Cell-mediated Hepatitis B Virus Persistence through Inducing Endotoxemia in Mice. *J Clin Transl Hepatol* 2022; **10**: 17-25 [PMID: 35233369 DOI: 10.14218/JCTH.2020.00161]
58 **Lin RS**, Lee FY, Lee SD, Tsai YT, Lin HC, Lu RH, Hsu WC, Huang CC, Wang SS, Lo KJ. Endotoxemia in patients with chronic liver diseases: relationship to severity of liver diseases, presence of esophageal varices, and hyperdynamic circulation. *J Hepatol* 1995; **22**: 165-172 [PMID: 7790704 DOI: 10.1016/0168-8278(95)80424-2]
59 **Wang X**, Chen L, Wang H, Cai W, Xie Q. Modulation of bile acid profile by gut microbiota in chronic hepatitis B. *J Cell Mol Med* 2020; **24**: 2573-2581 [PMID: 31925905 DOI: 10.1111/jcmm.14951]
60 **Kakiyama G**, Pandak WM, Gillevet PM, Hylemon PB, Heuman DM, Daita K, Takei H, Muto A, Nittono H, Ridlon JM, White MB, Noble NA, Monteith P, Fuchs M, Thacker LR, Sikaroodi M, Bajaj JS. Modulation of the fecal bile acid profile by gut microbiota in cirrhosis. *J Hepatol* 2013; **58**: 949-955 [PMID: 23333527 DOI: 10.1016/j.jhep.2013.01.003]
61 **Ma C**, Han M, Heinrich B, Fu Q, Zhang Q, Sandhu M, Agdashian D, Terabe M, Berzofsky JA, Fako V, Ritz T, Longerich T, Theriot CM, McCulloch JA, Roy S, Yuan W, Thovarai V, Sen SK, Ruchirawat M, Korangy F, Wang XW, Trinchieri G, Greten TF. Gut microbiome-mediated bile acid metabolism regulates liver cancer *via* NKT cells. *Science* 2018; **360** [PMID: 29798856 DOI: 10.1126/science.aan5931]
62 **Paludan SR**, Pradeu T, Masters SL, Mogensen TH. Constitutive immune mechanisms: mediators of host defence and immune regulation. *Nat Rev Immunol* 2021; **21**: 137-150 [PMID: 32782357 DOI: 10.1038/s41577-020-0391-5]
63 **Tacke F**. Targeting hepatic macrophages to treat liver diseases. *J Hepatol* 2017; **66**: 1300-1312 [PMID: 28267621 DOI: 10.1016/j.jhep.2017.02.026]
64 **Tacke F**, Zimmermann HW. Macrophage heterogeneity in liver injury and fibrosis. *J Hepatol* 2014; **60**: 1090-1096 [PMID: 24412603 DOI: 10.1016/j.jhep.2013.12.025]
65 **Żeromski J**, Kierepa A, Brzezicha B, Kowala-Piaskowska A, Mozer-Lisewska I. Pattern Recognition Receptors: Significance of Expression in the Liver. *Arch Immunol Ther Exp (Warsz)* 2020; **68**: 29 [PMID: 32944845 DOI: 10.1007/s00005-020-00595-1]
66 **Kassa Y**, Million Y, Gedefie A, Moges F. Alteration of Gut Microbiota and Its Impact on Immune Response in Patients with Chronic HBV Infection: A Review. *Infect Drug Resist* 2021; **14**: 2571-2578 [PMID: 34262302 DOI:

10.2147/IDR.S305901]
67 **Fitzgerald KA**, Kagan JC. Toll-like Receptors and the Control of Immunity. *Cell* 2020; **180**: 1044-1066 [PMID: 32164908 DOI: 10.1016/j.cell.2020.02.041]
68 **Friedman SL**. Mechanisms of hepatic fibrogenesis. *Gastroenterology* 2008; **134**: 1655-1669 [PMID: 18471545 DOI: 10.1053/j.gastro.2008.03.003]
69 **Iwaisako K**, Jiang C, Zhang M, Cong M, Moore-Morris TJ, Park TJ, Liu X, Xu J, Wang P, Paik YH, Meng F, Asagiri M, Murray LA, Hofmann AF, Iida T, Glass CK, Brenner DA, Kisseleva T. Origin of myofibroblasts in the fibrotic liver in mice. *Proc Natl Acad Sci U S A* 2014; **111**: E3297-E3305 [PMID: 25074909 DOI: 10.1073/pnas.1400062111]
70 **Senoo H**, Kojima N, Sato M. Vitamin A-storing cells (stellate cells). *Vitam Horm* 2007; **75**: 131-159 [PMID: 17368315 DOI: 10.1016/S0083-6729(06)75006-3]
71 **Xu F**, Liu C, Zhou D, Zhang L. TGF-β/SMAD Pathway and Its Regulation in Hepatic Fibrosis. *J Histochem Cytochem* 2016; **64**: 157-167 [PMID: 26747705 DOI: 10.1369/0022155415627681]
72 **Kendall TJ**, Hennedige S, Aucott RL, Hartland SN, Vernon MA, Benyon RC, Iredale JP. p75 Neurotrophin receptor signaling regulates hepatic myofibroblast proliferation and apoptosis in recovery from rodent liver fibrosis. *Hepatology* 2009; **49**: 901-910 [PMID: 19072833 DOI: 10.1002/hep.22701]
73 **Issa R**, Zhou X, Constandinou CM, Fallowfield J, Millward-Sadler H, Gaca MD, Sands E, Suliman I, Trim N, Knorr A, Arthur MJ, Benyon RC, Iredale JP. Spontaneous recovery from micronodular cirrhosis: evidence for incomplete resolution associated with matrix cross-linking. *Gastroenterology* 2004; **126**: 1795-1808 [PMID: 15188175 DOI: 10.1053/j.gastro.2004.03.009]
74 **Ginès P**, Krag A, Abraldes JG, Solà E, Fabrellas N, Kamath PS. Liver cirrhosis. *Lancet* 2021; **398**: 1359-1376 [PMID: 34543610 DOI: 10.1016/S0140-6736(21)01374-X]
75 **Arab JP**, Martin-Mateos RM, Shah VH. Gut-liver axis, cirrhosis and portal hypertension: the chicken and the egg. *Hepatol Int* 2018; **12**: 24-33 [PMID: 28550391 DOI: 10.1007/s12072-017-9798-x]
76 **Paratore M**, Santopaolo F, Cammarota G, Pompili M, Gasbarrini A, Ponziani FR. Fecal Microbiota Transplantation in Patients with HBV Infection or Other Chronic Liver Diseases: Update on Current Knowledge and Future Perspectives. *J Clin Med* 2021; **10** [PMID: 34204748 DOI: 10.3390/jcm10122605]
77 **European Association for the Study of the Liver**. EASL Clinical Practice Guidelines for the management of patients with decompensated cirrhosis. *J Hepatol* 2018; **69**: 406-460 [PMID: 29653741 DOI: 10.1016/j.jhep.2018.03.024]
78 **Dever JB**, Sheikh MY. Review article: spontaneous bacterial peritonitis--bacteriology, diagnosis, treatment, risk factors and prevention. *Aliment Pharmacol Ther* 2015; **41**: 1116-1131 [PMID: 25819304 DOI: 10.1111/apt.13172]
79 **Llovet JM**, Bartolí R, March F, Planas R, Viñado B, Cabré E, Arnal J, Coll P, Ausina V, Gassull MA. Translocated intestinal bacteria cause spontaneous bacterial peritonitis in cirrhotic rats: molecular epidemiologic evidence. *J Hepatol* 1998; **28**: 307-313 [PMID: 9580278 DOI: 10.1016/0168-8278(88)80018-7]
80 **Steib CJ**, Hartmann AC, v Hesler C, Benesic A, Hennenberg M, Bilzer M, Gerbes AL. Intraperitoneal LPS amplifies portal hypertension in rat liver fibrosis. *Lab Invest* 2010; **90**: 1024-1032 [PMID: 20212458 DOI: 10.1038/labinvest.2010.60]
81 **Wijdicks EF**. Hepatic Encephalopathy. *N Engl J Med* 2016; **375**: 1660-1670 [PMID: 27783916 DOI: 10.1056/NEJMra1600561]
82 **Gu X**, Lu Q, Zhang C, Tang Z, Chu L. Clinical Application and Progress of Fecal Microbiota Transplantation in Liver Diseases: A Review. *Semin Liver Dis* 2021; **41**: 495-506 [PMID: 34261137 DOI: 10.1055/s-0041-1732319]
83 Retraction notice to: Clinical significance of the best response during repeated transarterial chemoembolization in the treatment of hepatocellular carcinoma [J. Hepatol. 2014; 60: 1212–1218]. *J Hepatol* 2015; **62**: 252 [PMID: 25538970 DOI: 10.1016/j.jhep.2014.10.038]
84 **Ding JH**, Jin Z, Yang XX, Lou J, Shan WX, Hu YX, Du Q, Liao QS, Xie R, Xu JY. Role of gut microbiota *via* the gut-liver-brain axis in digestive diseases. *World J Gastroenterol* 2020; **26**: 6141-6162 [PMID: 33177790 DOI: 10.3748/wjg.v26.i40.6141]
85 **Bajaj JS**, Ridlon JM, Hylemon PB, Thacker LR, Heuman DM, Smith S, Sikaroodi M, Gillevet PM. Linkage of gut microbiome with cognition in hepatic encephalopathy. *Am J Physiol Gastrointest Liver Physiol* 2012; **302**: G168-G175 [PMID: 21940902 DOI: 10.1152/ajpgi.00190.2011]
86 **Ahluwalia V**, Betrapally NS, Hylemon PB, White MB, Gillevet PM, Unser AB, Fagan A, Daita K, Heuman DM, Zhou H, Sikaroodi M, Bajaj JS. Impaired Gut-Liver-Brain Axis in Patients with Cirrhosis. *Sci Rep* 2016; **6**: 26800 [PMID: 27225869 DOI: 10.1038/srep26800]
87 **Johnson KV**, Foster KR. Why does the microbiome affect behaviour? *Nat Rev Microbiol* 2018; **16**: 647-655 [PMID: 29691482 DOI: 10.1038/s41579-018-0014-3]
88 **Peng CY**, Chien RN, Liaw YF. Hepatitis B virus-related decompensated liver cirrhosis: benefits of antiviral therapy. *J Hepatol* 2012; **57**: 442-450 [PMID: 22504333 DOI: 10.1016/j.jhep.2012.02.033]
89 **Caraceni P**, Vargas V, Solà E, Alessandria C, de Wit K, Trebicka J, Angeli P, Mookerjee RP, Durand F, Pose E, Krag A, Bajaj JS, Beuers U, Ginès P; Liverhope Consortium. The Use of Rifaximin in Patients With Cirrhosis. *Hepatology* 2021; **74**: 1660-1673 [PMID: 33421158 DOI: 10.1002/hep.31708]
90 **Woodhouse CA**, Patel VC, Singanayagam A, Shawcross DL. Review article: the gut microbiome as a therapeutic target in the pathogenesis and treatment of chronic liver disease. *Aliment Pharmacol Ther* 2018; **47**: 192-202 [PMID: 29083037 DOI: 10.1111/apt.14397]
91 **European Association For The Study Of The Liver**. EASL clinical practice guidelines: Management of chronic hepatitis B virus infection. *J Hepatol* 2012; **57**: 167-185 [PMID: 22436845 DOI: 10.1016/j.jhep.2012.02.010]
92 **Lok AS**, McMahon BJ, Brown RS Jr, Wong JB, Ahmed AT, Farah W, Almasri J, Alahdab F, Benkhadra K, Mouchli MA, Singh S, Mohamed EA, Abu Dabrh AM, Prokop LJ, Wang Z, Murad MH, Mohammed K. Antiviral therapy for chronic hepatitis B viral infection in adults: A systematic review and meta-analysis. *Hepatology* 2016; **63**: 284-306 [PMID: 26566246 DOI: 10.1002/hep.28280]
93 **Marcellin P**, Gane E, Buti M, Afdhal N, Sievert W, Jacobson IM, Washington MK, Germanidis G, Flaherty JF, Aguilar

Schall R, Bornstein JD, Kitrinos KM, Subramanian GM, McHutchison JG, Heathcote EJ. Regression of cirrhosis during treatment with tenofovir disoproxil fumarate for chronic hepatitis B: a 5-year open-label follow-up study. *Lancet* 2013; **381**: 468-475 [PMID: 23234725 DOI: 10.1016/S0140-6736(12)61425-1]

94 **Liaw YF**. Reversal of cirrhosis: an achievable goal of hepatitis B antiviral therapy. *J Hepatol* 2013; **59**: 880-881 [PMID: 23673137 DOI: 10.1016/j.jhep.2013.05.007]

95 **Su TH**, Hu TH, Chen CY, Huang YH, Chuang WL, Lin CC, Wang CC, Su WW, Chen MY, Peng CY, Chien RN, Huang YW, Wang HY, Lin CL, Yang SS, Chen TM, Mo LR, Hsu SJ, Tseng KC, Hsieh TY, Suk FM, Hu CT, Bair MJ, Liang CC, Lei YC, Tseng TC, Chen CL, Kao JH; C-TEAM study group and the Taiwan Liver Diseases Consortium. Four-year entecavir therapy reduces hepatocellular carcinoma, cirrhotic events and mortality in chronic hepatitis B patients. *Liver Int* 2016; **36**: 1755-1764 [PMID: 27634134 DOI: 10.1111/liv.13253]

96 **Li X**, Wu S, Du Y, Yang L, Li Y, Hong B. Entecavir therapy reverses gut microbiota dysbiosis induced by hepatitis B virus infection in a mouse model. *Int J Antimicrob Agents* 2020; **56**: 106000 [PMID: 32360229 DOI: 10.1016/j.ijantimicag.2020.106000]

97 **Lu YX**, He CZ, Wang YX, Ai ZS, Liang P, Yang CQ. Effect of Entecavir on the Intestinal Microflora in Patients with Chronic Hepatitis B: A Controlled Cross-Sectional and Longitudinal Real-World Study. *Infect Dis Ther* 2021; **10**: 241-252 [PMID: 33111216 DOI: 10.1007/s40121-020-00355-w]

98 **Lu YX**, Chang YZ, Liang P, Yang CQ. Effect of Additional Clostridium butyricum on the Intestinal Flora of Chronic Hepatitis B Patients Treated with Entecavir. *Infect Dis Ther* 2021; **10**: 1519-1530 [PMID: 34132991 DOI: 10.1007/s40121-021-00463-1]

99 **Alimirah M**, Sadiq O, Gordon SC. Novel Therapies in Hepatic Encephalopathy. *Clin Liver Dis* 2020; **24**: 303-315 [PMID: 32245535 DOI: 10.1016/j.cld.2020.01.009]

100 **Scarpignato C**, Pelosini I. Rifaximin, a poorly absorbed antibiotic: pharmacology and clinical potential. *Chemotherapy* 2005; **51** Suppl 1: 36-66 [PMID: 15855748]

101 **Bajaj JS**, Heuman DM, Wade JB, Gibson DP, Saeian K, Wegelin JA, Hafeezullah M, Bell DE, Sterling RK, Stravitz RT, Fuchs M, Luketic V, Sanyal AJ. Rifaximin improves driving simulator performance in a randomized trial of patients with minimal hepatic encephalopathy. *Gastroenterology* 2011; **140**: 478-487.e1 [PMID: 20849805 DOI: 10.1053/j.gastro.2010.08.061]

102 **Kimer N**, Pedersen JS, Tavenier J, Christensen JE, Busk TM, Hobolth L, Krag A, Al-Soud WA, Mortensen MS, Sørensen SJ, Møller S, Bendtsen F; members of the CoRif study group. Rifaximin has minor effects on bacterial composition, inflammation, and bacterial translocation in cirrhosis: A randomized trial. *J Gastroenterol Hepatol* 2018; **33**: 307-314 [PMID: 28671712 DOI: 10.1111/jgh.13852]

103 **Kaji K**, Saikawa S, Takaya H, Fujinaga Y, Furukawa M, Kitagawa K, Ozutsumi T, Kaya D, Tsuji Y, Sawada Y, Kawaratani H, Moriya K, Namisaki T, Akahane T, Mitoro A, Yoshiji H. Rifaximin Alleviates Endotoxemia with Decreased Serum Levels of Soluble CD163 and Mannose Receptor and Partial Modification of Gut Microbiota in Cirrhotic Patients. *Antibiotics (Basel)* 2020; **9** [PMID: 32235367 DOI: 10.3390/antibiotics9040145]

104 **Bajaj JS**, Heuman DM, Sanyal AJ, Hylemon PB, Sterling RK, Stravitz RT, Fuchs M, Ridlon JM, Daita K, Monteith P, Noble NA, White MB, Fisher A, Sikaroodi M, Rangwala H, Gillevet PM. Modulation of the metabiome by rifaximin in patients with cirrhosis and minimal hepatic encephalopathy. *PLoS One* 2013; **8**: e60042 [PMID: 23565181 DOI: 10.1371/journal.pone.0060042]

105 **Kang DJ**, Kakiyama G, Betrapally NS, Herzog J, Nittono H, Hylemon PB, Zhou H, Carroll I, Yang J, Gillevet PM, Jiao C, Takei H, Pandak WM, Iida T, Heuman DM, Fan S, Fiehn O, Kurosawa T, Sikaroodi M, Sartor RB, Bajaj JS. Rifaximin Exerts Beneficial Effects Independent of its Ability to Alter Microbiota Composition. *Clin Transl Gastroenterol* 2016; **7**: e187 [PMID: 27560928 DOI: 10.1038/ctg.2016.44]

106 **Bezirtzoglou E**, Stavropoulou E. Immunology and probiotic impact of the newborn and young children intestinal microflora. *Anaerobe* 2011; **17**: 369-374 [PMID: 21515397 DOI: 10.1016/j.anaerobe.2011.03.010]

107 **Stavropoulou E**, Bezirtzoglou E. Probiotics in Medicine: A Long Debate. *Front Immunol* 2020; **11**: 2192 [PMID: 33072084 DOI: 10.3389/fimmu.2020.02192]

108 **Lai HH**, Chiu CH, Kong MS, Chang CJ, Chen CC. Probiotic *Lactobacillus casei*: Effective for Managing Childhood Diarrhea by Altering Gut Microbiota and Attenuating Fecal Inflammatory Markers. *Nutrients* 2019; **11** [PMID: 31126062 DOI: 10.3390/nu11051150]

109 **Korpela K**, Salonen A, Vepsäläinen O, Suomalainen M, Kolmeder C, Varjosalo M, Miettinen S, Kukkonen K, Savilahti E, Kuitunen M, de Vos WM. Probiotic supplementation restores normal microbiota composition and function in antibiotic-treated and in caesarean-born infants. *Microbiome* 2018; **6**: 182 [PMID: 30326954 DOI: 10.1186/s40168-018-0567-4]

110 **Zhang Y**, Gu Y, Ren H, Wang S, Zhong H, Zhao X, Ma J, Gu X, Xue Y, Huang S, Yang J, Chen L, Chen G, Qu S, Liang J, Qin L, Huang Q, Peng Y, Li Q, Wang X, Kong P, Hou G, Gao M, Shi Z, Li X, Qiu Y, Zou Y, Yang H, Wang J, Xu G, Lai S, Li J, Ning G, Wang W. Gut microbiome-related effects of berberine and probiotics on type 2 diabetes (the PREMOTE study). *Nat Commun* 2020; **11**: 5015 [PMID: 33024120 DOI: 10.1038/s41467-020-18414-8]

111 **Sergeev IN**, Aljutaily T, Walton G, Huarte E. Effects of Synbiotic Supplement on Human Gut Microbiota, Body Composition and Weight Loss in Obesity. *Nutrients* 2020; **12** [PMID: 31952249 DOI: 10.3390/nu12010222]

112 **Kang DW**, Adams JB, Gregory AC, Borody T, Chittick L, Fasano A, Khoruts A, Geis E, Maldonado J, McDonough-Means S, Pollard EL, Roux S, Sadowsky MJ, Lipson KS, Sullivan MB, Caporaso JG, Krajmalnik-Brown R. Microbiota Transfer Therapy alters gut ecosystem and improves gastrointestinal and autism symptoms: an open-label study. *Microbiome* 2017; **5**: 10 [PMID: 28122648 DOI: 10.1186/s40168-016-0225-7]

113 **Chahwan B**, Kwan S, Isik A, van Hemert S, Burke C, Roberts L. Gut feelings: A randomised, triple-blind, placebo-controlled trial of probiotics for depressive symptoms. *J Affect Disord* 2019; **253**: 317-326 [PMID: 31078831 DOI: 10.1016/j.jad.2019.04.097]

114 **Bagga D**, Reichert JL, Koschutnig K, Aigner CS, Holzer P, Koskinen K, Moissl-Eichinger C, Schöpf V. Probiotics drive gut microbiome triggering emotional brain signatures. *Gut Microbes* 2018; **9**: 486-496 [PMID: 29723105 DOI:

10.1080/19490976.2018.1460015]
115 **Ziada DH**, Soliman HH, El Yamany SA, Hamisa MF, Hasan AM. Can Lactobacillus acidophilus improve minimal hepatic encephalopathy? *Arab J Gastroenterol* 2013; **14**: 116-122 [PMID: 24206740 DOI: 10.1016/j.ajg.2013.08.002]
116 **Xia X**, Chen J, Xia J, Wang B, Liu H, Yang L, Wang Y, Ling Z. Role of probiotics in the treatment of minimal hepatic encephalopathy in patients with HBV-induced liver cirrhosis. *J Int Med Res* 2018; **46**: 3596-3604 [PMID: 29806520 DOI: 10.1177/0300060518776064]
117 **Agrawal A**, Sharma BC, Sharma P, Sarin SK. Secondary prophylaxis of hepatic encephalopathy in cirrhosis: an open-label, randomized controlled trial of lactulose, probiotics, and no therapy. *Am J Gastroenterol* 2012; **107**: 1043-1050 [PMID: 22710579 DOI: 10.1038/ajg.2012.113]
118 **Thilakarathna WPDW**, Rupasinghe HPV, Ridgway ND. Mechanisms by Which Probiotic Bacteria Attenuate the Risk of Hepatocellular Carcinoma. *Int J Mol Sci* 2021; **22** [PMID: 33807605 DOI: 10.3390/ijms22052606]
119 **Ozdemir T**, Fedorec AJH, Danino T, Barnes CP. Synthetic Biology and Engineered Live Biotherapeutics: Toward Increasing System Complexity. *Cell Syst* 2018; **7**: 5-16 [PMID: 30048620 DOI: 10.1016/j.cels.2018.06.008]
120 **Nicaise C**, Prozzi D, Viaene E, Moreno C, Gustot T, Quertinmont E, Demetter P, Suain V, Goffin P, Devière J, Hols P. Control of acute, chronic, and constitutive hyperammonemia by wild-type and genetically engineered Lactobacillus plantarum in rodents. *Hepatology* 2008; **48**: 1184-1192 [PMID: 18697211 DOI: 10.1002/hep.22445]
121 **Kurtz CB**, Millet YA, Puurunen MK, Perreault M, Charbonneau MR, Isabella VM, Kotula JW, Antipov E, Dagon Y, Denney WS, Wagner DA, West KA, Degar AJ, Brennan AM, Miller PF. An engineered *E. coli* Nissle improves hyperammonemia and survival in mice and shows dose-dependent exposure in healthy humans. *Sci Transl Med* 2019; **11** [PMID: 30651324 DOI: 10.1126/scitranslmed.aau7975]
122 **Ochoa-Sanchez R**, Oliveira MM, Tremblay M, Petrazzo G, Pant A, Bosoi CR, Perreault M, Querbes W, Kurtz CB, Rose CF. Genetically engineered E. coli Nissle attenuates hyperammonemia and prevents memory impairment in bile-duct ligated rats. *Liver Int* 2021; **41**: 1020-1032 [PMID: 33548108 DOI: 10.1111/liv.14815]
123 **Khoruts A**, Sadowsky MJ. Understanding the mechanisms of faecal microbiota transplantation. *Nat Rev Gastroenterol Hepatol* 2016; **13**: 508-516 [PMID: 27329806 DOI: 10.1038/nrgastro.2016.98]
124 **Marotz CA**, Zarrinpar A. Treating Obesity and Metabolic Syndrome with Fecal Microbiota Transplantation. *Yale J Biol Med* 2016; **89**: 383-388 [PMID: 27698622]
125 **Niina A**, Kibe R, Suzuki R, Yuchi Y, Teshima T, Matsumoto H, Kataoka Y, Koyama H. Fecal microbiota transplantation as a new treatment for canine inflammatory bowel disease. *Biosci Microbiota Food Health* 2021; **40**: 98-104 [PMID: 33996366 DOI: 10.12938/bmfh.2020-049]
126 **Geng S**, Cheng S, Li Y, Wen Z, Ma X, Jiang X, Wang Y, Han X. Faecal Microbiota Transplantation Reduces Susceptibility to Epithelial Injury and Modulates Tryptophan Metabolism of the Microbial Community in a Piglet Model. *J Crohns Colitis* 2018; **12**: 1359-1374 [PMID: 30010734 DOI: 10.1093/ecco-jcc/jjy103]
127 **Sun MF**, Zhu YL, Zhou ZL, Jia XB, Xu YD, Yang Q, Cui C, Shen YQ. Neuroprotective effects of fecal microbiota transplantation on MPTP-induced Parkinson's disease mice: Gut microbiota, glial reaction and TLR4/TNF-α signaling pathway. *Brain Behav Immun* 2018; **70**: 48-60 [PMID: 29471030 DOI: 10.1016/j.bbi.2018.02.005]
128 **Kelly CR**, Khoruts A, Staley C, Sadowsky MJ, Abd M, Alani M, Bakow B, Curran P, McKenney J, Tisch A, Reinert SE, Machan JT, Brandt LJ. Effect of Fecal Microbiota Transplantation on Recurrence in Multiply Recurrent Clostridium difficile Infection: A Randomized Trial. *Ann Intern Med* 2016; **165**: 609-616 [PMID: 27547925 DOI: 10.7326/M16-0271]
129 **Khanna S**, Vazquez-Baeza Y, González A, Weiss S, Schmidt B, Muñiz-Pedrogo DA, Rainey JF 3rd, Kammer P, Nelson H, Sadowsky M, Khoruts A, Farrugia SL, Knight R, Pardi DS, Kashyap PC. Changes in microbial ecology after fecal microbiota transplantation for recurrent C. difficile infection affected by underlying inflammatory bowel disease. *Microbiome* 2017; **5**: 55 [PMID: 28506317 DOI: 10.1186/s40168-017-0269-3]
130 **Mocanu V**, Zhang Z, Deehan EC, Kao DH, Hotte N, Karmali S, Birch DW, Samarasinghe KK, Walter J, Madsen KL. Fecal microbial transplantation and fiber supplementation in patients with severe obesity and metabolic syndrome: a randomized double-blind, placebo-controlled phase 2 trial. *Nat Med* 2021; **27**: 1272-1279 [PMID: 34226737 DOI: 10.1038/s41591-021-01399-2]
131 **Suez J**, Zmora N, Zilberman-Schapira G, Mor U, Dori-Bachash M, Bashiardes S, Zur M, Regev-Lehavi D, Ben-Zeev Brik R, Federici S, Horn M, Cohen Y, Moor AE, Zeevi D, Korem T, Kotler E, Harmelin A, Itzkovitz S, Maharshak N, Shibolet O, Pevsner-Fischer M, Shapiro H, Sharon I, Halpern Z, Segal E, Elinav E. Post-Antibiotic Gut Mucosal Microbiome Reconstitution Is Impaired by Probiotics and Improved by Autologous FMT. *Cell* 2018; **174**: 1406-1423.e16 [PMID: 30193113 DOI: 10.1016/j.cell.2018.08.047]
132 **Ren YD**, Ye ZS, Yang LZ, Jin LX, Wei WJ, Deng YY, Chen XX, Xiao CX, Yu XF, Xu HZ, Xu LZ, Tang YN, Zhou F, Wang XL, Chen MY, Chen LG, Hong MZ, Ren JL, Pan JS. Fecal microbiota transplantation induces hepatitis B virus e-antigen (HBeAg) clearance in patients with positive HBeAg after long-term antiviral therapy. *Hepatology* 2017; **65**: 1765-1768 [PMID: 28027582 DOI: 10.1002/hep.29008]
133 **Chauhan A**, Kumar R, Sharma S, Mahanta M, Vayuuru SK, Nayak B, Kumar S, Shalimar. Fecal Microbiota Transplantation in Hepatitis B e Antigen-Positive Chronic Hepatitis B Patients: A Pilot Study. *Dig Dis Sci* 2021; **66**: 873-880 [PMID: 32279172 DOI: 10.1007/s10620-020-06246-x]
134 **Liu R**, Kang JD, Sartor RB, Sikaroodi M, Fagan A, Gavis EA, Zhou H, Hylemon PB, Herzog JW, Li X, Lippman RH, Gonzalez-Maeso J, Wade JB, Ghosh S, Gurley E, Gillevet PM, Bajaj JS. Neuroinflammation in Murine Cirrhosis Is Dependent on the Gut Microbiome and Is Attenuated by Fecal Transplant. *Hepatology* 2020; **71**: 611-626 [PMID: 31220352 DOI: 10.1002/hep.30827]
135 **Bajaj JS**, Kassam Z, Fagan A, Gavis EA, Liu E, Cox IJ, Kheradman R, Heuman D, Wang J, Gurry T, Williams R, Sikaroodi M, Fuchs M, Alm E, John B, Thacker LR, Riva A, Smith M, Taylor-Robinson SD, Gillevet PM. Fecal microbiota transplant from a rational stool donor improves hepatic encephalopathy: A randomized clinical trial. *Hepatology* 2017; **66**: 1727-1738 [PMID: 28586116 DOI: 10.1002/hep.29306]
136 **Bajaj JS**, Salzman NH, Acharya C, Sterling RK, White MB, Gavis EA, Fagan A, Hayward M, Holtz ML, Matherly S, Lee H, Osman M, Siddiqui MS, Fuchs M, Puri P, Sikaroodi M, Gillevet PM. Fecal Microbial Transplant Capsules Are Safe in

Hepatic Encephalopathy: A Phase 1, Randomized, Placebo-Controlled Trial. *Hepatology* 2019; **70**: 1690-1703 [PMID: 31038755 DOI: 10.1002/hep.30690]

137 **Shen TC**, Albenberg L, Bittinger K, Chehoud C, Chen YY, Judge CA, Chau L, Ni J, Sheng M, Lin A, Wilkins BJ, Buza EL, Lewis JD, Daikhin Y, Nissim I, Yudkoff M, Bushman FD, Wu GD. Engineering the gut microbiota to treat hyperammonemia. *J Clin Invest* 2015; **125**: 2841-2850 [PMID: 26098218 DOI: 10.1172/JCI79214]

138 **Liu J**, Zhai C, Rho JR, Lee S, Heo HJ, Kim S, Kim HJ, Hong ST. Treatment of Hyperammonemia by Transplanting a Symbiotic Pair of Intestinal Microbes. *Front Cell Infect Microbiol* 2021; **11**: 696044 [PMID: 35071025 DOI: 10.3389/fcimb.2021.696044]

139 **Ponziani FR**, Gerardi V, Pecere S, D'Aversa F, Lopetuso L, Zocco MA, Pompili M, Gasbarrini A. Effect of rifaximin on gut microbiota composition in advanced liver disease and its complications. *World J Gastroenterol* 2015; **21**: 12322-12333 [PMID: 26604640 DOI: 10.3748/wjg.v21.i43.12322]

140 **Zeng Y**, Chen S, Fu Y, Wu W, Chen T, Chen J, Yang B, Ou Q. Gut microbiota dysbiosis in patients with hepatitis B virus-induced chronic liver disease covering chronic hepatitis, liver cirrhosis and hepatocellular carcinoma. *J Viral Hepat* 2020; **27**: 143-155 [PMID: 31600845 DOI: 10.1111/jvh.13216]

141 **Kang Y**, Cai Y. Gut microbiota and hepatitis-B-virus-induced chronic liver disease: implications for faecal microbiota transplantation therapy. *J Hosp Infect* 2017; **96**: 342-348 [PMID: 28545829 DOI: 10.1016/j.jhin.2017.04.007]

142 **Xu M**, Wang B, Fu Y, Chen Y, Yang F, Lu H, Xu J, Li L. Changes of fecal Bifidobacterium species in adult patients with hepatitis B virus-induced chronic liver disease. *Microb Ecol* 2012; **63**: 304-313 [PMID: 21814872 DOI: 10.1007/s00248-011-9925-5]

143 **Wu ZW**, Lu HF, Wu J, Zuo J, Chen P, Sheng JF, Zheng SS, Li LJ. Assessment of the fecal lactobacilli population in patients with hepatitis B virus-related decompensated cirrhosis and hepatitis B cirrhosis treated with liver transplant. *Microb Ecol* 2012; **63**: 929-937 [PMID: 21965156 DOI: 10.1007/s00248-011-9945-1]

144 **Lutz P**, Parcina M, Bekeredjian-Ding I, Nischalke HD, Nattermann J, Sauerbruch T, Hoerauf A, Strassburg CP, Spengler U. Impact of rifaximin on the frequency and characteristics of spontaneous bacterial peritonitis in patients with liver cirrhosis and ascites. *PLoS One* 2014; **9**: e93909 [PMID: 24714550 DOI: 10.1371/journal.pone.0093909]

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MINIREVIEWS

Combination approaches in hepatocellular carcinoma: How systemic treatment can benefit candidates to locoregional modalities

Leonardo Gomes da Fonseca, Raphael L C Araujo

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Abstract

The management of hepatocellular carcinoma (HCC) is challenging because most patients have underlying cirrhosis, and the treatment provides, historically, a limited impact on the natural history of patients with advanced-stage disease. Additionally, recurrence rates are high for those patients who receive local and locoregional modalities, such as surgical (resection and transplantation) or image-guided (ablation and intra-arterial) therapies. Translational research has led to new concepts that are reshaping the current clinical practice. Substantial advancements were achieved in the understanding of the hallmarks that drive hepatocarcinogenesis. This has primed a successful incorporation of novel agents with different targets, such as anti-angiogenic drugs, targeted-therapies, and immune-checkpoint inhibitors. Although clinical trials have proven efficacy of systemic agents in advanced stage disease, there is no conclusive evidence to support their use in combination with loco-regional therapy. While novel local modalities are being incorporated (*e.g.*, radioembolization, microwave ablation, and irreversible electroporation), emerging data indicate that locoregional treatments may induce tumor microenvironment changes, such as hyperexpression of growth factors, release of tumor antigens, infiltration of cytotoxic lymphocytes, and modulation of adaptative and innate immune response. Past trials that evaluated the use of antiangiogenic drugs in the adjuvant setting after ablation or chemoembolization fail to demonstrate a substantial improvement. Current efforts are directed to investigate the role of immunotherapy-based regimens in this context. The present review aims to describe the current landscape of systemic and locoregional treatments for HCC, present evidence to support combination approaches, and address future perspectives.

Key Words: Liver cancer; Hepatocellular carcinoma; Immunotherapy; Systemic therapy; Ablation; Embolization

Core Tip: Management of hepatocellular carcinoma (HCC) is based on stages defined by tumor burden, liver function, and performance status. With the advent of more effective systemic treatments, such as immunotherapy and immunotherapy-based combinations, patients with advanced stage disease have better outcomes. The migration of systemic treatment to earlier stages, in combination with locoregional therapies, are expected to improve the outcomes and cure rates. Currently, the research field is moving towards an increasing interest in combining locoregional and systemic treatments for HCC.

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INTRODUCTION

Hepatocellular carcinoma (HCC) is a highly fatal disease, representing the fourth cause of cancer-related mortality worldwide[1]. This situation is attributed to the challenging management of patients with HCC *per se* in concomitance with an underlying liver disease and cirrhosis-related complications. Moreover, a significant proportion of patients are diagnosed with advanced stage disease, not amenable to curative options. Finally, even patients who are treated with local therapies, such as surgery and ablation, present high rates of recurrence, reaching up to 70% in 5 years[2].

Treatment options for HCC are selected based on liver function, performance status, and tumor burden (size, number of lesions, metastatic spread, and vascular invasion). These patient- and tumor-centered characteristics define the stage of the cancer, each with a different prognosis[3]. Thus, clinical practice guidelines according to both tumor presentation and liver function integrate the available evidence based on well-delineated clinical trials and recommend treatment strategies for each stage[2,4] (Figure 1).

Liver resection, local ablation, and liver transplantation are recommended for very-early and early-stage disease and are considered curative-intent treatments for HCC. Intra-arterial therapies, such as transarterial chemoembolization (TACE), are indicated for cases classified as intermediate stage (multinodular liver disease beyond Milan-criteria with preserved liver function), and systemic treatments are used for patients with advanced stage disease[3]. Therapeutic management of HCC is evolving rapidly with the incorporation of novel locoregional techniques and the increasing number of systemic agents tested in clinical trials, including immunotherapy. Each treatment approach is directed to treat HCC at different stages, and there is a lack of evidence to support the use of combined systemic and locoregional treatments. Therefore, the biological rational behind combination strategies and the urgent need to improve outcomes for this lethal disease prime intense research activity in both basic and clinical fields. This review aims to address the current landscape of systemic and locoregional treatments and the future perspectives regarding combined approaches.

SYSTEMIC TREATMENT FOR HCC: CURRENT LANDSCAPE AND PERSPECTIVES

Systemic treatment is recommended for patients with advanced stage disease (preserved liver function, performance status 0-2, metastatic spread, and/or macrovascular invasion) and for patients in earlier stages who have contra-indications or progressed after locoregional modalities according to the concept of treatment stage migration[3].

The natural history of advanced HCC is poor, with a median overall survival of 4-8 mo without active treatments[5]. Since 2008, the use of agents that target hallmarks of hepatocarcinogenesis are gradually improving prognosis and achieving substantial clinical benefit for HCC patients. Sorafenib, a multikinase inhibitor, has been shown to improve overall survival over placebo in the SHARP and Asia-Pacific trials and became the first approved drug for advanced HCC[6,7]. After almost a decade, other drugs of the same class were tested with positive results in phase III trials, such as lenvatinib (non-inferior to sorafenib in first-line)[8], regorafenib (superior over placebo in second-line for patients who tolerated sorafenib)[9], and cabozantinib (superior over placebo after sorafenib failure)[10]. These drugs

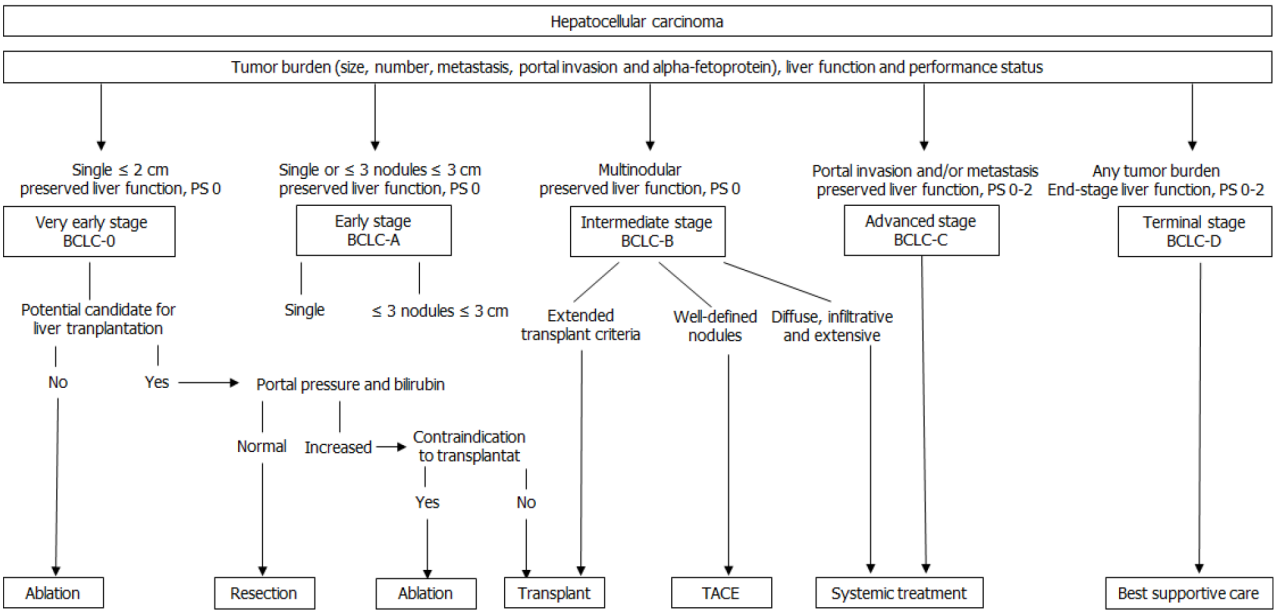

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Figure 1 Treatment algorithm for hepatocellular carcinoma based on level-1 evidence. The algorithm establishes five stages according to liver function, tumor burden, and performance status. Each stage is linked to first-line treatment recommendation, although individual decisions can be defined according to patient profile and available treatment options. Adapted from Ref. [3]. BCLC: Barcelona clinic liver cancer; TACE: Transarterial chemoembolization.

share common targets, such as vascular endothelial growth factor receptor (VEGFR) and platelet-derived growth factor receptor, while some of them present, individually, more direct actions against specific targets, such as the case of lenvatinib against fibroblast growth factor receptor, and cabozantinib against mesenchymal-epithelial transition (MET) receptor. Additionally, ramucirumab, a monoclonal antibody against VEGFR, was shown to improve overall survival over placebo in patients who present with alpha-fetoprotein (AFP) ≥ 400 ng/mL after sorafenib failure and is also approved for clinical use [11]. Generally, these drugs present antiangiogenic activity and promote clinical benefit by delaying tumor progression rather than inducing a substantial reduction in tumor burden. Trials with these drugs are consistent in showing a statically significant, but modest, increase in overall survival, with survivals around 11-13 mo in the first line, a response rate of less than 10% (except for lenvatinib, with a response rate of 18.8% in the REFLECT trial), and a range of class-related adverse events, such as fatigue, arterial hypertension, skin reaction, and diarrhea[12].

In the past years, the advent of immune-based treatments, such as immune checkpoint inhibitors, brought huge advances in HCC management. The biological background for the use of immunotherapy in HCC is based on the remarkable immunotolerance of the liver due to the high antigenic load derived from the enteral-portal circulation. Additionally, HCC develops in a microenvironment of chronic inflammation and underlying liver disease. Low infiltration of CD8 + T lymphocytes, responsible for the antitumor immune response, and a marked presence of exhausted lymphocytes and regulatory T lymphocytes are described in HCC, contributing to an immunosuppressive and procarcinogenic microenvironment[13,14].

The immune checkpoint inhibitors aimed at boosting anti-tumoral immunity in the priming phase by the recognition of antigens presented by dendritic cells to lymphocytes (anti-CTLA4 agents) and in the effector phase of T-CD8 cytotoxic cells against tumor cells (anti-PD1, anti-PDL1) were tested in phase I/II trials showing durable response rates of around 15%-20%. Therefore, pembrolizumab and nivolumab (anti-PD1 drugs) received approval for use in second-line treatments[15,16]. However, comparative trials showed that immune checkpoint inhibitors as monotherapy did not improve outcomes comparing to the available treatments. In a phase III trial, nivolumab was not proven to improve survival compared to sorafenib in the first line[17] and pembrolizumab did not achieve statistical superiority over placebo in the second-line[18].

The use of combinations of agents with different mechanisms of action can improve results of immunotherapy through additive effect and because VEGF can enhance the immunosuppression of the tumor microenvironment by inhibiting the function of effector T cells, increasing the recruitment of regulatory T cells and myeloid-derived suppressor cells[19]. This combination was explored in the IMBRAVE150 trial, which randomized patients to receive sorafenib *vs* a combination of bevacizumab (an anti-VEGF antibody) and atezolizumab (an anti-PD-L1 antibody). This trial demonstrated a significant improvement in overall survival (19.2 mo *vs* 13.4 mo), and also in progression-free survival and response rate[20,21]. IMBRAVE150 trial was a landmark in HCC management and marked the transition towards the use of combined systemic therapies (using dual-immunotherapy or antian-

giogenic plus immunotherapy combinations). Several combinations showed encouraging results in phases I and II trials, such as lenvatinib plus pembrolizumab[22], pembrolizumab plus regorafenib[23], nivolumab plus ipilimumab[24], and nivolumab, ipilimumab, and cabozantinib[25]. These preliminary results suggested that combinations may yield response rates of more than 20% according to RECIST criteria, which seems to compare favorably to the rates with multikinase inhibitors. However, it is important to point out some limitations of early phase trials. Firstly, non-comparative trials are not adequate to draw definitive conclusions. Besides, the response rate is not a surrogate marker for survival benefit in HCC, even more considering that RECIST may not capture the spectrum and patterns of progression and response in patients under immunotherapy.

Recently, durvalumab and tremelimumab as a combination treatment was announced to yield survival benefit over sorafenib in a phase III trial and is expected to be incorporated in the first-line setting as an alternative to atezolizumab plus bevacizumab[26,27]. Currently, other phase III trials are awaiting results in the context of combination therapies for advanced HCC (Table 1).

ROLE OF LOCOREGIONAL THERAPIES IN HCC: IS THERE ROOM FOR IMPROVING OUTCOMES?

Liver resection is indicated in early HCC, regardless of the presence of cirrhosis, since the liver function remains compensated, and is rarely is does the patient have a clinically significant portal hypertension. However, the risk of liver decompensation after resection in patients with restricted hepatic functional reserve is a concern, especially in cases requiring major hepatectomies (3 or more segments)[2,28,29]. On the other side, liver transplantation has the advantage of treating both cirrhosis and offer curative-intent treatment for HCC. Thus, liver transplantation decreases both the risk of recurrence, since the most recurrence is in the liver, and *de novo* lesions by removing the cirrhotic liver. But its application in clinical practice is hampered by organ shortage, complexity, heterogenous availability in different worldwide regions, and the risk of progression on waiting list[30].

In patients with liver-only disease not amenable to surgical modalities, locoregional interventional procedures play a key role. Locoregional treatment is defined as imaging-guided tumor directed procedures, and it is estimated that 50% of patients with HCC might receive any of these treatments during the course of the disease[31,32]. Basically, there are two groups of locoregional therapies: (1) Ablative therapies; or (2) Intra-arterial therapies. Although both modalities are consolidated with high level evidence, emerging approaches and techniques are being increasingly adopted and are expected to improve clinical outcomes. Besides, the risk of recurrence and progression in patients treated with locoregional therapies has led to intense research activity towards combination with systemic treatment [2,4].

ABLATIVE THERAPIES

Current evidence of ablative methods in HCC

Ablative therapies induce tumor destruction through different mechanisms according to the method: Chemical [percutaneous ethanol injection (PEI)], thermal [radiofrequency ablation (RFA), microwave (MWA), and cryoablation], and short pulses of high voltage (irreversible electroporation). In general, ablative techniques are considered a curative therapy for HCC < 2-3 cm and is associated with complete responses in 70%-90% of the cases, although recurrences occur in approximately 50% of the cases within 5 years[33-35].

The most used and recommended method is RFA, which has shown survival superiority over PEI in randomized trials[33]. Tumor size, number of nodules, and Child-Pugh class are associated with prognosis in patients treated with RFA[36-38]. Comparative studies *vs* resection demonstrated that RFA seems to be inferior to surgery in tumors > 3 cm[39]. Limitations to RFA include proximity to large vessels (due to heat effect), size, and location.

MWA is another technique that has been increasingly used and has the advantage of achieving a faster heating over a larger volume and being less susceptible to heat sink effect. Most studies that addressed comparisons between RFA and MWA showed similar efficacy, with a trend toward best results with MWA in tumors > 3 cm[40-42].

Alternative techniques, such as IRE and cryoablation, are under active research, with a small series of studies showing encouraging results[43,44]. IRE has the advantage of causing less thermal damage to adjacent tissues. However, more data are still required for IRE and cryoablation in order to fully incorporate these techniques into clinical practice guidelines.

Combination of ablative and systemic treatment: rationale and current evidence

The high risk of local and distant recurrence after ablation indicates the need for adjuvant strategies to improve cure rates. Features, such as large tumors, multinodularity, and vascular invasion (macroscopic

Table 1 Clinical trials in advanced hepatocellular carcinoma with combination of systemic treatment

Study name	Design	Experimental arm	Median overall survival, mo	Response rate-RECIST, %	Grade 3 - 4 treatment-related adverse events
IMBRAVE 150[21]	Phase III; First-line	Atezolizumab + Bevacizumab	19.2	30%	36%
KEYNOTE 524/Study 116[22]	Phase Ib; First-line	Pembrolizumab + Lenvatinib	22	36%	67%
REG-PEMBRO-HCC [23]	Phase Ib; First-line	Pembrolizumab + Regorafenib	26.5	32%	86%
CHECKMATE 040[24]	Phase II; Second-line	Nivolumab + Ipilimumab (arm A)	22.8	32%	53%
STUDY 22[27]	Phase II; Second-line	Durvalumab + Tremelimumab	18.7	24%	35.1%
CHECKMATE-040[25]	Phase II; First and second-line	Nivolumab + Ipilimumab + Cabozantinib	Not-reached	26%	71%

or microscopic), are significantly related to higher recurrence rates. Novel markers, such as genetic signatures, circulating microRNA, circulating non-coding RNA, circulating cell-free DNA, gut microbiota, and circulating tumor cells, have also been shown to predict the risk of recurrence[45-47].

To evaluate the impact of multikinase inhibitors with antiangiogenic activity combined with ablation, the STORM trial randomized patients treated with resection (n = 900) or ablation (n = 214) to sorafenib or placebo for a total of 4 years. Unfortunately, there was no difference in recurrence-free survival between groups, with a median to time to recurrence of 33.3 mo in the sorafenib group and 33.7 mo for the placebo group (HR = 0.94, P = 0.26)[48]. Thus, the positive results obtained with sorafenib in advanced disease have not been reproduced in the context of adjuvant post-resection or ablation. Other drugs used in the advanced stage, such as cabozantinib, regorafenib, ramucirumab, and lenvatinib have not been evaluated in patients after ablation. In sum, VEGFR-directed therapies as monotherapy do not seem to improve the results of ablation.

Recently, the immune features of localized HCC and the response to local treatment are being investigated[49-51]. The concentration of intratumor CD3 + and CD8 + T cells and the expression of programmed death ligand 1 (PD-L1) by immune and tumor cells appear to be associated with HCC aggressiveness and risk of recurrence after curative treatments, such as ablation and surgery[13]. The high expression of PD1 can lead a condition of exhausted CD8 + impairing cytotoxicity and decreasing of pro-inflammatory cytokines production and anti-tumor ability[13].

Ablation can induce stimulate inflammatory and cytokine production within the treated site. The tumor debris released upon ablation represent a tumor antigen repertoire that can boost antitumoral immunologic response. Indeed, tumor antigens can be found in dendritic cells in lymph nodes following ablation[52]. This background indicates a potential role for immune checkpoint inhibitors directed to PD1 and PD-L1 in combination with ablative therapies (Figure 2). A phase I trial including 32 patients treated with tremelimumab and ablation showed accumulation of CD8+ cells, suggesting immune system activation[53]. Preliminary results of the NIVOLVE trial were presented in 2021. In this phase II single arm trial, 55 patients after surgery (n = 33) or ablation (n = 22) received nivolumab for 12 mo. The median recurrence-free survival was 26 mo. In this trial, the authors suggested that patients with tumor-infiltrating lymphocytes, positive PD-L1, and negative staining for beta-catenin tend to develop fewer recurrences[54]; however, larger and comparative studies are needed to confirm these findings. Currently, several active trials (recruiting or waiting results) are addressing the role of immune-checkpoint inhibitors in combination with anti-VEGFR agents or alone after curative treatments, such as ablation (Table 2).

INTRA-ARTERIAL THERAPIES

Current evidence of intra-arterial therapy in HCC

Intra-arterial therapies harness the arterial supply of HCC that differs from the liver parenchyma, which receives its blood supply predominantly from the portal vein. This group of therapies are composed of transarterial embolization (TAE), chemoembolization (TACE), and selective internal radiotherapy (SIRT) or radioembolization (TARE).

The mechanism of action of TAE is based on the obstruction of the blood supply causing ischemia, while TACE adds a cytotoxic agent (most commonly doxorubicin) and a carrier agent (commonly lipiodol). Drug-eluting bead (DEB)-TACE uses microspheres carrying cytotoxic agents which are associated with a sustained drug release and, theoretically, a lower systemic exposure to chemotherapy

Table 2 Clinical trials approaching combination of systemic treatment and ablation enrolling, recruiting, or waiting for final results

Trial	Trial registration	Drug	Control	*n*	Disease stage	Local treatment	Expected termination	Primary end-point
CHECKMATE 9DX	NCT03383458	Nivolumab	Placebo	530	Early/intermediate	Surgery/Ablation	2025	Recurrence-free survival
KEYNOTE 937	NCT03867084	Pembrolizumab	Placebo	950	Early/intermediate	Surgery/Ablation	2025	Recurrence-free survival and overall survival
IMBRAVE 050	NCT04102098	Atezolizumab + Bevacizumab	Surveillance	662	Early/intermediate /advanced	Surgery/Ablation	2027	Recurrence-free survival
EMERALD-2	NCT03847428	Durvalumab + Bevacizumab	Placebo	888	Early/intermediate	Surgery/Ablation	2024	Recurrence-free survival

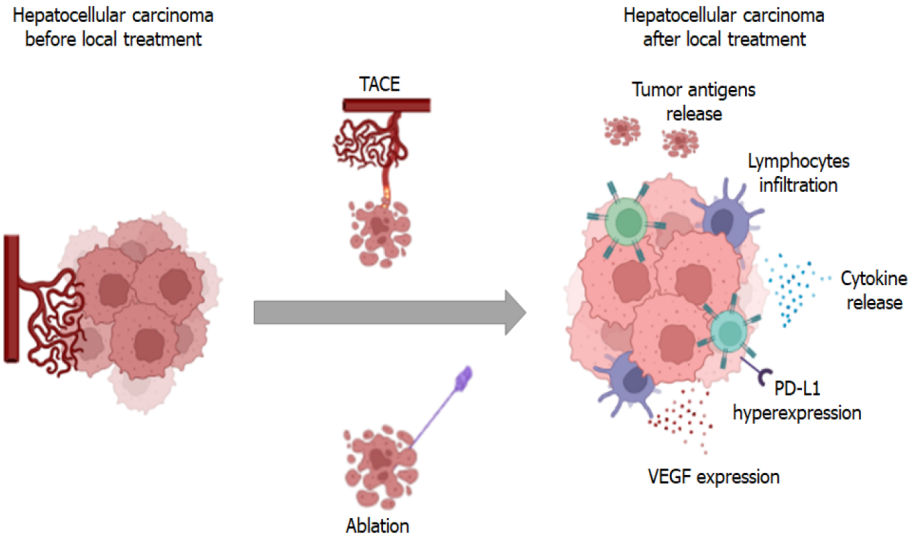

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Figure 2 Mechanisms of tumor microenvironment changes after locoregional therapies. After local interventions, tumor immune microenvironment may change with a pronounced release of tumor antigens, cytokines, lymphocyte infiltration, and hyperexpression of vascular endothelial growth factor. TACE: Transarterial chemoembolization; PD-L1: Programmed death ligand 1; VEGF: Vascular endothelial growth factor.

[55].

Based on randomized clinical trials of TACE *vs* no active treatment and also in a large meta-analysis, this is considered the standard of care for patients with intermediate-stage [Barcelona clinic liver cancer (BCLC)-B: Multinodular disease, preserved liver function, and performance status of 0][56,57]. In published cohorts with selected patients, the median overall survival with TACE reaches 48 mo[58]. There is no definitive evidence for the superiority of TACE over TAE, although data from meta-analysis suggests that the former is associated with better outcomes[57]. A prospective trial demonstrated that DEB-TACE presents lower treatment-related toxicities and a trend towards a better objective response rate compared to conventional TACE[59].

Guidelines recommend that patients who present progression with infiltrative tumors, vascular invasion, metastatic spread, or absence of a significant response after two TACEs should be considered for systemic treatment[2,4]. The risk of liver deterioration should be carefully monitored after TACE due to the prognostic impact of liver decompensation in patients with HCC[60]. The decision to declare TACE failure and switch to systemic treatment is heterogenous in different parts of the world, and many scores have been proposed to help in this decision, although some of them still require further validation in a population treated strictly according to the BCLC algorithm[61-65]. For example, the hepatoma-arterial embolization (HAP) score includes albumin, AFP, bilirubin and tumor diameter and was concepted to predict prognosis before the first TACE in a heterogeneous cohort with around 30% of BCLC-C patients[64]. The Assessment for Retreatment with TACE (ART) score was designed to evaluate benefit of a 2nd TACE and assess Child-Pugh score, AST levels, and tumor response after the first TACE. The ART score was originally developed in a cohort that included around 20% of patients with impaired liver function (Child-Pugh B8 or more)[66].

With the availability of more effective systemic agents, the migration from TACE to systemic treatment is a key decision in the management of HCC and should not be delayed at a point of

irreversible deterioration of liver function or performance status.

TARE has been increasingly studied in HCC and has become a common practice in many centers. Data comparing TACE to TARE demonstrated in patients with early or intermediate stage a longer time to progression favoring TARE, but no survival benefit[67]. Although encouraging, there is no solid evidence that TARE should became standard of care in patients eligible to intra-arterial therapies.

A recent single-arm retrospective study including patients with solitary HCC ≤ 8 cm reported objective response rates of 88.3% and a 3-years survival of 86.6%. In this study TARE served as a neoadjuvant therapy for transplantation or resection in 21% and 6.8% of patients, respectively[68].

In uncontrolled trials, TARE was suggested to be safe in patients with portal vein invasion[69]. However, randomized trials in the setting of advanced stage HCC did not prove superiority of TARE over sorafenib in overall survival[70,71]. Therefore, TARE is not recommended as a standard therapy in patients classified as BCLC-C. There is a need to better explore the role of TARE in HCC in prospective and randomized trials, especially for subgroups of patients requiring downstaging, segmental portal vein thrombosis, and using rigorous dosimetry.

Combination of intra-arterial and systemic treatment: rationale and current evidence

Although TACE is the standard treatment for intermediate stage, only around 50% of patients present objective response rate and most of them will eventually present distant and local progression[72]. This has led to studies focused on improving the outcomes for HCC patients treated with TACE by combining multikinase inhibitors.

The rational for testing these combinations comes from the demonstration of increased intratumorally micro vessel density and VEGF expression in residual tumors after TACE, suggesting that TACE may stimulate tumor angiogenesis[73]. However, antiangiogenic drugs, such as sorafenib[74,75], brivanib [76], and bevacizumab[77], failed to demonstrated survival benefit in combination with TACE in controlled trials.

Recently, TACTIS trials randomized patients to TACE alone or combined with sorafenib and showed that sorafenib improved progression-free survival (25.2 mo *vs* 13.5 mo; P = 0.006), but with no significant impact on overall survival (36.2 mo *vs* 30.8 mo; P = 0.40)[78,79]. Currently, available evidence fails to encourage the use of multikinase inhibitors in combination with TACE as a standard practice, but there is a growing enthusiasm with the use of immunotherapy in this setting.

Similar to ablative treatments, intra-arterial therapies may modulate innate and adaptative immunity by releasing cellular debris and inflammatory cytokines and inducing T-cell responses, what is suggested to be related to improved prognosis[80,81] (Figure 2). A recent study compared HCC tissue from patients who were previously submitted to TACE to patients not treated with TACE and found that the former had a lower density of immune-exhausted effector cytotoxic and T-regs with an upregulation of pro-inflammatory pathways[82]. Considering this background, strategies aimed at blocking negative regulators such as PD-1 and CTLA4 are promising and are being investigated in phase III clinical trials (Table 3).

TARE also seems to modulate immunological microenvironment in HCC. A study that compared findings before and after TARE showed that tumor-infiltrating lymphocytes after TARE exhibited signs of immune activation, such as higher expression of granzyme-B and infiltration of CD8+Tcells, NK cells, and antigen-presenting cells[83,84]. Initial data on the combination of nivolumab and SIRT showed favorable safety profile, but efficacy data are pending[85,86].

Besides the biological background, the use of systemic treatment in earlier stages (combined with locoregional treatment) may benefit those patients who would present clinical deterioration or liver function worsening during TACE-refractoriness and would not found fit to receive further systemic treatment in that situation.

CONCLUSION

The treatment landscape of HCC management evolved rapidly in the past years. The most relevant achievements took place in the management of advanced stage disease. The median overall survival in pivotal clinical trials shifted from 5-8 mo in placebo arms[6], to almost 20 mo with immunotherapy combinations[21]. Still, some questions remain to be answer regarding the identification of predictive biomarkers and the optimal sequence in first-, second-, and later lines[87-89].

On the other, the field evolved modestly in the setting of local and locoregional stages, comprising early and intermediate stages. Patients in the early stage are offered surgical resection, liver transplantation, or ablation. Due to organ shortage and strict criteria to transplant, surgery and ablation has been widely used. Unfortunately, their recurrence rates are high and determine results in long-term mortality[33-35]. Although attempts were made, no systemic treatment has improved outcomes in early stage. Robust evidence supports those immunological changes occur after tumor ablation and the use immunotherapy is encouraging.

Patients in the intermediate stage have historically been offered TACE with the aim to achieve tumor control. Similarly, most of them will eventually present local or distant progression and will be

Table 3 Clinical trials approaching combination of systemic treatment and intra-arterial actually enrolling, recruiting or waiting for final results

Trial	Trial registration	Drug	Control	*n*	Disease stage	Local treatment	Expected termination	Primary end-point
EMERALD-1	NCT03778957	Durvalumab + Bevacizumab plus TACE	TACE plusplacebo	600	Intermediate/advanced	TACE	2024	Progression-free survival
TACE-3	NCT04268888	Nivolumab plus DEB-TACE	DEB- TACE	522	Intermediate	DEB-TACE	2026	Overall survival
LEAP-012	NCT04246177	Lenvatinib plus Pembrol-izumab plus cTACE	cTACE	950	Intermediate	Surgery/Ablation	2029	Overall survival and progression-free survival
CheckMate 74 W	NCT04340193	Nivolumab plusIpilimumab/placebo plus cTACE	cTACE plusplacebo	765	Intermediate	Surgery/Ablation	Non-available	Time-to-progression and overall survival

c: Conventional; DEB: Drug-eluting beads; TACE: Transarterial chemoembolization.

considered for systemic treatment. The concomitant use of immunotherapy-based systemic agents and intra-arterial therapies may enhance anti-tumor immune response and impact positively on the outcomes. Several clinical trials are eagerly awaited.

The most promising ongoing trials approach the combination of TACE and immunotherapy, with the aim to significantly delay time-to-progression and, hopefully, increase cure rates for patients who achieve complete response with locoregional treatment. Similarly, trials that test adjuvant immunotherapy after ablation may increase cure rates by reducing the risk of recurrence and -*de novo* tumors. It is likely that biomarkers and subsets of clinical features will emerge from these trials and will support the selection of patients that will derive more benefit from combination approaches.

Finally, it is key to consider that if active trials succeed in showing a better anti-tumor efficacy with combination of locoregional and systemic treatment, a longer survival of patients with HCC will be expected. Furthermore, this will highlight the burden of cirrhosis-related complications as a competing risk of mortality and the importance of multidisciplinary teams as the standard approach for HCC.

FOOTNOTES

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REFERENCES

1 **Llovet JM**, Zucman-Rossi J, Pikarsky E, Sangro B, Schwartz M, Sherman M, Gores G. Hepatocellular carcinoma. *Nat Rev Dis Primers* 2016; **2**: 16018 [PMID: 27158749 DOI: 10.1038/nrdp.2016.18]

2 **European Association for the Study of the Liver**. EASL Clinical Practice Guidelines: Management of hepatocellular carcinoma. *J Hepatol* 2018; **69**: 182-236 [PMID: 29628281 DOI: 10.1016/j.jhep.2018.03.019]

3 **Reig M**, Forner A, Rimola J, Ferrer-Fàbrega J, Burrel M, Garcia-Criado Á, Kelley RK, Galle PR, Mazzaferro V, Salem R, Sangro B, Singal AG, Vogel A, Fuster J, Ayuso C, Bruix J. BCLC strategy for prognosis prediction and treatment recommendation: The 2022 update. *J Hepatol* 2022; **76**: 681-693 [PMID: 34801630 DOI: 10.1016/j.jhep.2021.11.018]

4 **Heimbach JK**, Kulik LM, Finn RS, Sirlin CB, Abecassis MM, Roberts LR, Zhu AX, Murad MH, Marrero JA. AASLD guidelines for the treatment of hepatocellular carcinoma. *Hepatology* 2018; **67**: 358-380 [PMID: 28130846 DOI: 10.1002/hep.29086]

5 **Llovet JM**, Bustamante J, Castells A, Vilana R, Ayuso Mdel C, Sala M, Brú C, Rodés J, Bruix J. Natural history of untreated nonsurgical hepatocellular carcinoma: rationale for the design and evaluation of therapeutic trials. *Hepatology* 1999; **29**: 62-67 [PMID: 9862851 DOI: 10.1002/hep.510290145]

6 **Llovet JM**, Ricci S, Mazzaferro V, Hilgard P, Gane E, Blanc JF, de Oliveira AC, Santoro A, Raoul JL, Forner A, Schwartz M, Porta C, Zeuzem S, Bolondi L, Greten TF, Galle PR, Seitz JF, Borbath I, Häussinger D, Giannaris T, Shan M, Moscovici M, Voliotis D, Bruix J; SHARP Investigators Study Group. Sorafenib in advanced hepatocellular carcinoma. *N Engl J Med* 2008; **359**: 378-390 [PMID: 18650514 DOI: 10.1056/NEJMoa0708857]

7 **Cheng AL**, Kang YK, Chen Z, Tsao CJ, Qin S, Kim JS, Luo R, Feng J, Ye S, Yang TS, Xu J, Sun Y, Liang H, Liu J, Wang J, Tak WY, Pan H, Burock K, Zou J, Voliotis D, Guan Z. Efficacy and safety of sorafenib in patients in the Asia-Pacific region with advanced hepatocellular carcinoma: a phase III randomised, double-blind, placebo-controlled trial. *Lancet Oncol* 2009; **10**: 25-34 [PMID: 19095497 DOI: 10.1016/S1470-2045(08)70285-7]

8 **Kudo M**, Finn RS, Qin S, Han KH, Ikeda K, Piscaglia F, Baron A, Park JW, Han G, Jassem J, Blanc JF, Vogel A, Komov D, Evans TRJ, Lopez C, Dutcus C, Guo M, Saito K, Kraljevic S, Tamai T, Ren M, Cheng AL. Lenvatinib versus sorafenib in first-line treatment of patients with unresectable hepatocellular carcinoma: a randomised phase 3 non-inferiority trial. *Lancet* 2018; **391**: 1163-1173 [PMID: 29433850 DOI: 10.1016/S0140-6736(18)30207-1]

9 **Bruix J**, Qin S, Merle P, Granito A, Huang YH, Bodoky G, Pracht M, Yokosuka O, Rosmorduc O, Breder V, Gerolami R, Masi G, Ross PJ, Song T, Bronowicki JP, Ollivier-Hourmand I, Kudo M, Cheng AL, Llovet JM, Finn RS, LeBerre MA, Baumhauer A, Meinhardt G, Han G; RESORCE Investigators. Regorafenib for patients with hepatocellular carcinoma who progressed on sorafenib treatment (RESORCE): a randomised, double-blind, placebo-controlled, phase 3 trial. *Lancet* 2017; **389**: 56-66 [PMID: 27932229 DOI: 10.1016/S0140-6736(16)32453-9]

10 **Abou-Alfa GK**, Meyer T, Cheng AL, El-Khoueiry AB, Rimassa L, Ryoo BY, Cicin I, Merle P, Chen Y, Park JW, Blanc JF, Bolondi L, Klümpen HJ, Chan SL, Zagonel V, Pressiani T, Ryu MH, Venook AP, Hessel C, Borgman-Hagey AE, Schwab G, Kelley RK. Cabozantinib in Patients with Advanced and Progressing Hepatocellular Carcinoma. *N Engl J Med* 2018; **379**: 54-63 [PMID: 29972759 DOI: 10.1056/NEJMoa1717002]

11 **Zhu AX**, Park JO, Ryoo BY, Yen CJ, Poon R, Pastorelli D, Blanc JF, Chung HC, Baron AD, Pfiffer TE, Okusaka T, Kubackova K, Trojan J, Sastre J, Chau I, Chang SC, Abada PB, Yang L, Schwartz JD, Kudo M; REACH Trial Investigators. Ramucirumab versus placebo as second-line treatment in patients with advanced hepatocellular carcinoma following first-line therapy with sorafenib (REACH): a randomised, double-blind, multicentre, phase 3 trial. *Lancet Oncol* 2015; **16**: 859-870 [PMID: 26095784 DOI: 10.1016/S1470-2045(15)00050-9]

12 **da Fonseca LG**, Reig M, Bruix J. Tyrosine Kinase Inhibitors and Hepatocellular Carcinoma. *Clin Liver Dis* 2020; **24**: 719-737 [PMID: 33012455 DOI: 10.1016/j.cld.2020.07.012]

13 **Ma J**, Zheng B, Goswami S, Meng L, Zhang D, Cao C, Li T, Zhu F, Ma L, Zhang Z, Zhang S, Duan M, Chen Q, Gao Q, Zhang X. $PD1^{Hi}$ $CD8^{+}$ T cells correlate with exhausted signature and poor clinical outcome in hepatocellular carcinoma. *J Immunother Cancer* 2019; **7**: 331 [PMID: 31783783 DOI: 10.1186/s40425-019-0814-7]

14 **Makarova-Rusher OV**, Medina-Echeverz J, Duffy AG, Greten TF. The yin and yang of evasion and immune activation in HCC. *J Hepatol* 2015; **62**: 1420-1429 [PMID: 25733155 DOI: 10.1016/j.jhep.2015.02.038]

15 **Zhu AX**, Finn RS, Edeline J, Cattan S, Ogasawara S, Palmer D, Verslype C, Zagonel V, Fartoux L, Vogel A, Sarker D, Verset G, Chan SL, Knox J, Daniele B, Webber AL, Ebbinghaus SW, Ma J, Siegel AB, Cheng AL, Kudo M; KEYNOTE-224 investigators. Pembrolizumab in patients with advanced hepatocellular carcinoma previously treated with sorafenib (KEYNOTE-224): a non-randomised, open-label phase 2 trial. *Lancet Oncol* 2018; **19**: 940-952 [PMID: 29875066 DOI: 10.1016/S1470-2045(18)30351-6]

16 **El-Khoueiry AB**, Sangro B, Yau T, Crocenzi TS, Kudo M, Hsu C, Kim TY, Choo SP, Trojan J, Welling TH Rd, Meyer T, Kang YK, Yeo W, Chopra A, Anderson J, Dela Cruz C, Lang L, Neely J, Tang H, Dastani HB, Melero I. Nivolumab in patients with advanced hepatocellular carcinoma (CheckMate 040): an open-label, non-comparative, phase 1/2 dose escalation and expansion trial. *Lancet* 2017; **389**: 2492-2502 [PMID: 28434648 DOI: 10.1016/S0140-6736(17)31046-2]

17 **Yau T**, Park JW, Finn RS, Cheng AL, Mathurin P, Edeline J, Kudo M, Harding JJ, Merle P, Rosmorduc O, Wyrwicz L, Schott E, Choo SP, Kelley RK, Sieghart W, Assenat E, Zaucha R, Furuse J, Abou-Alfa GK, El-Khoueiry AB, Melero I, Begic D, Chen G, Neely J, Wisniewski T, Tschaika M, Sangro B. Nivolumab versus sorafenib in advanced hepatocellular carcinoma (CheckMate 459): a randomised, multicentre, open-label, phase 3 trial. *Lancet Oncol* 2022; **23**: 77-90 [PMID: 34914889 DOI: 10.1016/S1470-2045(21)00604-5]

18 **Finn RS**, Ryoo BY, Merle P, Kudo M, Bouattour M, Lim HY, Breder V, Edeline J, Chao Y, Ogasawara S, Yau T, Garrido M, Chan SL, Knox J, Daniele B, Ebbinghaus SW, Chen E, Siegel AB, Zhu AX, Cheng AL; KEYNOTE-240 investigators. Pembrolizumab As Second-Line Therapy in Patients With Advanced Hepatocellular Carcinoma in KEYNOTE-240: A Randomized, Double-Blind, Phase III Trial. *J Clin Oncol* 2020; **38**: 193-202 [PMID: 31790344 DOI: 10.1200/JCO.19.01307]

19 **Hegde PS**, Wallin JJ, Mancao C. Predictive markers of anti-VEGF and emerging role of angiogenesis inhibitors as immunotherapeutics. *Semin Cancer Biol* 2018; **52**: 117-124 [PMID: 29229461 DOI: 10.1016/j.semcancer.2017.12.002]

20 **Cheng AL**, Qin S, Ikeda M, Galle PR, Ducreux M, Kim TY, Lim HY, Kudo M, Breder V, Merle P, Kaseb AO, Li D, Verret W, Ma N, Nicholas A, Wang Y, Li L, Zhu AX, Finn RS. Updated efficacy and safety data from IMbrave150: Atezolizumab plus bevacizumab vs. sorafenib for unresectable hepatocellular carcinoma. *J Hepatol* 2022; **76**: 862-873 [PMID: 34902530 DOI: 10.1016/j.jhep.2021.11.030]

21 **Finn RS**, Qin S, Ikeda M, Galle PR, Ducreux M, Kim TY, Kudo M, Breder V, Merle P, Kaseb AO, Li D, Verret W, Xu DZ, Hernandez S, Liu J, Huang C, Mulla S, Wang Y, Lim HY, Zhu AX, Cheng AL; IMbrave150 Investigators.

Atezolizumab plus Bevacizumab in Unresectable Hepatocellular Carcinoma. *N Engl J Med* 2020; **382**: 1894-1905 [PMID: 32402160 DOI: 10.1056/NEJMoa1915745]

22 **Finn RS**, Ikeda M, Zhu AX, Sung MW, Baron AD, Kudo M, Okusaka T, Kobayashi M, Kumada H, Kaneko S, Pracht M, Mamontov K, Meyer T, Kubota T, Dutcus CE, Saito K, Siegel AB, Dubrovsky L, Mody K, Llovet JM. Phase Ib Study of Lenvatinib Plus Pembrolizumab in Patients With Unresectable Hepatocellular Carcinoma. *J Clin Oncol* 2020; **38**: 2960-2970 [PMID: 32716739 DOI: 10.1200/JCO.20.00808]

23 **El-Khoueiry AB**, Kim RD, Harris WP, Sung MW, Galle PR. Phase Ib study of regorafenib (REG) plus pembrolizumab (PEMBRO) for first-line treatment of advanced hepatocellular carcinoma (HCC). *J Clin Oncol* 2020; **38**: 564-564 [DOI: 10.1200/JCO.2020.38.4_suppl.564]

24 **Yau T**, Kang YK, Kim TY, El-Khoueiry AB, Santoro A, Sangro B, Melero I, Kudo M, Hou MM, Matilla A, Tovoli F, Knox JJ, Ruth He A, El-Rayes BF, Acosta-Rivera M, Lim HY, Neely J, Shen Y, Wisniewski T, Anderson J, Hsu C. Efficacy and Safety of Nivolumab Plus Ipilimumab in Patients With Advanced Hepatocellular Carcinoma Previously Treated With Sorafenib: The CheckMate 040 Randomized Clinical Trial. *JAMA Oncol* 2020; **6**: e204564 [PMID: 33001135 DOI: 10.1001/jamaoncol.2020.4564]

25 **Yau T**, Zagonel V, Santoro A, Acosta-Rivera M, Choo SP, Matilla A, He AR, Gracián AC, El-Khoueiry AB, Sangro B, Eldawy T, Bruix J, Frassineti G, Vaccaro GM, Tschaika M, Scheffold C, Shen Y, Neely J, Piscaglia F. Nivolumab (NIVO) + ipilimumab (IPI) + cabozantinib (CABO) combination therapy in patients (pts) with advanced hepatocellular carcinoma (aHCC): Results from CheckMate 040. *J Clin Oncol* 2020; **38**: 478-478 [DOI: 10.1200/jco.2020.38.4_suppl.478]

26 **AstraZeneca**. Imfinzi plus tremelimumab significantly improved overall survival in HIMALAYA Phase III trial in 1st-line unresectable liver cancer. [cited January 2, 2022] Available from: https://www.astrazeneca.com/media-centre/press-releases/2021/imfinzi-and-tremelimumab-improved-os-in-liver-cancer.html

27 **Kelley RK**, Sangro B, Harris W, Ikeda M, Okusaka T, Kang YK, Qin S, Tai DW, Lim HY, Yau T, Yong WP, Cheng AL, Gasbarrini A, Damian S, Bruix J, Borad M, Bendell J, Kim TY, Standifer N, He P, Makowsky M, Negro A, Kudo M, Abou-Alfa GK. Safety, Efficacy, and Pharmacodynamics of Tremelimumab Plus Durvalumab for Patients With Unresectable Hepatocellular Carcinoma: Randomized Expansion of a Phase I/II Study. *J Clin Oncol* 2021; **39**: 2991-3001 [PMID: 34292792 DOI: 10.1200/JCO.20.03555]

28 **Makary MS**, Ramsell S, Miller E, Beal EW, Dowell JD. Hepatocellular carcinoma locoregional therapies: Outcomes and future horizons. *World J Gastroenterol* 2021; **27**: 7462-7479 [PMID: 34887643 DOI: 10.3748/wjg.v27.i43.7462]

29 **Kong FH**, Miao XY, Zou H, Xiong L, Wen Y, Chen B, Liu X, Zhou JJ. End-stage liver disease score and future liver remnant volume predict post-hepatectomy liver failure in hepatocellular carcinoma. *World J Clin Cases* 2019; **7**: 3734-3741 [PMID: 31799298 DOI: 10.12998/wjcc.v7.i22.3734]

30 **Bhoori S**, Mazzaferro V. Current challenges in liver transplantation for hepatocellular carcinoma. *Best Pract Res Clin Gastroenterol* 2014; **28**: 867-879 [PMID: 25260314 DOI: 10.1016/j.bpg.2014.08.001]

31 **Park JW**, Chen M, Colombo M, Roberts LR, Schwartz M, Chen PJ, Kudo M, Johnson P, Wagner S, Orsini LS, Sherman M. Global patterns of hepatocellular carcinoma management from diagnosis to death: the BRIDGE Study. *Liver Int* 2015; **35**: 2155-2166 [PMID: 25752327 DOI: 10.1111/liv.12818]

32 **Llovet JM**, Kelley RK, Villanueva A, Singal AG, Pikarsky E, Roayaie S, Lencioni R, Koike K, Zucman-Rossi J, Finn RS. Hepatocellular carcinoma. *Nat Rev Dis Primers* 2021; **7**: 6 [PMID: 33479224 DOI: 10.1038/s41572-020-00240-3]

33 **Cho YK**, Kim JK, Kim MY, Rhim H, Han JK. Systematic review of randomized trials for hepatocellular carcinoma treated with percutaneous ablation therapies. *Hepatology* 2009; **49**: 453-459 [PMID: 19065676 DOI: 10.1002/hep.22648]

34 **Breen DJ**, Lencioni R. Image-guided ablation of primary liver and renal tumours. *Nat Rev Clin Oncol* 2015; **12**: 175-186 [PMID: 25601446 DOI: 10.1038/nrclinonc.2014.237]

35 **Kloeckner R**, Galle PR, Bruix J. Local and Regional Therapies for Hepatocellular Carcinoma. *Hepatology* 2021; **73** Suppl 1: 137-149 [PMID: 32557715 DOI: 10.1002/hep.31424]

36 **Yao XS**, Yan D, Jiang XX, Li X, Zeng HY, Li H. Short-term outcomes of radiofrequency ablation for hepatocellular carcinoma using cone-beam computed tomography for planning and image guidance. *World J Clin Cases* 2021; **9**: 1580-1591 [PMID: 33728301 DOI: 10.12998/wjcc.v9.i7.1580]

37 **Lee DH**, Lee JM, Lee JY, Kim SH, Yoon JH, Kim YJ, Han JK, Choi BI. Radiofrequency ablation of hepatocellular carcinoma as first-line treatment: long-term results and prognostic factors in 162 patients with cirrhosis. *Radiology* 2014; **270**: 900-909 [PMID: 24475823 DOI: 10.1148/radiol.13130940]

38 **N'Kontchou G**, Mahamoudi A, Aout M, Ganne-Carrié N, Grando V, Coderc E, Vicaut E, Trinchet JC, Sellier N, Beaugrand M, Seror O. Radiofrequency ablation of hepatocellular carcinoma: long-term results and prognostic factors in 235 Western patients with cirrhosis. *Hepatology* 2009; **50**: 1475-1483 [PMID: 19731239 DOI: 10.1002/hep.23181]

39 **Xia Y**, Li J, Liu G, Wang K, Qian G, Lu Z, Yang T, Yan Z, Lei Z, Si A, Wan X, Zhang H, Gao C, Cheng Z, Pawlik TM, Wang H, Lau WY, Wu M, Shen F. Long-term Effects of Repeat Hepatectomy vs Percutaneous Radiofrequency Ablation Among Patients With Recurrent Hepatocellular Carcinoma: A Randomized Clinical Trial. *JAMA Oncol* 2020; **6**: 255-263 [PMID: 31774468 DOI: 10.1001/jamaoncol.2019.4477]

40 **Glassberg MB**, Ghosh S, Clymer JW, Qadeer RA, Ferko NC, Sadeghirad B, Wright GW, Amaral JF. Microwave ablation compared with radiofrequency ablation for treatment of hepatocellular carcinoma and liver metastases: a systematic review and meta-analysis. *Onco Targets Ther* 2019; **12**: 6407-6438 [PMID: 31496742 DOI: 10.2147/OTT.S204340]

41 **Tan W**, Deng Q, Lin S, Wang Y, Xu G. Comparison of microwave ablation and radiofrequency ablation for hepatocellular carcinoma: a systematic review and meta-analysis. *Int J Hyperthermia* 2019; **36**: 264-272 [PMID: 30676100 DOI: 10.1080/02656736.2018.1562571]

42 **Harari CM**, Magagna M, Bedoya M, Lee FT Jr, Lubner MG, Hinshaw JL, Ziemlewicz T, Brace CL. Microwave Ablation: Comparison of Simultaneous and Sequential Activation of Multiple Antennas in Liver Model Systems. *Radiology* 2016; **278**: 95-103 [PMID: 26133361 DOI: 10.1148/radiol.2015142151]

43 **Sutter O**, Calvo J, N'Kontchou G, Nault JC, Ourabia R, Nahon P, Ganne-Carrié N, Bourcier V, Zentar N, Bouhafs F, Sellier N, Diallo A, Seror O. Safety and Efficacy of Irreversible Electroporation for the Treatment of Hepatocellular Carcinoma Not Amenable to Thermal Ablation Techniques: A Retrospective Single-Center Case Series. *Radiology* 2017;

284: 877-886 [PMID: 28453431 DOI: 10.1148/radiol.2017161413]
44 **Wang C**, Wang H, Yang W, Hu K, Xie H, Hu KQ, Bai W, Dong Z, Lu Y, Zeng Z, Lou M, Gao X, Chang X, An L, Qu J, Li J, Yang Y. Multicenter randomized controlled trial of percutaneous cryoablation versus radiofrequency ablation in hepatocellular carcinoma. *Hepatology* 2015; **61**: 1579-1590 [PMID: 25284802 DOI: 10.1002/hep.27548]
45 **Dapito DH**, Mencin A, Gwak GY, Pradere JP, Jang MK, Mederacke I, Caviglia JM, Khiabanian H, Adeyemi A, Bataller R, Lefkowitch JH, Bower M, Friedman R, Sartor RB, Rabadan R, Schwabe RF. Promotion of hepatocellular carcinoma by the intestinal microbiota and TLR4. *Cancer Cell* 2012; **21**: 504-516 [PMID: 22516259 DOI: 10.1016/j.ccr.2012.02.007]
46 **Portolani N**, Coniglio A, Ghidoni S, Giovanelli M, Benetti A, Tiberio GA, Giulini SM. Early and late recurrence after liver resection for hepatocellular carcinoma: prognostic and therapeutic implications. *Ann Surg* 2006; **243**: 229-235 [PMID: 16432356 DOI: 10.1097/01.sla.0000197706.21803.a1]
47 **Okajima W**, Komatsu S, Ichikawa D, Miyamae M, Ohashi T, Imamura T, Kiuchi J, Nishibeppu K, Arita T, Konishi H, Shiozaki A, Morimura R, Ikoma H, Okamoto K, Otsuji E. Liquid biopsy in patients with hepatocellular carcinoma: Circulating tumor cells and cell-free nucleic acids. *World J Gastroenterol* 2017; **23**: 5650-5668 [PMID: 28883691 DOI: 10.3748/wjg.v23.i31.5650]
48 **Bruix J**, Takayama T, Mazzaferro V, Chau GY, Yang J, Kudo M, Cai J, Poon RT, Han KH, Tak WY, Lee HC, Song T, Roayaie S, Bolondi L, Lee KS, Makuuchi M, Souza F, Berre MA, Meinhardt G, Llovet JM; STORM investigators. Adjuvant sorafenib for hepatocellular carcinoma after resection or ablation (STORM): a phase 3, randomised, double-blind, placebo-controlled trial. *Lancet Oncol* 2015; **16**: 1344-1354 [PMID: 26361969 DOI: 10.1016/S1470-2045(15)00198-9]
49 **Pu T**, Li ZH, Jiang D, Chen JM, Guo Q, Cai M, Chen ZX, Xie K, Zhao YJ, Liu FB. Nomogram based on inflammation-related markers for predicting survival of patients undergoing hepatectomy for hepatocellular carcinoma. *World J Clin Cases* 2021; **9**: 11193-11207 [PMID: 35071550 DOI: 10.12998/wjcc.v9.i36.11193]
50 **Liu P**, Tan XZ, Zhang T, Gu QB, Mao XH, Li YC, He YQ. Prediction of microvascular invasion in solitary hepatocellular carcinoma ≤ 5 cm based on computed tomography radiomics. *World J Gastroenterol* 2021; **27**: 2015-2024 [PMID: 34007136 DOI: 10.3748/wjg.v27.i17.2015]
51 **Tian YL**, Ji JJ, Chen LN, Cui XL, Liu ST, Mao L, Qiu YD, Li BB. Risk factors for long-term prognosis of hepatocellular carcinoma patients after anatomic hepatectomy. *World J Clin Cases* 2020; **8**: 713-722 [PMID: 32149055 DOI: 10.12998/wjcc.v8.i4.713]
52 **den Brok MH**, Sutmuller RP, Nierkens S, Bennink EJ, Toonen LW, Figdor CG, Ruers TJ, Adema GJ. Synergy between in situ cryoablation and TLR9 stimulation results in a highly effective in vivo dendritic cell vaccine. *Cancer Res* 2006; **66**: 7285-7292 [PMID: 16849578 DOI: 10.1158/0008-5472.CAN-06-0206]
53 **Duffy AG**, Ulahannan SV, Makorova-Rusher O, Rahma O, Wedemeyer H, Pratt D, Davis JL, Hughes MS, Heller T, ElGindi M, Uppala A, Korangy F, Kleiner DE, Figg WD, Venzon D, Steinberg SM, Venkatesan AM, Krishnasamy V, Abi-Jaoudeh N, Levy E, Wood BJ, Greten TF. Tremelimumab in combination with ablation in patients with advanced hepatocellular carcinoma. *J Hepatol* 2017; **66**: 545-551 [PMID: 27816492 DOI: 10.1016/j.jhep.2016.10.029]
54 **Kudo M**, Ueshima K, Nakahira S, Nishida N, Ida H, Minami Y, Nakai T, Wada H, Kubo S, Ohkawa K, Morishita A, Nomi T, Ishida K, Kobayashi S, Umeda M, Tsurusaki M, Chiba Y, Yoshimura K, Sakai K, Nishio K. Adjuvant nivolumab for hepatocellular carcinoma (HCC) after surgical resection (SR) or radiofrequency ablation (RFA) (NIVOLVE): A phase 2 prospective multicenter single-arm trial and exploratory biomarker analysis. *J Clin Oncol* 2021; **39**: 4070-4070 [DOI: 10.1200/JCO.2021.39.15_suppl.4070]
55 **Salem R**, Lewandowski RJ. Chemoembolization and radioembolization for hepatocellular carcinoma. *Clin Gastroenterol Hepatol* 2013; **11**: 604-11; quiz e43 [PMID: 23357493 DOI: 10.1016/j.cgh.2012.12.039]
56 **Llovet JM**, Real MI, Montaña X, Planas R, Coll S, Aponte J, Ayuso C, Sala M, Muchart J, Solà R, Rodés J, Bruix J; Barcelona Liver Cancer Group. Arterial embolisation or chemoembolisation versus symptomatic treatment in patients with unresectable hepatocellular carcinoma: a randomised controlled trial. *Lancet* 2002; **359**: 1734-1739 [PMID: 12049862 DOI: 10.1016/s0140-6736(02)08649-x]
57 **Llovet JM**, Bruix J. Systematic review of randomized trials for unresectable hepatocellular carcinoma: Chemoembolization improves survival. *Hepatology* 2003; **37**: 429-442 [PMID: 12540794 DOI: 10.1053/jhep.2003.50047]
58 **Burrel M**, Reig M, Forner A, Barrufet M, de Lope CR, Tremosini S, Ayuso C, Llovet JM, Real MI, Bruix J. Survival of patients with hepatocellular carcinoma treated by transarterial chemoembolisation (TACE) using Drug Eluting Beads. Implications for clinical practice and trial design. *J Hepatol* 2012; **56**: 1330-1335 [PMID: 22314428 DOI: 10.1016/j.jhep.2012.01.008]
59 **Lammer J**, Malagari K, Vogl T, Pilleul F, Denys A, Watkinson A, Pitton M, Sergent G, Pfammatter T, Terraz S, Benhamou Y, Avajon Y, Gruenberger T, Pomoni M, Langenberger H, Schuchmann M, Dumortier J, Mueller C, Chevallier P, Lencioni R; PRECISION V Investigators. Prospective randomized study of doxorubicin-eluting-bead embolization in the treatment of hepatocellular carcinoma: results of the PRECISION V study. *Cardiovasc Intervent Radiol* 2010; **33**: 41-52 [PMID: 19908093 DOI: 10.1007/s00270-009-9711-7]
60 **Pipa-Muñiz M**, Sanmartino S, Mesa A, Álvarez-Navascués C, González-Diéguez ML, Cadahía V, Rodríguez JE, Vega F, Rodríguez M, Costilla-García SM, Varela M. The development of early ascites is associated with shorter overall survival in patients with hepatocellular carcinoma treated with drug-eluting embolic chemoembolization. *BMC Gastroenterol* 2020; **20**: 166 [PMID: 32487071 DOI: 10.1186/s12876-020-01307-x]
61 **Teyateeti A**, Mahvash A, Long J, Abdelsalam M, Avritscher R, Kaseb A, Odisio B, Ravizzini G, Surasi D, Teyateeti A, Macapinlac H, Kappadath SC. Disease control and failure patterns of unresectable hepatocellular carcinoma following transarterial radioembolization with yttrium-90 microspheres and with/without sorafenib. *World J Gastroenterol* 2021; **27**: 8166-8181 [PMID: 35068861 DOI: 10.3748/wjg.v27.i47.8166]
62 **Romano F**, Chiarelli M, Garancini M, Scotti M, Zago M, Cioffi G, De Simone M, Cioffi U. Rethinking the Barcelona clinic liver cancer guidelines: Intermediate stage and Child-Pugh B patients are suitable for surgery? *World J Gastroenterol* 2021; **27**: 2784-2794 [PMID: 34135554 DOI: 10.3748/wjg.v27.i21.2784]
63 **Inchingolo R**, Acquafredda F, Tedeschi M, Laera L, Surico G, Surgo A, Fiorentino A, Spiliopoulos S, de'Angelis N, Memeo R. Worldwide management of hepatocellular carcinoma during the COVID-19 pandemic. *World J Gastroenterol*

2021; **27**: 3780-3789 [PMID: 34321843 DOI: 10.3748/wjg.v27.i25.3780]

64 **Kadalayil L**, Benini R, Pallan L, O'Beirne J, Marelli L, Yu D, Hackshaw A, Fox R, Johnson P, Burroughs AK, Palmer DH, Meyer T. A simple prognostic scoring system for patients receiving transarterial embolisation for hepatocellular cancer. *Ann Oncol* 2013; **24**: 2565-2570 [PMID: 23857958 DOI: 10.1093/annonc/mdt247]

65 **Raoul JL**, Forner A, Bolondi L, Cheung TT, Kloeckner R, de Baere T. Updated use of TACE for hepatocellular carcinoma treatment: How and when to use it based on clinical evidence. *Cancer Treat Rev* 2019; **72**: 28-36 [PMID: 30447470 DOI: 10.1016/j.ctrv.2018.11.002]

66 **Sieghart W**, Hucke F, Pinter M, Graziadei I, Vogel W, Müller C, Heinzl H, Trauner M, Peck-Radosavljevic M. The ART of decision making: retreatment with transarterial chemoembolization in patients with hepatocellular carcinoma. *Hepatology* 2013; **57**: 2261-2273 [PMID: 23316013 DOI: 10.1002/hep.26256]

67 **Salem R**, Gordon AC, Mouli S, Hickey R, Kallini J, Gabr A, Mulcahy MF, Baker T, Abecassis M, Miller FH, Yaghmai V, Sato K, Desai K, Thornburg B, Benson AB, Rademaker A, Ganger D, Kulik L, Lewandowski RJ. Y90 Radioembolization Significantly Prolongs Time to Progression Compared With Chemoembolization in Patients With Hepatocellular Carcinoma. *Gastroenterology* 2016; **151**: 1155-1163.e2 [PMID: 27575820 DOI: 10.1053/j.gastro.2016.08.029]

68 **Salem R**, Johnson GE, Kim E, Riaz A, Bishay V, Boucher E, Fowers K, Lewandowski R, Padia SA. Yttrium-90 Radioembolization for the Treatment of Solitary, Unresectable HCC: The LEGACY Study. *Hepatology* 2021; **74**: 2342-2352 [PMID: 33739462 DOI: 10.1002/hep.31819]

69 **Vilgrain V**, Pereira H, Assenat E, Guiu B, Ilonca AD, Pageaux GP, Sibert A, Bouattour M, Lebtahi R, Allaham W, Barraud H, Laurent V, Mathias E, Bronowicki JP, Tasu JP, Perdrisot R, Silvain C, Gerolami R, Mundler O, Seitz JF, Vidal V, Aubé C, Oberti F, Couturier O, Brenot-Rossi I, Raoul JL, Sarran A, Costentin C, Itti E, Luciani A, Adam R, Lewin M, Samuel D, Ronot M, Dinut A, Castera L, Chatellier G; SARAH Trial Group. Efficacy and safety of selective internal radiotherapy with yttrium-90 resin microspheres compared with sorafenib in locally advanced and inoperable hepatocellular carcinoma (SARAH): an open-label randomised controlled phase 3 trial. *Lancet Oncol* 2017; **18**: 1624-1636 [PMID: 29107679 DOI: 10.1016/S1470-2045(17)30683-6]

70 **Ricke J**, Klümpen HJ, Amthauer H, Bargellini I, Bartenstein P, de Toni EN, Gasbarrini A, Pech M, Peck-Radosavljevic M, Popovič P, Rosmorduc O, Schott E, Seidensticker M, Verslype C, Sangro B, Malfertheiner P. Impact of combined selective internal radiation therapy and sorafenib on survival in advanced hepatocellular carcinoma. *J Hepatol* 2019; **71**: 1164-1174 [PMID: 31421157 DOI: 10.1016/j.jhep.2019.08.006]

71 **Chow PKH**, Gandhi M, Tan SB, Khin MW, Khasbazar A, Ong J, Choo SP, Cheow PC, Chotipanich C, Lim K, Lesmana LA, Manuaba TW, Yoong BK, Raj A, Law CS, Cua IHY, Lobo RR, Teh CSC, Kim YH, Jong YW, Han HS, Bae SH, Yoon HK, Lee RC, Hung CF, Peng CY, Liang PC, Bartlett A, Kok KYY, Thng CH, Low AS, Goh ASW, Tay KH, Lo RHG, Goh BKP, Ng DCE, Lekurwale G, Liew WM, Gebski V, Mak KSW, Soo KC; Asia-Pacific Hepatocellular Carcinoma Trials Group. SIRveNIB: Selective Internal Radiation Therapy Versus Sorafenib in Asia-Pacific Patients With Hepatocellular Carcinoma. *J Clin Oncol* 2018; **36**: 1913-1921 [PMID: 29498924 DOI: 10.1200/JCO.2017.76.0892]

72 **Kwak MS**, Lee JH, Yoon JH, Yu SJ, Cho EJ, Jang ES, Kim YJ, Lee HS. Risk factors, clinical features, and prognosis of the hepatocellular carcinoma with peritoneal metastasis. *Dig Dis Sci* 2012; **57**: 813-819 [PMID: 22147252 DOI: 10.1007/s10620-011-1995-1]

73 **Wang B**, Xu H, Gao ZQ, Ning HF, Sun YQ, Cao GW. Increased expression of vascular endothelial growth factor in hepatocellular carcinoma after transcatheter arterial chemoembolization. *Acta Radiol* 2008; **49**: 523-529 [PMID: 18568538 DOI: 10.1080/02841850801958890]

74 **Meyer T**, Fox R, Ma YT, Ross PJ, James MW, Sturgess R, Stubbs C, Stocken DD, Wall L, Watkinson A, Hacking N, Evans TRJ, Collins P, Hubner RA, Cunningham D, Primrose JN, Johnson PJ, Palmer DH. Sorafenib in combination with transarterial chemoembolisation in patients with unresectable hepatocellular carcinoma (TACE 2): a randomised placebo-controlled, double-blind, phase 3 trial. *Lancet Gastroenterol Hepatol* 2017; **2**: 565-575 [PMID: 28648803 DOI: 10.1016/S2468-1253(17)30156-5]

75 **Lencioni R**, Llovet JM, Han G, Tak WY, Yang J, Guglielmi A, Paik SW, Reig M, Kim DY, Chau GY, Luca A, Del Arbol LR, Leberre MA, Niu W, Nicholson K, Meinhardt G, Bruix J. Sorafenib or placebo plus TACE with doxorubicin-eluting beads for intermediate stage HCC: The SPACE trial. *J Hepatol* 2016; **64**: 1090-1098 [PMID: 26809111 DOI: 10.1016/j.jhep.2016.01.012]

76 **Kudo M**, Han G, Finn RS, Poon RT, Blanc JF, Yan L, Yang J, Lu L, Tak WY, Yu X, Lee JH, Lin SM, Wu C, Tanwandee T, Shao G, Walters IB, Dela Cruz C, Poulart V, Wang JH. Brivanib as adjuvant therapy to transarterial chemoembolization in patients with hepatocellular carcinoma: A randomized phase III trial. *Hepatology* 2014; **60**: 1697-1707 [PMID: 24996197 DOI: 10.1002/hep.27290]

77 **Buijs M**, Reyes DK, Pawlik TM, Blackford AL, Salem R, Messersmith WA, Weekes CD, Mulcahy M, Kamel IR, Geschwind JF. Phase 2 trial of concurrent bevacizumab and transhepatic arterial chemoembolization in patients with unresectable hepatocellular carcinoma. *Cancer* 2013; **119**: 1042-1049 [PMID: 23132335 DOI: 10.1002/cncr.27859]

78 **Kudo M**, Ueshima K, Ikeda M, Torimura T, Aikata H, Izumi N, Yamasaki T, Hino K, Kuzuya T, Isoda N, Yasui K, Aino H, Ido A, Kawabe N, Nakao K, Wada Y, Yoshimura K, Okusaka T, Furuse J, Arai Y. TACTICS: Final overall survival (OS) data from a randomized, open label, multicenter, phase II trial of transcatheter arterial chemoembolization (TACE) therapy in combination with sorafenib as compared with TACE alone in patients (pts) with hepatocellular carcinoma (HCC). *J Clin Oncol* 2021; **39**: 270-270 [DOI: 10.1200/JCO.2021.39.3_suppl.270]

79 **Kudo M**, Ueshima K, Ikeda M, Torimura T, Tanabe N, Aikata H, Izumi N, Yamasaki T, Nojiri S, Hino K, Tsumura H, Kuzuya T, Isoda N, Yasui K, Aino H, Ido A, Kawabe N, Nakao K, Wada Y, Yokosuka O, Yoshimura K, Okusaka T, Furuse J, Kokudo N, Okita K, Johnson PJ, Arai Y; TACTICS study group. Randomised, multicentre prospective trial of transarterial chemoembolisation (TACE) plus sorafenib as compared with TACE alone in patients with hepatocellular carcinoma: TACTICS trial. *Gut* 2020; **69**: 1492-1501 [PMID: 31801872 DOI: 10.1136/gutjnl-2019-318934]

80 **Tampaki M**, Ionas E, Hadziyannis E, Deutsch M, Malagari K, Koskinas J. Association of TIM-3 with BCLC Stage, Serum PD-L1 Detection, and Response to Transarterial Chemoembolization in Patients with Hepatocellular Carcinoma. *Cancers (Basel)* 2020; **12** [PMID: 31952209 DOI: 10.3390/cancers12010212]

81 **Singh P**, Toom S, Avula A, Kumar V, Rahma OE. The Immune Modulation Effect of Locoregional Therapies and Its Potential Synergy with Immunotherapy in Hepatocellular Carcinoma. *J Hepatocell Carcinoma* 2020; **7**: 11-17 [PMID: 32104669 DOI: 10.2147/JHC.S187121]

82 **Pinato DJ**, Murray SM, Forner A, Kaneko T, Fessas P, Toniutto P, Mínguez B, Cacciato V, Avellini C, Diaz A, Boyton RJ, Altmann DM, Goldin RD, Akarca AU, Marafioti T, Mauri FA, Casagrande E, Grillo F, Giannini E, Bhoori S, Mazzaferro V. Trans-arterial chemoembolization as a loco-regional inducer of immunogenic cell death in hepatocellular carcinoma: implications for immunotherapy. *J Immunother Cancer* 2021; **9** [PMID: 34593621 DOI: 10.1136/jitc-2021-003311]

83 **Craciun L**, de Wind R, Demetter P, Lucidi V, Bohlok A, Michiels S, Bouazza F, Vouche M, Tancredi I, Verset G, Garaud S, Naveaux C, Galdon MG, Gallo KW, Hendlisz A, Derijckere ID, Flamen P, Larsimont D, Donckier V. Retrospective analysis of the immunogenic effects of intra-arterial locoregional therapies in hepatocellular carcinoma: a rationale for combining selective internal radiation therapy (SIRT) and immunotherapy. *BMC Cancer* 2020; **20**: 135 [PMID: 32075608 DOI: 10.1186/s12885-020-6613-1]

84 **Chew V**, Lee YH, Pan L, Nasir NJM, Lim CJ, Chua C, Lai L, Hazirah SN, Lim TKH, Goh BKP, Chung A, Lo RHG, Ng D, Filarca RLF, Albani S, Chow PKH. Immune activation underlies a sustained clinical response to Yttrium-90 radioembolisation in hepatocellular carcinoma. *Gut* 2019; **68**: 335-346 [PMID: 29440463 DOI: 10.1136/gutjnl-2017-315485]

85 **Tai D**, Loke K, Gogna A, Kaya NA, Tan SH, Hennedige T, Ng D, Irani F, Lee J, Lim JQ, Too CW, Ng MCH, Tham CK, Lam J, Koo SL, Chong HS, Goh GB, Huang HL, Venkatanarasimha N, Lo R, Chow PKH, Goh BKP, Chung A, Toh HC, Thng CH, Lim TKH, Yeong J, Zhai W, Chan CY, Choo SP. Radioembolisation with Y90-resin microspheres followed by nivolumab for advanced hepatocellular carcinoma (CA 209-678): a single arm, single centre, phase 2 trial. *Lancet Gastroenterol Hepatol* 2021; **6**: 1025-1035 [PMID: 34695377 DOI: 10.1016/S2468-1253(21)00305-8]

86 **Berberabe T**. SIRT Administered With Nivolumab Demonstrates Positive Safety, Tolerability Results in HCC. *Targeted Therapies Oncol* 2020; **9**: 24

87 **Han Y**, Zhi WH, Xu F, Zhang CB, Huang XQ, Luo JF. Selection of first-line systemic therapies for advanced hepatocellular carcinoma: A network meta-analysis of randomized controlled trials. *World J Gastroenterol* 2021; **27**: 2415-2433 [PMID: 34040331 DOI: 10.3748/wjg.v27.i19.2415]

88 **Long HY**, Huang TY, Xie XY, Long JT, Liu BX. Treatment strategies for hepatocellular carcinoma with extrahepatic metastasis. *World J Clin Cases* 2021; **9**: 5754-5768 [PMID: 34368295 DOI: 10.12998/wjcc.v9.i21.5754]

89 **Piñero F**, da Fonseca LG. Trial eligibility in advanced hepatocellular carcinoma: Does it support clinical practice in underrepresented subgroups? *World J Gastroenterol* 2021; **27**: 3429-3439 [PMID: 34239261 DOI: 10.3748/wjg.v27.i24.3429]

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MINIREVIEWS

Update on endoscopic ultrasound-guided liver biopsy

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Abstract

Endoscopic ultrasound guided liver biopsy (EUS-LB) has emerged as a minimally-invasive alternative to the traditional (percutaneous or transjugular) liver biopsy techniques for the diagnosis of liver parenchymal diseases. Potentially, EUS-LB combines the advantages of percutaneous and transjugular liver biopsy in addressing focused sampling in addition to measuring portal pressure. Additionally, EUS-LB facilitates access to both the lobes of the liver which is not considered with the traditional percutaneous liver biopsy. Multiple studies have compared EUS-LB with conventional liver biopsy and reported comparable diagnostic yield, increased acquisition of complete portal tracts, and longer specimen length as compared to the traditional approaches. EUS-LB is associated with lesser post-procedural pain and shorter recovery time, while providing lower risk of complications when compared to traditional liver biopsy. Innovations in needle types, needle sizes and suction techniques have aimed at further optimizing the EUS-LB technique. This review article updates current literature with focus on the variations in the technique and equipment used for EUS-LB, and compares EUS-LB with traditional methods of liver biopsy.

Key Words: Endoscopic ultrasound guided liver biopsy; Liver biopsy; Percutaneous liver biopsy; Transjugular liver biopsy; Liver parenchymal disease; Portal pressure gradient

Core Tip: Endoscopic ultrasound guided liver biopsy (EUS-LB) has emerged as a minimally invasive alternative to the traditional (percutaneous or transjugular) liver biopsy techniques for the diagnosis of liver parenchymal diseases. EUS-LB facilitates access to both the lobes of the liver and allows measurement of portal pressure. EUS-LB has comparable diagnostic accuracy, increased yield of complete portal tracts, and longer specimen length compared to the traditional approaches. Innovations in needle technology and variations in suction have further optimized the EUS-LB technique. This review article updates current literature comparing EUS-LB to traditional liver biopsy and advances in this field.

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INTRODUCTION

Liver biopsy is helpful in diagnosis of parenchymal pathologies such as alcoholic liver disease, autoimmune hepatitis, viral hepatitis, metabolic liver diseases (non-alcoholic fatty liver disease, alfa-1 anti-trypsin deficiency, Wilson disease, hemochromatosis, Gaucher's disease, *etc.*), drug-induced liver injury and infiltrative liver disease (*i.e.*, malignancy, abscess, sarcoidosis, *etc.*). Tissue examination also allows for diagnosis of rare overlapping liver diseases. The liver biopsy has traditionally been obtained *via* two routes: percutaneous liver biopsy (PC-LB) and transjugular liver biopsy (TJ-LB). In recent years, endoscopic technique and hardware advancement have led to the rise of endoscopic ultrasound-guided liver biopsy (EUS-LB). There is changing epidemiology of liver disease with increased global incidence of non-alcoholic fatty liver disease over the last two decades; it has reached to an estimated global prevalence of 25%[1]. This increased prevalence has led to advancements in lesser or non-invasive diagnostic tests such as ultrasound-elastography and MRI-proton density fat fraction[2]. Although these tests provide greater diagnostic accuracy compared to traditional peripheral blood laboratory tests, liver biopsy remains the gold standard for diagnosing focal lesions and parenchymal liver disease[3]. In this review, we will compare EUS-LB with traditional liver biopsy and highlight its, advantages and disadvantages in context of changing epidemiology of liver disease. Further, we will summarize the latest advancements on EUS-LB, focusing on technique, needle types/size, and suction type.

METHODS OF LIVER BIOPSY

The first mode of acquisition of liver tissue was PC-LB, as it provides the most direct route to access the liver. Percutaneous needle aspiration biopsies have been performed since the late 19th century and popularized in the 1930s[4]. Initial PC-LB techniques used percussion to guide needle placement; however, modern PC-LB is done under ultrasound- or fluoroscopic-image guidance[5]. If hepatomegaly is present, a subcostal route for PC-LB is preferred; however, a transthoracic approach is employed in the absence of hepatomegaly. In the early days of PC-LB, interventionists preferred 14 gauge (G) or 16 G needles to provide large, intact tissue samples. In recent years, spring-loaded 18 G and 20 G needles have been common[6]. PC-LB is usually done while the patient is awake with local anesthesia, but conscious sedation is often used. Common complications of PC-LB include pain (74%), minor bleeding (30%), and infection at the biopsy site[7,8]. As the liver capsule is punctured to obtain biopsy, intraperitoneal hemorrhage is a severe, albeit rare complication[7]. Other less-common complications include pneumothorax and hemothorax if the transthoracic approach is employed (0.1%-0.9%)[7-9]. Contraindications to PC-LB include significant ascites, large body-habitus, and severe coagulopathy[5]. As it has been a long-standing method of biopsy, PC-LB is widely available. Furthermore, given its short procedure time and the fact that general anesthesia is not required, PC-LB provides a cost-effective approach to diagnosing liver diseases. Drawbacks to PC-LB include access to only the right hepatic lobe and a limited view of surrounding anatomy. Further, if real-time imaging is not used for guidance, there is an increased risk of serious complications[10].

More recently, TJ-LB has been developed to obtain liver samples in patients with contraindications to PC-LB. In this technique, an interventional radiologist cannulates the internal jugular vein and accesses one of the hepatic vein under fluoroscopic guidance. Biopsy of the right hepatic lobe is preferred because of its size and the acute angulation of the veins with the inferior vena cava[11]. This procedure can be performed in patients with high body mass index, and significant ascites. Additionally, as the liver capsule is not punctured, it is the preferred method in those with profound coagulopathies[11]. This technique provides the added benefit of assessing the hepatic venous pressure gradient (HVPG), which is an indirect measure of the absence, presence and degree of portal hypertension. HVPG

measurement will be compared to EUS-guided measurement of portal pressure in the discussion of future research and practice below. Complications from TJ-LB include pain, hematoma, hemobilia, arterial aneurysm, and major hemorrhage[11]. In addition to subverting contraindications posed by the PC-LB, advantages of TJ-LB include lack of capsular puncture and the ability to obtain a portal pressure gradient measurement[7,10,11]. One of the limitation of TJ-LB is inability to obtain tissue from a focal lesion, due to limited view of surrounding structures[10].

Endoscopic ultrasound-guided fine-needle aspiration was first done in 1993 and EUS-LB was first described by Mathew in 2007[12,13]. EUS-LB poses several advantages over the traditional LB techniques. Firstly, EUS-LB allows access to both lobes of the liver (except in patients with Roux-en-Y or gastric bypass anatomy)[10]. Secondly, like TJ-LB, EUS-LB enables a practitioner to obtain liver tissue regardless of body habitus or ascites. The most advantageous aspects of EUS-LB, when compared to PC-LB and TJ-LB, lie in its real-time multi-dimensional evaluative abilities. Those undergoing workup for liver disease often require endoscopic evaluation – the advent of EUS-LB posed a solution to facilitate patients to undergo multiple endoscopic procedures in the same session. With EUS, a liver biopsy can be performed along with simultaneous evaluation for varices (esophageal and gastric) and portal pressure measurement. Further, the use of EUS in close proximity to the liver allows for better visualization of liver lesions for targeted liver biopsies[10]. In fact, multiple studies have shown a diagnostic accuracy between 85%-90% for solid liver masses using EUS-guided FNB[14-16]. Use of ultrasound-guided technique also allows practitioners to avoid critical structures during biopsy procurement. The drawbacks to EUS-LB mirror some of the drawbacks found in PC-LB and TJ-LB. Although the number of EUS-trained practitioners have grown rapidly over the past ten years in the United States, this procedure is less widely accessible when compared to PC-LB[10]. Further, unlike TJ-LB, EUS-LB does require capsular puncture and is not readily performed in patients with severe coagulopathy. Additionally, EUS-LB does require moderate or deep sedation, as with TJ-LB.

METHODS OF LIVER BIOPSY - COMPARISONS

Recovery times and complications

With the advent of multiple modalities for obtaining liver biopsies, recent studies have comparatively evaluated these approaches. The comparison between each of the three liver biopsy methods is summarized in Table 1. With regards to length of recovery, EUS-LB patients (n = 30) experienced a shorter post-procedural monitoring time (3 h) when compared to those (n = 60) undergoing PC-LB (4.2 h, P = 0.004)[17]. A recent study done in 2021 showed the mean recovery time for those undergoing EUS-LB was 90 min *vs* 141.3 min (P = 0.004) for TJ-LB[18]. The most common minor complication in each of the three modes of liver biopsy is pain. In a 2020 study, EUS-LB had significantly less pain scores when compared to PC-LB (0/10 for EUS-LB *vs* 3.5 for PC-LB, P = 0.0009)[17]. When comparing pain in EUS-LB and TJ-LB patients, one 2019 study found a significantly higher incidence of post-procedural pain in TJ-LB patients compared to EUS-LB patients, 8% *vs* 0% (P < 0.0001), respectively[19]. Adverse events with regard to liver biopsy include, but are not limited to, severe hemorrhage, hemobilia, abdominal wall injury, and intraperitoneal injury. Although no studies have directly compared adverse event rates between the three liver biopsy modalities, adverse event rates appear to be similar across each modality – EUS-LB: 1%-2.3%, PC-LB: 0.9%-3.1%, TJ-LB: 0.56%-6.5%[18-22].

Specimen adequacy

Regarding histological adequacy of liver biopsy samples, the American Association for the Study of Liver Diseases recommends a tissue length of between 2-3 cm with greater-than-or-equal-to 11 complete portal tracts (CPT)[23]. Systematic reviews have shown a total specimen length for PC-LB to be 17.7mm (± 5.5 mm), TJ-LB: 13.5 mm (± 4.5 mm), and EUS-LB 36.9 mm (± 6.2 mm)[20,24]. CPT numbers in these same studies revealed an aggregate CPT for PC-LB of 7.7 (± 3.4), TJ-LB: 6.8 (± 2.3), and EUS-LB: 9.0 (± 3.1)[20,24]. A recent 2021 study compared each liver biopsy technique head-to-head and found that EUS-LB (n = 53) had a significantly greater mean aggregate length (22.95 mm) compared to PC-LB (n = 20) (14.5 mm) (P = 0.03) and TJ-LB (n = 20) (14.6 mm) (P = 0.02)[18]. Further, both EUS-LB and TJ-LB provided a greater number of CPTs when compared to PC-LB: EUS-LB (19.36), TJ-LB (20.2), PC-LB (9.1) (EUS-LB *vs* PC-LB: P < 0.0001, EUS-LB *vs* TJ-LB P = 1)[18].

TECHNIQUE OF EUS-GUIDED LIVER BIOPSY

Since the inception of EUS-LB in 2007 with a Tru-Cut core biopsy needle (QuickCore, Cook Medical, Winston Salem, NC, United States), multiple studies have aimed at optimizing EUS-LB technique[13]. These changes coincided with procedural upgrades and improvements in needle technology. Incorporating these advancements into EUS-LB comes with technical (skills and training) and logical challenges (cost and availability). However, the essential technique of EUS-LB remains unchanged.

Table 1 Comparison of liver biopsy methods[1]

	Percutaneous liver biopsy	Transjugular liver biopsy	Endoscopic ultrasound-guided liver biopsy
Most common form of anesthesia	Local	Moderate sedation	Moderate sedation, deep sedation
Imaging	Fluoroscopy, ultrasound	Fluoroscopy	Ultrasound
Capsular puncture	Yes	No	Yes
Ability to evaluate focal hepatic lesions	No	No	Yes
Liver lobe access	Right	Right	Right, left
Contraindications	INR > 1.5, body habitus, significant ascites	Venous thrombosis, cholangitis	INR > 1.5
Post-procedural pain	+++	+	+
Post-procedural minor bleeding	+	+	+
Adverse event rate	+	+	+
Post-procedural recovery time	+++	++	+
Specimen length	+	+	+++
Total complete portal tracts	+	++	+++
Procedure cost	+	++	+++
Institutional availability	+++	++	+

[1]Information adapted using the Ref. [10,17,18,21].
+: Low/less; ++: Medium; +++: High/most; INR: International normalized ratio.

General technique

Patients undergoing EUS-LB are either moderately sedated with short-acting benzodiazepines and opiates or deeply sedated with propofol, per the availability of dedicated anesthetists. The patients are prone positioned and a linear array echo-endoscope is inserted for endo-sonography. Once the area of interest is identified, the stylet is removed, and a needle primed (with heparin or saline) or unprimed (air) is inserted[6,10]. Per endoscopist and center preference, the needle should be attached either to a wet suction (saline or heparin) or a dry suction (air). Before needle puncture, color Doppler should be used to ensure there are no vascular structures in the trajectory of the needle. Suction should be applied *via* a syringe once adequate liver parenchymal penetration is achieved (depending on the needle selection). Figure 1 shows the echoendoscopic image of a needle passing through into the left hepatic lobe. In 1 pass, actuations or fanning back-and-forth motions can be done with continued suction to allow for improved tissue acquisition[25]. Multiple such passes can be done overall to increase tissue acquisition. After each pass, the needle should be removed from the echo-endoscope and the tissue should be stored in formalin solution directly for preservation[6,10]. This gross tissue can be analyzed for adequacy and should guide the decision if more passes are required. Following the procedure, patients can be discharged after 1-2 h observation in the recovery unit.

Accessing liver parenchyma requires identification of endoscopic gastric landmarks. The left lobe of liver adjoins the gastroesophageal junction can be accessed through the gastroesophageal junction in the proximal stomach. The right lobe of the liver can be accessed through the duodenal bulb[26]. Among patients with altered anatomy, EUS-LB is limited. For Roux-en-Y gastric bypass patients, only left liver lobe biopsy is feasible through the transgastric approach[10].

Suction

Suction can be applied in a wet or dry fashion. In dry suction, an empty 10 cc or 20 cc syringe is applied to maintain suction after passing the needle into the liver parenchyma. In wet suction, the needle lumen is primed with fluid, causing negative pressure at the needle tip. As outlined above, wet suction utilizes either saline or heparin. A 2018 study compared wet heparin suction technique with dry suction technique. The study showed significantly increased aggregate specimen length (wet: 49.2 mm, dry: 23.9 mm, P = 0.003) and number of CPTs (7.0 *vs* 4.0, P = 0.01) for wet suction compared to dry suction techniques[27]. With wet suction, the sample is suspended in a column of fluid—leading to reduced shearing forces as it is not in contact with the needle wall. This likely leads to increased CPTs and intact specimen length for wet suction technique. To our knowledge, no studies directly comparing wet suction with saline *vs* heparin have been performed.

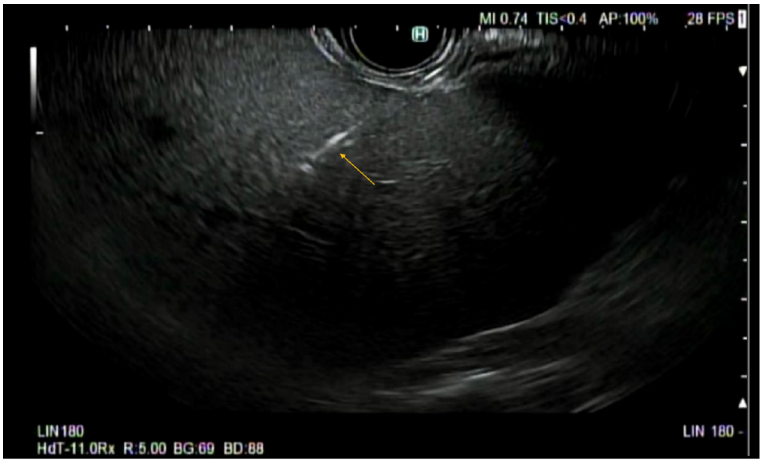

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Figure 1 Needle for endoscopic ultrasound guided liver biopsy accessing left lobe of the liver. Orange arrow denotes needle. Image obtained by Krishna SG at the Ohio State University Wexner Medical Center Division of Gastroenterology, Hepatology, and Nutrition.

In contrast, specimens can be obtained without applying suction *via* stylet, in a "slow-pull" technique. A 2020 meta-analysis showed that FNB with a slow-pull technique provided similar total specimen length (44.3 mm *vs* 53.9 mm, $P = 0.40$) when compared to suction application; however, the slow-pull technique provided improved CPT than suction (30 *vs* 14.6, $P < 0.001$)[28]. Authors hypothesize that this stems from reduced fragmentation of the tissue specimen as it is subjected to an environment of less negative pressure with the slow-pull technique.

Needle pass/Actuation

Needle pass refers to the number of times a needle is introduced into the liver parenchyma through puncture of the liver capsule, while actuation refers to the number of back-and-forth motions are made in a specified needle pass. Through our literature review, it appears the 1-pass 1-actuation technique is the most common mode of EUS-LB acquisition[25,29]. There are few studies that have been done comparing needle pass and needle actuation with regards to pain, adverse events, and specimen quality. A 2021 study showed that EUS-LB using a 1-pass, 3-actuation technique ($n = 40$) provided longer liver cores with more CPTs than a 1-to-1 technique ($n = 40$), with an equivalent safety profile: CPT (24.5 *vs* 17.25, $P < 0.008$), aggregate specimen length (12.85 cm *vs* 6.89 cm $P < 0.001$)[25].

Needle selection: Size

When considering needle selection, a EUS-LB practitioner must take needle size and type into account. With regards to needle size, multiple studies have been done to determine the optimal needle size for EUS-LB. In the early days of EUS-LB, large 14G – 16 G spring-loaded cutting needles were used; however, these needles had varied diagnostic yields ranging from 29%-100%[30,31]. As time progressed, researchers found that smaller-gauge needles provided better results than their 14-16 G counterparts. In a 2017 study, 18 G percutaneous, 19 G FNA, 19G FNB, and 22 G FNB needles were compared on human cadaveric tissue to test for tissue adequacy. The study showed that a 19 G needle provided a significantly greater number of CPTs when compared to its 22 G and 18 G counterparts (6.2 *vs* 3.5 *vs* 3.2, respectively, $P < 0.001$ for both comparisons)[32]. In a similar study in 2019 on fresh bovine liver, 19 G and 20 G fork-tip needles yielded similar CPTs and total specimen length; however, both the 19 G and 20 G fork-tip needles significantly outperformed their 22 G fork-tip counterpart in terms of CPTs and specimen length[33].

More recent *in vivo* studies have confirmed the superiority of 19 G needles when compared to 22 G needles. A recent (2021) study showed that 19 G Franseen tip needle performed better than a 22 G Franseen tip needle in terms of specimen adequacy (19 G: 81.5%, 22 G: 66.7%, $P < 0.01$)[34]. The researchers posit that the 22 G samples had greater fragmentation, leading to reduced intact specimen length and CPT, reducing histological adequacy. The advantage of 19 G compared to 22 G needle was also shown in core needles, where 19 G core needles had a greater CPT (8.8 *vs* 3, $P < 0.0001$), longer core length (2.5 cm *vs* 1.1 cm, $P < 0.0001$), and a higher rate of pathological diagnostic samples (85% *vs* 10%, $P < 0.0001$) when compared to 22 G core needles[35].

Needle selection: Tip and design

As noted earlier, large spring-loaded cutting needles were initially used to perform EUS-LB. As EUS-LB grew, practitioners noted that fine-needle biopsy (FNB) needles may provide better samples in the endoscopic setting. In 2015, Dewitt *et al*[36] showed that a 19 G FNB histology needle performed better

than a 19 G spring-loaded cutting needle and provided longer specimens (19.4 mm *vs* 4.3 mm, P = 0.0001), greater CPTs (10.4 *vs* 1.3, P = 0.0004), and a higher percent of diagnostic histology (85% *vs* 57%, P = 0.006)[36].

Holding needle gauge constant, Franseen needle types have shown to be superior than their counterparts in recent studies. A 2019 study showed that a 19 G Franseen needle provided lengthier specimens when compared to a 19 G FNA needle for *in vivo* EUS-LB (2.09 cm *vs* 1.47 cm, P < 0.001), more CPTs (42.6 *vs* 18.1, P < 0.001), and similar pain scores[37]. In the late portion of the last decade, EUS-LB has been most commonly performed with either Franseen or Fork-Tip biopsy needles. Multiple studies have compared the effectiveness with regards to specimen quality and adverse events between these two needles. A 2020 study showed a similar adverse event and abdominal pain profile between the use of 19 G Franseen and fork-tip needles[29]. The Franseen needle, however, had a significantly greater total specimen length when compared to the fork-tip needle (3.1 cm *vs* 2.7 cm, P = 0.01) and greater total CPTs (24.0 *vs* 19.55, P < 0.01)[29]. The Franseen tip has also shown equal-to-superior diagnostic yield when compared to fork-tip, with one study showing nonsignificant-difference in histological adequacy rates but an increased number of CPTs (14.4 *vs* 9.5, P < 0.001) and another study showing a higher diagnostic yield of 97.2% for Franseen tip compared to 79.4% for fork-tip (P < 0.001)[38,39].

FUTURE RESEARCH AND PRACTICE

Portal pressure gradient

In trained hands it has become possible to obtain accurate measures of portal pressure gradients by various approaches[36]. The measure of the portal pressure gradient is primarily done through the transjugular approach. Although described as the gold standard, the hepatic venous pressure gradient (HVPG) is actually an indirect measure of portal pressure calculated by subtracting the free hepatic venous pressure from the wedge hepatic venous pressure[40]. In contrast, EUS-guided measurement of portal pressure is a direct measurement of sinusoidal pressure rather than an estimate, and may prove to be more accurate[40]. To obtain EUS-guided measurement of portal pressure, endoscopists first puncture the hepatic vein *via* a transgastric transhepatic approach, with the needle hooked up to a digital manometer *via* non-compressible tubing[41]. Once obtaining hepatic vein pressure averages, endoscopists then turn their attention to the portal vein, which is accessed in a transgastric transhepatic approach usually at the umbilical portion of the portal vein. Once the portal vein pressure is obtained, the portal pressure gradient is calculated by subtracting the mean portal vein pressure from the mean hepatic vein pressure[41]. These readings are usually obtained with a small-gauge (25 G) FNA needle [40,41].

Since the HVPG can be obtained without performing a liver biopsy, it is a very safe procedure when performed alone, mainly imparting the risk associated with right internal jugular access[9,11]. Therefore, it is essential to ensure that EUS-guided measurement of portal pressure is performed with specific indications to ensure safety. For example, patients with splenomegaly may benefit from EUS-LB and EUS-guided measurement of portal pressure to confirm whether cirrhosis and portal hypertension are the underlying causes of splenomegaly. However, if a patient has esophageal or gastric varices, it can be safely assumed that the portal pressure is 10 mmHg or higher and the patient has clinically significant portal hypertension[40]. That information should be used to determine whether EUS-guided measurement of portal pressure is warranted at the time of the procedure.

Concomitant EUS-LB with other endoscopic procedures

EUS-LB provides an exciting new method for liver tissue acquisition. A novel application is for special patient populations such as liver transplant recipients (LTRs). Laboratory abnormalities in liver transplant patients often requires an extensive workup including imaging, liver biopsy, and ERCP. Combining EUS-LB with ERCP can potentially provide a "one-stop-shop" for evaluation in this patient population. This will hopefully reduce the time, cost, and healthcare resources needed to accurately diagnose and treat post-transplant liver function abnormalities. Our group at Ohio State University, has recently submitted a case series of 12 consecutive LTRs with abnormal liver function tests (Han S, Jalil S, Groce JR, Krishna SG, Lara L, Lee P, Limkemann A, Papachristou GI, Mumtaz K. Feasibility of Single-Session EUS-guided Liver Biopsy and ERCP in Liver Transplant Recipients with Abnormal Liver Function Tests), who underwent concomitant EUS-LB and ERCP. In this case series, tissue adequacy was obtained in 100% of patients with the most common diagnoses including anastomotic stricture (75%) and T-cell mediated rejection (66.7%). Seven (58.3%) patients had dual diagnoses of T-cell mediated rejection and anastomotic stricture. There were no 30 d adverse events. Authors concluded that **s**ingle-session EUS-guided LB and ERCP for the evaluation of elevated liver function tests in LTRs appears to be safe and feasible, but larger studies are needed to verify these findings.

Similarly, a recent study published by Hajifathalian *et al*[42] on concomitant EUS-LB and portal pressure gradient measurement reported 24 (100%) and 23 (96%) patients had successful portosystemic gradient (PSG) measurement and EUS-liver biopsy, respectively. Analysis revealed a significant

association between both PSG and liver stiffness measured on transient elastography (P = 0.01) and FIB-4 score (P = 0.02). There was no significant correlation between the fibrosis stage on histology and measured PSG (P = 0.56)[42].

Lastly, EUS-LB has been discussed anecdotally as a more effective option in pediatric population, as it may reduce patient anxiety when compared to PC-LB or TJ-LB – although, to our knowledge, formal studies in pediatric populations are yet to be done.

CONCLUSION

Overall, EUS-LB provides unique advantages when compared to its counterparts: ability to evaluate the GI tract and pancreato-biliary system, while obtaining access to both lobes of the liver. It also has proven to produce more histologically/pathologically complete samples than PC-LB and TJ-LB. EUS-LB appears to be a safe, diagnostic modality for obtaining liver tissue. Concomitant EUS-LB, EUS portal pressure gradient measurement and other endoscopic procedure (simple endoscopy and ERCP) is another emerging area in the field of endo-hepatology.

FOOTNOTES

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REFERENCES

1 **Younossi ZM**, Koenig AB, Abdelatif D, Fazel Y, Henry L, Wymer M. Global epidemiology of nonalcoholic fatty liver disease-Meta-analytic assessment of prevalence, incidence, and outcomes. *Hepatology* 2016; **64**: 73-84 [PMID: 26707365 DOI: 10.1002/hep.28431]
2 **Piazzolla VA**, Mangia A. Noninvasive Diagnosis of NAFLD and NASH. *Cells* 2020; **9** [PMID: 32316690 DOI: 10.3390/cells9041005]
3 **Castera L**, Friedrich-Rust M, Loomba R. Noninvasive Assessment of Liver Disease in Patients With Nonalcoholic Fatty Liver Disease. *Gastroenterology* 2019; **156**: 1264-1281.e4 [PMID: 30660725 DOI: 10.1053/j.gastro.2018.12.036]
4 **McLoughlin MJ**, Ho CS, Tao LC. Percutaneous needle aspiration biopsy. *Can Med Assoc J* 1978; **119**: 1324-1328 [PMID: 367550]
5 **Gilmore IT**, Burroughs A, Murray-Lyon IM, Williams R, Jenkins D, Hopkins A. Indications, methods, and outcomes of percutaneous liver biopsy in England and Wales: an audit by the British Society of Gastroenterology and the Royal College of Physicians of London. *Gut* 1995; **36**: 437-441 [PMID: 7698705 DOI: 10.1136/gut.36.3.437]
6 **Diehl DL**. Endoscopic Ultrasound-guided Liver Biopsy. *Gastrointest Endosc Clin N Am* 2019; **29**: 173-186 [PMID: 30846147 DOI: 10.1016/j.giec.2018.11.002]
7 **Shah AR**, Al-Hanayneh M, Chowdhry M, Bilal M, Singh S. Endoscopic ultrasound guided liver biopsy for parenchymal liver disease. *World J Hepatol* 2019; **11**: 335-343 [PMID: 31114638 DOI: 10.4254/wjh.v11.i4.335]
8 **Chi H**, Hansen BE, Tang WY, Schouten JN, Sprengers D, Taimr P, Janssen HL, de Knegt RJ. Multiple biopsy passes and the risk of complications of percutaneous liver biopsy. *Eur J Gastroenterol Hepatol* 2017; **29**: 36-41 [PMID: 27556687 DOI: 10.1097/MEG.0000000000000731]
9 **Lavian JD**, Thornton LM, Zybulewski A, Kim E, Nowakowski SF, Ranade M, Patel RS, Lookstein RA, Fischman A, Bishay V. Safety of percutaneous versus transjugular liver biopsy: A propensity score matched analysis. *Eur J Radiol* 2020;

133: 109399 [PMID: 33202374 DOI: 10.1016/j.ejrad.2020.109399]
10 **Madhok IK**, Parsa N, Nieto JM. Endoscopic Ultrasound-Guided Liver Biopsy. *Clin Liver Dis* 2022; **26**: 127-138 [PMID: 34802658 DOI: 10.1016/j.cld.2021.09.002]
11 **Kalambokis G**, Manousou P, Vibhakorn S, Marelli L, Cholongitas E, Senzolo M, Patch D, Burroughs AK. Transjugular liver biopsy--indications, adequacy, quality of specimens, and complications--a systematic review. *J Hepatol* 2007; **47**: 284-294 [PMID: 17561303 DOI: 10.1016/j.jhep.2007.05.001]
12 **Gress FG**. The Early History of Interventional Endoscopic Ultrasound. *Gastrointest Endosc Clin N Am* 2017; **27**: 547-550 [PMID: 28918797 DOI: 10.1016/j.giec.2017.06.015]
13 **Mathew A**. EUS-guided routine liver biopsy in selected patients. *Am J Gastroenterol* 2007; **102**: 2354-2355 [PMID: 17897349 DOI: 10.1111/j.1572-0241.2007.01353_7.x]
14 **Chon HK**, Yang HC, Choi KH, Kim TH. Endoscopic Ultrasound-Guided Liver Biopsy Using a Core Needle for Hepatic Solid Mass. *Clin Endosc* 2019; **52**: 340-346 [PMID: 31302987 DOI: 10.5946/ce.2018.175]
15 **Lee YN**, Moon JH, Kim HK, Choi HJ, Choi MH, Kim DC, Lee TH, Cha SW, Kim SG, Kim YS. Usefulness of endoscopic ultrasound-guided sampling using core biopsy needle as a percutaneous biopsy rescue for diagnosis of solid liver mass: Combined histological-cytological analysis. *J Gastroenterol Hepatol* 2015; **30**: 1161-1166 [PMID: 25684303 DOI: 10.1111/jgh.12922]
16 **Kongkam P**, Nalinthassanai N, Prueksapanich P, Sanpavat A, Cañones AR, Luangsukrerk T, Angsuwatcharakon P, Ridtitid W, Kullavanijaya P, Treeprasertsuk S, Rerknimitr R. A comparison of the antegrade core trap and reverse bevel needles for EUS-guided fine-needle biopsy sampling of liver mass: a prospective randomized cross over study. *HPB (Oxford)* 2022; **24**: 797-805 [PMID: 34794898 DOI: 10.1016/j.hpb.2021.10.009]
17 **Ali AH**, Panchal S, Rao DS, Gan Y, Al-Juboori A, Samiullah S, Ibdah JA, Hammoud GM. The efficacy and safety of endoscopic ultrasound-guided liver biopsy versus percutaneous liver biopsy in patients with chronic liver disease: a retrospective single-center study. *J Ultrasound* 2020; **23**: 157-167 [PMID: 32141043 DOI: 10.1007/s40477-020-00436-z]
18 **Patel HK**, Therapondos G, Galliano G, Romero R, Evans J, Cohen A, Mubarak MF, Shah JN, Chafic AHE. Endoscopic Ultrasound Guided Liver Biopsy Using Newer 19G FNB Needles Compared to Percutaneous and Trans-jugular Liver Biopsy: A Tertiary Center Experience. *Tech Innov Gastrointest Endosc* 2022; **24**: 127-135 [DOI: 10.1016/j.tige.2021.11.009]
19 **Shuja A**, Alkhasawneh A, Fialho A, Shukri A, Harris C, Smotherman C, Malespin M, de Melo SW Jr. Comparison of EUS-guided versus percutaneous and transjugular approaches for the performance of liver biopsies. *Dig Liver Dis* 2019; **51**: 826-830 [PMID: 30755347 DOI: 10.1016/j.dld.2019.01.006]
20 **Dhar J**, Samanta J. Role of endoscopic ultrasound in the field of hepatology: Recent advances and future trends. *World J Hepatol* 2021; **13**: 1459-1483 [PMID: 34904024 DOI: 10.4254/wjh.v13.i11.1459]
21 **Facciorusso A**, Crinò SF, Ramai D, Fabbri C, Mangiavillano B, Lisotti A, Muscatiello N, Cotsoglou C, Fusaroli P. Diagnostic yield of endoscopic ultrasound-guided liver biopsy in comparison to percutaneous liver biopsy: a systematic review and meta-analysis. *Expert Rev Gastroenterol Hepatol* 2022; **16**: 51-57 [PMID: 34918578 DOI: 10.1080/17474124.2022.2020645]
22 **Johnson KD**, Laoveeravat P, Yee EU, Perisetti A, Thandassery RB, Tharian B. Endoscopic ultrasound guided liver biopsy: Recent evidence. *World J Gastrointest Endosc* 2020; **12**: 83-97 [PMID: 32218888 DOI: 10.4253/wjge.v12.i3.83]
23 **Rockey DC**, Caldwell SH, Goodman ZD, Nelson RC, Smith AD; American Association for the Study of Liver Diseases. Liver biopsy. *Hepatology* 2009; **49**: 1017-1044 [PMID: 19243014 DOI: 10.1002/hep.22742]
24 **Cholongitas E**, Senzolo M, Standish R, Marelli L, Quaglia A, Patch D, Dhillon AP, Burroughs AK. A systematic review of the quality of liver biopsy specimens. *Am J Clin Pathol* 2006; **125**: 710-721 [PMID: 16707372 DOI: 10.1309/W3XC-NT4H-KFBN-2G0B]
25 **Ching-Companioni RA**, Johal AS, Confer BD, Forster E, Khara HS, Diehl DL. Single-pass 1-needle actuation versus single-pass 3-needle actuation technique for EUS-guided liver biopsy sampling: a randomized prospective trial (with video). *Gastrointest Endosc* 2021; **94**: 551-558 [PMID: 33771557 DOI: 10.1016/j.gie.2021.03.023]
26 **Bhatia V**, Hijioka S, Hara K, Mizuno N, Imaoka H, Yamao K. Endoscopic ultrasound description of liver segmentation and anatomy. *Dig Endosc* 2014; **26**: 482-490 [PMID: 24355092 DOI: 10.1111/den.12216]
27 **Mok SRS**, Diehl DL, Johal AS, Khara HS, Confer BD, Mudireddy PR, Kirchner HL, Chen ZE. A prospective pilot comparison of wet and dry heparinized suction for EUS-guided liver biopsy (with videos). *Gastrointest Endosc* 2018; **88**: 919-925 [PMID: 30120956 DOI: 10.1016/j.gie.2018.07.036]
28 **Baran B**, Kale S, Patil P, Kannadath B, Ramireddy S, Badillo R, DaVee RT, Thosani N. Endoscopic ultrasound-guided parenchymal liver biopsy: a systematic review and meta-analysis. *Surg Endosc* 2021; **35**: 5546-5557 [PMID: 33052529 DOI: 10.1007/s00464-020-08053-x]
29 **Nieto J**, Khaleel H, Challita Y, Jimenez M, Baron TH, Walters L, Hathaway K, Patel K, Lankarani A, Herman M, Holloman D, Saab S. EUS-guided fine-needle core liver biopsy sampling using a novel 19-gauge needle with modified 1-pass, 1 actuation wet suction technique. *Gastrointest Endosc* 2018; **87**: 469-475 [PMID: 28551024 DOI: 10.1016/j.gie.2017.05.013]
30 **Dewitt J**, McGreevy K, Cummings O, Sherman S, Leblanc JK, McHenry L, Al-Haddad M, Chalasani N. Initial experience with EUS-guided Tru-cut biopsy of benign liver disease. *Gastrointest Endosc* 2009; **69**: 535-542 [PMID: 19231495 DOI: 10.1016/j.gie.2008.09.056]
31 **Gleeson FC**, Clayton AC, Zhang L, Clain JE, Gores GJ, Rajan E, Smyrk TC, Topazian MD, Wang KK, Wiersema MJ, Levy MJ. Adequacy of endoscopic ultrasound core needle biopsy specimen of nonmalignant hepatic parenchymal disease. *Clin Gastroenterol Hepatol* 2008; **6**: 1437-1440 [PMID: 19081532 DOI: 10.1016/j.cgh.2008.07.015]
32 **Schulman AR**, Thompson CC, Odze R, Chan WW, Ryou M. Optimizing EUS-guided liver biopsy sampling: comprehensive assessment of needle types and tissue acquisition techniques. *Gastrointest Endosc* 2017; **85**: 419-426 [PMID: 27530070 DOI: 10.1016/j.gie.2016.07.065]
33 **Eskandari A**, Koo P, Bang H, Gui D, Urayama S. Comparison of Endoscopic Ultrasound Biopsy Needles for Endoscopic Ultrasound-Guided Liver Biopsy. *Clin Endosc* 2019; **52**: 347-352 [PMID: 31288504 DOI: 10.5946/ce.2019.005]

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

35 **Shah RM**, Schmidt J, John E, Rastegari S, Acharya P, Kedia P. Superior Specimen and Diagnostic Accuracy with Endoscopic Ultrasound-Guided Liver Biopsies Using 19-Gauge versus 22-Gauge Core Needles. *Clin Endosc* 2021; **54**: 739-744 [PMID: 33189102 DOI: 10.5946/ce.2020.212]

36 **DeWitt J**, Cho CM, Lin J, Al-Haddad M, Canto MI, Salamone A, Hruban RH, Messallam AA, Khashab MA. Comparison of EUS-guided tissue acquisition using two different 19-gauge core biopsy needles: a multicenter, prospective, randomized, and blinded study. *Endosc Int Open* 2015; **3**: E471-E478 [PMID: 26528504 DOI: 10.1055/s-0034-1392222]

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

38 **Aggarwal SN**, Magdaleno T, Klocksieben F, MacFarlan JE, Goonewardene S, Zator Z, Shah S, Shah HN. A prospective, head-to-head comparison of 2 EUS-guided liver biopsy needles in vivo. *Gastrointest Endosc* 2021; **93**: 1133-1138 [PMID: 33045222 DOI: 10.1016/j.gie.2020.09.050]

39 **Hashimoto R**, Lee DP, Samarasena JB, Chandan VS, Guo W, Lee JG, Chang KJ. Comparison of Two Specialized Histology Needles for Endoscopic Ultrasound (EUS)-Guided Liver Biopsy: A Pilot Study. *Dig Dis Sci* 2021; **66**: 1700-1706 [PMID: 32556821 DOI: 10.1007/s10620-020-06391-3]

40 **Rudnick SR**, Conway JD, Russo MW. Current state of endohepatology: Diagnosis and treatment of portal hypertension and its complications with endoscopic ultrasound. *World J Hepatol* 2021; **13**: 887-895 [PMID: 34552695 DOI: 10.4254/wjh.v13.i8.887]

41 **Samarasena JB**, Chang KJ. Endoscopic Ultrasound-Guided Interventions for the Measurement and Treatment of Portal Hypertension. *Gastrointest Endosc Clin N Am* 2019; **29**: 311-320 [PMID: 30846155 DOI: 10.1016/j.giec.2018.12.004]

42 **Hajifathalian K**, Westerveld D, Kaplan A, Dawod E, Herr A, Ianelli M, Saggese A, Kumar S, Fortune BE, Sharaiha RZ. Simultaneous EUS-guided portosystemic pressure measurement and liver biopsy sampling correlate with clinically meaningful outcomes. *Gastrointest Endosc* 2022; **95**: 703-710 [PMID: 34890694 DOI: 10.1016/j.gie.2021.11.037]

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MINIREVIEWS

Non-alcoholic fatty liver disease-related hepatocellular carcinoma: Is there a role for immunotherapy?

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Abstract

Hepatocellular carcinoma (HCC) is among the most common cancers and it is a major cause of cancer-related deaths. Non-alcoholic fatty liver disease (NAFLD) affects approximately one fourth of individuals worldwide and it is becoming one of the most important causes of HCC. The pathogenic mechanisms leading to NAFLD-related HCC are complex and not completely understood. However, metabolic, fibrogenic, oncogenic, inflammatory and immunological pathways seem to be involved. First-line therapy of advanced HCC has recently undergone major changes, since the combination of atezolizumab and bevacizumab was

proven to increase survival when compared to sorafenib. Other immune-oncology drugs are also demonstrating promising results in patients with advanced HCC when compared to traditional systemic therapy. However, initial studies raised concerns that the advantages of immunotherapy might depend on the underlying liver disease, which seems to be particularly important in NAFLD-related HCC, as these tumors might not benefit from it. This article will review the mechanisms of NAFLD-related hepatocarcinogenesis, with an emphasis on its immune aspects, the efficacy of traditional systemic therapy for advanced NAFLD-related HCC, and the most recent data on the role of immunotherapy for this specific group of patients, showing that the management of this condition should be individualized and that a general recommendation cannot be made at this time.

Key Words: Non-alcoholic fatty liver disease; Hepatocellular carcinoma; Hepatocarcinogenesis; Immunology; Immunotherapy; Tyrosine kinase inhibitors

Core Tip: Non-alcoholic fatty liver disease (NAFLD) is an important cause of hepatocellular carcinoma (HCC). While the treatment of advanced HCC has recently undergone a revolution led by immunotherapy, concerns have been raised that NAFLD-related HCC might not respond well to these new therapies. This review will approach the pathophysiology of NAFLD-related liver cancer, the evidence on traditional systemic treatments and the most recent data on immunotherapy for this particular group of patients, showing that the management of this condition should be individualized.

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INTRODUCTION

The incidence of liver cancer worldwide was 11.6/100000 individuals in 2020 and its mortality rate reached 10.7/100000 in the same year, making it the sixth most common cancer and the second cause of cancer-related mortality[1]. Furthermore, it is estimated that its incidence rate will continue to increase until 2030[2]. Hepatocellular carcinoma (HCC) is by far the most common among primary liver cancers [3,4].

Non-alcoholic fatty liver disease (NAFLD) has a striking prevalence of 25% worldwide[5], which highlights its relevance as an underlying cause of HCC[6]. In the year of 2019, for instance, NAFLD was estimated to be associated with 36300 new cases of HCC, as well as with 34700 HCC-related deaths[7]. As it would be expected, the burden of NAFLD as a cause of HCC seems to be growing. It is estimated that the age-standardized incidence rate of NAFLD-related liver cancer will raise from 0.92 to 1.18/100000 individuals between 2018 and 2030[2]. Moreover, while a multinational cohort study demonstrated that NAFLD was responsible for 9% of HCC cases followed between 2005 and 2015 in South America (an area with high prevalence of NAFLD)[8], the same group verified that it was responsible for 34% of cases followed between 2019 and 2020[9].

Cirrhosis in general predisposes to HCC, and the annual incidence of HCC in patients with NAFLD-related cirrhosis ranges between 0.5% and 2.6%[10-13]. However, some specific mechanisms associated with NAFLD, which will be further discussed in this article, also allow for the development of HCC in the absence of cirrhosis, making NAFLD itself etiologically associated to hepatocarcinogenesis[6,14-16]. According to a meta-analysis, approximately 38% of NAFLD-related HCCs would develop in non-cirrhotic settings[17]. Still, it must be highlighted that the risk of developing HCC is much lower in patients with NAFLD who do not have cirrhosis than in those with NAFLD-related cirrhosis, as it has been recently shown in another meta-analysis (incidence of 0.03 *vs* 3.78/100 person-years)[18].

Significant progress has been recently made in the treatment of advanced HCC, when immunotherapy was proven superior to the systemic therapy used for over a decade[19,20]. Table 1 shows a summary of the most important randomized controlled trials on systemic therapy for advanced HCC.

Nevertheless, initial studies raised concerns that the advantages of immunotherapy might depend on the underlying liver disease, and that patients with NAFLD-related HCC might benefit less from it[21,22]. Considering the epidemiological importance of NAFLD-related HCC, this article aims to review the

Table 1 Summary of randomized controlled trials on systemic therapy for advanced hepatocellular carcinoma

Ref.	Interventions	Mechanisms of action	Main results
Llovet *et al* [57]	Sorafenib × placebo	TKI × placebo	Increased OS with sorafenib
Cheng *et al* [56]	Sorafenib × placebo	TKI × placebo	Increased OS with sorafenib
Bruix[1] et al [59]	Regorafenib × placebo	TKI × placebo	Increased OS with regorafenib
Kudo *et al* [61]	Lenvatinib × sorafenib	TKI × TKI	Non-inferior OS
Abou-Alfa[2] et al[58]	Cabozantinib × placebo	TKI × placebo	Increased OS with cabozantinib
Zhu[1,3] et al [60]	Ramucirumab × placebo	Anti-VEGF receptor 2 × placebo	Increased OS with ramucirumab
Yau *et al*[73]	Nivolumab × sorafenib	Anti-PD-1 × TKI	No increase in OS[4]
Finn[1] et al [74]	Pembrolizumab × placebo	Anti-PD-L1 × placebo	No increase in OS[4]
Finn *et al*[19]	Atezolizumab + bevacizumab × sorafenib	Anti-PD-L1 + anti-VEGF × TKI	Increased OS with atezolizumab + bevacizumab
Kelley *et al* [79]	Atezolizumab + cabozantinib × sorafenib × cabozantinib	Anti-PD-L1 + TKI × TKI × TKI	No increase in OS[4]
Abou-Alfa *et al*[82]	Tremelimumab + durvalumab × durvalumab × sorafenib	Anti-CTLA-4 + anti-PD-L1 × anti-PD-L1 × TKI	Increased OS with tremelimumab + durvalumab (× sorafenib). Non-inferior OS with durvalumab (× sorafenib)

[1]2nd line treatment.
[2]2nd or 3rd line treatment.
[3]Individuals with alpha-fetoprotein ≥ 400 ng/mL.
[4]Primary endpoint not reached.
TKI: Tyrosine kinase inhibitor; OS: Overall survival; VEGF: Vascular endothelial growth factor; PD-1: Programmed cell death protein 1; PD-L1: Programmed cell death ligand-1; CTLA-4: Cytotoxic T-lymphocyte-associated protein 4.

mechanisms behind NAFLD-related hepatocarcinogenesis, with an emphasis on its immune aspects, the knowledge gathered over the years on the efficacy of traditional systemic therapy for advanced NAFLD-related HCC, and the most up-to-date evidence on the role of immunotherapy for this specific group of patients.

PATHOPHYSIOLOGY OF NAFLD-RELATED HCC

The underlying pathogenic mechanisms leading to NAFLD-related HCC are complex and not completely understood[10,23,24]. Metabolic dysfunction as well as inflammatory, fibrogenic and oncogenic pathways are involved, which are in turn modulated by a myriad of factors. These include, among others, genetic and epigenetic changes, different degrees of dysregulation of multiorgan metabolic and endocrine signaling, altered immunologic responses and changes in gut microbiota[24,25].

A disturbed systemic metabolic milieu characterized mainly by insulin resistance and a low-grade inflammatory state seems to be a key in promoting carcinogenesis in a fat-laden liver. Both type 2 diabetes and obesity are known risk factors for NAFLD-related HCC, which may act additively with other metabolic syndrome traits (*i.e.*, dyslipidemia and hypertension) in increasing the risk of HCC development[25,26]. However, the precise molecular mechanisms underpinning the connection between metabolic dysfunction and HCC remain incompletely understood. Metabolic dysfunction-related changes in lipid metabolism (including uncontrolled lipolysis in adipose tissue leading to fatty acid overflow towards the liver and stimulation of hepatic de novo lipogenesis) lead to hepatocyte lipid overload that, in turn, triggers some compensatory adaptations such as downregulation of carnitine palmitoyltransferase 2, which may promote hepatocarcinogenesis. Accumulation of acylcarnitine has been suggested as a potential mechanism in this regard[27]. Also, changes in sterol regulatory element-binding proteins transcription factors, key regulators of sterol and fatty acid biosynthesis, may contribute through interaction with tumor suppressor genes such as p53[28].

Importantly, lipid overload also leads to the occurrence of oxidative stress, endoplasmic reticulum stress, mitochondrial dysfunction, cell injury and cell death, all triggering inflammatory responses and activating fibrogenic processes[29]. Moreover, numerous signaling pathways are also dysregulated, including activation of interleukin (IL)-6/Janus kinase/signal transducer and activator of transcription (STAT) pathway and insulin-like growth factor, among others[30].

Of note, in the setting of a chronic inflammatory microenvironment, death of hepatocytes by different mechanisms (necrosis, apoptosis, pyroptosis and other forms of the cell death) determines a compensatory hepatocyte proliferation. This proliferative response is associated with genetic and epigenetic changes that play a pivotal role in hepatocarcinogenesis. Among these genetic changes, different studies have identified mutations in the telomerase reverse transcriptase promoter, *p53* gene and wingless-related integration site (Wnt)/β-catenin signaling pathway genes as the most consistent genetic changes during HCC development[26]. Finally, epigenetic modifications have been shown to be involved in HCC progression in the context of NAFLD. A broad range of changes in chromatin remodeling, histone alterations, DNA methylation, and noncoding RNA expression has been described and reviewed recently[31]. Such processes have diagnostic, prognostic, and therapeutic implications[32].

Experimental evidence generated in mouse models and some human data strongly suggest that altered gut microbiota may be an important contributing factor to HCC development in the setting of NAFLD/nonalcoholic steatohepatitis (NASH)[33]. The occurrence of dysbiosis is common in NAFLD/NASH, as well as in its commonly associated comorbidities, such as obesity and type 2 diabetes. Nevertheless, although microbiome changes have been characterized in different studies involving subjects with NASH or NAFLD-related cirrhosis, there is considerable variability across different reports, and only few studies have focused specifically on NAFLD-related HCC. Ponziani *et al* [34] showed that cirrhotic NAFLD-related HCC patients had a microbiome profile enriched in Bacteroides and Ruminococcaceae compared to patients with NAFLD-related cirrhosis without HCC [34]. This microbiota profile was associated with increased levels of some circulating proinflammatory cytokines. However, it remains unclear if this is just an epiphenomenon or has pathogenetic relevance.

Mechanistically, dysbiosis leads to altered intestinal permeability, which in turn contributes to hepatic inflammation *via* toll-like receptor 4 (TLR4) stimulation by bacterial derived products. Sustained hepatic inflammation triggered by this pathway can then contribute to hepatocarcinogenesis through multiple mechanisms[23]. Consistent with this proposed mechanism, TLR4 ablation has been shown to prevent HCC development in NASH models[35]. Additionally, changes in gut microbiota critically influence bile acid metabolism. Thus, animals fed with a NASH-promoting diet exhibit a change in their bile acid pool composition, which become enriched in secondary bile acids, particularly deoxycholic acid[33]. Moreover, microbiome changes may induce the transition of hepatic stellate cells (HSCs) in the stroma into a senescence-associated secretory phenotype, which are cells associated with suppression of antitumor immunity[36].

Specific genetic polymorphisms that influence NAFLD susceptibility and severity, such as patatin-like phospholipase domain containing protein 3 (PNPLA3), transmembrane 6 superfamily member 2 and membrane bound O-acetyltransferase domain containing 7, have been linked to a higher risk for NAFLD-related HCC[26]. Of these polymorphisms, the I148M variant of PNPLA3 is one of striking relevance in HCC development[37]. Carriage of this variant critically influences triacylglycerol remodeling in hepatocytes, and recent evidence shows that it also influences fibrogenesis and carcinogenesis by impairing retinol release from HSCs[38].

NAFLD-related HCC generally emerges in the setting of cirrhosis, but may arise also in a non-cirrhotic liver. Some pathways have been shown to play a role in this scenario. Increased STAT-3 signaling has been shown to be associated to the development of HCC independently of NASH and fibrosis. Also, disruption of circadian homeostasis has been shown to be related to HCC development independently of cirrhosis[39].

In summary, pathobiological mechanisms of hepatocarcinogenesis result from a complex interaction of the above-mentioned factors leading to uncontrolled proliferative responses, dysregulation of DNA-damage-response pathways, activation of survival signaling, angiogenesis, evasion of immune surveillance, genomic instability and ultimately to cancer development. It is likely that, in NAFLD patients, mechanisms leading to HCC vary from one subject to another given the heterogenous population grouped under the acronym[40]. Precision medicine approaches based on advanced biomarkers may help identify those at higher risk of developing HCC in order to include them in surveillance programs or, once diagnosed, may allow treatment with targeted therapies.

IMMUNE ASPECTS OF NAFLD-RELATED HCC

Emerging evidence suggests that the chronic inflammatory process of NAFLD and NASH sets the basis for the development of HCC[41]. In addition, there seems to be a role for innate and adaptive immune cells in the pathogenesis of NAFLD-related HCC (Figure 1). However, the immune mechanisms that are specific to NAFLD as the underlying cause of HCC are largely unclear. For example, numerous studies have demonstrated the critical involvement of hepatic macrophages, including resident Kupffer cells

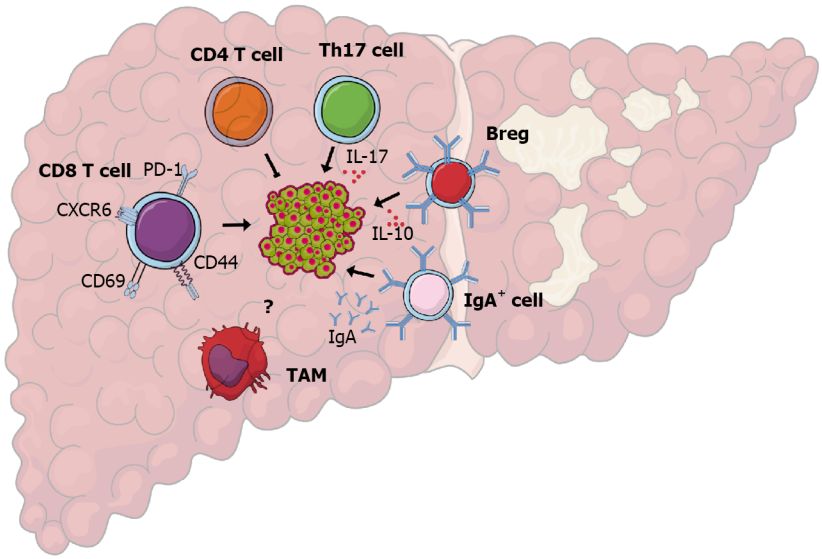

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Figure 1 Immune mechanisms in nonalcoholic fatty liver disease-related hepatocellular carcinoma pathogenesis. Studies using human specimens and mouse models have shown that activated non-alcoholic steatohepatitis-associated, programmed cell death protein 1 + CD8 T cells can cause non-specific cell death of hepatocytes and promote hepatocellular carcinoma development. T helper 17 cell-derived interleukin-17, immunoglobulin A-producing cells promote non-alcoholic steatohepatitis-derived hepatocellular carcinoma development, while an overall loss of CD4 T cells may increase tumor burden size. Bregs are abundant in hepatocellular carcinoma and promote disease progression, but are not specific to nonalcoholic fatty liver disease-related hepatocellular carcinoma. The role of tumor associated macrophages in the progression of nonalcoholic fatty liver disease-related hepatocellular carcinoma is unclear. Figure created in the Mind the Graph platform, available at www.mindthegraph.com. Th: T helper; PD-1: Programmed cell death protein 1; IL: Interleukin; Bregs: Regulatory B cells; IgA: Immunoglobulin A; CXCR6: C-X-C Motif Chemokine Receptor 6; TAM: Tumor associated macrophages.

and recruited monocyte-derived macrophages in the pathogenesis of NASH[42]. The activation of Kupffer cells is crucial for tumor development in the early stage of chemical-induced carcinogenesis[43] and tumor-associated macrophages play a prominent role in promoting HCC once a primary tumor is established[44]. Nevertheless, the role of hepatic macrophages in NAFLD-related HCC has not been investigated yet.

Regarding the role of adaptive immunity as a central player in the progression of NAFLD-related HCC, several reports have shown increased recruitment of adaptive immune cells such as T and B lymphocytes into the liver of these patients. For instance, whole-exome sequencing of livers from patients with NAFLD-related HCC shows enrichment of gene signatures associated with T lymphocytes suggesting increased hepatic T cell accumulation[45]. Notably, studies show that HCC cases from NAFLD-related cirrhosis display features of immune exhaustion, indicating a state of dysfunction likely due to stimulation by tumor antigens[45].

Experimental murine models of NAFLD-related HCC have shed light on the potential mechanisms by which T cells regulate HCC development. In a mouse model of NAFLD-related HCC that recapitulates metabolic disease, intrahepatic CD8 T cells become activated and express CD44 and CD69, suggesting that they can directly induce liver damage through interactions with hepatocytes[46]. Indeed, genetic ablation of CD8 T cells in this mouse model ameliorates liver damage, NASH, and HCC development, suggesting that CD8 T cells directly instigate disease progression[46]. The increased T cell infiltration in NAFLD-related HCC has been attributed to the inactivation of T cell protein tyrosine phosphatase in hepatocytes and increased expression of T cell chemoattractants[47]. Mechanistically, NASH programs CD8 T cells to acquire an activated exhausted phenotype and express increased levels of programmed cell death protein 1 (PD-1) in response to metabolic signals as well as to IL-15, instigating their non-specific killing of hepatocytes and disease progression[48]. In addition to exhaustion markers, NASH-derived PD-1 positive CD8 T cells express high levels of effector function molecules such as tumor necrosis factor α, interferon γ, and granzyme[21]. The increased activity of exhausted PD-1 positive CD8 T cells in the NASH liver is not limited to accelerating liver damage but can result in impaired immune surveillance and subsequent HCC[21].

In contrast to the accumulation of CD8 T cells, lipid dysregulation has been shown to cause a loss of CD4 T cells in tumor-free and tumor-bearing environments in the liver of mice with NASH induced by a methionine and choline-deficient L-amino acid-defined diet[49]. Importantly, depletion of total CD4 T cells promoted hepatic carcinogenesis in a MYC oncogene model of HCC, suggesting that the overall function of CD4 T cells is to provide antitumor immunity[49]. However, nutrient overload induces a subset of CD4 T cells known as T helper-17 cells that produce large amounts of IL-17A to promote tumorigenesis in NAFLD-related HCC[50]. Overall, these findings suggest that the exhaustion of hepatic CD8 T cells and the loss of CD4 T cells in NASH compromise the hepatic antitumor surveillance

and increase the susceptibility to HCC.

In addition to T cells, emerging evidence suggests that immunoglobulin A (IgA)-producing B cells play a critical role in the progression of NAFLD-related HCC. In humans, the number of tumor-infiltrating B cells correlates with tumor progression[51]. Likewise, patients with HCC that have fewer tumor-infiltrating plasma cells present a better prognosis[52]. Feeding a high fat diet to major urinary protein-urokinase-type plasminogen activator transgenic mice, a reliable model of NAFLD-related HCC, instigates IgA-producing cells to interfere with the protective role of anti-tumor cytotoxic CD8 T cells[53]. The IgA positive B cells inhibit the activity of CD8 T cells through the expression of programmed death-ligand 1 (PD-L1) and the production of the immunosuppressive cytokine IL-10[53]. Regulatory B cells, a subset of B cells, can also inhibit anti-tumor immunity through IL-10 production and promote HCC growth through direct interactions with the tumor cells[54]. As B cells promote NAFLD through the production of pro-inflammatory cytokines and regulation of intrahepatic T cells in NASH[55], future studies are needed to clarify the precise role of liver B cells in NAFLD-related HCC.

TRADITIONAL SYSTEMIC TREATMENT FOR ADVANCED NAFLD-RELATED HCC

Systemic therapy for HCC is relatively young in comparison to other cancers, becoming available only after 2007, with the arrival of sorafenib, a multikinase inhibitor that showed improved survival against placebo in both the SHARP and Asia-Pacific trials[56,57]. For 10 years, sorafenib was the only available systemic therapy for HCC, until the field expanded significantly over the last 5 years with trials showing benefit from regorafenib, lenvatinib, cabozantinib and ramucirumab[58-61]. All of these studies were performed on HCCs from multiple etiologies, but primarily viral hepatitis-related tumors. Recently, attention has shifted to the importance of HCC etiology as a predictor of response to systemic therapy. This issue has been most evident in HCC immunotherapy, but potentially applies to other systemic therapies as well.

NAFLD-related HCC represents a unique conundrum for systemic therapy, as metabolic issues beyond that of HCC may play a role[62]. Individuals with NAFLD-related HCC are more likely to be obese, and are more likely to be on hormone-related medications, both of which could affect the pharmacokinetics of systemic therapy. Indeed, a small study showed that patients on sorafenib who were receiving metformin for diabetes mellitus had worse survival than those on insulin, regardless of the cause of HCC[63].

Due to the temporal dynamics of HCC systemic therapy in the last 15 years, most of the available data is from sorafenib studies. Sorafenib is known to perform best when used in patients with viral hepatitis-related HCC, particularly hepatitis C infection. However, most studies addressed hepatitis C *vs* alcohol-related HCC, or viral hepatitis *vs* other causes of HCC, clouding the understanding of the role of sorafenib in NAFLD-related HCC specifically[64-66]. An international study, presented only in abstract form, found similar overall survival (OS) in patients treated with sorafenib with NAFLD-related HCC *vs* other causes [hazard ratio (HR): 0.94, 95% confidence interval (CI): 0.76-1.16, $P = 0.57$]. However, the NAFLD portion of the study included only 187 patients (3.5% of the total cohort), and further details are awaited with the full peer-reviewed version[67]. A recent retrospective subgroup analysis including 483 NAFLD-related HCC patients treated with sorafenib also found no significant difference in univariate analysis of survival between NAFLD and other etiologies (10.2 mo *vs* 12.3 mo; HR: 1.06; 95%CI: 0.83-1.34, $P = 0.63$)[68].

Information on NAFLD-related HCC for other non-immune forms of systemic therapy remains rather elusive, although very recent studies are shedding some light on the field. The initial trials for regorafenib (RESORCE), cabozantinib (CELESTIAL) and ramucirumab (REACH-2) included very limited numbers of patients with NAFLD-related HCC (6.6%, 9.3% and 7.9% respectively), and most subgroup analyses did not specifically evaluate NAFLD-related HCC[58-60]. The CELESTIAL trial for cabozantinib indicated benefit of the drug across all subgroups of etiologic factors and found no difference for OS between viral hepatitis-related HCC and other etiologies (HR: 0.76, 95%CI: 0.63-0.92 *vs* HR: 0.72, 95%CI: 0.54-0.96). However, the "other etiologies" group included all non-viral hepatitis-related tumors, with the NASH proportion conforming 43 patients. Also, this trial evaluated cabozantinib as second line therapy[58].

More recently, retrospective studies have evaluated lenvatinib in NAFLD-related HCC, suggesting an advantage for this multikinase inhibitor in this group of patients. A multicenter analysis of 236 NAFLD-related HCCs treated with lenvatinib (19% of the entire cohort) found a better OS for NAFLD-related HCC compared to other etiologies (22.2 mo *vs* 15.1 mo, HR: 0.69, 95%CI: 0.56-0.85, $P = 0.0006$)[68]. A Japanese study (also retrospective) of lenvatinib in unresectable tumors involving 103 NAFLD-related HCCs found no difference in OS compared to other etiologies, but the progression free survival (PFS) was better in the NAFLD group (median 9.3 mo *vs* 7.5 mo, $P = 0.01$)[69]. Furthermore, the retrospective multicenter ELEVATOR study, in which 200 patients were treated with lenvatinib, observed a similar survival in patients with viral and non-viral (NASH and alcoholic steatohepatitis) related HCC[70].

This early evidence suggests that sorafenib or cabozantinib are not particularly better (or worse) for NAFLD-related HCC, and that the use of lenvatinib might pose an advantage, with no specific data, so

far, for regorafenib. Nevertheless, it should be noted that better studies are needed in order to draw significant conclusions.

IMMUNOTHERAPY FOR ADVANCED NAFLD-RELATED HCC

In recent years, besides several tyrosine kinase inhibitors and a monoclonal antibody against vascular endothelial growth factor (VEGF) receptor-2 being added to the treatment armamentarium, immunotherapy with immune checkpoint blockers has been extensively investigated in patients with HCC[71]. Nivolumab, pembrolizumab, and the combination of nivolumab plus ipilimumab have been approved by the United States Food and Drug Administration (FDA) based on very promising phase II studies [72], but nivolumab and pembrolizumab failed to reach their primary endpoints in subsequent phase III trials in first- and second-line, respectively[73,74].

Atezolizumab plus bevacizumab

Overexpression of VEGF has been associated with liver cancer development and progression, and small phase II studies have shown modest anti-tumor efficacy of bevacizumab as monotherapy in advanced HCC[75]. In addition to the antiangiogenic effects, there is increasing evidence that anti-VEGF therapies may enhance the efficacy of anti-PD-1 and anti-PD-L1 by reversing VEGF-mediated immunosuppression and promoting T-cell infiltration in tumors[76]. Based on these observations, the PD-L1 inhibitor atezolizumab was evaluated together with the VEGF inhibitor bevacizumab in the phase III IMbrave150 study. The combination significantly improved all efficacy endpoints with a statistically significant and clinically meaningful survival benefit compared to sorafenib[19,77]. Side effect profiles of the drugs were in line with their respective mechanism of action and no new safety signals were observed. Additionally, patient-reported outcomes revealed that deteriorations in quality of life were significantly delayed in the combination arm compared to the sorafenib arm[78]. The combination has been approved by the FDA and by the European Medicines Agency (EMA), and it is currently regarded as the standard of care in first-line therapy of advanced HCC.

Atezolizumab plus cabozantinib

In the COSMIC-312 trial, in which 837 patients with HCC were randomized to be treated with atezolizumab plus cabozantinib, sorafenib in monotherapy or cabozantinib in monotherapy (2:1:1), PFS was 6.8 mo for the group receiving combination therapy, compared to 4.2 mo for the group under sorafenib (HR: 0.63, 95%CI: 0.44-0.91, P = 0.0012). Regarding OS, an interim analysis did not demonstrate a significant difference between combination treatment and monotherapy with sorafenib (HR: 0.90, 95%CI: 0.69-1.18, P = 0.438). Finally, the interim analysis on the comparison between cabozantinib and sorafenib showed a tendency favoring cabozantinib regarding PFS (HR: 0.71, 95%CI: 0.51-1.01)[79].

Dual checkpoint inhibition

Two antibodies against cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) have been investigated in advanced HCC. The combination of nivolumab and ipilimumab has already shown improved OS in HCC patients in the phase I/II CheckMate040 study[80,81]. The pure immune-oncology (IO) combination achieved a high overall response rate with a promising OS of almost 23 mo. The most common immune related adverse events were rash, hepatitis, and adrenal insufficiency. Based on these results, the FDA approved nivolumab plus ipilimumab as second-line treatment for patients with advanced HCC previously treated with sorafenib. Currently, the checkmate 9DW study evaluates the efficacy of nivolumab plus ipilimumab compared to sorafenib or lenvatinib as first-line therapy.

Similar to ipilimumab, tremelimumab has also been studied as an anti-CTLA-4 antibody in HCC. Based on promising phase II data, the combination of durvalumab and tremelimumab was compared with durvalumab in monotherapy or sorafenib in monotherapy as first-line therapy for unresectable HCC in the HIMALAYA trial. Regarding the comparison between combination therapy and monotherapy with sorafenib, there was a significant improvement in OS favoring combination therapy (16.4 mo *vs* 13.8 mo, HR: 0.78, 95%CI: 0.65-0.92, P = 0.0035), and durvalumab plus tremelimumab may be approved by the FDA and EMA in the near future as an additional option for first-line treatment in HCC. Finally, monotherapy with durvalumab was noninferior to sorafenib regarding OS (HR: 0.86, 95%CI: 0.73-1.03)[82].

Etiology of liver disease and immunotherapy

A very recent study suggested that clinicians might need to consider the underlying liver disease for treatment selection in HCC. Pfister *et al*[21] provided provocative preclinical and limited clinical evidence that patients with NAFLD-related HCC may benefit less from immune checkpoint inhibition than patients with viral hepatitis-related HCC. Regarding preclinical data, authors evaluated immunotherapy in mice with NAFLD-related HCC. Despite the hepatic abundance of PD-1 positive CD8 T cells with features of exhaustion and effector functions in mice with NASH, which might be suggestive of a good response to immunotherapy, none of the pre-existing tumors regressed with treatment, and there

was evidence indicating that immunotherapy could even play a role in hepatocarcinogenesis in mice with NASH. In the same publication, a meta-analysis of three randomized controlled phase III trials failed to demonstrate evidence of survival improvement for patients with non-viral hepatitis-related HCC receiving immunotherapy. The authors also investigated a cohort of 130 HCC cases, in which 13 were NAFLD-related and had OS after immunotherapy of 5.4 mo, significantly lower than that of patients with HCCs related to other causes of liver disease (11.0 mo, P = 0.023). Finally, authors validated these findings in yet another cohort of 118 individuals with HCC, in which 11 were NAFLD-related and had OS of 8.8 mo, while patients with HCC related to other causes of liver disease had OS of 17.7 mo (P = 0.034)[21]. Nevertheless, important remarks on this study have been made in an editorial, which highlighted that patients with NASH were not separated from those with other non-viral hepatitis etiologies of liver disease in the meta-analysis presented in the paper, and that the small number of patients evaluated in the cohorts must be kept in mind when considering their results[83].

In the same line, both the Checkmate-459 and the IMBrave150 trials found that HR for OS was worse in patients with non-viral hepatitis-related HCC compared with patients with viral hepatitis-related cancer. However, neither study distinguished between patients with NASH and alcoholic steatohepatitis. In addition, it should be noted that the poor HRs in the non-viral hepatitis group were not solely due to the shorter survival in the IO arm, but also to a surprisingly long survival in the sorafenib arm of both trials[19,84]. Similarly, in the COSMIC-312 trial, when a subgroup analysis on etiology of the underlying liver disease was performed, the benefit of atezolizumab plus cabozantinib on PFS seemed to be driven by the hepatitis B-related HCC group, and there was no significant difference between combination therapy and sorafenib for patients with non-viral hepatitis related-HCC (HR: 0.92, 95%CI: 0.60-1.41)[79].

Another recent meta-analysis of randomized controlled trials helped fuel this debate, when it also demonstrated that immune checkpoint inhibitors were significantly more effective for viral hepatitis-related HCCs than for tumors associated with other kinds of liver disease. On the other hand, the etiology of liver disease did not seem to interfere with the efficacy of tyrosine-kinase inhibitors or anti-VEGF therapies[22].

In contrast to these observations, underlying liver disease (viral hepatitis *vs* alcohol *vs* NASH) did not significantly impact PFS and OS in a recent real-world cohort treated with atezolizumab and bevacizumab[85]. Moreover, in the HIMALAYA trial, the benefit of the combination of durvalumab and tremelimumab on OS persisted in the subgroup analysis in patients with non-viral hepatitis related-HCC (HR: 0.74, 95%CI: 0.57-0.95)[82].

Perhaps the decision to start immunotherapy might be guided by prognostic biomarkers, rather than solely by the etiology of liver disease. Several predictive biomarkers for immunotherapy response have been proposed, including PD-L1 expression[86] and evidence of activated Wnt/β-catenin signaling[87], but currently no validated biomarker exists to guide treatment decisions in HCC patients undergoing immunotherapy. The recently proposed CRAFITY score (C reactive protein and alpha-fetoprotein in ImmunoTherapY) may be an easy-to-use tool to predict outcomes of patients undergoing immunotherapy for HCC, regardless of Child-Pugh class and performance status[88].

CONCLUSION

As the field moves forward, and with early evidence suggesting a potential decreased response of NAFLD-related HCC to immunotherapy, studies of non-immune systemic therapy for this group of patients should become more frequent and more robust. Moreover, randomized controlled trials evaluating immunotherapy in this particular group of patients are of the utmost importance. Multiple issues should be taken into account in such studies, including the strict definition of NAFLD, metabolic associated fatty liver disease or NASH, the heterogeneity of this group which includes lean NAFLD, NAFLD related to metabolic syndrome and others, as well as the impact of other co-morbidities and medication profiles.

FOOTNOTES

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REFERENCES

1 **World Health Organization**. Global Cancer Observatory-GLOBOCAN 2020. [cited 14 February 2022]. Available from: https://gco.iarc.fr/
2 **Liu Z**, Xu K, Jiang Y, Cai N, Fan J, Mao X, Suo C, Jin L, Zhang T, Chen X. Global trend of aetiology-based primary liver cancer incidence from 1990 to 2030: a modelling study. *Int J Epidemiol* 2021; **50**: 128-142 [PMID: 33349860 DOI: 10.1093/ije/dyaa196]
3 **McGlynn KA**, Petrick JL, El-Serag HB. Epidemiology of Hepatocellular Carcinoma. *Hepatology* 2021; **73** Suppl 1: 4-13 [PMID: 32319693 DOI: 10.1002/hep.31288]
4 **Arnold M**, Abnet CC, Neale RE, Vignat J, Giovannucci EL, McGlynn KA, Bray F. Global Burden of 5 Major Types of Gastrointestinal Cancer. *Gastroenterology* 2020; **159**: 335-349.e15 [PMID: 32247694 DOI: 10.1053/j.gastro.2020.02.068]
5 **Younossi ZM**, Koenig AB, Abdelatif D, Fazel Y, Henry L, Wymer M. Global epidemiology of nonalcoholic fatty liver disease-Meta-analytic assessment of prevalence, incidence, and outcomes. *Hepatology* 2016; **64**: 73-84 [PMID: 26707365 DOI: 10.1002/hep.28431]
6 **Anstee QM**, Reeves HL, Kotsiliti E, Govaere O, Heikenwalder M. From NASH to HCC: current concepts and future challenges. *Nat Rev Gastroenterol Hepatol* 2019; **16**: 411-428 [PMID: 31028350 DOI: 10.1038/s41575-019-0145-7]
7 **GBD 2019 Diseases and Injuries Collaborators**. Global burden of 369 diseases and injuries in 204 countries and territories, 1990-2019: a systematic analysis for the Global Burden of Disease Study 2019. *Lancet* 2020; **396**: 1204-1222 [PMID: 33069326 DOI: 10.1016/S0140-6736(20)30925-9]
8 **Debes JD**, Chan AJ, Balderramo D, Kikuchi L, Gonzalez Ballerga E, Prieto JE, Tapias M, Idrovo V, Davalos MB, Cairo F, Barreyro FJ, Paredes S, Hernandez N, Avendaño K, Diaz Ferrer J, Yang JD, Carrera E, Garcia JA, Mattos AZ, Hirsch BS, Gonçalves PT, Carrilho FJ, Roberts LR. Hepatocellular carcinoma in South America: Evaluation of risk factors, demographics and therapy. *Liver Int* 2018; **38**: 136-143 [PMID: 28640517 DOI: 10.1111/liv.13502]
9 **Farah M**, Diaz Ferrer J, Baca EL, Mattos A, Arrese M, Prieto Ortiz JE, Balderramo D, Carrera E, Boonstra A, Debes JD. Changing epidemiology of hepatocellular carcinoma in South America: a report from the ESCALON Network. *Hepatology* 2021; **74** Suppl 1: 681A
10 **Banini BA**, Sanyal AJ. NAFLD-related HCC. *Adv Cancer Res* 2021; **149**: 143-169 [PMID: 33579423 DOI: 10.1016/bs.acr.2020.11.001]
11 **Ascha MS**, Hanouneh IA, Lopez R, Tamimi TA, Feldstein AF, Zein NN. The incidence and risk factors of hepatocellular carcinoma in patients with nonalcoholic steatohepatitis. *Hepatology* 2010; **51**: 1972-1978 [PMID: 20209604 DOI: 10.1002/hep.23527]
12 **Yatsuji S**, Hashimoto E, Tobari M, Taniai M, Tokushige K, Shiratori K. Clinical features and outcomes of cirrhosis due to non-alcoholic steatohepatitis compared with cirrhosis caused by chronic hepatitis C. *J Gastroenterol Hepatol* 2009; **24**: 248-254 [PMID: 19032450 DOI: 10.1111/j.1440-1746.2008.05640.x]
13 **Amarapurkar DN**, Dharod M, Gautam S, Patel N. Risk of development of hepatocellular carcinoma in patients with NASH-related cirrhosis. *Trop Gastroenterol* 2013; **34**: 159-163 [PMID: 24851525 DOI: 10.7869/tg.120]
14 **Levrero M**, Zucman-Rossi J. Mechanisms of HBV-induced hepatocellular carcinoma. *J Hepatol* 2016; **64**: S84-S101 [PMID: 27084040 DOI: 10.1016/j.jhep.2016.02.021]
15 **Geh D**, Manas DM, Reeves HL. Hepatocellular carcinoma in non-alcoholic fatty liver disease-a review of an emerging challenge facing clinicians. *Hepatobiliary Surg Nutr* 2021; **10**: 59-75 [PMID: 33575290 DOI: 10.21037/hbsn.2019.08.08]
16 **Villanueva A**. Hepatocellular Carcinoma. *N Engl J Med* 2019; **380**: 1450-1462 [PMID: 30970190 DOI: 10.1056/NEJMra1713263]
17 **Stine JG**, Wentworth BJ, Zimmet A, Rinella ME, Loomba R, Caldwell SH, Argo CK. Systematic review with meta-analysis: risk of hepatocellular carcinoma in non-alcoholic steatohepatitis without cirrhosis compared to other liver

diseases. *Aliment Pharmacol Ther* 2018; **48**: 696-703 [PMID: 30136293 DOI: 10.1111/apt.14937]

18 **Orci LA**, Sanduzzi-Zamparelli M, Caballol B, Sapena V, Colucci N, Torres F, Bruix J, Reig M, Toso C. Incidence of Hepatocellular Carcinoma in Patients With Nonalcoholic Fatty Liver Disease: A Systematic Review, Meta-analysis, and Meta-regression. *Clin Gastroenterol Hepatol* 2022; **20**: 283-292.e10 [PMID: 33965578 DOI: 10.1016/j.cgh.2021.05.002]

19 **Finn RS**, Qin S, Ikeda M, Galle PR, Ducreux M, Kim TY, Kudo M, Breder V, Merle P, Kaseb AO, Li D, Verret W, Xu DZ, Hernandez S, Liu J, Huang C, Mulla S, Wang Y, Lim HY, Zhu AX, Cheng AL; IMbrave150 Investigators. Atezolizumab plus Bevacizumab in Unresectable Hepatocellular Carcinoma. *N Engl J Med* 2020; **382**: 1894-1905 [PMID: 32402160 DOI: 10.1056/NEJMoa1915745]

20 **Reig M**, Forner A, Rimola J, Ferrer-Fàbrega J, Burrel M, Garcia-Criado Á, Kelley RK, Galle PR, Mazzaferro V, Salem R, Sangro B, Singal AG, Vogel A, Fuster J, Ayuso C, Bruix J. BCLC strategy for prognosis prediction and treatment recommendation: The 2022 update. *J Hepatol* 2022; **76**: 681-693 [PMID: 34801630 DOI: 10.1016/j.jhep.2021.11.018]

21 **Pfister D**, Núñez NG, Pinyol R, Govaere O, Pinter M, Szydlowska M, Gupta R, Qiu M, Deczkowska A, Weiner A, Müller F, Sinha A, Friebel E, Engleitner T, Lenggenhager D, Moncsek A, Heide D, Stirm K, Kosla J, Kotsiliti E, Leone V, Dudek M, Yousuf S, Inverso D, Singh I, Teijeiro A, Castet F, Montironi C, Haber PK, Tiniakos D, Bedossa P, Cockell S, Younes R, Vacca M, Marra F, Schattenberg JM, Allison M, Bugianesi E, Ratziu V, Pressiani T, D'Alessio A, Personeni N, Rimassa L, Daly AK, Scheiner B, Pomej K, Kirstein MM, Vogel A, Peck-Radosavljevic M, Hucke F, Finkelmeier F, Waidmann O, Trojan J, Schulze K, Wege H, Koch S, Weinmann A, Bueter M, Rössler F, Siebenhüner A, De Dosso S, Mallm JP, Umansky V, Jugold M, Luedde T, Schietinger A, Schirmacher P, Emu B, Augustin HG, Billeter A, Müller-Stich B, Kikuchi H, Duda DG, Kütting F, Waldschmidt DT, Ebert MP, Rahbari N, Mei HE, Schulz AR, Ringelhan M, Malek N, Spahn S, Bitzer M, Ruiz de Galarreta M, Lujambio A, Dufour JF, Marron TU, Kaseb A, Kudo M, Huang YH, Djouder N, Wolter K, Zender L, Marche PN, Decaens T, Pinato DJ, Rad R, Mertens JC, Weber A, Unger K, Meissner F, Roth S, Jilkova ZM, Claassen M, Anstee QM, Amit I, Knolle P, Becher B, Llovet JM, Heikenwalder M. NASH limits anti-tumour surveillance in immunotherapy-treated HCC. *Nature* 2021; **592**: 450-456 [PMID: 33762733 DOI: 10.1038/s41586-021-03362-0]

22 **Haber PK**, Puigvehí M, Castet F, Lourdusamy V, Montal R, Tabrizian P, Buckstein M, Kim E, Villanueva A, Schwartz M, Llovet JM. Evidence-Based Management of Hepatocellular Carcinoma: Systematic Review and Meta-analysis of Randomized Controlled Trials (2002-2020). *Gastroenterology* 2021; **161**: 879-898 [PMID: 34126063 DOI: 10.1053/j.gastro.2021.06.008]

23 **Shetty K**, Chen J, Shin JH, Jogunoori W, Mishra L. Pathogenesis of hepatocellular carcinoma development in non alcoholic fatty liver disease. *Curr Hepatol Rep* 2015; **14**: 119-127 [PMID: 26114083 DOI: 10.1007/s11901-015-0260-z]

24 **Kanda T**, Goto T, Hirotsu Y, Masuzaki R, Moriyama M, Omata M. Molecular Mechanisms: Connections between Nonalcoholic Fatty Liver Disease, Steatohepatitis and Hepatocellular Carcinoma. *Int J Mol Sci* 2020; **21** [PMID: 32102237 DOI: 10.3390/ijms21041525]

25 **Wegermann K**, Hyun J, Diehl AM. Molecular Mechanisms Linking Nonalcoholic Steatohepatitis to Cancer. *Clin Liver Dis (Hoboken)* 2021; **17**: 6-10 [PMID: 33552478 DOI: 10.1002/cld.1006]

26 **Valenti L**, Pedica F, Colombo M. Distinctive features of hepatocellular carcinoma in non-alcoholic fatty liver disease. *Dig Liver Dis* 2022; **54**: 154-163 [PMID: 34294580 DOI: 10.1016/j.dld.2021.06.023]

27 **Fujiwara N**, Nakagawa H, Enooku K, Kudo Y, Hayata Y, Nakatsuka T, Tanaka Y, Tateishi R, Hikiba Y, Misumi K, Tanaka M, Hayashi A, Shibahara J, Fukayama M, Arita J, Hasegawa K, Hirschfield H, Hoshida Y, Hirata Y, Otsuka M, Tateishi K, Koike K. CPT2 downregulation adapts HCC to lipid-rich environment and promotes carcinogenesis *via* acylcarnitine accumulation in obesity. *Gut* 2018; **67**: 1493-1504 [PMID: 29437870 DOI: 10.1136/gutjnl-2017-315193]

28 **Wu H**, Ng R, Chen X, Steer CJ, Song G. MicroRNA-21 is a potential link between non-alcoholic fatty liver disease and hepatocellular carcinoma *via* modulation of the HBP1-p53-Srebp1c pathway. *Gut* 2016; **65**: 1850-1860 [PMID: 26282675 DOI: 10.1136/gutjnl-2014-308430]

29 **Schuster S**, Cabrera D, Arrese M, Feldstein AE. Triggering and resolution of inflammation in NASH. *Nat Rev Gastroenterol Hepatol* 2018; **15**: 349-364 [PMID: 29740166 DOI: 10.1038/s41575-018-0009-6]

30 **Garcia-Lezana T**, Lopez-Canovas JL, Villanueva A. Signaling pathways in hepatocellular carcinoma. *Adv Cancer Res* 2021; **149**: 63-101 [PMID: 33579428 DOI: 10.1016/bs.acr.2020.10.002]

31 **Nagaraju GP**, Dariya B, Kasa P, Peela S, El-Rayes BF. Epigenetics in hepatocellular carcinoma. *Semin Cancer Biol* 2021 [PMID: 34324953 DOI: 10.1016/j.semcancer.2021.07.017]

32 **Geh D**, Anstee QM, Reeves HL. NAFLD-Associated HCC: Progress and Opportunities. *J Hepatocell Carcinoma* 2021; **8**: 223-239 [PMID: 33854987 DOI: 10.2147/JHC.S272213]

33 **Akkız H**. The Gut Microbiome and Hepatocellular Carcinoma. *J Gastrointest Cancer* 2021; **52**: 1314-1319 [PMID: 34870783 DOI: 10.1007/s12029-021-00748-1]

34 **Ponziani FR**, Bhoori S, Castelli C, Putignani L, Rivoltini L, Del Chierico F, Sanguinetti M, Morelli D, Paroni Sterbini F, Petito V, Reddel S, Calvani R, Camisaschi C, Picca A, Tuccitto A, Gasbarrini A, Pompili M, Mazzaferro V. Hepatocellular Carcinoma Is Associated With Gut Microbiota Profile and Inflammation in Nonalcoholic Fatty Liver Disease. *Hepatology* 2019; **69**: 107-120 [PMID: 29665135 DOI: 10.1002/hep.30036]

35 **Miura K**, Ishioka M, Minami S, Horie Y, Ohshima S, Goto T, Ohnishi H. Toll-like Receptor 4 on Macrophage Promotes the Development of Steatohepatitis-related Hepatocellular Carcinoma in Mice. *J Biol Chem* 2016; **291**: 11504-11517 [PMID: 27022031 DOI: 10.1074/jbc.M115.709048]

36 **Yoshimoto S**, Loo TM, Atarashi K, Kanda H, Sato S, Oyadomari S, Iwakura Y, Oshima K, Morita H, Hattori M, Honda K, Ishikawa Y, Hara E, Ohtani N. Obesity-induced gut microbial metabolite promotes liver cancer through senescence secretome. *Nature* 2013; **499**: 97-101 [PMID: 23803760 DOI: 10.1038/nature12347]

37 **Liu YL**, Patman GL, Leathart JB, Piguet AC, Burt AD, Dufour JF, Day CP, Daly AK, Reeves HL, Anstee QM. Carriage of the PNPLA3 rs738409 C >G polymorphism confers an increased risk of non-alcoholic fatty liver disease associated hepatocellular carcinoma. *J Hepatol* 2014; **61**: 75-81 [PMID: 24607626 DOI: 10.1016/j.jhep.2014.02.030]

38 **Bruschi FV**, Claudel T, Tardelli M, Caligiuri A, Stulnig TM, Marra F, Trauner M. The PNPLA3 I148M variant modulates the fibrogenic phenotype of human hepatic stellate cells. *Hepatology* 2017; **65**: 1875-1890 [PMID: 28073161 DOI:

10.1002/hep.29041]
39 **Kettner NM**, Voicu H, Finegold MJ, Coarfa C, Sreekumar A, Putluri N, Katchy CA, Lee C, Moore DD, Fu L. Circadian Homeostasis of Liver Metabolism Suppresses Hepatocarcinogenesis. *Cancer Cell* 2016; **30**: 909-924 [PMID: 27889186 DOI: 10.1016/j.ccell.2016.10.007]
40 **Arrese M**, Arab JP, Barrera F, Kaufmann B, Valenti L, Feldstein AE. Insights into Nonalcoholic Fatty-Liver Disease Heterogeneity. *Semin Liver Dis* 2021; **41**: 421-434 [PMID: 34233370 DOI: 10.1055/s-0041-1730927]
41 **Giraud J**, Chalopin D, Blanc JF, Saleh M. Hepatocellular Carcinoma Immune Landscape and the Potential of Immunotherapies. *Front Immunol* 2021; **12**: 655697 [PMID: 33815418 DOI: 10.3389/fimmu.2021.655697]
42 **Krenkel O**, Tacke F. Liver macrophages in tissue homeostasis and disease. *Nat Rev Immunol* 2017; **17**: 306-321 [PMID: 28317925 DOI: 10.1038/nri.2017.11]
43 **Wu J**, Li J, Salcedo R, Mivechi NF, Trinchieri G, Horuzsko A. The proinflammatory myeloid cell receptor TREM-1 controls Kupffer cell activation and development of hepatocellular carcinoma. *Cancer Res* 2012; **72**: 3977-3986 [PMID: 22719066 DOI: 10.1158/0008-5472.CAN-12-0938]
44 **Noy R**, Pollard JW. Tumor-associated macrophages: from mechanisms to therapy. *Immunity* 2014; **41**: 49-61 [PMID: 25035953 DOI: 10.1016/j.immuni.2014.06.010]
45 **Pinyol R**, Torrecilla S, Wang H, Montironi C, Piqué-Gili M, Torres-Martin M, Wei-Qiang L, Willoughby CE, Ramadori P, Andreu-Oller C, Taik P, Lee YA, Moeini A, Peix J, Faure-Dupuy S, Riedl T, Schuehle S, Oliveira CP, Alves VA, Boffetta P, Lachenmayer A, Roessler S, Minguez B, Schirmacher P, Dufour JF, Thung SN, Reeves HL, Carrilho FJ, Chang C, Uzilov AV, Heikenwalder M, Sanyal A, Friedman SL, Sia D, Llovet JM. Molecular characterisation of hepatocellular carcinoma in patients with non-alcoholic steatohepatitis. *J Hepatol* 2021; **75**: 865-878 [PMID: 33992698 DOI: 10.1016/j.jhep.2021.04.049]
46 **Wolf MJ**, Adili A, Piotrowitz K, Abdullah Z, Boege Y, Stemmer K, Ringelhan M, Simonavicius N, Egger M, Wohlleber D, Lorentzen A, Einer C, Schulz S, Clavel T, Protzer U, Thiele C, Zischka H, Moch H, Tschöp M, Tumanov AV, Haller D, Unger K, Karin M, Kopf M, Knolle P, Weber A, Heikenwalder M. Metabolic activation of intrahepatic CD8+ T cells and NKT cells causes nonalcoholic steatohepatitis and liver cancer *via* cross-talk with hepatocytes. *Cancer Cell* 2014; **26**: 549-564 [PMID: 25314080 DOI: 10.1016/j.ccell.2014.09.003]
47 **Grohmann M**, Wiede F, Dodd GT, Gurzov EN, Ooi GJ, Butt T, Rasmiena AA, Kaur S, Gulati T, Goh PK, Treloar AE, Archer S, Brown WA, Muller M, Watt MJ, Ohara O, McLean CA, Tiganis T. Obesity Drives STAT-1-Dependent NASH and STAT-3-Dependent HCC. *Cell* 2018; **175**: 1289-1306.e20 [PMID: 30454647 DOI: 10.1016/j.cell.2018.09.053]
48 **Dudek M**, Pfister D, Donakonda S, Filpe P, Schneider A, Laschinger M, Hartmann D, Hüser N, Meiser P, Bayerl F, Inverso D, Wigger J, Sebode M, Öllinger R, Rad R, Hegenbarth S, Anton M, Guillot A, Bowman A, Heide D, Müller F, Ramadori P, Leone V, Garcia-Caceres C, Gruber T, Seifert G, Kabat AM, Mallm JP, Reider S, Effenberger M, Roth S, Billeter AT, Müller-Stich B, Pearce EJ, Koch-Nolte F, Käser R, Tilg H, Thimme R, Boettler T, Tacke F, Dufour JF, Haller D, Murray PJ, Heeren R, Zehn D, Böttcher JP, Heikenwälder M, Knolle PA. Auto-aggressive CXCR6^{+} CD8 T cells cause liver immune pathology in NASH. *Nature* 2021; **592**: 444-449 [PMID: 33762736 DOI: 10.1038/s41586-021-03233-8]
49 **Ma C**, Kesarwala AH, Eggert T, Medina-Echeverz J, Kleiner DE, Jin P, Stroncek DF, Terabe M, Kapoor V, ElGindi M, Han M, Thornton AM, Zhang H, Egger M, Luo J, Felsher DW, McVicar DW, Weber A, Heikenwalder M, Greten TF. NAFLD causes selective CD4(+) T lymphocyte loss and promotes hepatocarcinogenesis. *Nature* 2016; **531**: 253-257 [PMID: 26934227 DOI: 10.1038/nature16969]
50 **Gomes AL**, Teijeiro A, Burén S, Tummala KS, Yilmaz M, Waisman A, Theurillat JP, Perna C, Djouder N. Metabolic Inflammation-Associated IL-17A Causes Non-alcoholic Steatohepatitis and Hepatocellular Carcinoma. *Cancer Cell* 2016; **30**: 161-175 [PMID: 27411590 DOI: 10.1016/j.ccell.2016.05.020]
51 **Faggioli F**, Palagano E, Di Tommaso L, Donadon M, Marrella V, Recordati C, Mantero S, Villa A, Vezzoni P, Cassani B. B lymphocytes limit senescence-driven fibrosis resolution and favor hepatocarcinogenesis in mouse liver injury. *Hepatology* 2018; **67**: 1970-1985 [PMID: 29105104 DOI: 10.1002/hep.29636]
52 **Zhang S**, Liu Z, Wu D, Chen L, Xie L. Single-Cell RNA-Seq Analysis Reveals Microenvironmental Infiltration of Plasma Cells and Hepatocytic Prognostic Markers in HCC With Cirrhosis. *Front Oncol* 2020; **10**: 596318 [PMID: 33224891 DOI: 10.3389/fonc.2020.596318]
53 **Shalapour S**, Lin XJ, Bastian IN, Brain J, Burt AD, Aksenov AA, Vrbanac AF, Li W, Perkins A, Matsutani T, Zhong Z, Dhar D, Navas-Molina JA, Xu J, Loomba R, Downes M, Yu RT, Evans RM, Dorrestein PC, Knight R, Benner C, Anstee QM, Karin M. Inflammation-induced IgA+ cells dismantle anti-liver cancer immunity. *Nature* 2017; **551**: 340-345 [PMID: 29144460 DOI: 10.1038/nature24302]
54 **Shao Y**, Lo CM, Ling CC, Liu XB, Ng KT, Chu AC, Ma YY, Li CX, Fan ST, Man K. Regulatory B cells accelerate hepatocellular carcinoma progression *via* CD40/CD154 signaling pathway. *Cancer Lett* 2014; **355**: 264-272 [PMID: 25301451 DOI: 10.1016/j.canlet.2014.09.026]
55 **Barrow F**, Khan S, Fredrickson G, Wang H, Dietsche K, Parthiban P, Robert S, Kaiser T, Winer S, Herman A, Adeyi O, Mouzaki M, Khoruts A, Hogquist KA, Staley C, Winer DA, Revelo XS. Microbiota-Driven Activation of Intrahepatic B Cells Aggravates NASH Through Innate and Adaptive Signaling. *Hepatology* 2021; **74**: 704-722 [PMID: 33609303 DOI: 10.1002/hep.31755]
56 **Cheng AL**, Kang YK, Chen Z, Tsao CJ, Qin S, Kim JS, Luo R, Feng J, Ye S, Yang TS, Xu J, Sun Y, Liang H, Liu J, Wang J, Tak WY, Pan H, Burock K, Zou J, Voliotis D, Guan Z. Efficacy and safety of sorafenib in patients in the Asia-Pacific region with advanced hepatocellular carcinoma: a phase III randomised, double-blind, placebo-controlled trial. *Lancet Oncol* 2009; **10**: 25-34 [PMID: 19095497 DOI: 10.1016/S1470-2045(08)70285-7]
57 **Llovet JM**, Ricci S, Mazzaferro V, Hilgard P, Gane E, Blanc JF, de Oliveira AC, Santoro A, Raoul JL, Forner A, Schwartz M, Porta C, Zeuzem S, Bolondi L, Greten TF, Galle PR, Seitz JF, Borbath I, Häussinger D, Giannaris T, Shan M, Moscovici M, Voliotis D, Bruix J; SHARP Investigators Study Group. Sorafenib in advanced hepatocellular carcinoma. *N Engl J Med* 2008; **359**: 378-390 [PMID: 18650514 DOI: 10.1056/NEJMoa0708857]
58 **Abou-Alfa GK**, Meyer T, Cheng AL, El-Khoueiry AB, Rimassa L, Ryoo BY, Cicin I, Merle P, Chen Y, Park JW, Blanc JF, Bolondi L, Klümpen HJ, Chan SL, Zagonel V, Pressiani T, Ryu MH, Venook AP, Hessel C, Borgman-Hagey AE,

Schwab G, Kelley RK. Cabozantinib in Patients with Advanced and Progressing Hepatocellular Carcinoma. *N Engl J Med* 2018; **379**: 54-63 [PMID: 29972759 DOI: 10.1056/NEJMoa1717002]

59 **Bruix J**, Qin S, Merle P, Granito A, Huang YH, Bodoky G, Pracht M, Yokosuka O, Rosmorduc O, Breder V, Gerolami R, Masi G, Ross PJ, Song T, Bronowicki JP, Ollivier-Hourmand I, Kudo M, Cheng AL, Llovet JM, Finn RS, LeBerre MA, Baumhauer A, Meinhardt G, Han G; RESORCE Investigators. Regorafenib for patients with hepatocellular carcinoma who progressed on sorafenib treatment (RESORCE): a randomised, double-blind, placebo-controlled, phase 3 trial. *Lancet* 2017; **389**: 56-66 [PMID: 27932229 DOI: 10.1016/S0140-6736(16)32453-9]

60 **Zhu AX**, Kang YK, Yen CJ, Finn RS, Galle PR, Llovet JM, Assenat E, Brandi G, Pracht M, Lim HY, Rau KM, Motomura K, Ohno I, Merle P, Daniele B, Shin DB, Gerken G, Borg C, Hiriart JB, Okusaka T, Morimoto M, Hsu Y, Abada PB, Kudo M; REACH-2 study investigators. Ramucirumab after sorafenib in patients with advanced hepatocellular carcinoma and increased α-fetoprotein concentrations (REACH-2): a randomised, double-blind, placebo-controlled, phase 3 trial. *Lancet Oncol* 2019; **20**: 282-296 [PMID: 30665869 DOI: 10.1016/S1470-2045(18)30937-9]

61 **Kudo M**, Finn RS, Qin S, Han KH, Ikeda K, Piscaglia F, Baron A, Park JW, Han G, Jassem J, Blanc JF, Vogel A, Komov D, Evans TRJ, Lopez C, Dutcus C, Guo M, Saito K, Kraljevic S, Tamai T, Ren M, Cheng AL. Lenvatinib *vs* sorafenib in first-line treatment of patients with unresectable hepatocellular carcinoma: a randomised phase 3 non-inferiority trial. *Lancet* 2018; **391**: 1163-1173 [PMID: 29433850 DOI: 10.1016/S0140-6736(18)30207-1]

62 **Foerster F**, Gairing SJ, Müller L, Galle PR. NAFLD-driven HCC: Safety and efficacy of current and emerging treatment options. *J Hepatol* 2022; **76**: 446-457 [PMID: 34555422 DOI: 10.1016/j.jhep.2021.09.007]

63 **Casadei Gardini A**, Faloppi L, De Matteis S, Foschi FG, Silvestris N, Tovoli F, Palmieri V, Marisi G, Brunetti O, Vespasiani-Gentilucci U, Perrone G, Valgiusti M, Granato AM, Ercolani G, Negrini G, Tamburini E, Aprile G, Passardi A, Santini D, Cascinu S, Frassineti GL, Scartozzi M. Metformin and insulin impact on clinical outcome in patients with advanced hepatocellular carcinoma receiving sorafenib: Validation study and biological rationale. *Eur J Cancer* 2017; **86**: 106-114 [PMID: 28985579 DOI: 10.1016/j.ejca.2017.09.003]

64 **Bruix J**, Cheng AL, Meinhardt G, Nakajima K, De Sanctis Y, Llovet J. Prognostic factors and predictors of sorafenib benefit in patients with hepatocellular carcinoma: Analysis of two phase III studies. *J Hepatol* 2017; **67**: 999-1008 [PMID: 28687477 DOI: 10.1016/j.jhep.2017.06.026]

65 **Bruix J**, Raoul JL, Sherman M, Mazzaferro V, Bolondi L, Craxi A, Galle PR, Santoro A, Beaugrand M, Sangiovanni A, Porta C, Gerken G, Marrero JA, Nadel A, Shan M, Moscovici M, Voliotis D, Llovet JM. Efficacy and safety of sorafenib in patients with advanced hepatocellular carcinoma: subanalyses of a phase III trial. *J Hepatol* 2012; **57**: 821-829 [PMID: 22727733 DOI: 10.1016/j.jhep.2012.06.014]

66 **Leathers JS**, Balderramo D, Prieto J, Diehl F, Gonzalez-Ballerga E, Ferreiro MR, Carrera E, Barreyro F, Diaz-Ferrer J, Singh D, Mattos AZ, Carrilho F, Debes JD. Sorafenib for Treatment of Hepatocellular Carcinoma: A Survival Analysis From the South American Liver Research Network. *J Clin Gastroenterol* 2019; **53**: 464-469 [PMID: 29952857 DOI: 10.1097/MCG.0000000000001085]

67 **Howell J**, Samani A, Mannan B, Hajiev S, Aval LM, Abdelmalak R, Tam VC, Bettinger D, Thimme R, Taddei TH, Kaplan DE, Seidensticker M, Sharma R. Impact of NAFLD on clinical outcomes in hepatocellular carcinoma treated with sorafenib: An international cohort study. *J Clin Oncol* 2021; **39** Suppl 3: 289 [DOI: 10.1200/JCO.2021.39.3_suppl.289]

68 **Rimini M**, Kudo M, Tada T, Shigeo S, Kang W, Suda G, Jefremow A, Burgio V, Iavarone M, Tortora R, Marra F, Lonardi S, Tamburini E, Piscaglia F, Masi G, Cabibbo G, Foschi FG, Silletta M, Kumada T, Iwamoto H, Aoki T, Goh MJ, Sakamoto N, Siebler J, Hiraoka A, Niizeki T, Ueshima K, Sho T, Atsukawa M, Hirooka M, Tsuji K, Ishikawa T, Takaguchi K, Kariyama K, Itobayashi E, Tajiri K, Shimada N, Shibata H, Ochi H, Yasuda S, Toyoda H, Fukunishi S, Ohama H, Kawata K, Tani J, Nakamura S, Nouso K, Tsutsui A, Nagano T, Takaaki T, Itokawa N, Okubo T, Arai T, Imai M, Joko K, Koizumi Y, Hiasa Y, Cucchetti A, Ratti F, Aldrighetti L, Cascinu S, Casadei-Gardini A. Nonalcoholic steatohepatitis in hepatocarcinoma: new insights about its prognostic role in patients treated with lenvatinib. *ESMO Open* 2021; **6**: 100330 [PMID: 34847382 DOI: 10.1016/j.esmoop.2021.100330]

69 **Hiraoka A**, Kumada T, Tada T, Tani J, Kariyama K, Fukunishi S, Atsukawa M, Hirooka M, Tsuji K, Ishikawa T, Takaguchi K, Itobayashi E, Tajiri K, Shimada N, Shibata H, Ochi H, Kawata K, Yasuda S, Toyoda H, Aoki T, Tanaka T, Ohama H, Nouso K, Tsutsui A, Nagano T, Itokawa N, Arai T, Okubo T, Imai M, Koizumi Y, Nakamura S, Joko K, Hiasa Y, Kudo M; Real-life Practice Experts for HCC (RELPEC) Study Group and HCC 48 Group (hepatocellular carcinoma experts from 48 clinics in Japan). Efficacy of lenvatinib for unresectable hepatocellular carcinoma based on background liver disease etiology: multi-center retrospective study. *Sci Rep* 2021; **11**: 16663 [PMID: 34404856 DOI: 10.1038/s41598-021-96089-x]

70 **Welland S**, Leyh C, Finkelmeier F, Jefremow A, Shmanko K, Gonzalez-Carmona MA, Kandulski A, Jeliazkova P, Best J, Fründt TW, Djanani A, Pangerl M, Maieron A, Greil R, Fricke C, Sookthai D, Günther R, Schmiderer A, Wege H, Venerito M, Ehmer U, Müller M, Strassburg CP, Weinmann A, Siebler J, Waidmann O, Lange CM, Saborowski A, Vogel A. Real-World Data for Lenvatinib in Hepatocellular Carcinoma (ELEVATOR): A retrospective multicenter study. *Liver Cancer* 2022 [DOI: 10.1159/000521746]

71 **Vogel A**, Martinelli E; ESMO Guidelines Committee. Electronic address: clinicalguidelines@esmo.org; ESMO Guidelines Committee. Updated treatment recommendations for hepatocellular carcinoma (HCC) from the ESMO Clinical Practice Guidelines. *Ann Oncol* 2021; **32**: 801-805 [PMID: 33716105 DOI: 10.1016/j.annonc.2021.02.014]

72 **Díaz-González Á**, Sanduzzi-Zamparelli M, da Fonseca LG, Di Costanzo GG, Alves R, Iavarone M, Leal C, Sacco R, Matilla AM, Hernández-Guerra M, Aballay Soteras G, Wörns MA, Pinter M, Varela M, Ladekarl M, Chagas AL, Mínguez B, Arenas JI, Granito A, Sánchez-Torrijos Y, Rojas Á, Rodríguez de Lope C, Alvares-da-Silva MR, Pascual S, Rimassa L, Lledó JL, Huertas C, Sangro B, Giannini EG, Delgado M, Vergara M, Perelló C, Lue A, Sala M, Gallego A, Coll S, Hernáez T, Piñero F, Pereira G, França A, Marín J, Anders M, Mello V, Lozano M, Nault JC, Menéndez J, García Juárez I, Bruix J, Reig M. International and multicenter real-world study of sorafenib-treated patients with hepatocellular carcinoma under dialysis. *Liver Int* 2020; **40**: 1467-1476 [PMID: 32170821 DOI: 10.1111/liv.14436]

73 **Yau T**, Park JW, Finn RS, Cheng AL, Mathurin P, Edeline J, Kudo M, Harding JJ, Merle P, Rosmorduc O, Wyrwicz L, Schott E, Choo SP, Kelley RK, Sieghart W, Assenat E, Zaucha R, Furuse J, Abou-Alfa GK, El-Khoueiry AB, Melero I,

Begic D, Chen G, Neely J, Wisniewski T, Tschaika M, Sangro B. Nivolumab *vs* sorafenib in advanced hepatocellular carcinoma (CheckMate 459): a randomised, multicentre, open-label, phase 3 trial. *Lancet Oncol* 2022; **23**: 77-90 [PMID: 34914889 DOI: 10.1016/S1470-2045(21)00604-5]

74 **Finn RS**, Ryoo BY, Merle P, Kudo M, Bouattour M, Lim HY, Breder V, Edeline J, Chao Y, Ogasawara S, Yau T, Garrido M, Chan SL, Knox J, Daniele B, Ebbinghaus SW, Chen E, Siegel AB, Zhu AX, Cheng AL; KEYNOTE-240 investigators. Pembrolizumab As Second-Line Therapy in Patients With Advanced Hepatocellular Carcinoma in KEYNOTE-240: A Randomized, Double-Blind, Phase III Trial. *J Clin Oncol* 2020; **38**: 193-202 [PMID: 31790344 DOI: 10.1200/JCO.19.01307]

75 **Siegel AB**, Cohen EI, Ocean A, Lehrer D, Goldenberg A, Knox JJ, Chen H, Clark-Garvey S, Weinberg A, Mandeli J, Christos P, Mazumdar M, Popa E, Brown RS Jr, Rafii S, Schwartz JD. Phase II trial evaluating the clinical and biologic effects of bevacizumab in unresectable hepatocellular carcinoma. *J Clin Oncol* 2008; **26**: 2992-2998 [PMID: 18565886 DOI: 10.1200/JCO.2007.15.9947]

76 **Hughes PE**, Caenepeel S, Wu LC. Targeted Therapy and Checkpoint Immunotherapy Combinations for the Treatment of Cancer. *Trends Immunol* 2016; **37**: 462-476 [PMID: 27216414 DOI: 10.1016/j.it.2016.04.010]

77 **Finn RS**, Qin S, Ikeda M, Galle PR, Ducreux M, Kim TY, Lim HY, Kudo M, Breder VV, Merle P, Kaseb AO, Li D, Verret W, Shao H, Liu J, Li L, Zhu AX, Cheng AL. IMbrave150: Updated overall survival (OS) data from a global, randomized, open-label phase III study of atezolizumab (atezo) + bevacizumab (bev) *vs* sorafenib (sor) in patients (pts) with unresectable hepatocellular carcinoma (HCC). *J Clin Oncol* 2021; **39** Suppl 3: 267 [DOI: 10.1200/JCO.2021.39.3_suppl.267]

78 **Galle PR**, Finn RS, Qin S, Ikeda M, Zhu AX, Kim TY, Kudo M, Breder V, Merle P, Kaseb A, Li D, Mulla S, Verret W, Xu DZ, Hernandez S, Ding B, Liu J, Huang C, Lim HY, Cheng AL, Ducreux M. Patient-reported outcomes with atezolizumab plus bevacizumab *vs* sorafenib in patients with unresectable hepatocellular carcinoma (IMbrave150): an open-label, randomised, phase 3 trial. *Lancet Oncol* 2021; **22**: 991-1001 [PMID: 34051880 DOI: 10.1016/S1470-2045(21)00151-0]

79 **Kelley R**, Yau T, Cheng A, Kaseb A, Qin A, Zhu AX, Chan S, Sukeepaisarnjaroen W, Breder V, Verset G, Gane E, Borbath I, Gomez Rangel JD, Merle P, Benzaghou FM, Banerjee K, Hazra S, Fawcett J, Rimassa L. Cabozantinib (C) plus atezolizumab (A) *vs* sorafenib (S) as first-line systemic treatment for advanced hepatocellular carcinoma (aHCC): Results from the randomized phase III COSMIC-312 trial. *Ann Oncol* 2022; **33**: 114-116 [DOI: 10.1016/j.annonc.2021.10.008]

80 **El-Khoueiry AB**, Sangro B, Yau T, Crocenzi TS, Kudo M, Hsu C, Kim TY, Choo SP, Trojan J, Welling TH Rd, Meyer T, Kang YK, Yeo W, Chopra A, Anderson J, Dela Cruz C, Lang L, Neely J, Tang H, Dastani HB, Melero I. Nivolumab in patients with advanced hepatocellular carcinoma (CheckMate 040): an open-label, non-comparative, phase 1/2 dose escalation and expansion trial. *Lancet* 2017; **389**: 2492-2502 [PMID: 28434648 DOI: 10.1016/S0140-6736(17)31046-2]

81 **Yau T**, Kang YK, Kim TY, El-Khoueiry AB, Santoro A, Sangro B, Melero I, Kudo M, Hou MM, Matilla A, Tovoli F, Knox JJ, Ruth He A, El-Rayes BF, Acosta-Rivera M, Lim HY, Neely J, Shen Y, Wisniewski T, Anderson J, Hsu C. Efficacy and Safety of Nivolumab Plus Ipilimumab in Patients With Advanced Hepatocellular Carcinoma Previously Treated With Sorafenib: The CheckMate 040 Randomized Clinical Trial. *JAMA Oncol* 2020; **6**: e204564 [PMID: 33001135 DOI: 10.1001/jamaoncol.2020.4564]

82 **Abou-Alfa G**, Chan S, Kudo M, Lau G, Kelley RK, Furuse J, Sukeepaisarnjaroen W, Kang YK, Dao TV, De Toni EN, Rimassa L, Breder VV, Vasilyev A, Heurgue A, Tam V, Mody K, Thungappa SC, He P, Negro A, Sangro B. Phase 3 randomized, open-label, multicenter study of tremelimumab (T) and durvalumab (D) as first-line therapy in patients (pts) with unresectable hepatocellular carcinoma (uHCC): HIMALAYA. *J Clin Oncol* 2022; **40** Suppl 4: 379

83 **Kelley RK**, Greten TF. Hepatocellular Carcinoma - Origins and Outcomes. *N Engl J Med* 2021; **385**: 280-282 [PMID: 34260842 DOI: 10.1056/NEJMcibr2106594]

84 **Yau T**, Park JW, Finn RS, Cheng AL, Mathurin P, Edeline J, Kudo M, Han KH, Harding JJ, Merle P, Rosmorduc O, Wyrwicz L, Schott E, Choo SP, Kelley RK, Begic D, Chen G, Neely J, Anderson J, Sangro B. CheckMate 459: A randomized, multi-center phase III study of nivolumab (NIVO) *vs* sorafenib (SOR) as first-line (1L) treatment in patients (pts) with advanced hepatocellular carcinoma (aHCC). *Ann Oncol* 2019; **30** Suppl 5 [DOI: 10.1093/annonc/mdz394.029]

85 **de Castro T**, Jochheim LS, Bathon M, Welland S, Scheiner B, Shmanko K, Roessler D, Ben Khaled N, Jeschke M, Ludwig JM, Marquardt JU, Weinmann A, Pinter M, Lange CM, Vogel A, Saborowski A. Atezolizumab and bevacizumab in patients with advanced hepatocellular carcinoma with impaired liver function and prior systemic therapy: a real-world experience. *Ther Adv Med Oncol* 2022; **14**: 17588359221080298 [PMID: 35251317 DOI: 10.1177/17588359221080298]

86 **Sangro B**, Melero I, Wadhawan S, Finn RS, Abou-Alfa GK, Cheng AL, Yau T, Furuse J, Park JW, Boyd Z, Tang HT, Shen Y, Tschaika M, Neely J, El-Khoueiry A. Association of inflammatory biomarkers with clinical outcomes in nivolumab-treated patients with advanced hepatocellular carcinoma. *J Hepatol* 2020; **73**: 1460-1469 [PMID: 32710922 DOI: 10.1016/j.jhep.2020.07.026]

87 **Sia D**, Jiao Y, Martinez-Quetglas I, Kuchuk O, Villacorta-Martin C, Castro de Moura M, Putra J, Camprecios G, Bassaganyas L, Akers N, Losic B, Waxman S, Thung SN, Mazzaferro V, Esteller M, Friedman SL, Schwartz M, Villanueva A, Llovet JM. Identification of an Immune-specific Class of Hepatocellular Carcinoma, Based on Molecular Features. *Gastroenterology* 2017; **153**: 812-826 [PMID: 28624577 DOI: 10.1053/j.gastro.2017.06.007]

88 **Scheiner B**, Pomej K, Kirstein MM, Hucke F, Finkelmeier F, Waidmann O, Himmelsbach V, Schulze K, von Felden J, Fründt TW, Stadler M, Heinzl H, Shmanko K, Spahn S, Radu P, Siebenhüner AR, Mertens JC, Rahbari NN, Kütting F, Waldschmidt DT, Ebert MP, Teufel A, De Dosso S, Pinato DJ, Pressiani T, Meischl T, Balcar L, Müller C, Mandorfer M, Reiberger T, Trauner M, Personeni N, Rimassa L, Bitzer M, Trojan J, Weinmann A, Wege H, Dufour JF, Peck-Radosavljevic M, Vogel A, Pinter M. Prognosis of patients with hepatocellular carcinoma treated with immunotherapy - development and validation of the CRAFITY score. *J Hepatol* 2022; **76**: 353-363 [PMID: 34648895 DOI: 10.1016/j.jhep.2021.09.035]

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MINIREVIEWS

Potassium-competitive acid blockers and gastroesophageal reflux disease

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Abstract

Proton pump inhibitors (PPIs), the most commonly used antisecretory medications in the management of reflux illness, virtually eliminate elective surgery for ulcer disease, and relegate anti-reflux surgery to patients with gastroesophageal reflux disease (GERD) who are inadequately managed by medical therapy. However, PPI medications still leave some therapeutic demands of GERD unmet. Furthermore, up to 40%-55% of daily PPI users have chronic symptoms, due to PPI refractoriness. Potassium-competitive acid blockers (P-CABs) transcend many of the problems and limits of PPIs, delivering quick, powerful, and extended acid suppression and allowing for treatment of numerous unmet needs. Recently, it has become clear that compromised mucosal integrity plays a role in the etiology of GERD. As a result, esophageal mucosal protection has emerged as a novel and potential treatment approach. An increasing body of research demonstrates that when P-CABs are used as primary drugs or add-on drugs (to regular treatment), they provide a considerable extra benefit, particularly in alleviating symptoms that do not respond to PPI therapy.

Key Words: Potassium-competitive acid blocker; Gastroesophageal reflux disease; Proton pump inhibitors; Treatment outcome; Proton pump inhibitor-refractory patients; Esophageal mucosal resistance

Core Tip: The potassium-competitive acid blocker (P-CAB) has been discovered as a possible acid suppression therapeutic option. At the enzyme level, P-CABs compete with K^+ to suppress acid formation; the binding location of these compounds is separate from the probable pocket that K^+ occupies. When the P-CAB binds to the enzyme, it stops K^+ from attaching and activating it. According to clinical trials, P-CABs are extremely selective for gastric H^+, K^+-ATPase, restricting stomach acid production while acting quickly. Such medicines, which can have a rapid onset of action and a longer duration, may offer considerable advantages to individuals suffering from gastroesophageal reflux disease and other acid-related disorders.

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INTRODUCTION

Gastroesophageal reflux, the reflux of stomach contents into the esophagus, usually occurs after large and fatty meals. Nonetheless, under normal settings, efficient esophageal cleaning mechanisms return the bulk of the refluxed material to the stomach, and symptoms do not occur. However, when stomach reflux is severe or physically aggressive, it causes symptoms and difficulties, as well as a decrease in quality of life, thus creating gastroesophageal reflux disease (GERD)[1,2]. Since the 1990s, the prevalence of GERD has grown consistently, particularly in East Asia and North America (2.5%-7.8%, and 10%-20%, respectively)[3-5]. Heartburn and regurgitation are common esophageal symptoms, accompanied by consequent chest discomfort and dysphagia. Chronic cough, hoarseness of voice, and asthma are other extra-esophageal symptoms that have been linked to GERD in population-based research. The three GERD phenotypes are erosive esophagitis (EE), non-erosive reflux disease (NERD), and Barrett's esophagitis. Their responses to therapy vary greatly. Of note, the majority (70%) of GERD patients have an endoscopically normal mucosa and are regarded as having NERD.

Pharmacologic, endoscopic, and surgical options for GERD treatment are now available. The most often recommended drugs for GERD treatment in clinical practice are proton pump inhibitors (PPIs) and histamine-2 receptor antagonists. Other, less potent agents typically used to treat mild or intermittent symptoms or as adjunct therapies include antacids, sucralfate, baclofen, alginate, and prokinetics[6,7]. The goals of GERD management include symptom relief, esophageal inflammation healing, esophageal inflammation maintenance and prevention, and quality of life enhancement. PPIs have long been thought to be the basis of GERD treatment. Because of their significant and consistent antisecretory action, PPIs have proven particularly useful in treating EE, managing symptoms, and preventing GERD-related complications such as esophageal ulcers, esophageal bleeding, and peptic stricture. Overall, PPIs have been regarded as a relatively safe class of drugs, with many patients taking them for an extended time. Despite the success of PPIs in addressing many aspects of GERD, there are still many unmet needs[8-10]. Advanced EE, NERD, overnight heartburn, maintenance therapy, and refractory GERD are among them.

Furthermore, PPIs are ineffective for postprandial heartburn and are currently not recommended for atypical or extra-esophageal GERD presentations as well as GERD complications[11,12]. Importantly, prolonged PPI medications have been linked to side effects, generating worries among physicians and patients alike about their safety profile. This review looks at the development and quality of present and potential acid suppression medications, focusing on the potassium-competitive acid blocker (P-CAB) class and exploring their clinical studies.

ROLE OF POTASSIUM ION IN ACID SECRETION

Gastric acid is essential for food and water purification and digestion. Gastric juice has an extremely low pH (pH = 1) because parietal cells in the oxyntic mucosa of the stomach secrete H^+ and Cl^- ions to make hydrochloric acid. These cells secrete 1-2 L of hydrochloric acid every day. The action of gastric H^+/K^+-ATPase in the apical membrane of parietal cells promotes a very high concentration of H^+ in the lumen compared to plasma. The potassium ion plays an essential role in activating gastric H^+/K^+-ATPase and is required for the enzyme to function. At rest, H^+/K^+-ATPase is confined to tubulovesicular regions of a parietal cell with low K^+ concentrations and membranes that are impermeable to K^+. As a result, the enzyme is incapable of activating and transporting H^+ ions. When the parietal cell is stimulated, the tubulovesicular components merge with the cell's apical membrane. After being exposed to K^+-containing luminal fluid, the H^+/K^+-ATPase enzyme can begin to exchange H^+ for K^+[13-15]. Due to its

important involvement in the production of gastric juice, K^+ is a possible therapeutic target for acid blockers. One technique is to block the K^+ channels that allow ions to flow through the apical membrane of parietal cells, while another is to compete with K^+ at the level of the parietal cells' H^+/K^+-ATPase.

P-CABs

The surface of the stomach H^+/K^+-ATPase (the proton pump) is exposed to the extremely acidic parietal cell canaliculus, which has a high affinity for K^+ during acid secretion. Given the significance of the cation for enzyme activity, drugs that compete for K^+ binding have the potential to inhibit acid secretion. The mechanism of action of P-CAB is based on this premise. P-CABs were initially developed in the 1980s and have been studied by many pharmaceutical companies worldwide due to their ability to block the proton pump quickly, efficiently, and reversibly. Schering-Plough was one of those companies that developed a prototype P-CAB, SCH28080. Although the mechanism of action was not fully known at that time, this drug was discovered to lower stomach acid output in humans[16,17]. SCH28080 was later demonstrated to inhibit gastric H^+/K^+-ATPase by competing with K^+[18,19]. However, clinical development of SCH28080 was halted due to hepatotoxicity produced by repeated treatment. This condition sparked research into several kinds of SCH28080 derivatives, such as imidazopyridine derivatives (BY841), imidazo-naphthyridine derivatives (soraprazan), imidazo-thienopyridines (SPI-447), quinolone derivatives (SK&F96067)[20-23], pyrrolo-pyridazine derivatives (CS-526), pyrimidine derivatives (revaprazan), and pyrrole derivatives [vonoprazanm (VPZ)][24-26].

These novel antisecretory medicines vary from PPIs in that they compete with K^+ and cause a dose-dependent selective and reversible inhibition of the proton pump. Because they aren't prodrugs that need to be activated in parietal cells like PPIs, they start working right away, and control of acid secretion begins within the first day of treatment after starting the first dose. In addition, their dissociation from the proton pump is sluggish, and they can stay in the stomach mucosa for up to 24 h. As a result, unlike PPIs, which are less effective at night, their acid inhibitory action persists throughout the day and night[27,28]. Table 1 compares the key differences in the mechanisms of action of P-CABs and PPIs.

ACTION MECHANISM OF P-CABs

P-CABs are classified into many chemical classes (Figure 1). They are a varied class of medications while having a similar mechanism of action. P-CABs are lipophilic, weak bases with limited pH stability and high pKa values. They may concentrate in acidic environments because of the combination of these properties. In the parietal cell canaliculi (pH = 1), a P-CAB with pKa of 6.0 would be 100000-fold larger than in the plasma (pH = 7.4). *In vitro* and *in vivo* investigations using AZD0865 and revaprazan showed a concentrated P-CAB in the gastric mucosa[29-31]. By binding ionically to the enzyme, P-CAB inhibits gastric H^+/K^+-ATPase and prevents further activation by K^+. P-CABs are expected to bind at or near the K^+ binding site, preventing the K^+ from accessing the site (Figure 2). Although these novel drugs exhibit quick and potent antisecretory action, not all of their attractive pharmacodynamic features have translated into therapeutic advantages due to their hepatotoxicity and insufficient efficacy. In addition, linaprazan (AZD0865) failed to outperform standard-dose PPIs in the treatment of peptic ulcers and reflux esophagitis (RE)[32,33]. Soraprazan (BY359) and CS526 (R105366) have fulfilled their proof of effectiveness and safety goals in phase II tests, but data on these medicines have not been published[34].

PHARMACOKINETICS AND PHARMACODYNAMICS OF P-CABs

P-CABs reach peak plasma concentrations quickly after oral administration, in part because they are acid-stable and may be administered as immediate-release formulations. In healthy men, a single dose of revaprazan (100 to 200 mg) resulted in peak plasma concentrations at 1.7 to 1.8 h, which declined to a mean elimination time (T1/2) of 2.2 h to 2.4 h with repeated administration. The pharmacokinetics and concentration-time profiles of revaprazan were comparable following repeated administration (on day 7) to those reported after the initial dosage on day 1[35]. In 2004, Yu *et al*[36] conducted a single-blind, dose-rising, parallel-group, randomized, placebo-controlled trial in 46 healthy subjects. In the single-dose research, plasma concentrations of revaprazan attained peak values 1.3 h to 2.5 h after administration and thereafter decreased mono-exponentially with a terminal T1/2 of 2.2 h to 2.4 h in dosage groups up to 200 mg. Revaprazan has linear pharmacokinetic properties, with negligible accumulation after numerous doses. The onset of pharmacological effects was quick, with the greatest benefits shown on the first day of treatment with repeated doses. They concluded that revaprazan was safe, well-tolerated, and efficiently suppressed acid secretion by increasing intragastric pH in a dose-dependent manner.

Table 1 Main differences in the mechanisms of action between proton pump inhibitors and potassium-competitive acid blockers[12]

Proton pump inhibitors	Potassium-competitive acid blockers
Prodrug requires transformation to the active form, sulphenamide	Direct action on the H^+/K^+ ATPase after protonation
Sulphenamide binds covalently to H^+/K^+-ATPase	P-CABs bind competitively to the K^+ binding site of H^+/K^+-ATPase
Irreversible binding to the proton pump	Reversible binding to the proton pump
The full effect after repeated doses	The full effect after the first dose
PK affected by genetic polymorphism	PK not affected by genetic polymorphism
PD effect more significant during the daytime	PD effect lasting for both the daytime and nocturnal hours
Meal-dependent antisecretory activity	Meal-independent antisecretory activity
Concentrate in parietal cell acid space (1000-fold higher than in plasma)	Super concentrates in parietal cell acid space (100000-fold higher than in plasma)
Duration of effect related to the half-life of the sulphenamide-enzyme complex	Duration of effect related to the half-life of the drug in plasma

P-CAB: Potassium-competitive acid blocker; PD: Pharmacodynamic; PK: Pharmacokinetic.

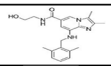
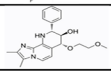
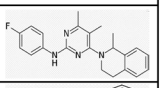
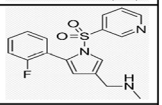
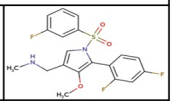
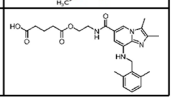
P-CABs	Chemical class	Development phase	Company	Chemical structure
P-CABs whose development has been stopped				
Linaprazan (AZD0865)	Imidazopyridine	Stopped after phase III	AstraZeneca	
CS526 (R105266)	Pyrrolopyridazine	Stopped after phase I	Sankyo and Ube/ Novartis	
Soraprazan (BY359)	Imidazonaphthyridine	Stopped after phase II	Altana	
Available P-CABs in the market				
Revaprazan (YH1885)	Pyrimidine	Marketed in South Korea and India	Yuhan	
Vonoprazan (TAK-438)	Pyrrole	Marketed in Japan; Phase III in Europe /United States	Takeda and Phathom	
Tegoprazan (RQ-00000004)	Benzimidazole	Marketed in South Korea	Raqualia	
P-CABs under active study				
Fexuprazan (DWP14012)	Pyrrole	Phase III in South Korea	Daewoong	
X842 (Linaprazan pro-drug)	Imidazopyridine	Phase II in Europe	Cinclus Pharma	

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Figure 1 Differences in chemical classes and chemical structures of potassium-competitive acid blockers[12]. P-CAB: Potassium-competitive acid blocker.

In 2015, Sakurai *et al*[37] conducted 2 phase I, single rising-dose, randomized, double-blind, placebo-controlled trials in 84 volunteers from Japan using 1-120 mg VPZ and 63 healthy males from the United Kingdom using 1-40 mg VPZ to evaluate the pharmacokinetics, pharmacodynamics, safety, and tolerability. They discovered that VPZ plasma concentration-time profiles exhibited fast absorption at all doses, with a median T_{max} of up to 2 h. $T_{1/2}$ was predicted to be up to 9 h. The acid suppression effect was dose-dependent, with a 24-h intragastric pH 4 holding time for 40 mg VPZ being 92% in Japanese males and 87% in British males. They determined that single oral doses of 20-120 mg VPZ suppressed gastric acid production in healthy male participants in a quick, deep, and 24-h manner. Furthermore, VPZ was well tolerated at all dosages tested, suggesting that it might be used as an alternate therapy for

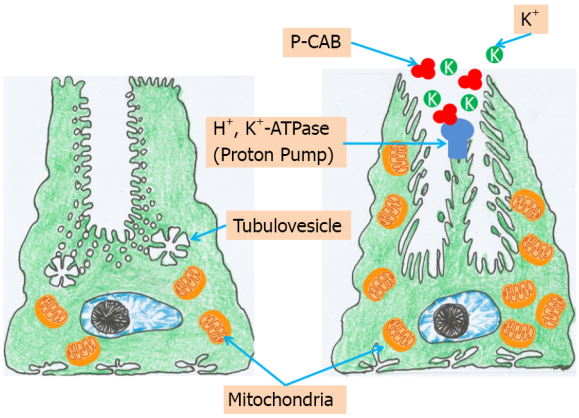

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Figure 2 Potassium-competitive acid blockers inhibit gastric H⁺/K⁺-ATPase by binding ionically to the enzyme and prevent further activation by the K⁺[7]. P-CAB: Potassium-competitive acid blocker.

acid-related diseases.

Recently, Han *et al*[38] performed a phase I, randomized, placebo-controlled study in 56 healthy volunteers without *Helicobacter pylori* infection to evaluate the safety, tolerability, pharmacokinetics, and pharmacodynamics of tegoprazan (TPZ). They found that TPZ was well tolerated. The majority of the adverse events were mild and resolved without any long-term consequences. On day 7, despite multiple dosages of TPZ, there was no accumulation in the plasma. The pharmacodynamics study found that TPZ suppressed gastric acid rapidly in a dose-dependent manner. They concluded that TPZ was well tolerated and demonstrated rapid and potent gastric acid suppression.

Sunwoo *et al*[39] conducted a randomized, double-blind, double-dummy, placebo-controlled study to evaluate a single ascending dose and multiple ascending doses of fexuprazan (FPZ) in 120 healthy male subjects without *Helicobacter pylori* (*H. pylori*) infection. They discovered that FPZ was well tolerated and suppressed gastric acid output for 24 h after delivery. In the multiple ascending doses trial, FPZ plasma concentrations grew in a dose-proportional way, but it did not appreciably accumulate in the plasma in the single ascending dosage study. They concluded that FPZ showed an immediate, long-lasting gastric acid suppression effect and was safe.

In 2020, Hwang *et al*[40] conducted a single- and multiple-dose, randomized, double-blind, placebo-controlled trial to elucidate the pharmacodynamics, pharmacokinetics, and safety of FPZ among healthy volunteers of Korean, Caucasian, and Japanese descent. FPZ (40, 60, or 80 mg for Koreans; 40 or 80 mg for Caucasians; 20, 40, or 80 mg for Japanese) or a placebo were given to ten participants in each group at random. Gastric acid suppression was shown to be consistent across ethnic groups. After successive doses of 40 mg, the mean percentages of time when the intragastric pH was over 4 in Korean, Caucasian, and Japanese participants were 64.3 percent, 62.8 percent, and 70.3 percent, respectively. Furthermore, the 80 mg dose that could effectively suppress gastric secretion was 94.8%, 90.6%, and 90.6% for the Korean, Caucasian, and Japanese subjects, respectively. They determined that FPZ suppressed stomach acid in the same way in Korean, Caucasian, and Japanese patients, and that the pharmacokinetic and pharmacodynamic correlations, as well as the safety, were the same in all three ethnic groups. The FPZ could be utilized regardless of ethnicity.

X842 is a linaprazan prodrug that is currently under development. X842 has a $T_{1/2}$ of 10 h and enables improved intragastric pH regulation over 24 h. Linaprazan was established in a phase I study that used a single dose or multiple escalating dosage design to assess the drug's safety and tolerability in healthy volunteers as the primary endpoint[41]. X842 was shown to be safe and well tolerated by the participants in the study. During the trial, no severe or significant adverse events were recorded. When both pharmacodynamics and pharmacokinetics were evaluated, precise dose linearity was identified. The mean median intragastric pH at each X842 dosage was never less than 4.

P-CABs EFFICACY IN GERD

VPZ efficacy in GERD

In 2015, Ashida *et al*[42] undertook a parallel-group, dose-ranging, multicenter, randomized, double-blind trial in 732 patients with EE to assess the effectiveness and safety of VPZ *vs* lansoprazole (LPZ). The eligible EE individuals were randomized 1:1:1:1:1 to undergo an 8-wk therapy with LPZ 30 mg, VPZ 5 mg, VPZ 10 mg, VPZ 20 mg, and VPZ 40 mg. They discovered that with VPZ 5, 10, 20, and 40 mg,

and LPZ 30 mg, the proportion of healed EE individuals at week 4 was 92.3%, 92.5%, 94.4%, 97.0% and 93.2%, respectively. When corrected for baseline Los Angeles (LA) grades A/B and C/D, all VPZ regimens were non-inferior to LPZ 30 mg therapy. With VPZ 5, 10, 20, 40 mg, and LPZ 30 mg, the proportions of healed EE individuals were 87.3%, 86.4%, 100%, 96.0%, and 87.0%, respectively, for LA grades C/D. The incidence of adverse events was the same in all groups. They indicated that VPZ was efficacious and comparable to LPZ in the treatment of EE. For severe EE (LA grades C/D), they advised VPZ doses of 20 mg or more.

Furthermore, Ashida *et al*[43] performed another parallel-group comparison, multicenter, randomized, double-blind trial to evaluate the non-inferiority, long-term efficacy, and safety as maintenance therapy of VPZ 20 mg compared with LPZ 30 mg in 409 endoscopically confirmed EE patients (LA grades A-D). They discovered that the proportion of patients with healed EE up to week 8 for VPZ (203/205) and 95.5% for LPZ (190/199) was 99.0% for VPZ (203/205) and 95.5% for LPZ (190/199), demonstrating the non-inferiority of VPZ 20 mg therapy. There were a few EE recurrences (< 10%) for long-term maintenance evaluation in patients treated with VPZ 10 mg or 20 mg. They stated that the comparative trial demonstrated the non-inferiority of VPZ 20 mg to LPZ 30 mg in EE, and that VPZ was well tolerated and efficacious among long-term maintenance EE patients (Table 2).

In 2017, Iwakiri *et al*[44] conducted a randomized, double-blind, multicenter trial of VPZ 20 mg or 40 mg in 19 patients with PPI-resistant (LPZ 30 mg) EE to measure stomach and esophageal pH over 24 h. Patients with endoscopically proven PPI-resistant EE received VPZ 20 mg (9 cases) or VPZ 40 mg (10 cases) for 8 wk after a 7- to 14-d run-in period with LPZ 30 mg therapy. The percentage of stomach pH 4 [pH 4 holding time ratio (HTR)] increased significantly in both groups from baseline: In the 20 mg group, it increased from 73.21% to 96.46%, and in the 40 mg group, it increased from 69.97% to 100.00%. There were no significant increases in esophageal pH 4 HTRs from baseline. After 8 wk of treatment, the healing rate in patients with baseline EE grades A-D was 60.0% in the 20 mg group and 71.4% in the 40 mg group. They concluded that VPZ 20 mg and 40 mg substantially suppressed gastric acid secretion over 24 h with considerably higher gastric pH at 4 HTR, resulting in an EE repair rate greater than 60%.

In the same year, Yamashita *et al*[45] conducted a study to evaluate the effects of VPZ and PPIs in 8 RE patients using multichannel intraluminal impedance-pH. They found that the mucosal lesions in 7/8 patients (87.5%) with persistent gastric mucosal injury after completing an 8-wk standard PPI therapy had entirely healed after VPZ therapy. From 26.5% to 78.0%, there was a considerable rise in stomach pH > 4 HTR. In addition, a decrease in esophageal pH of 4 HTR was seen, although it was not statistically significant. The overall number of reflux events, comprising acid and proximal reflux episodes, as well as the time it took for acid to clear, were both dramatically reduced. They concluded that VPZ should be used in patients with PPI-refractory RE.

In 2018, Ashida *et al*[46] conducted a study to compare VPZ 10 mg (n = 202) and 20 mg (n = 204) with LPZ 15 mg (n = 201) as maintenance treatment in 607 patients with healed EE. EE recurrence rates with LPZ 15 mg, VPZ 10 mg, and VPZ 20 mg were 16.8%, 5.1%, and 2.0%, respectively, throughout a 24-wk maintenance period. VPZ was shown to be non-inferior to 15 mg LPZ. The 10 mg and 20 mg VPZ were significantly lower than the LPZ at 15 mg. The EE recurrence rate, on the other hand, did not change substantially between the two VPZ dosages. They determined that VPZ 10 mg and 20 mg were non-inferior to LPZ 15 mg as maintenance treatment for individuals with healed EE.

A retrospective cohort study that recruited 55 patients with symptomatic GERD (NERD = 30, EE = 25) treated with VPZ 10 mg who had been followed for more than 1 year was conducted by Shinozaki *et al*[47]. They discovered that taking VPZ 10 mg for one month relieved GERD symptoms in 89% of patients and was maintained in 82% after one year without further treatment. In 47% of cases, 1-year maintenance treatment resulted in long-term relief of GERD symptoms. Nine of the forty-nine responders experienced a return of GERD symptoms, while VPZ dosage escalation relieved symptoms in 67% (6/9) of patients. Postprandial discomfort, postprandial distress, constipation, and diarrhea significantly decreased after 1 m and remained stable after a year. After 1 year of therapy, the endoscopic healing rate of EE was 95%. They concluded that 1 year of VPZ therapy reduces GERD symptoms significantly, and endoscopic healing of EE is good. VPZ is an efficient and beneficial long-term treatment for GERD.

In 2019, Oshima *et al*[48] conducted a randomized, placebo-controlled experiment in 32 patients with endoscopically proven EE who had heartburn at least once a week to see how quickly VPZ and LPZ relieve heartburn. For 14 d, the patients were given either VPZ 20 mg or LPZ 30 mg before breakfast. They found that heartburn was relieved earlier with VPZ than with LPZ. Furthermore, on day 1, VPZ and LPZ totally cured heartburn in 31.3% and 12.5% of patients, respectively. With VPZ medication, significantly more patients experienced full nighttime heartburn resolution compared with LPZ treatment. They concluded that during the first week of therapy, VPZ provided more prolonged heartburn relief than LPZ.

Recently, Akiyama *et al*[49] studied the efficacy of VPZ 20 mg in 13 patients with PPI-refractory GERD who exhibited continued pathological esophageal acid exposure (EAE). Among 13 patients who were observed by multichannel intraluminal impedance-pH at baseline (PPI treatment) and after VPZ 20 mg therapy, the median gastric acid exposure times of the VPZ group were lower than those of the PPI group, both during daytime and nocturnal observations. Furthermore, during the 24-h monitoring period, VPZ 20 mg treatment resulted in lower median EAE values (4.5%) than PPI therapy (10.6%).

Table 2 Clinical studies regarding the efficacy of potassium-competitive acid blocker in gastroesophageal reflux disease treatment

Ref.	Country/region	Study design	Patients, *n*	Diagnosis	Treatment groups	Duration of treatment, wk	Clinical outcomes
Ashida *et al*[42]	Japan	Multicenter, randomized, double-blind	732	EE	LPZ 30 mg; VPZ 5 mg; VPZ 10 mg; VPZ 20 mg; VPZ 40 mg	8	All VPZ regimens were non-inferior to LPZ 30 mg treatment. The proportions of healed EE subjects were 87.3%, 86.4%, 100%, 96.0%, and 87.0% with VPZ 5, 10, 20, 40 mg, and LPZ 30 mg, respectively
Ashida *et al*[43]	Japan	Multicenter, randomized, double-blind	607	EE	LPZ 15 mg; VPZ 10 mg; VPZ 20 mg	24	The EE recurrence rates were 16.8%, 5.1%, and 2.0% with LPZ 15 mg, VPZ 10 mg, and VPZ 20 mg, respectively, over the 24-wk maintenance period
Shinozaki *et al*[47]	Japan	Retrospective cohort	55	NERD = 30; EE = 25	VPZ 10 mg	4	The VPZ 10 mg for 1-mo alleviated GERD symptoms in 89% and were sustained in 82% after 1 yr without further therapy
Oshima *et al*[48]	Japan	Randomized placebo control	32	EE	LPZ 30 mg; VPZ 20 mg	2	The heartburn was relieved earlier with VPZ than with LPZ, and it was completely relieved in 31.3% and 12.5% of patients on day 1 with VPZ and LPZ, respectively
Akiyama *et al*[49]	Japan	Retrospective cohort with prospective study	13	PPI-refractory GERD	PPIs switch to VPZ 20 mg	8	The median gastric acid exposure times of the VPZ 20 mg were lower than the median gastric acid exposure times of the PPI treatment in both daytime and nocturnal observations. The VPZ 20 mg outperforms PPIs in stomach acid suppression, EAE control, symptom alleviation, and esophagitis healing in patients with PPI-refractory GERD
Mizuno *et al*[50]	Japan	Open-label, single-center, prospective study	50	PPI-refractory RE	PPIs switch to VPZ 20 mg for 4 wk and VPZ 10 mg	48	VPZ 10 mg prevented the recurrence of esophageal mucosal breaks in 43 of 50 (86.0%) patients. VPZ 10 mg is clinically efficacious for the long-term maintenance of healed RE
Xiao *et al* [51]	China, South Korea, Taiwan, Malaysia	Phase III, double-blind, multicenter study	481	EE	LPZ 30 mg; VPZ 20 mg	8	The 8-wk EE healing rates in the VPZ and LPZ groups were 92.4% and 91.3%, respectively. VPZ 20 mg was not inferior to LPZ 30 mg in EE healing at 8 wk
Okanobu *et al*[52]	Japan	Randomized control study	73	EE	VPZ 10 mg; VPZ 20 mg	Each dose for 4 wk and VPZ 10 mg for 8 wk	VPZ 10 mg had the same result as VPZ 20 mg in mucosal healing and symptom reduction at 4 wk and throughout the trial
Matsuda *et al*[53]	Japan	Multicenter, randomized, cross-over study	122	Erosive GERD	VPZ 10 mg; LPZ 15 mg	Every 2nd day for 4 wk and cross-over for more 4 wk	GERD symptoms were significantly reduced with VPZ 10 mg every other day, as measured by the FSSG and the gastrointestinal symptom rating scale
Lee *et al* [54]	South Korea	Randomized, double-blind, parallel-group comparison study	302	EE	TPZ 50 mg; TPZ 100 mg; EPZ 40 mg	4 and 8	At week 8, the cumulative healing rates for TPZ 50 mg, TPZ 100 mg, and EPZ 40 mg were 98.9%, 98.9%, and 98.9%, respectively
Kim *et al* [55]	South Korea	Phase III, double-blind, placebo-controlled, multicenter study	324	NERD	TPZ 50 mg; TPZ 100 mg; placebo	4	The proportions of heartburn-free days and full-resolution rates of heartburn were significantly higher in both TPZ groups than in the placebo group
Lee *et al* [56]	South Korea	Phase III, multicenter, randomized, double-blind trial	260	EE	FPZ 40 mg; EPZ 40 mg	8	FPZ 40 mg was non-inferior to EPZ 40 mg. FPZ 40 mg provided better symptom relief in patients with moderate to severe heartburn, with the effect lasting throughout the night

EAE: Esophageal acid exposure; EE: Erosive esophagitis; EPZ: Esomeprazole; FPZ: Fexuprazan; FSSG: Frequency scale for the symptoms of gastroesophageal reflux disease; GERD: Gastroesophageal reflux disease; LPZ: Lansoprazole; NERD: Non-erosive reflux disease; PPI: Proton pump

inhibitor; RE: Reflux esophagitis; TPZ: Tegoprazan; VPZ: Vonoprazan.

EAE normalization occurred in 46% of VPZ-treated people, and it was connected to complete stomach acid reduction (P = 0.005). Reflux symptoms (P = 0.01) and EE (P = 0.01) improved after switching to VPZ 20 mg. They concluded that VPZ 20 mg outperforms PPIs in stomach acid suppression, EAE control, symptom alleviation, and esophagitis healing in patients with PPI-refractory GERD.

Mizuno *et al*[50] carried out a study to assess the effectiveness of VPZ 10 mg as a maintenance treatment for healed RE in 50 patients who completed 48-wk maintenance therapy. Maintenance therapy with VPZ 10 mg at 48 wk avoided the recurrence of esophageal mucosal breaches in 43 of 50 patients (86.0%). Throughout the 48-wk maintenance therapy, the symptomatic non-relapse rate for acid reflux-related symptom score on the frequency scale for GERD symptoms and acid reflux score on the gastrointestinal symptom rating scale was 70.0% and 72.0%, respectively. They determined that VPZ 10 mg is clinically efficacious for the long-term maintenance of healed RE.

In 2020, Xiao *et al*[51] conducted a phase III, double-blind, multicenter study in 468 endoscopically confirmed EE patients and randomly assigned them to take either VPZ 20 mg (238) or LPZ 30 mg (230) once daily for 8 wk. They found that the 8-wk EE healing rates in the VPZ and LPZ groups were 92.4% and 91.3%, respectively. Moreover, in patients with a baseline LA classification of C/D, VPZ had higher 2-wk, 4-wk, and 8-wk EE healing rates than LPZ. Overall, the rates of EE healing appeared to be greater with VPZ than with LPZ. They concluded that VPZ is not inferior to LPZ in EE healing at 8 wk, and the two treatment groups had identical safety results.

Last year, Okanobu *et al*[52] performed a randomized control study to evaluate the efficacy of VPZ 10 mg (n = 36) compared with VPZ 20 mg (n = 37) in 73 patients with EE. The patients were given each dose for four weeks as an initial treatment, followed by eight weeks of maintenance therapy with VPZ 10 mg. The endoscopic healing rates of the 20 mg and 10 mg groups were 94.6% and 94.4%, respectively, after four wk. In both treatment groups, the frequency scale for GERD symptoms decreased significantly, from 13 to 4 and 14 to 3, respectively, in the 20 mg and 10 mg groups. The scores have plummeted to 2 after 12 wk. They determined that after 4 wk and throughout the experiment, VPZ 10 mg medication had a similar therapeutic response to VPZ 20 mg treatment in terms of mucosal repair and symptom reduction. These results were also the same in LA classification grade A/B patients but not in grade C/D patients.

A prospective, multicenter, open-label, randomized cross-over trial with two periods was conducted by Matsuda *et al*[53] to clarify the efficacy and superiority of VPZ 10 mg every other day over LPZ 15 mg in the maintenance management of 122 erosive GERD patients. They observed that 93.6% of the VPZ group and 82.1% of the LPZ group had well-controlled symptoms, with a significant difference (P = 0.003) using McNemar's test. The VPZ-LPZ and LPZ-VPZ groups, respectively, had 96.7% and 80.0% of patients well managed throughout the first four weeks (P = 0.007). For the second 4 wk, 94.4% of patients in the VPZ-LPZ and 76.7% of patients in the LPZ-VPZ groups were well controlled following 6 consecutive days a week (P = 0.009). They found that taking VPZ 10 mg every other day decreased GERD symptoms considerably, as indicated by the frequency scale for the gastrointestinal symptom rating scale and GERD symptoms.

TPZ efficacy in GERD

Lee *et al*[54] conducted a parallel-group, multicenter, randomized, double-blind comparative trial in 302 Korean patients with endoscopically confirmed EE in 2019. The patients were randomly assigned to receive TPZ 50 mg, TPZ 100 mg, or EPZ 40 mg for 4 wk or 8 wk. They confirmed that at week 8, the cumulative healing rates for TPZ 50 mg, TPZ 100 mg, and EPZ 40 mg were 98.9% (91/92), 98.9% (90/91), and 98.9% (87/88), respectively. Both TPZ dosages were non-inferior to EPZ 40 mg, and TPZ was well tolerated.

Kim *et al*[55] undertook a multicenter, double-blind, placebo-controlled, phase III trial in 324 Korean patients with NERD in 2021 to investigate the safety and effectiveness characteristics of TPZ relative to placebo. They were randomized into three groups with TPZ 50 mg, TPZ 100 mg, and placebo once daily for 4 wk. After 4 wk of therapy with TPZ 50 mg, TPZ 100 mg, or placebo, 42.5% (45/106), 48.5% (48/99), and 24.2% (24/99) of patients achieved full relief of significant symptoms. TPZ 50 mg and 100 mg performed better than the placebo by a substantial statistical difference. Both the TPZ and placebo groups had significantly greater percentages of heartburn-free days and full-resolution rates of heartburn. Furthermore, no apparent change in the occurrence of treatment-emergent adverse events was observed.

FPZ efficacy in GERD

In a phase III, multicenter, randomized, double-blind trial, FPZ, the antisecretory action of a pyrrole derivative with a quick and full onset was tested. A total of 260 adult patients with endoscopically proven EE (LA grades A to D) were given either FPZ 40 mg or EPZ 40 mg *per* day. The cumulative proportion of patients with healed mucosal breaks, as evaluated by endoscopy, was the main outcome

measure at week 8. The healing rate, symptoms, and quality of life were assessed at week 4, and it was discovered that FPZ was non-inferior to EPZ, with the groups having identical cumulative healing rates at 8 wk and equivalent rates at 4 wk. In individuals with moderate to severe heartburn, FPZ provided superior symptom alleviation that lasted throughout the night. The medication was well tolerated, with similar rates of side effects across treatment groups[56].

SAFETY OF P-CABs

Despite the fact that PPIs are one of the safest pharmacological classes available and have been used for almost 30 years, the number of papers on delayed-release PPI safety has skyrocketed, with many extensively discussed subjects appearing in high-profile journals[57,58]. Clinical studies and subsequent meta-analyses have revealed that VPZ has outstanding short and medium-term safety when compared to the performance of PPIs[42-53,59,60]. Because serum gastrin and pepsinogen I levels in healthy volunteers and GERD patients mirrored the antisecretory effect of VPZ, hypergastrinemia associated with long-term therapy may be a concern[61,62]. Clinical studies and subsequent meta-analyses have revealed that VPZ has outstanding short and medium-term safety when compared to the performance of PPIs[63]. Long-term PPI therapy has been associated with dysbiosis and changes in the gut microbiome, and similar changes have now been reported with VPZ[64-66]. Lipopolysaccharide biosynthesis proteins and lipopolysaccharide biosynthesis were the most dramatically elevated pathways in response to VPZ. They are most likely caused by an increase in intraluminal pH and are analogous to *H. pylori* responses to external pH changes. Because lipopolysaccharide is a potent immune response stimulator generated by Gram-negative bacteria, these data imply that VPZ may increase bacterial growth[67-69].

CONCLUSION

Many of the disadvantages and limitations of delayed-release PPIs are overcome by P-CABs. Mucosal healing in acid-related disorders is linked to the duration and degree of acid suppression, and also the length of treatment. Given the challenges in achieving good symptom management, particularly at night, with presently available delayed-release PPIs once daily, this novel family of medicines, P-CABs, delivers immediate, powerful, and extended acid suppression. They have the potential to address many of the unmet therapeutic needs in GERD, such as obtaining immediate heartburn relief and fast and specific healing of severe RE. The benefits of long-term acid suppression extend to *H. pylori* eradication, where intragastric pH control, especially at night, is crucial. Moreover, VPZ may be an optimum dual therapy as a straightforward, dependable, and successful first-line treatment for GERD. More thorough evaluations of VPZ, TPZ, and FPZ are needed, especially in Europe and North America. After extensive worldwide use of P-CABs, clinicians will justify whether it is effective, safe, and superior to currently available treatment with PPIs. In our opinion, VPZ or other P-CABs should be reserved for difficult-to-treat acid-related illnesses and unmet requirements, where the benefit-to-risk ratio is predicted to be the best.

FOOTNOTES

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REFERENCES

1 **Tack J**, Pandolfino JE. Pathophysiology of Gastroesophageal Reflux Disease. *Gastroenterology* 2018; **154**: 277-288 [PMID: 29037470 DOI: 10.1053/j.gastro.2017.09.047]
2 **Chatila AT**, Nguyen MTT, Krill T, Roark R, Bilal M, Reep G. Natural history, pathophysiology and evaluation of gastroesophageal reflux disease. *Dis Mon* 2020; **66**: 100848 [PMID: 30803725 DOI: 10.1016/j.disamonth.2019.02.001]
3 **El-Serag HB**, Sweet S, Winchester CC, Dent J. Update on the epidemiology of gastro-oesophageal reflux disease: a systematic review. *Gut* 2014; **63**: 871-880 [PMID: 23853213 DOI: 10.1136/gutjnl-2012-304269]
4 **Richter JE**, Rubenstein JH. Presentation and Epidemiology of Gastroesophageal Reflux Disease. *Gastroenterology* 2018; **154**: 267-276 [PMID: 28780072 DOI: 10.1053/j.gastro.2017.07.045]
5 **Fock KM**, Talley N, Goh KL, Sugano K, Katelaris P, Holtmann G, Pandolfino JE, Sharma P, Ang TL, Hongo M, Wu J, Chen M, Choi MG, Law NM, Sheu BS, Zhang J, Ho KY, Sollano J, Rani AA, Kositchaiwat C, Bhatia S. Asia-Pacific consensus on the management of gastro-oesophageal reflux disease: an update focusing on refractory reflux disease and Barrett's oesophagus. *Gut* 2016; **65**: 1402-1415 [PMID: 27261337 DOI: 10.1136/gutjnl-2016-311715]
6 **Hershcovici T**, Fass R. Gastro-oesophageal reflux disease: beyond proton pump inhibitor therapy. *Drugs* 2011; **71**: 2381-2389 [PMID: 22117130 DOI: 10.2165/11597300-000000000-00000]
7 **Shibli F**, Kitayama Y, Fass R. Novel Therapies for Gastroesophageal Reflux Disease: Beyond Proton Pump Inhibitors. *Curr Gastroenterol Rep* 2020; **22**: 16 [PMID: 32185589 DOI: 10.1007/s11894-020-0753-y]
8 **Spechler SJ**. Evaluation and Treatment of Patients with Persistent Reflux Symptoms Despite Proton Pump Inhibitor Treatment. *Gastroenterol Clin North Am* 2020; **49**: 437-450 [PMID: 32718563 DOI: 10.1016/j.gtc.2020.04.003]
9 **Wijarnpreecha K**, Thongprayoon C, Chesdachai S, Panjawatanana P, Ungprasert P, Cheungpasitporn W. Associations of Proton-Pump Inhibitors and H2 Receptor Antagonists with Chronic Kidney Disease: A Meta-Analysis. *Dig Dis Sci* 2017; **62**: 2821-2827 [PMID: 28836158 DOI: 10.1007/s10620-017-4725-5]
10 **Yadlapati R**, DeLay K. Proton Pump Inhibitor-Refractory Gastroesophageal Reflux Disease. *Med Clin North Am* 2019; **103**: 15-27 [PMID: 30466671 DOI: 10.1016/j.mcna.2018.08.002]
11 **Moore JM**, Vaezi MF. Extraesophageal manifestations of gastroesophageal reflux disease: real or imagined? *Curr Opin Gastroenterol* 2010; **26**: 389-394 [PMID: 20473157 DOI: 10.1097/MOG.0b013e32833adc8d]
12 **Rettura F**, Bronzini F, Campigotto M, Lambiase C, Pancetti A, Berti G, Marchi S, de Bortoli N, Zerbib F, Savarino E, Bellini M. Refractory Gastroesophageal Reflux Disease: A Management Update. *Front Med (Lausanne)* 2021; **8**: 765061 [PMID: 34790683 DOI: 10.3389/fmed.2021.765061]
13 **Engevik AC**, Kaji I, Goldenring JR. The Physiology of the Gastric Parietal Cell. *Physiol Rev* 2020; **100**: 573-602 [PMID: 31670611 DOI: 10.1152/physrev.00016.2019]
14 **DuBose TD Jr**, Codina J. H,K-ATPase. *Curr Opin Nephrol Hypertens* 1996; **5**: 411-416 [PMID: 8937809 DOI: 10.1097/00041552-199609000-00006]
15 **Sakai H**, Fujii T, Takeguchi N. Proton-Potassium (H(+)/K(+)) ATPases: Properties and Roles in Health and Diseases. *Met Ions Life Sci* 2016; **16**: 459-483 [PMID: 26860309 DOI: 10.1007/978-3-319-21756-7_13]
16 **Ene MD**, Khan-Daneshmend T, Roberts CJ. A study of the inhibitory effects of SCH 28080 on gastric secretion in man. *Br J Pharmacol* 1982; **76**: 389-391 [PMID: 7104515 DOI: 10.1111/j.1476-5381.1982.tb09232.x]
17 **Long JF**, Chiu PJ, Derelanko MJ, Steinberg M. Gastric antisecretory and cytoprotective activities of SCH 28080. *J Pharmacol Exp Ther* 1983; **226**: 114-120 [PMID: 6864535]
18 **Beil W**, Hackbarth I, Sewing KF. Mechanism of gastric antisecretory effect of SCH 28080. *Br J Pharmacol* 1986; **88**: 19-23 [PMID: 3011171 DOI: 10.1111/j.1476-5381.1986.tb09466.x]
19 **Kaunitz JD**, Sachs G. Identification of a vanadate-sensitive potassium-dependent proton pump from rabbit colon. *J Biol Chem* 1986; **261**: 14005-14010 [PMID: 3021724]
20 **Keeling DJ**, Malcolm RC, Laing SM, Ife RJ, Leach CA. SK&F 96067 is a reversible, lumenally acting inhibitor of the gastric (H+ + K+)-ATPase. *Biochem Pharmacol* 1991; **42**: 123-130 [PMID: 1648919 DOI: 10.1016/0006-2952(91)90690-7]
21 **Tsukimi Y**, Ushiro T, Yamazaki T, Ishikawa H, Hirase J, Narita M, Nishigaito T, Banno K, Ichihara T, Tanaka H. Studies on the mechanism of action of the gastric H+,K(+)-ATPase inhibitor SPI-447. *Jpn J Pharmacol* 2000; **82**: 21-28 [PMID: 10874584 DOI: 10.1254/jjp.82.21]
22 **Wurst W**, Hartmann M. Current status of acid pump antagonists (reversible PPIs). *Yale J Biol Med* 1996; **69**: 233-243 [PMID: 9165692]
23 **Simon WA**, Herrmann M, Klein T, Shin JM, Huber R, Senn-Bilfinger J, Postius S. Soraprazan: setting new standards in inhibition of gastric acid secretion. *J Pharmacol Exp Ther* 2007; **321**: 866-874 [PMID: 17369284 DOI: 10.1124/jpet.107.120428]
24 **Ito K**, Kinoshita K, Tomizawa A, Morikawa-Inomata Y, Inaba F, Fujita Y, Tabata K, Shibakawa N. The effect of subchronic administration of 7-(4-fluorobenzyloxy)-2,3-dimethyl-1-{[(1S,2S)-2-methylcyclopropyl]methyl}-1H-pyrrolo[2,3-d]pyridazine (CS-526), a novel acid pump antagonist, on gastric acid secretion and gastrin levels in rats. *J Pharmacol Exp Ther* 2008; **326**: 163-170 [PMID: 18411413 DOI: 10.1124/jpet.108.137299]
25 **Mori H**, Tonai-Kachi H, Ochi Y, Taniguchi Y, Ohshiro H, Takahashi N, Aihara T, Hirao A, Kato T, Sakakibara M, Kurebayashi Y. N-(2-hydroxyethyl)-N,2-dimethyl-8-{[(4R)-5-methyl-3,4-dihydro-2H-chromen-4-yl]amino}imidazo[1,2-a]pyridine-6-carboxamide (PF-03716556), a novel, potent, and selective acid pump antagonist for the treatment of gastroesophageal reflux disease. *J Pharmacol Exp Ther* 2009; **328**: 671-679 [PMID: 18981288 DOI: 10.1124/jpet.108.146415]
26 **Arikawa Y**, Nishida H, Kurasawa O, Hasuoka A, Hirase K, Inatomi N, Hori Y, Matsukawa J, Imanishi A, Kondo M, Tarui N, Hamada T, Takagi T, Takeuchi T, Kajino M. Discovery of a novel pyrrole derivative 1-[5-(2-fluorophenyl)-1-(pyridin-3-ylsulfonyl)-1H-pyrrol-3-yl]-N-methylmethanamine fumarate (TAK-438) as a potassium-competitive acid blocker (P-CAB). *J Med Chem* 2012; **55**: 4446-4456 [PMID: 22512618 DOI: 10.1021/jm300318t]

27 **Scarpignato C**, Pelosini I, Di Mario F. Acid suppression therapy: where do we go from here? *Dig Dis* 2006; **24**: 11-46 [PMID: 16699262 DOI: 10.1159/000091298]

28 **Savarino V**, Marabotto E, Zentilin P, Demarzo MG, de Bortoli N, Savarino E. Pharmacological Management of Gastro-Esophageal Reflux Disease: An Update of the State-of-the-Art. *Drug Des Devel Ther* 2021; **15**: 1609-1621 [PMID: 33907381 DOI: 10.2147/DDDT.S306371]

29 **Gedda K**, Briving C, Svensson K, Maxvall I, Andersson K. Mechanism of action of AZD0865, a K+-competitive inhibitor of gastric H+,K+-ATPase. *Biochem Pharmacol* 2007; **73**: 198-205 [PMID: 17081503 DOI: 10.1016/j.bcp.2006.09.020]

30 **Kirchhoff P**, Andersson K, Socrates T, Sidani S, Kosiek O, Geibel JP. Characteristics of the K+-competitive H+,K+-ATPase inhibitor AZD0865 in isolated rat gastric glands. *Am J Physiol Gastrointest Liver Physiol* 2006; **291**: G838-G843 [PMID: 16798725 DOI: 10.1152/ajpgi.00120.2006]

31 **Sunwoo J**, Ji SC, Oh J, Ban MS, Nam JY, Kim B, Song GS, Yu KS, Jang IJ, Lee S. Pharmacodynamics of tegoprazan and revaprazan after single and multiple oral doses in healthy subjects. *Aliment Pharmacol Ther* 2020; **52**: 1640-1647 [PMID: 33131095 DOI: 10.1111/apt.16121]

32 **Kahrilas PJ**, Dent J, Lauritsen K, Malfertheiner P, Denison H, Franzén S, Hasselgren G. A randomized, comparative study of three doses of AZD0865 and esomeprazole for healing of reflux esophagitis. *Clin Gastroenterol Hepatol* 2007; **5**: 1385-1391 [PMID: 17950677 DOI: 10.1016/j.cgh.2007.08.014]

33 **Dent J**, Kahrilas PJ, Hatlebakk J, Vakil N, Denison H, Franzén S, Lundborg P. A randomized, comparative trial of a potassium-competitive acid blocker (AZD0865) and esomeprazole for the treatment of patients with nonerosive reflux disease. *Am J Gastroenterol* 2008; **103**: 20-26 [PMID: 18184117 DOI: 10.1111/j.1572-0241.2007.01544.x]

34 **Metz DC**, Vakil N, Keeffe EB, Lichtenstein GR. Advances in gastrointestinal pharmacotherapy. *Clin Gastroenterol Hepatol* 2005; **3**: 1167-1179 [PMID: 16361040 DOI: 10.1016/s1542-3565(05)00895-5]

35 **Kim HK**, Park SH, Cheung DY, Cho YS, Kim JI, Kim SS, Chae HS, Kim JK, Chung IS. Clinical trial: inhibitory effect of revaprazan on gastric acid secretion in healthy male subjects. *J Gastroenterol Hepatol* 2010; **25**: 1618-1625 [PMID: 20880169 DOI: 10.1111/j.1440-1746.2010.06408.x]

36 **Yu KS**, Bae KS, Shon JH, Cho JY, Yi SY, Chung JY, Lim HS, Jang IJ, Shin SG, Song KS, Moon BS. Pharmacokinetic and pharmacodynamic evaluation of a novel proton pump inhibitor, YH1885, in healthy volunteers. *J Clin Pharmacol* 2004; **44**: 73-82 [PMID: 14681344 DOI: 10.1177/0091270003261321]

37 **Sakurai Y**, Nishimura A, Kennedy G, Hibberd M, Jenkins R, Okamoto H, Yoneyama T, Jenkins H, Ashida K, Irie S, Täubel J. Safety, Tolerability, Pharmacokinetics, and Pharmacodynamics of Single Rising TAK-438 (Vonoprazan) Doses in Healthy Male Japanese/non-Japanese Subjects. *Clin Transl Gastroenterol* 2015; **6**: e94 [PMID: 26111126 DOI: 10.1038/ctg.2015.18]

38 **Han S**, Choi HY, Kim YH, Nam JY, Kim B, Song GS, Lim HS, Bae KS. Randomised clinical trial: safety, tolerability, pharmacokinetics, and pharmacodynamics of single and multiple oral doses of tegoprazan (CJ-12420), a novel potassium-competitive acid blocker, in healthy male subjects. *Aliment Pharmacol Ther* 2019; **50**: 751-759 [PMID: 31437865 DOI: 10.1111/apt.15438]

39 **Sunwoo J**, Oh J, Moon SJ, Ji SC, Lee SH, Yu KS, Kim HS, Lee A, Jang IJ. Safety, tolerability, pharmacodynamics and pharmacokinetics of DWP14012, a novel potassium-competitive acid blocker, in healthy male subjects. *Aliment Pharmacol Ther* 2018; **48**: 206-218 [PMID: 29863280 DOI: 10.1111/apt.14818]

40 **Hwang JG**, Jeon I, Park SA, Lee A, Yu KS, Jang IJ, Lee S. Pharmacodynamics and pharmacokinetics of DWP14012 (fexuprazan) in healthy subjects with different ethnicities. *Aliment Pharmacol Ther* 2020; **52**: 1648-1657 [PMID: 33111337 DOI: 10.1111/apt.16131]

41 **Unge P**, Andersson K. A first-in-human, open-label, healthy volunteer study of the new P-CAB X842 demonstrating 24h acid control for treatment of acid related diseases. *Gastroenterology* 2017; **154**: S238 [DOI: 10.1016/S0016-5085(18)31174-0]

42 **Ashida K**, Sakurai Y, Nishimura A, Kudou K, Hiramatsu N, Umegaki E, Iwakiri K, Chiba T. Randomised clinical trial: a dose-ranging study of vonoprazan, a novel potassium-competitive acid blocker, vs. lansoprazole for the treatment of erosive oesophagitis. *Aliment Pharmacol Ther* 2015; **42**: 685-695 [PMID: 26201312 DOI: 10.1111/apt.13331]

43 **Ashida K**, Sakurai Y, Hori T, Kudou K, Nishimura A, Hiramatsu N, Umegaki E, Iwakiri K. Randomised clinical trial: vonoprazan, a novel potassium-competitive acid blocker, vs. lansoprazole for the healing of erosive oesophagitis. *Aliment Pharmacol Ther* 2016; **43**: 240-251 [PMID: 26559637 DOI: 10.1111/apt.13461]

44 **Iwakiri K**, Sakurai Y, Shiino M, Okamoto H, Kudou K, Nishimura A, Hiramatsu N, Umegaki E, Ashida K. A randomized, double-blind study to evaluate the acid-inhibitory effect of vonoprazan (20 mg and 40 mg) in patients with proton-pump inhibitor-resistant erosive esophagitis. *Therap Adv Gastroenterol* 2017; **10**: 439-451 [PMID: 28567114 DOI: 10.1177/1756283X17705329]

45 **Yamashita H**, Kanamori A, Kano C, Hashimura H, Matsumoto K, Tsujimae M, Yoshizaki T, Momose K, Obata D, Eguchi T, Fujita M, Okada A. The Effects of Switching to Vonoprazan, a Novel Potassium-Competitive Acid Blocker, on Gastric Acidity and Reflux Patterns in Patients with Erosive Esophagitis Refractory to Proton Pump Inhibitors. *Digestion* 2017; **96**: 52-59 [PMID: 28662503 DOI: 10.1159/000478255]

46 **Ashida K**, Iwakiri K, Hiramatsu N, Sakurai Y, Hori T, Kudou K, Nishimura A, Umegaki E. Maintenance for healed erosive esophagitis: Phase III comparison of vonoprazan with lansoprazole. *World J Gastroenterol* 2018; **24**: 1550-1561 [PMID: 29662293 DOI: 10.3748/wjg.v24.i14.1550]

47 **Shinozaki S**, Osawa H, Kobayashi Y, Sakamoto H, Hayashi Y, Miura Y, Kawarai Lefor A, Yamamoto H. Long-term outcomes of patients with symptomatic gastroesophageal reflux disease treated with vonoprazan. *Scand J Gastroenterol* 2018; **53**: 897-904 [PMID: 30056768 DOI: 10.1080/00365521.2018.1486883]

48 **Oshima T**, Arai E, Taki M, Kondo T, Tomita T, Fukui H, Watari J, Miwa H. Randomised clinical trial: vonoprazan *vs* lansoprazole for the initial relief of heartburn in patients with erosive oesophagitis. *Aliment Pharmacol Ther* 2019; **49**: 140-146 [PMID: 30589965 DOI: 10.1111/apt.15062]

49 **Akiyama J**, Hosaka H, Kuribayashi S, Moriyasu S, Hisada Y, Okubo H, Watanabe K, Imbe K, Nagata N, Kojima Y, Yokoi C, Uemura N, Shimoyama Y, Kawamura O, Yamada M, Kusano M. Efficacy of Vonoprazan, a Novel Potassium-

Competitive Acid Blocker, in Patients with Proton Pump Inhibitor-Refractory Acid Reflux. *Digestion* 2020; **101**: 174-183 [PMID: 30897577 DOI: 10.1159/000497775]

50 **Mizuno H**, Nishino M, Yamada K, Kamiyamamoto S, Hinoue Y. Efficacy of Vonoprazan for 48-Week Maintenance Therapy of Patients with Healed Reflux Esophagitis. *Digestion* 2020; **101**: 411-421 [PMID: 31129668 DOI: 10.1159/000500399]

51 **Xiao Y**, Zhang S, Dai N, Fei G, Goh KL, Chun HJ, Sheu BS, Chong CF, Funao N, Zhou W, Chen M. Phase III, randomised, double-blind, multicentre study to evaluate the efficacy and safety of vonoprazan compared with lansoprazole in Asian patients with erosive oesophagitis. *Gut* 2020; **69**: 224-230 [PMID: 31409606 DOI: 10.1136/gutjnl-2019-318365]

52 **Okanobu H**, Kohno T, Mouri R, Hatsushika Y, Yamashita Y, Miyaki E, Fukuhara T, Okazaki A, Sakano A, Urabe A, Takaki S, Mori N, Tsuji K, Ochi H, Furukawa Y. Efficacy of vonoprazan 10 mg compared with 20 mg for the initial treatment in patients with erosive esophagitis: a randomized pilot study. *Esophagus* 2021; **18**: 669-675 [PMID: 33221955 DOI: 10.1007/s10388-020-00798-7]

53 **Matsuda S**, Kato M, Sakakibara Y, Hamada H, Sasaki Y, Mori H, Hirai Y, Inoue S, Toyokawa T, Kagaya T, Kuwai T, Esaka N, Yamashita H, Watanabe N, Matsumoto M, Fujii H, Demura M, Kubo K, Mabe K, Harada N. A study for every second day administration of vonoprazan for maintenance treatment of erosive GERD (ESD von GERD): a multicenter randomized cross-over study. *J Gastroenterol* 2022; **57**: 133-143 [PMID: 35092498 DOI: 10.1007/s00535-022-01850-2]

54 **Lee KJ**, Son BK, Kim GH, Jung HK, Jung HY, Chung IK, Sung IK, Kim JI, Kim JH, Lee JS, Kwon JG, Park JH, Huh KC, Park KS, Park MI, Kim N, Lee OY, Jee SR, Lee SK, Youn SJ, Kim SK, Lee ST, Hong SJ, Choi SC, Kim TN, Youn YH, Park HJ, Kang MJ, Park CH, Kim BT, Youn S, Song GS, Rhee PL. Randomised phase 3 trial: tegoprazan, a novel potassium-competitive acid blocker, vs. esomeprazole in patients with erosive oesophagitis. *Aliment Pharmacol Ther* 2019; **49**: 864-872 [PMID: 30843245 DOI: 10.1111/apt.15185]

55 **Kim SH**, Cho KB, Chun HJ, Lee SW, Kwon JG, Lee DH, Kim SG, Jung HY, Kim JW, Lee JS, Park H, Choi SC, Jee SR, Kim HS, Ko KH, Park SJ, Lee YC, Park SH, Kim AR, Kim EJ, Park HW, Kim BT, Song GS. Randomised clinical trial: comparison of tegoprazan and placebo in non-erosive reflux disease. *Aliment Pharmacol Ther* 2021; **54**: 402-411 [PMID: 34227708 DOI: 10.1111/apt.16477]

56 **Lee OY**, Chun HJ, Kim JJ, Kim JI, Kim SK, Lee SW, Park KS, Lee KL, Choi SC, Jang JY. A phase 3, non-inferiority randomized controlled trial with fexuprazan, a novel potassium competitive acid blocker vs. esomeprazole in patients with erosive esophagitis. *Gastroenterology* 2020; **158**: S1072

57 **Vaezi MF**, Yang YX, Howden CW. Complications of Proton Pump Inhibitor Therapy. *Gastroenterology* 2017; **153**: 35-48 [PMID: 28528705 DOI: 10.1053/j.gastro.2017.04.047]

58 **Malfertheiner P**, Kandulski A, Venerito M. Proton-pump inhibitors: understanding the complications and risks. *Nat Rev Gastroenterol Hepatol* 2017; **14**: 697-710 [PMID: 28930292 DOI: 10.1038/nrgastro.2017.117]

59 **Cheng Y**, Liu J, Tan X, Dai Y, Xie C, Li X, Lu Q, Kou F, Jiang H, Li J. Direct Comparison of the Efficacy and Safety of Vonoprazan Versus Proton-Pump Inhibitors for Gastroesophageal Reflux Disease: A Systematic Review and Meta-Analysis. *Dig Dis Sci* 2021; **66**: 19-28 [PMID: 32095968 DOI: 10.1007/s10620-020-06141-5]

60 **Miwa H**, Igarashi A, Teng L, Uda A, Deguchi H, Tango T. Systematic review with network meta-analysis: indirect comparison of the efficacy of vonoprazan and proton-pump inhibitors for maintenance treatment of gastroesophageal reflux disease. *J Gastroenterol* 2019; **54**: 718-729 [PMID: 30919071 DOI: 10.1007/s00535-019-01572-y]

61 **Kojima Y**, Takeuchi T, Sanomura M, Higashino K, Kojima K, Fukumoto K, Takata K, Sakamoto H, Sakaguchi M, Tominaga K, Higuchi K. Does the Novel Potassium-Competitive Acid Blocker Vonoprazan Cause More Hypergastrinemia than Conventional Proton Pump Inhibitors? *Digestion* 2018; **97**: 70-75 [PMID: 29393198 DOI: 10.1159/000484217]

62 **Uemura N**, Kinoshita Y, Haruma K, Yao T, Kushima R, Kanoo T. Rationale and design of the VISION study: a randomized, open-label study to evaluate the long-term safety of vonoprazan as maintenance treatment in patients with erosive esophagitis. *Clin Exp Gastroenterol* 2018; **11**: 51-56 [PMID: 29416369 DOI: 10.2147/CEG.S144149]

63 **Hafiz RA**, Wong C, Paynter S, David M, Peeters G. The Risk of Community-Acquired Enteric Infection in Proton Pump Inhibitor Therapy: Systematic Review and Meta-analysis. *Ann Pharmacother* 2018; **52**: 613-622 [PMID: 29457492 DOI: 10.1177/1060028018760569]

64 **Naito Y**, Kashiwagi K, Takagi T, Andoh A, Inoue R. Intestinal Dysbiosis Secondary to Proton-Pump Inhibitor Use. *Digestion* 2018; **97**: 195-204 [PMID: 29316555 DOI: 10.1159/000481813]

65 **Singh A**, Cresci GA, Kirby DF. Proton Pump Inhibitors: Risks and Rewards and Emerging Consequences to the Gut Microbiome. *Nutr Clin Pract* 2018; **33**: 614-624 [PMID: 30071147 DOI: 10.1002/ncp.10181]

66 **Kakiuchi T**, Yamamoto K, Imamura I, Hashiguchi K, Kawakubo H, Yamaguchi D, Fujioka Y, Okuda M. Gut microbiota changes related to Helicobacter pylori eradication with vonoprazan containing triple therapy among adolescents: a prospective multicenter study. *Sci Rep* 2021; **11**: 755 [PMID: 33436953 DOI: 10.1038/s41598-020-80802-3]

67 **Otsuka T**, Sugimoto M, Inoue R, Ohno M, Ban H, Nishida A, Inatomi O, Takahashi S, Naito Y, Andoh A. Influence of potassium-competitive acid blocker on the gut microbiome of *Helicobacter pylori*-negative healthy individuals. *Gut* 2017; **66**: 1723-1725 [PMID: 27965281 DOI: 10.1136/gutjnl-2016-313312]

68 **Marcus EA**, Inatomi N, Nagami GT, Sachs G, Scott DR. The effects of varying acidity on Helicobacter pylori growth and the bactericidal efficacy of ampicillin. *Aliment Pharmacol Ther* 2012; **36**: 972-979 [PMID: 23009227 DOI: 10.1111/apt.12059]

69 **Cochet F**, Peri F. The Role of Carbohydrates in the Lipopolysaccharide (LPS)/Toll-Like Receptor 4 (TLR4) Signalling. *Int J Mol Sci* 2017; **18** [PMID: 29099761 DOI: 10.3390/ijms18112318]

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MINIREVIEWS

Making the case for multidisciplinary pediatric aerodigestive programs

Sohit P Kanotra, Rebecca Weiner, Riad Rahhal

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Abstract

Multidisciplinary pediatric aerodigestive centers have been proposed to address the needs of children with complex multi-system problems affecting the respiratory and upper gastrointestinal tracts. The setup of a multidisciplinary service allows for the complex coordination needed between different subspecialties. This allows for rapid communication and family-centered decision making and agreement on further diagnostic and/or therapeutic next steps such as offering triple endoscopy when indicated. Triple endoscopy entails performing rigid upper airway assessment, flexible bronchoscopy and upper gastrointestinal endoscopy and has been linked to reduced time to diagnosis/treatment, reduced costs and anesthesia exposure. This review summarizes the available literature on the structure and benefits of multidisciplinary pediatric aerodigestive services.

Key Words: Aerodigestive; Multidisciplinary; Pediatric; Triple endoscopy

Core Tip: Multidisciplinary pediatric aerodigestive programs serve patients with multisystem problems affecting the respiratory and upper gastrointestinal tracts and require the participation and expertise of essential subspecialists including gastroenterologists. This setup allows for coordinated diagnostic and therapeutic interventions such as triple endoscopy. Benefits include reducing time to diagnosis and/or treatment, reducing healthcare costs and limiting exposure to radiation and anesthesia, providing a strong example of value-based healthcare delivery.

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INTRODUCTION

Multidisciplinary programs for complex medical conditions such as cystic fibrosis and asthma have been shown to optimize the value of healthcare delivery by improving outcomes and reducing cost[1,2]. There is growing literature supporting this concept for aerodigestive programs[3,4]. Multidisciplinary pediatric aerodigestive programs have been proposed to address the specific needs of children with complex multi-system problems affecting the respiratory and upper gastrointestinal (GI) tracts. Conditions managed by aerodigestive programs are diverse and may include structural or physiological airway disease, chronic parenchymal lung disease, lung injury from aspiration or infection, dysphagia, and esophageal mucosal, motility and anatomic abnormalities[1,5]. One main aim of this coordinated interdisciplinary approach is to allow for expedited patient evaluation in a single clinical setting, including diagnostic and therapeutic interventions. Several benefits have been attributed to aerodigestive programs, as opposed to independent evaluations by different subspecialists at different times. Benefits include shorter time to diagnosis, reduced radiation and anesthesia exposure and lower healthcare related costs. The purpose of the review is to shed light on the structure, services and impacts of aerodigestive programs and to highlight the need for gastroenterology expertise and involvement within these programs.

Advances in the care of critically ill infants and children have created a growing population of patients with complex chronic multi-system diseases requiring a collaborative multispecialty approach to their management[6]. This includes multidisciplinary aerodigestive programs that focus on children with a combination of multiple and interrelated congenital and/or acquired conditions affecting airway, breathing, feeding, and concerns about growth/nutrition (Table 1). Key service components of an aerodigestive program include a pediatric otolaryngologist, pediatric pulmonologist and pediatric gastroenterologist along with a speech language pathologist, social worker and nutritionist. The first consensus statement on aerodigestive programs, published in 2018, provided a definition of an aerodigestive patient, specified needed participation of vital pediatric disciplines and their levels of expertise, and highlighted essential care components, assessments and therapies including endoscopic procedures [5].

Results of a survey published in 2019 noted the number of pediatric aerodigestive programs to be rapidly proliferating with diffuse geographic presence throughout the United States, with the largest subset operating in academic centers[7]. This was a recent phenomenon as a considerable percent of these programs had been operating for just a few years.

AERODIGESTIVE PROGRAM SERVICES

Aerodigestive programs not only offer expertise from different specialties in a single setting, but also enable the coordination of evaluation and intervention. Depending on patient presentation and the differential diagnoses, management includes education, discussion of potential treatment options and risks and benefits of treatment modalities and further testing. Evaluation can include a list of airway and GI interventions offered individually or in combination.

From the airway perspective, evaluation can include awake in-office flexible laryngoscopy and swallow assessment. Further workup may include drug induced sleep endoscopy, direct laryngoscopy and rigid bronchoscopy, while therapeutic interventions targeting specific pathology such as endoscopic repair of laryngeal cleft (Figure 1), laser excision of cysts, balloon dilation of subglottic stenosis and more advanced open airway interventions. These procedures have benefits and risks which should be taken in consideration and clearly discussed with caregivers. Some evaluation can take place without sedation while others require various levels of sedation, often performed in the operating room with the assistance of anesthesia services.

Table 1 Common aerodigestive problems (with overall among subspecialists)

Pulmonary	Otolaryngology	Gastroenterology
Chronic cough	Tracheostomy dependence	Dysphagia
Stridor	Stridor	Feeding intolerance
Wheezing	Noisy breathing	Gastroesophageal reflux disease
Recurrent pneumonia	Recurrent croup	Eosinophilic esophagitis
Tracheomalacia	Laryngeal cleft	Esophageal stricture
Bronchomalacia	Laryngeal stenosis	Congenital esophageal anomalies
Chronic lung disease	Laryngomalacia	Malnutrition
Need for ventilatory support	Vocal cord paralysis	Feeding tube dependence
	Subglottic stenosis	
	Tracheal stenosis	

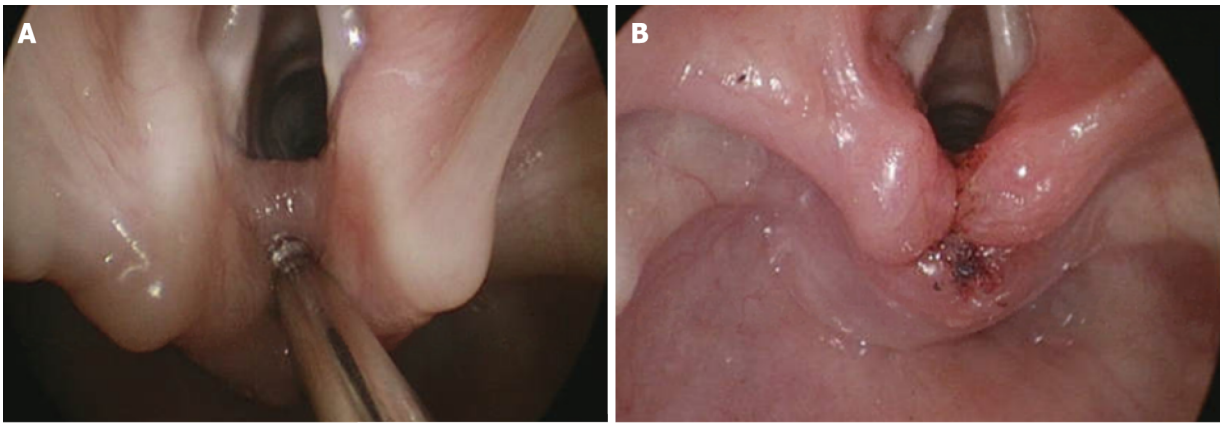

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Figure 1 Endoscopic assessment and management for a laryngeal cleft which can contribute to aspiration. A: Probing of interaytenoid region to assess for cleft; B: Post laryngeal cleft repair using laser and suturing.

Awake in-office flexible laryngoscopy allows for a non-sedated evaluation of the upper airway. This allows for the assessment of upper airway dynamics and abnormalities such as vocal cord lesions and vocal cord movement, tongue base obstruction, hypopharyngeal collapse, adenoid or tonsillar hypertrophy and laryngomalacia. Assessment of dysphagia and aspiration can also be undertaken during in-office flexible laryngoscopy, *via* a procedure referred to as Fiberoptic Endoscopic Evaluation of Swallowing. Even though an invaluable tool for assessment of airway and swallowing abnormalities in the children, in-office flexible laryngoscopy has some disadvantages including limited assessment of structures below the vocal cords and dependence on patient compliance. In addition, therapeutic airway interventions are not possible in the office setting.

Drug induced sleep endoscopy (DISE) allows for upper airway evaluation using a flexible endoscope while a patient is in a pharmacologically induced sleep-like state. During DISE, an endoscope is passed through the nares to assess the nasopharynx, oropharynx, larynx, and in some cases the trachea. The procedure is often carried out either with propofol or dexmedetomidine sedation, which simulates natural sleep and can demonstrate lesions causing obstruction that may not be evident on an awake in-office flexible laryngoscopy[8].

Rigid endoscopy is considered the gold standard for diagnostic airway assessment of structures below the vocal cords, including trachea and bronchi. It offers better optics, ability to size areas of narrowing and provides an opportunity to perform a variety of interventions (Figure 2). This procedure requires patient sedation and thus is not hampered by patient tolerance or compliance. Spontaneous breathing can be maintained, which allows for examination of the dynamic status of the airway. The sedated status allows for surgical interventions such as biopsy, laryngeal cleft injection or suturing, epiglottopexy, balloon dilation, vocal cord injection, and laryngotracheal reconstruction[9,10].

Upper GI evaluation may include video fluoroscopic evaluation of swallowing, contrast imaging for esophageal strictures, impedance pH testing for gastroesophageal reflux disease (GERD), esophageal manometry for motility disorders and mucosal endoscopic assessment for esophagitis including eosino-

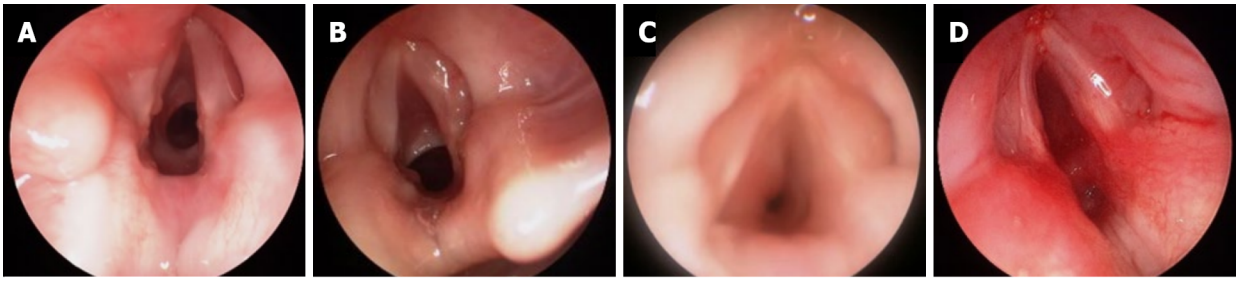

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Figure 2 Endoscopic grading of subglottic stenosis. A: Grade I: 0%-50% obstruction; B: Grade II: 51%-70% obstruction; C: Grade III: 71%-99%; D: Grade IV: No detectable lumen.

philic esophagitis (EoE). Aerodigestive patients often have feeding problems and are at high risk for malnutrition, necessitating feeding tube placement and use[11]. Some patients can be managed with nasogastric and gastrostomy tubes while others require jejunal feeds due to persistent GERD and/or intolerance to intragastric feeds. The choice, placement and maintenance of feeding tubes can be managed by a pediatric gastroenterologist within an aerodigestive program[12].

Specific patient categories presenting to an aerodigestive clinic may have a particularly higher risk for upper GI abnormalities contributing the symptoms. These include patients previously treated for esophageal atresia and tracheoesophageal fistula (Figure 3). Common complications among these patients include esophagitis[13], recurrent respiratory tract infection, tracheomalacia and poor growth with increased healthcare utilization[14-16]. Pediatric gastroenterology input can be crucial to evaluate for anatomic and mucosal esophageal abnormalities and for persistent tracheoesophageal fistula, which can be difficult to diagnosis. Simultaneous tracheal and esophageal endoscopic assessment can help in challenging cases when imaging modalities are not definitive (Figure 4).

In some patients, flexible bronchoscopy, flexible upper GI endoscopy, direct laryngoscopy, and rigid bronchoscopy are indicated and can be offered in combination aptly named as "triple endoscopy". A triple endoscopy provides efficient, organized and thorough evaluation of the patient's disorders without requiring multiple, separate procedures under anesthesia. This allows for complementary modalities for evaluation of upper GI and airway structures and dynamics and facilitates real-time discovery, discussion, and planning amongst all the specialists involved, allowing for excellent, patient-centric care. A consensus on the core data elements to be gathered during triple endoscopy has been published[17]. From the gastroenterology standpoint, the importance of assessing for EoE is highlighted due to potential contribution to aerodigestive symptoms. An upper endoscopy should therefore be considered as part of a triple endoscopy in patients presenting with symptoms potentailly arising from esophageal mucosal disease (especially EoE) as well as anatomic GI disorders (such as esophageal strictures). Another common indication for triple endoscopy is in the preoperative evaluation of children undergoing open airway reconstructive surgery (like patients with tracheostomies moving towards decannulation). Triple endoscopy has also shown high yield in the evaluation of chronic cough, aspiration, apparent life-threatening episodes, recurrent croup and recurrent pneumonias. Triple endoscopy can assess factors that can predict outcomes of open airway reconstruction such as laryngo-tracheal reconstruction, cricotracheal resections and tracheal resections, allowing for risk modification to optimize patient outcomes. Triple endoscopy serves to provide a comprehensive assessment including evaluating grade of subglottic stenosis, ruling out secondary airway lesions (such as tracheobron-chomalacia). Active esophageal disorders, like EoE and GERD, have been linked to unfavorable effect outcomes of open airway reconstruction so it is important for identify these conditions and optimize their management[18,19]. In this patient population, flexible bronchoscopy with bronchoalveolar lavage provides evidence of bacterial colonization which can direct prophylactic antibiotics. Patients with tracheostomies, frequently evaluated by aerodigestive programs, commonly have airway colonization with *Pseudomonas aeruginosa*. Pseudomonal wound infection during open airway reconstruction surgeries leads to increased morbidity[20,21]. Implementation of culture directed antibiotics prior to or during open airway reconstruction surgery can therefore improve outcomes. Studies have demonstrated that specific subsets of aerodigestive patients are more likely to have abnormal mucosal histology on upper GI endoscopy and/or abnormal impedance pH testing[22,23]. This includes those with previously diagnosed asthma, feeding difficulty, and esophageal atresia with or without tracheoesophageal fistula, with 40%-50% yield on upper GI testing[22]. Findings noted included reflux esophagitis and EoE. It is important to note that in many studies a large percent of patients (> 50%) were on acid suppressive therapy which may have treated underlying proton pump inhibitor responsive eosinophilia (now considered a subset of EoE) which requires prolonged treatment and monitoring[24].

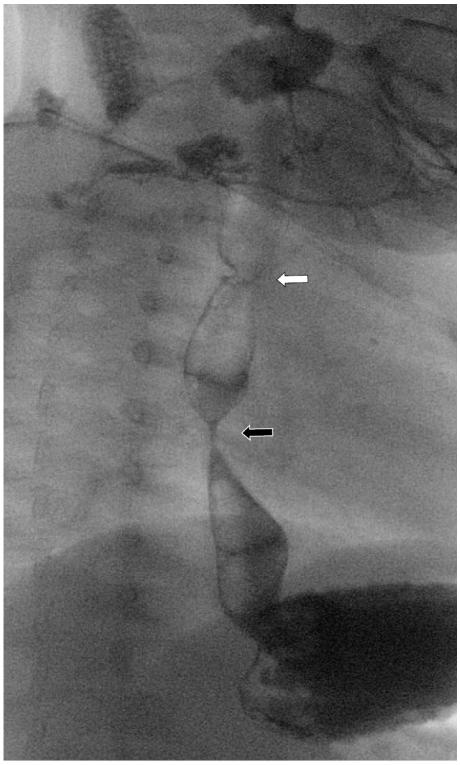

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Figure 3 Fluoroscopic esophageal evaluation in high-risk patients. Esophagram in 10-month-old with repaired esophageal atresia presenting with feeding difficulty and poor growth, showing previously unrecognized distal esophageal congenital stricture (black arrow), far below the surgical repair site (white arrow).

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Figure 4 Simultaneous tracheal and esophageal endoscopy in a patient with history of esophageal atresia to assess for persistent tracheoesophageal fistula.

AERODIGESTIVE PROGRAM OUTCOMES

As aerodigestive programs expand, there has been more emphasis on measuring program impact on patients, their families and the healthcare system. Clinical outcomes typically followed can include documented improvement in swallowing (control of aspiration), frequency of respiratory infections, rate of decannulation in patients with tracheostomies and overall patient mortality. Other important outcomes studied include time to reach a diagnosis and/or initiate treatment, impact of health-related quality of life, healthcare costs and utilization.

Studies have demonstrated numerous positive impacts of aerodigestive programs. These include lower technical direct cost (by as much as 70%) and fewer inpatient days after enrolling patients in an aerodigestive program[3]. Frequency of unnecessary evaluation (swallow studies and impedance pH testing) dropped when such programs were established[24,25]. Aerodigestive programs have been linked to improved patient quality of life and reduced caregiver burden[11]. Studies have also noted

shortened time to diagnosis (6 d *vs* 150 d, $P < 0.001$), fewer specialist consultations, lower hospital charges, less radiation exposure and reduced anesthesia exposure when aerodigestive programs were established[4,26]. The benefit of reducing anesthesia exposure is of particular interest in infants and young children in terms of diminishing neurocognitive risks associated with repeated exposure to anesthetics[27].

Further advantages of triple endoscopy include a significant reduction in mean anesthesia time (54 min *vs* 89 min, $P < 0.0001$) compared to having the 3 procedures done separately[28]. Charges and direct costs for triple endoscopy were also significantly lower as compared to performing these as separate procedures[28]. It is important to note that not all patients who present to an aerodigestive require a triple endoscopy. Patients with isolated surgical pathology such as cystic lesions (for example vallecular cyst) are likely to only require otolaryngologist intervention only.

CONCLUSION

Aerodigestive programs have specific requirements for essential subspecialist participation and expertise which includes gastroenterology services. These programs offer numerous advantages in the management of complex multi-system problems affecting the respiratory and upper GI tracts especially with the diverse and overlapping contributions of conditions leading to a range of patient signs and symptoms. The setup of an aerodigestive program allows for diagnostic and therapeutic interventions, offered individually or in combination. Benefits of aerodigestive programs have been demonstrated and include reducing time to diagnosis and/or initiating of treatment, reducing healthcare costs and limiting patient radiation and anesthesia exposure, providing a strong example of value-based healthcare.

FOOTNOTES

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REFERENCES

1 **Condren M**, Boger JA. Impact of a pediatric clinic-based multidisciplinary asthma education and management program. *J Pediatr Pharmacol Ther* 2005; **10**: 254-258 [PMID: 23118641 DOI: 10.5863/1551-6776-10.4.254]
2 **Sawicki GS**, Goss CH. Tackling the increasing complexity of CF care. *Pediatr Pulmonol* 2015; **50** Suppl 40: S74-S79 [PMID: 26335957 DOI: 10.1002/ppul.23244]
3 **Appachi S**, Banas A, Feinberg L, Henry D, Kenny D, Kraynack N, Rosneck A, Carl J, Krakovitz P. Association of Enrollment in an Aerodigestive Clinic With Reduced Hospital Stay for Children With Special Health Care Needs. *JAMA Otolaryngol Head Neck Surg* 2017; **143**: 1117-1121 [PMID: 28983551 DOI: 10.1001/jamaoto.2017.1743]
4 **Collaco JM**, Aherrera AD, Au Yeung KJ, Lefton-Greif MA, Hoch J, Skinner ML. Interdisciplinary pediatric aerodigestive care and reduction in health care costs and burden. *JAMA Otolaryngol Head Neck Surg* 2015; **141**: 101-105 [PMID: 25473812 DOI: 10.1001/jamaoto.2014.3057]
5 **Boesch RP**, Balakrishnan K, Acra S, Benscoter DT, Cofer SA, Collaco JM, Dahl JP, Daines CL, DeAlarcon A, DeBoer EM, Deterding RR, Friedlander JA, Gold BD, Grothe RM, Hart CK, Kazachkov M, Lefton-Greif MA, Miller CK, Moore PE, Pentiuk S, Peterson-Carmichael S, Piccione J, Prager JD, Putnam PE, Rosen R, Rutter MJ, Ryan MJ, Skinner ML,

Torres-Silva C, Wootten CT, Zur KB, Cotton RT, Wood RE. Structure and Functions of Pediatric Aerodigestive Programs: A Consensus Statement. *Pediatrics* 2018; **141** [PMID: 29437862 DOI: 10.1542/peds.2017-1701]

6 **Burns KH**, Casey PH, Lyle RE, Bird TM, Fussell JJ, Robbins JM. Increasing prevalence of medically complex children in US hospitals. *Pediatrics* 2010; **126**: 638-646 [PMID: 20855383 DOI: 10.1542/peds.2009-1658]

7 **Gumer L**, Rosen R, Gold BD, Chiou EH, Greifer M, Cohen S, Friedlander JA. Size and Prevalence of Pediatric Aerodigestive Programs in 2017. *J Pediatr Gastroenterol Nutr* 2019; **68**: e72-e76 [PMID: 31022094 DOI: 10.1097/MPG.0000000000002268]

8 **Arganbright JM**, Lee JC, Weatherly RA. Pediatric drug-induced sleep endoscopy: An updated review of the literature. *World J Otorhinolaryngol Head Neck Surg* 2021; **7**: 221-227 [PMID: 34430829 DOI: 10.1016/j.wjorl.2021.05.002]

9 **Wineski RE**, Panico E, Karas A, Rosen P, Van Diver B, Norwood TG, Grayson JW, Beltran-Ale G, Dimmitt R, Kassel R, Rogers A, Leonard M, Chapman A, Boehm L, Wiatrak B, Harris WT, Smith N. Optimal timing and technique for endoscopic management of dysphagia in pediatric aerodigestive patients. *Int J Pediatr Otorhinolaryngol* 2021; **150**: 110874 [PMID: 34392101 DOI: 10.1016/j.ijporl.2021.110874]

10 **Day KE**, Smith NJ, Kulbersh BD. Early surgical intervention in type I laryngeal cleft. *Int J Pediatr Otorhinolaryngol* 2016; **90**: 236-240 [PMID: 27729141 DOI: 10.1016/j.ijporl.2016.09.017]

11 **Kim S**, Park M, Kim E, Kim GE, Jung JH, Kim SY, Kim MJ, Kim DH, Park S, Koh H, Ho IG, Kim SK, Hwang S, Shin KH, Lee H, Lee B, Sohn MH, Rha DW, Kim KW. Development of a Multidisciplinary Aerodigestive Program: An Institutional Experience. *Children (Basel)* 2021; **8** [PMID: 34201876 DOI: 10.3390/children8070535]

12 **Al-Zubeidi D**, Demir H, Bishop WP, Rahhal RM. Gastrojejunal feeding tube use by gastroenterologists in a pediatric academic center. *J Pediatr Gastroenterol Nutr* 2013; **56**: 523-527 [PMID: 23254445 DOI: 10.1097/MPG.0b013e318282a8db]

13 **Tambucci R**, Rea F, Angelino G, Malamisura M, Mennini M, Riccardi C, Farello G, Valfré L, Dall'Oglio L, Markowitz JE, Fiocchi AG, De Angelis P. Eosinophilic esophagitis in esophageal atresia: Tertiary care experience of a "selective" approach for biopsy sampling. *World Allergy Organ J* 2020; **13**: 100116 [PMID: 32256942 DOI: 10.1016/j.waojou.2020.100116]

14 **Tuğba RG**, Tuğba ŞE, Ayşe TA, Zeynep RO, Pelin A, Cem K, Ramazan K. Review of Complications of Operated Esophageal Atresia and Tracheoesophageal Fistula Patients. *Turk Arch Pediatr* 2021; **56**: 380-385 [PMID: 35005734 DOI: 10.5152/TurkArchPediatr.2021.20125]

15 **Leibovitch L**, Zohar I, Maayan-Mazger A, Mazkereth R, Strauss T, Bilik R. Infants Born with Esophageal Atresia with or without Tracheo-Esophageal Fistula: Short- and Long-Term Outcomes. *Isr Med Assoc J* 2018; **20**: 161-166 [PMID: 29527854]

16 **Cartabuke RH**, Lopez R, Thota PN. Long-term esophageal and respiratory outcomes in children with esophageal atresia and tracheoesophageal fistula. *Gastroenterol Rep (Oxf)* 2016; **4**: 310-314 [PMID: 26475129 DOI: 10.1093/gastro/gov055]

17 **Boesch RP**, de Alarcon A, Piccione J, Prager J, Rosen R, Sidell DR, Wootten C, Balakrishnan K; Aerodigestive Research Collaborative. Consensus on Triple Endoscopy Data Elements Preparatory to Development of an Aerodigestive Registry. *Laryngoscope* 2022 [PMID: 35122443 DOI: 10.1002/lary.30038]

18 **Ludemann JP**, Hughes CA, Noah Z, Holinger LD. Complications of pediatric laryngotracheal reconstruction: prevention strategies. *Ann Otol Rhinol Laryngol* 1999; **108**: 1019-1026 [PMID: 10579227 DOI: 10.1177/000348949910801101]

19 **Halstead LA**. Gastroesophageal reflux: A critical factor in pediatric subglottic stenosis. *Otolaryngol Head Neck Surg* 1999; **120**: 683-688 [PMID: 10229593 DOI: 10.1053/hn.1999.v120.a91766]

20 **Sasaki CT**, Horiuchi M, Koss N. Tracheostomy-related subglottic stenosis: bacteriologic pathogenesis. *Laryngoscope* 1979; **89**: 857-865 [PMID: 449531 DOI: 10.1288/00005537-197906000-00001]

21 **Gray S**, Miller R, Myer CM 3rd, Cotton RT. Adjunctive measures for successful laryngotracheal reconstruction. *Ann Otol Rhinol Laryngol* 1987; **96**: 509-513 [PMID: 3674646 DOI: 10.1177/000348948709600506]

22 **DeBoer EM**, Kinder S, Duggar A, Prager JD, Soden J, Deterding RR, Ruiz AG, Jensen EL, Weinman J, Wine T, Fortunato JE, Friedlander JA. Evaluating the yield of gastrointestinal testing in pediatric patients in aerodigestive clinic. *Pediatr Pulmonol* 2018; **53**: 1517-1524 [PMID: 30288952 DOI: 10.1002/ppul.24170]

23 **Rosen R**, Amirault J, Johnston N, Haver K, Khatwa U, Rubinstein E, Nurko S. The utility of endoscopy and multichannel intraluminal impedance testing in children with cough and wheezing. *Pediatr Pulmonol* 2014; **49**: 1090-1096 [PMID: 24178927 DOI: 10.1002/ppul.22949]

24 **Hart CK**, de Alarcon A, Tabangin ME, Hamilton S, Rutter MJ, Pentiuk SP, Garza JM. Impedance probe testing prior to pediatric airway reconstruction. *Ann Otol Rhinol Laryngol* 2014; **123**: 641-646 [PMID: 24707014 DOI: 10.1177/0003489414528867]

25 **Wentland C**, Hersh C, Sally S, Fracchia MS, Hardy S, Liu B, Garcia JA, Hartnick CJ. Modified Best-Practice Algorithm to Reduce the Number of Postoperative Videofluoroscopic Swallow Studies in Patients With Type 1 Laryngeal Cleft Repair. *JAMA Otolaryngol Head Neck Surg* 2016; **142**: 851-856 [PMID: 27356238 DOI: 10.1001/jamaoto.2016.1252]

26 **Boesch RP**, Balakrishnan K, Grothe RM, Driscoll SW, Knoebel EE, Visscher SL, Cofer SA. Interdisciplinary aerodigestive care model improves risk, cost, and efficiency. *Int J Pediatr Otorhinolaryngol* 2018; **113**: 119-123 [PMID: 30173969 DOI: 10.1016/j.ijporl.2018.07.038]

27 **Gascon E**, Klauser P, Kiss JZ, Vutskits L. Potentially toxic effects of anaesthetics on the developing central nervous system. *Eur J Anaesthesiol* 2007; **24**: 213-224 [PMID: 17261215 DOI: 10.1017/s0265021506002365]

28 **Ruiz AG**, Bhatt JM, DeBoer EM, Friedlander J, Janosy N, Peterson MB, Wine T, Deterding R, Prager JD. Demonstrating the benefits of a multidisciplinary aerodigestive program. *Laryngoscope* 2020; **130**: 521-525 [PMID: 30919460 DOI: 10.1002/lary.27939]

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MINIREVIEWS

Therapeutic potential of mesenchymal stem cells in the treatment of acute liver failure

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Abstract

Acute liver failure (ALF) is a severe and life-threatening condition in which rapid deterioration of liver function develops in a patient who has no preexisting liver disease. Mesenchymal stem cells (MSCs) are immunoregulatory stem cells which are able to modulate phenotype and function of all immune cells that play pathogenic role in the development and progression of ALF. MSCs in juxtacrine and paracrine manner attenuate antigen-presenting properties of dendritic cells and macrophages, reduce production of inflammatory cytokines in T lymphocytes, suppress hepatotoxicity of natural killer T (NKT) cells and promote generation and expansion of immunosuppressive T, B and NKT regulatory cells in acutely inflamed liver. Due to their nano-sized dimension and lipid envelope, intravenously injected MSC-derived exosomes (MSC-Exos) may by-pass all biological barriers to deliver MSC-sourced immunoregulatoy factors directly into the liver-infiltrated immune cells and injured hepatocytes. Results obtained by us and others revealed that intravenous administration of MSCs and MSC-Exos efficiently attenuated detrimental immune response and acute inflammation in the liver, suggesting that MSCs and MSC-Exos could be considered as potentially new remedies in the immunotherapy of ALF. In this review, we emphasize the current knowledge about molecular and cellular mechanisms which are responsible for MSC-based modulation of liver-infiltrated immune cells and we discuss different insights regarding the therapeutic potential of MSCs in liver regeneration.

Key Words: Mesenchymal stem cells; Acute liver failure; Therapy; Immunomodulation; Regeneration

Core Tip: Due to their potent immunoregulatory, angiomodulatory and hepatoprotective properties, mesenchymal stem cells and their exosomes suppress detrimental immune response, prevent apoptosis and promote survival of injured hepatocytes which results in an enhanced repair and regeneration of acutely injured liver.

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INTRODUCTION

The liver is the largest solid organ in the human body. It consists of large sections (lobes) which are divided in a thousand, hexagonally-shaped small units (lobules)[1]. The lobule is the main structural unit of the liver consisting of a portal triad, hepatocytes and a central vein[1]. The liver is essential organ which performs many functions including bile production, removal of waste products and toxins from the bloodstream, regulation of blood clotting, storage of fat-soluble vitamins, minerals (copper, iron) and glycogen[1]. The liver conjugates bilirubin, handles cholesterol homeostasis, regulates sex hormone metabolism and produces proteins which are important for optimal functioning of endocrine, gastrointestinal, reproductive and immune system. Since liver regulates many vital functions, severe and massive liver injury results in the development of life-threatening multi-organ dysfunction[1].

Acute liver failure (ALF) is a clinical syndrome characterized by rapid deterioration of liver function followed by ascites, coagulopathy, hepatic encephalopathy and multi-organ failure which occur in a patient who has no preexisting liver disease[2]. An annual incidence of ALF is 5.5-6.2 cases per million population per year[3]. ALF is a consequence of severe drug-induced liver injury, hepatic ischemia, invasion of hepatotropic viruses and develops due to the generation of detrimental immune response against foreign or self-antigens, released from injured hepatocytes[4].

Pathogen-associated molecular patterns expressed on invading pathogens or damage-associated molecular patterns and alarmins released from damaged-hepatocytes activate liver resident professional antigen presenting cells [dendritic cells (DCs) and Kupffer cells][2,5]. Activated DCs present lipid antigens to the liver natural killer T (NKT) cells in CD1-dependent manner, enabling their activation and polarization in inflammatory, interferon gamma (IFN-γ)-producing NKT1 and interleukin (IL)-17-producing NKT17 cells[6]. Additionally, activated liver DCs capture microbial proteins and self-proteins released from injured hepatocytes, and present them to the antigen-specific naïve CD4+ and CD8+ T cells[7]. DC-derived IL-12 induce activation and differentiation of naïve CD4+T cells in effector, IFN-γ-producing Th1 cells while DC-sourced IL-1, IL-6 and IL-23 induce generation of IL-17 and IL-22-producing effector Th17 cells[7].

Activated Kupffer cells produce tumor necrosis factor alpha (TNF-α) and IL-1β which induce enhanced expression of E and P selectins on liver endothelial cells, enabling massive influx of circulating neutrophils and monocytes in the injured livers[2,5]. Additionally, activated Kupffer cells release Th1 and Th17 cell-attracting chemokines (CXCL3 and CCL20) and recruit effector CXCR3-expressing CD4+Th1 cells and CCR6-expressing CD4+Th17 cells in the inflamed livers[6]. NKT1/Th1 cell-derived IFN-γ and NKT17/Th17 cell-sourced IL-17 induce enhanced synthesis of hepatotoxic TNF-α , nitric oxide (NO) and reactive oxygen species in Kupffer cells and liver-infiltrated neutrophils, resulting in massive necrosis of hepatocytes[6,7].

Transplantation of bio-artificial livers and hepatocytes could provide support of liver function temporarily and are therefore used as therapeutic approaches for the treatment of ALF[8]. Restored liver function and reduced incidence of extra hepatic complications were observed in ALF patients who underwent transplantation of hepatocytes[2]. However, short-term viability of engrafted hepatocytes and immune rejection against major histocompatibility complex (MHC) miss-matched transplanted hepatic cells significantly limited therapeutic efficacy of liver transplantation. Immunosuppressive therapy managed to temporarily improve survival of ALF patients who underwent liver transplantation [8,9]. Nevertheless, continuous and long-term use of immunosuppressive drugs may induce severe and life-threatening immunosuppression which may be fatal for ALF-treated patients[9]. Accordingly, there is an urgent need for the development and therapeutic use of new immunoregulatory and hepatoprotective agent which could create immunosuppressive microenvironment in injured liver, suppress

detrimental immune response, alleviate liver inflammation and, at the same time, promote hepatocyte proliferation without causing severe, systemic immunosuppression.

Mesenchymal stem cells (MSCs) are adult stem cells that reside in almost all postnatal organs, where participate in the elimination of microbial pathogens, suppress detrimental immune response, promote survival of injured parenchymal cells and enhance tissue repair and regeneration[10]. A large number of experimental and clinical studies demonstrated therapeutic efficacy of MSCs and their secretome in the treatment of ALF[11,12]. MSCs were either directly transplanted in the injured livers or were infused *via* peripheral, portal, splenic veins and hepatic artery. Sang *et al*[13] and Cao *et al*[14] demonstrated that MSCs administered *via* portal vein had better capability to attenuate liver inflammation, reduce necrosis and promote liver regeneration than MSCs which were transplanted *via* hepatic artery, peripheral vein or MSCs which were directly injected in the damaged livers. Teshima and colleagues observed that MSCs which were transplanted *via* splenic vein more efficiently suppressed liver inflammation and enhanced liver regeneration than MSCs which were injected *via* peripheral vein[15]. Opposite to these findings were results reported by Sun *et al*[16] who showed that there was no significant difference in the regeneration of acutely injured livers of animals that received MSCs *via* hepatic artery, portal or peripheral vein, suggesting that route of MSCs injection was not crucially important for their therapeutic effects in the treatment of ALF.

mmUpon engraftment, transplanted MSCs in juxtacrine (cell-to-cell contact-dependent signaling) and paracrine manner [through the activity of MSC-sourced soluble factors: Prostaglandin E2 (PGE2), IL-10, transforming growth factor beta (TGF-β), indoleamine 2, 3 dyoxigenase (IDO), NO, hepatic growth factor (HGF), IL-1 receptor antagonist] modulate phenotype and function of all immune cells that play pathogenic role in the development and progression of acute liver injury[11,12]. MSCs also produce various growth and angiomodulatory factors [HGF, IL-6, vascular endothelial growth factor, basic fibroblast growth factor (bFGF)] which support survival and proliferation of injured hepatocytes[10,13]. Herewith, in this review article, we summarized current knowledge about molecular and cellular pathways that were responsible for hepatoprotective, immunoregulatory and angiomodulatory effects of MSCs and their secretome in the therapy of ALF. An extensive literature review was carried out in March 2022 across several databases (MEDLINE, EMBASE, Google Scholar, ClinicalTrials.gov). Keywords used in the selection were: "mesenchymal stem cells", "secretome", "exosomes", "acute liver injury", "immunomodulation", "immunosuppression", "hepatocytes", "differentiation", "ischemia", "angiomodulation", "therapeutic potential", "liver repair and regeneration". All journals were considered, and an initial search retrieved 125 articles. The abstracts of all these articles were subsequently reviewed by two of the authors (CRH and VV) to check their relevance to the subject of this manuscript. Eligible studies had to delineate the effects of MSCs and their secretome on hepatocyte survival, phenotype and function of liver-infiltrated immune cells, and their findings were analyzed in this review.

MSC-DEPENDENT MODULATION OF LIVER-INFILTRATED IMMUNE CELLS

A large number of experimental studies demonstrated that hepatoprotective effects of MSCs were mainly relied on the immunoregulatory properties of MSCs[17-20]. MSCs attenuated acute liver inflammation and promoted liver regeneration by inducing immunosuppressive phenotype of liver macrophages, DCs, T lymphocytes and NKT cells[21-24].

MOLECULAR MECHANISMS RESPONSIBLE FOR MSC-BASED SUPPRESSION OF INFLAMMATORY MACROPHAGES IN INJURED LIVERS

Wang and colleagues showed that beneficial effects of MSCs in the attenuation of ALF were relied on the suppression of inflammatory M1 macrophages which hold central function in initiating acute liver inflammation[17]. Murine BM-MSCs alleviated murine D-galactosamine (D-Gal)-induced ALF by inducing generation of immunosuppressive M2 phenotype in liver macrophages in PGE2-dependent manner (Figure 1). MSC-derived PGE2 bound to the EP4 receptor and inhibited TGF-β-activated kinase 1-driven activation of nucleotide-binding and oligomerization domain-like receptor 3 (NLRP3) inflammasome in Kupffer cells[17]. PGE2-dependent suppression of NLRP3 inflammasome in liver macrophages resulted in down-regulated secretion of inflammatory cytokines (IL-1β, IL-6 and IL-18) which led to the alleviation of acute liver inflammation[17]. Additionally, MSC-sourced PGE2 induced phosphorylation and activation of transcriptional factor STAT6 which modulated mammalian target of rapamycin (mTOR) signaling that resulted in the generation of immunosuppressive M2 phenotype in liver macrophages. By producing anti-inflammatory cytokines (IL-10 and TGF-β), M2 macrophages attenuated on-going inflammation and promoted repair and regeneration of D-Gal-injured livers[17]. Similar findings were reported by Miao and colleagues who provided evidence that PGE2-dependent repression of NLRP3 inflammasome in Kupffer cells was mainly responsible for beneficial effects of BM-

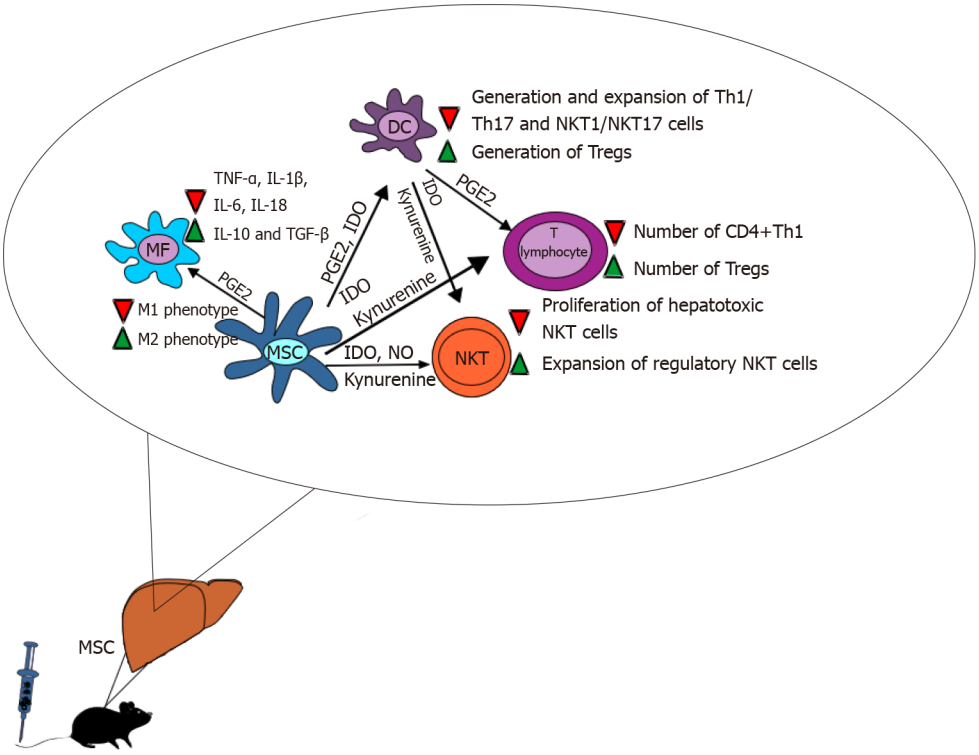

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Figure 1 Mesenchymal stem cells-based modulation of immune cells in acute liver injury. Mesenchymal stem cells (MSCs), in prostaglandin E2 (PGE2) dependent manner, prevent generation of inflammatory (M1) phenotype and induce generation of immunosuppressive (M2) phenotype in liver macrophages by down-regulating synthesis of inflammatory cytokines [tumor necrosis factor alpha, interleukin (IL)-1β, IL-6, IL-18] and by promoting production of anti-inflammatory cytokines (IL-10 and transforming growth factor beta). Increased indoleamine 2, 3 dyoxigenase (IDO) activity and enhanced secretion of PGE2 are mainly responsible for MSC-based generation of tolerogenic dendritic cells (DCs) which suppress expansion of Th1/Th17 lymphocytes and natural killer T (NKT) 1/NKT17 cells, but favor proliferation of immunosuppressive T regulatory cells (Tregs). MSC-generated tolerogenic DCs, in PGE2-dependent manner, suppress expansion of inflammatory CD4+ Th1 cells and induce expansion of Tregs, while in IDO/Kynurenine-dependent manner attenuate proliferation of hepatotoxic NKT cells and induce expansion of NKT regulatory cells (NKTregs). MSC-sourced nitric oxide, in autocrine manner, induces enhanced IDO activity in MSCs. MSC-derived IDO and Kynurenine suppress proliferation of inflammatory Th1 and NKT cells in the liver and were mainly responsible for MSC-dependent expansion of immunosuppressive Tregs and NKTregs in injured livers. MSCs: Mesenchymal stem cells; PGE2: Prostaglandin E2; TNF-α: Tumor necrosis factor alpha; IL: Interleukin; TGF-β: Transforming growth factor beta; IDO: Indoleamine 2, 3 dioxygenase; NKT: Natural killer T cells; Tregs: T regulatory cells; NKTregs: Natural killer T regulatory cells; NO: Nitric oxide.

MSCs in the attenuation of acute liver injury during lipopolysaccharide (LPS)-induced sepsis in mice [18]. BM-MSC-sourced PGE2 generated immunosuppressive phenotype and increased IL-10 production by modulating activity of extracellular signal-regulated kinase 1 (ERK1) in Kupffer cells[18].

Liu *et al*[19] who analyzed the frequency of F4/80-expressing macrophages in the livers of CCL4+MSC-treated mice confirmed that MSC-based therapy significantly altered phenotype and function of Kupffer cells in inflamed livers. During the initial phase of ALF (first 48 h after CCL4 injection), significantly higher number of inflammatory M1 macrophages was observed in injured livers while immunosuppressive M2 macrophages were dominant subpopulation of liver macrophages during the recovery phase (7 d after CCL4 injection)[19]. Intravenous injection of murine MSCs (5×10^5 cells/mice) which were obtained from compact bone attenuated liver inflammation and completely regenerate CCL4-injured livers. During the injured phase, the number of inflammatory monocyte-derived macrophages was significantly increased by MSC treatment while during the recovery phase of acute liver injury MSCs favored generation and expansion of immunosuppressive M2 macrophages, crucially contributed to the creation of immunosuppressive microenvironment in regenerated livers[19].

Hua *et al*[20] demonstrated that amnion-derived MSCs (A-MSCs) suppressed inducible nitric oxide synthase (iNOS) activity and NO production, attenuated synthesis of inflammatory cytokines (TNF-α, IL-6) and induced generation of anti-inflammatory phenotype in liver macrophages by promoting production of immunosuppressive IL-10. A-MSC-dependent modulation of liver macrophages was relied on modulation of autophagy, as evidenced by down-regulated expression of microtubule-associated protein 1 light chain 3 in A-MSC-treated Kupffer cells[20]. Single injection of A-MSCs (1×10^6 cells/mice) alleviated acetaminophen (APAP)-induced ALF in mice by inducing alternative activation of Kupffer cells. Liposome-induced macrophage depletion almost completely diminished beneficial effects of A-MSCs, indicating that macrophages were the main cellular targets of A-MSCs in the treatment of APAP-induced ALF[20].

THE CROSS-TALK BETWEEN MSCS, DCS, T LYMPHOCYTES AND NKT CELLS IN INJURED LIVERS

We and others demonstrated that MSCs and their secretome [MSC-derived conditioned medium (MSC-CM)] attenuated antigen-presenting properties of liver-infiltrated DCs, reducing their capacity for the generation and expansion of inflammatory Th1/Th17 and NKT1/NKT17 cells in injured livers[21-24]. MSC-sourced PGE2 and IDO were mainly responsible for the suppression of DC-dependent activation of liver T and NKT cells (Figure 1)[21-24].

Zhang *et al*[21] showed that intravenously injected BM-MSCs prevented progression of *Propionibacterium acnes* (*P. acnes*)-induced ALF in mice by inducing regulatory phenotype in liver DCs. MSCs-treated livers had normal morphology and structure without any signs of massive hepatocyte injury. MSCs down-regulated production of Th1 cell-attracting chemokines (CXCL9, CXCL10, CCL3, CCL21) which resulted in reduced influx of CXCR3 and CCR5-expressing effector CD4+ Th1 cells and CCR7-expressing memory CD4+ Th1 cells in the livers of *P. acnes*-treated mice[21]. Accordingly, only small number of liver-infiltrating lymphocytes was observed around the central veins of *P. acnes* + MSC-treated mice. Flow cytometry analysis of liver-infiltrated CD4+T cells revealed decreased expression of activating markers (CD44, CD69), indicating that MSCs suppressed activation of CD4+ T cells in *P. acnes* -treated animals[21]. MSCs attenuated ALF in mice by suppressing production of inflammatory and hepatotoxic cytokines (TNF-α and IFN-γ) in liver CD4+Th1 cells which was followed by the attenuation of systemic inflammation and by complete restoration of liver function[21]. Reduced number of CD4+ Th1 cells was accompanied with increased presence of FoxP3-expressing CD4+ CD25+ T regulatory cells (Tregs) in the livers of *P. acnes* + MSC-treated mice. Suppression of Th1 cell-driven liver inflammation was a consequence of MSC-dependent modulation of hepatic DCs[21]. DCs isolated from the livers of *P. acnes* + MSC-treated animals had regulatory phenotype, characterized by low expression of co-stimulatory molecules (CD80, CD86), decreased production of Th1 cell-related inflammatory cytokines (TNF-α, IL-1β, IL-12) and increased production of immunosuppressive cytokines (IL-10 and TGF-β) which induced generation and expansion of FoxP3-expressing CD4+ CD25+ Tregs in injured livers[21]. MSCs induced generation of regulatory phenotype in CD11c+ B220- precursor DCs through the activation of PI3K signaling pathway. By activating EP4 receptor, MSC-sourced PGE2 induced phosphorylation of PI3K and ERK1/2 kinases and elicited generation of tolerogenic, immunosuppressive phenotype in liver DCs[21]. Importantly, MSC-generated DCs possessed potent immunosuppressive and hepatoprotective properties. Passive transfer of MSC-generated regulatory DCs induced generation of hepatic Tregs, attenuated liver inflammation and prevented development of ALF in *P. acnes*-treated mice[21]. MSCs and DC-dependent expansion of Tregs was responsible for beneficial effects of MSCs since Treg depletion completely abrogated MSC-based attenuation of acute liver inflammation[21,22].

By using Concanavalin (Con A) and alpha-galactoceramide (α-GalCer)-induced hepatitis as murine models of ALF, we showed that MSC-dependent modulation of the cross-talk between DCs and NKT play important role in MSC-based attenuation of ALF[22-25]. Intravenously injected BM-MSCs significantly alleviated serum levels of AST and ALT, reduced apoptosis of hepatocytes and improved survival of Con A- and α-GalCer-treated mice[22-24]. Cellular make-up of the livers of MSC-treated animals revealed significantly reduced number of hepatotoxic, FasL, NKG2D and CD107-expressing NKT cells, indicating that MSCs suppressed cytotoxic properties of NKT cells[23]. The same phenomenon was observed in Con A- and α-GalCer-treated mice that received BM-MSC-CM, suggesting that MSCs suppressed NKT cell-driven liver inflammation in paracrine manner[22,23]. Among various MSC-derived immunosuppressive factors, NO and Kynurenine (IDO metabolite) were present in the highest concentration in BM-MSC-CM[23]. Additionally, inhibition of iNOS and IDO activity completely diminished hepatoprotective properties of MSCs, confirming the importance of NO and IDO/Kynurenine for MSC-dependent suppression of ALF[23,24]. MSC-sourced NO inhibited proliferation of hepatotoxic NKT cells while MSC-derived IDO induced generation of regulatory and immunosuppressive phenotype in NKT cells[23,25]. Additionally, MSC-sourced IDO prevented trans-differentiation of NKT regulatory cells (NKTregs) in inflammatory NKT17 cells by activating general control nonderepressible 2 (GCN2) kinase which suppressed protein kinase B/mTOR-dependent synthesis of inflammatory cytokines IL-17 and IL-22 in NKTregs[23,25].

In addition to their direct immunoregulatory effects on hepatotoic NKT cells, BM-MSCs and BM-MSC-CM suppressed effector functions of liver NKT cells indirectly, through the modulation of phenotype and function of hepatic DCs[25]. Liver DCs play important role in MSC-dependent suppression of liver NKT cells since their depletion abrogated hepatoprotective effects of MSCs and aggravated liver injury in MSC-treated mice[23,25]. MSCs attenuated antigen-presenting properties of hepatic DCs by down-regulating expression of MHC class II and co-stimulatory molecules on their membranes and by reducing synthesis of NKT1 and NKT17 cell- related inflammatory cytokines (TNF-α , IL-β, IL-12, IL-23, IFN-γ, IL-17)[22-25]. In similar manner as it was observed by Zhang *et al*[16], we also noticed that MSCs induced generation of regulatory and tolerogenic phenotype in hepatic DCs[22-25]. Increased IDO activity and enhanced production of anti-inflammatory cytokines (TGF-β and IL-10) were observed in the DCs which were isolated from the livers of Con A + MSCs and α-GalCer + MSCs-treated

mice[23]. MSC-generated regulatory DCs induced generation and expansion of IL-10 and TGF-β-producing NKTregs, contributing to the creation of immunosuppressive microenvironment in injured livers[24]. Additionally, BM-MSCs reduced presence of inflammatory, IFN-γ and TNF-α-producing NKT1 and IL-17-producing NKT17 cells in the livers of Con A + MSCs and α-GalCer + MSCs-treated mice which resulted in alleviated liver inflammation[24]. MSC-sourced IDO and its metabolite Kynurenine were mainly responsible for MSC-dependent generation of regulatory DCs[24,25]. Inhibition of IDO activity in MSCs attenuated their capacity for the generation of tolerogenic phenotype in hepatic DCs and diminished their hepatoprotective and immunosuppressive effects[24].

MSCS AS POTENTIALLY NEW THERAPEUTIC AGENTS IN REGENERATIVE HEPATOLOGY

MSCs represent heterogeneous population of immunoregulatory and angiomodulatory stem cells which possess enormous differentiation potential[25]. All subpopulations of MSCs spontaneously differentiate into the cells of mesodermal origin[25]. However, under appropriate conditions, MSCs may generate cells of ectodermal and endodermal origin, including hepatocyte-like cells (HLCs)[26-29].

Local tissue microenvironment, particularly oxygen supply and cytokines/growth factors released by parenchymal and tissue-resident immune cells modulate phenotype of MSCs[25,26]. MSCs are usually derived from bone marrow, adipose tissue, amniotic fluid, placenta, umbilical cord, dental pulp, compact bone and peripheral blood[10]. The development origin significantly affects potential for differentiation and other functional characteristics of tissue-specific MSCs[26]. BM-MSCs possess great genomic stability after long-term propagation[26]. An improved engraftment and increased life span are the main characteristics of adipose tissue-derived MSCs (AT-MSCs)[26]. Dental pulp-derived MSCs, amniotic fluid derived MSCs and placental derived MSCs have increased capacity for differentiation in neural cells, while BM-MSCs, AT-MSCs and umbilical cord derived MSCs (UC-MSCs) have great differentiation potential towards the cells of endodermal origin, including HLCs[26].

Accordingly, a large number of evidence demonstrated that human BM-MSC-, AT-MSC- and UC-MSC-derived HLCs represent valuable and promising cell source for the regeneration of injured livers [25-29].

Two step protocol is the most usually used for the generation of human MSC-derived HLCs[29]. After isolation from BM, AT and UC, MSCs are, under standard conditions (at 37 ℃ in a humidified atmosphere containing 5% CO_2), cultured in complete Dulbecco's modified Eagle's medium (DMEM) supplemented with fetal bovine serum (FBS) until they reach 75%-80% of confluence. Then, MSCs are cultured for 48 h in the FBS-free DMEM medium supplemented with endothelial growth factor (20 ng/mL) and basic fibroblast growth factor (bFGF, 10 ng/mL) to be committed towards endodermal lineage[29]. Afterwards, MSCs were seven days exposed to HLC step-1 differentiation medium, consisting of HGF (20 ng/mL), bFGF (10 ng/mL) and nicotin-amide (0.61 g/L). By binding to the c-met receptor on differentiated cells, HGF activates intracellular signaling molecules (Grb2-associated binder-1, son of sevenless), ERK kinase and mitogen-activated protein kinase which are important in the early stages of hepatogenesis[29]. Intracellular signaling elicited by HGF/c-met are enhanced by nicotin-amide which acts as selective kinase inhibitor that improves cell survival and proliferation during the initial stage of endodermal patterning. Finally, MSCs are cultured in HLC step-2 differentiation medium consisting of oncostatin (OSM, 20 ng/mL), dexamethasone (1 mmol/L) and insulin, transferrin and selenium (ITS, 50 mg/mL). OSM promotes maturation of hepatocytes, dexamethasone induces enhanced expression of liver-enriched transcription factors [CCAAT/enhancer-binding protein alpha (C/EBPα), hepatocyte nuclear factor (HNF)-4α], while ITS promotes the proliferation and survival of differentiated HLCs[29]. At the end of the differentiation process, HLCs generated from BM-, AT- and UC-MSCs, fully obtain hepatocyte-like polygonal morphology with cytoplasmic granulation and prominent centrally positioned nucleus[29]. Exposition to HLC differentiation mediums do not alter telomerase activity and do not induce genetic instability in human BM-, AT- and UC-MSC-derived HLCs[29].

MSC-derived HLCs had gene expression profile similar to fetal or adult hepatocytes[25-29]. During the early stage of differentiation, MSC-derived HLCs express several early hepatocyte differentiation markers [alfa-fetoprotein, cytokeratin (CK)7, GATA4, HNF1α, HNF3β, HNF4α, C/EBPα] while during the late stage of differentiation, MSC-derived HLCs express cell markers of mature hepatocytes (forkhead transcription factor which has a crucial role in hepatocyte differentiation), hepatocyte-specific gap junction protein connexin 32 and hepatocyte paraffin 1, localized in hepatocytes mitochondria). Finally, at the end of differentiation, MSC-derived HLCs are able to produce hepatocyte-specific proteins (albumin, fibrinogen, transferrin) and enzymes (cytochrome P450 subtypes 3A4 and 1A1, phosphoenolpyruvate carboxykinase 1 and carbamoylphosphate synthetase[26-29].

The analysis of functional characteristics of BM-, AT- and UC-MSCs-derived HLCs revealed that AT-MSC- and BM-MSC-derived HLCs had the higher capacity for glycogen storage than UC-MSCs-derived HLCs and their gene expressions were more consistent with the normal hepatocyte-differentiation profile[30]. Albumin gene expression increased progressively during the differentiation process of AT-

MSC- and BM-MSC-derived HLCs. In contrast, only transitory expression of albumin was observed in UC-MSC-derived HLCs (between day 7 and day 25). Additionally, HLCs which were generated from AT-MSCs and BM-MSCs displayed clusters arrangements and had increased proliferation rate compared to UC-MSC-derived HLCs which were not expanded as expected, hampering their potential for *in vivo* use[30]. Accordingly, only therapeutic potential of AT-MSC-derived HLCs and BM-MSC-derived HLCs was examined *in vivo*, in murine model of CCL4-induced acute liver injury[30]. Intravenously injected human AT-MSC-and BM-MSC-derived HLCs (1×10^6 cells), managed to completely regenerate CCL4-injured livers of immunodeficient mice. Hepatocytes isolated from the livers of CCL4-treated mice displayed normal morphology two weeks after the transplantation of human AT-MSC- and BM-MSC-derived HLCs. Importantly, hepatocytes in the regenerated livers of experimental mice express human albumin and CK19 confirming that they were generated from transplanted human AT-MSC- and BM-MSC-derived HLCs[30]. Accordingly, both AT-MSCs and BM-MSCs represent valuable cell source for the generation of functional HLCs which may repopulate and regenerate injured livers of ALF patients[30]. Nevertheless, it should be noted that progression of ALF is mainly a consequence of immune cell-dependent massive damage of hepatocytes[2,6]. Accordingly, detrimental immune response could induce injury of engrafted HLCs as well. Therefore, co-transplantation of MSC-derived HLCs and immunosuppressive MSCs should be explored as new therapeutic approach which could successfully regenerate injured livers of ALF patients.

THERAPEUTIC POTENTIAL OF MSC-EXOS IN THE TREATMENT OF ACUTE LIVER INJURY

Although results obtained in animal studies strongly suggest that MSCs could be considered as new therapeutic agents in regenerative hepatology, there are several safety issues which limit clinical use of MSCs[31]. Firstly, MSCs are not constitutively immunosuppressive cells[32]. MSCs may adopt phenotype and function under the influence of biological factors to which they are exposed. When MSCs engraft in the tissue with low level of TNF-α and IFN-γ, they obtain pro-inflammatory MSC1 phenotype and secrete large number of bioactive factors which aggravate on-going inflammation. On the contrary, when MSCs are exposed to the high levels of TNF-α and IFN-γ, they acquire immunosuppressive MSC2 phenotype and produce immunoregulatory factors that suppress inflammatory immune cells[32]. Since local tissue concentration of TNF-α and IFN-γ differ in initial and recovery phases of acute liver inflammation, there is a concern that MSCs which will be engrafted in the injured livers with low level of TNF-α and IFN-γ may obtain pro-inflammatory MSC1 phenotype and may aggravate on-going inflammation[31,32]. Additionally, TGF-β and bone morphogenetic protein, released by macrophages in inflamed and regenerated tissues, may induce spontaneous and unwanted chondrogenic and osteogenic differentiation of MSCs[33]. Possible unwanted differentiation of transplanted MSCs represents an important safety concern which limits clinical use of MSCs in regenerative hepatology[33].

MSC-Exos are nano-sized extracellular vesicles which contain all of MSC-sourced immunoregulatory and angiomodulatory factors[34]. Due to their nano-sized dimension and lipid envelope, intravenously injected MSC-Exos by-pass all biological barriers and deliver their cargo (miRNAs) anti-inflammatory cytokines, growth factors) directly into the target liver-infiltrated immune cells and injured hepatocytes. Since the majority of MSC-dependent beneficial effects in attenuation of liver inflammation were relied on the activity of MSCs-sourced bioactive factors[25], administration of MSC-Exos is considered as an alternative therapeutic approach to MSC-based therapy since it addresses all safety concerns related to the transplantation of MSCs[34]. A detailed proteomic analysis revealed that MSC-Exos contained proteins that regulate hepatocyte survival, oxidative stress, tissue remodeling, activation and migration of immune cells[34]. Accordingly, several experimental studies demonstrated therapeutic potential of MSC-Exos in the treatment of ALF[35-37].

After intravenous injection, MSC-Exos obtained from MB-MSC-Exos migrated in the liver, attenuated D-Gal/LPS-induced acute liver inflammation and improved survival of in D-Gal/LPS-treated mice suffering from ALF[35]. By delivering immunosuppressive molecules and trophic factors in inflamed livers [IL-8, angiopoietin-2, angiogenin, insulin-like growth factor binding protein-6 (IGFBP-6)] MB-MSC-Exos attenuated caspase-3-dependent apoptosis of injured hepatocytes[35]. MB-MSCs-sourced IL-8 inhibited TNF-α-induced activation of caspase-3 and prevented apoptosis of hepatocytes while angiopoietin-2, angiogenin and IGFBP-6 promoted survival of hepatocytes by preventing the progression of ischemia-induced injury in inflamed livers[35].

The importance of anti-apoptotic effects of MSC-Exos in the treatment of ALF was confirmed in murine model of CCL4-induced acute liver injury[36]. Jiang and colleagues demonstrated that hepato-protective effects of Exos isolated from the secretome of human UC-MSCs (UC-MSC-Exos) were relied on their capacity to inhibit caspase 3 and Bcl-2-associated X protein-driven apoptosis of hepatocytes [36]. Additionally, significant decrease of oxidase metabolite 8-hydroxy-2-deoxyguanosine was observed in CCL4-injured livers 24 h after administration of UC-MSC-Exos, indicating that UC-MSC-Exos-dependent inhibition of oxidative stress in injured hepatocytes was, at least partially, responsible

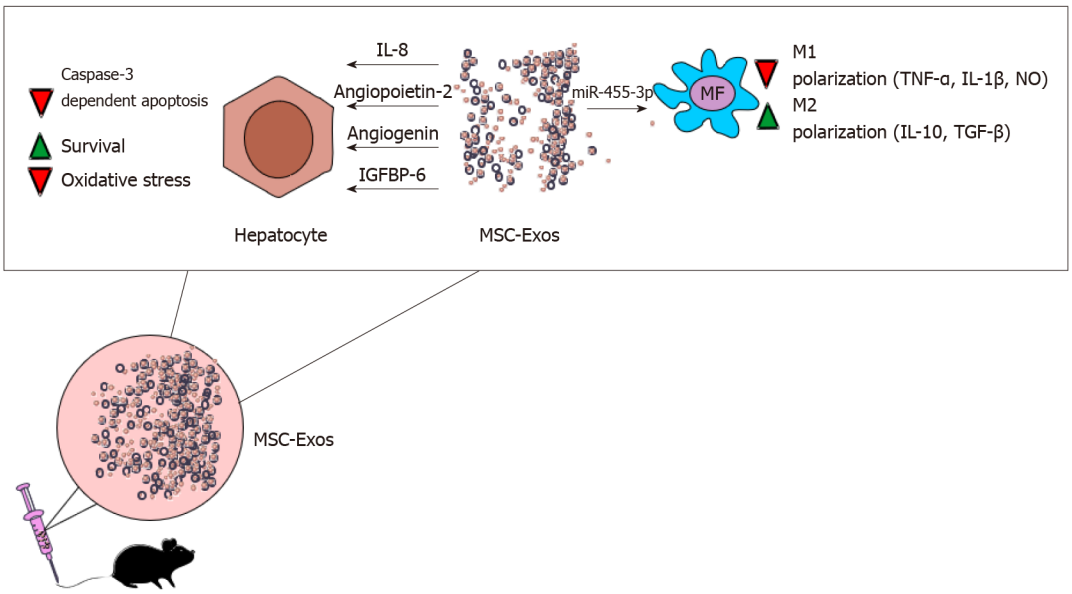

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Figure 2 Molecular mechanisms responsible for the beneficial effects of mesenchymal stem cell derived exosomes in attenuation of acute liver failure. By delivering interleukin (IL)-8, angiopoietin-2, angiogenin, insulin-like growth factor binding protein-6, mesenchymal stem cell derived exosomes (MSC-Exos) attenuate oxidative stress, suppress caspase-3-dependent apoptosis and promoted survival of hepatocytes in acutely injured livers. MSC-Exos, in miRNA-455-3p-dependent manner, alleviate on-going liver inflammation by inhibiting inflammatory, tumor necrosis factor alpha, IL-1β and nitric oxide-producing M1 macrophages and by promoting generation of immunosuppressive, transforming growth factor beta-β and IL-10-producing M2 macrophages. Abbreviations: MSC-Exos: Mesenchymal stem cell derived exosomes; IL: Interleukin; IGFBP6: Insulin like growth factor binding protein 6; TNF-α: Tumor necrosis factor alpha; NO: Nitric oxide.

for the alleviation of ALF in experimental mice[36].

As evidenced by Shao and colleagues, MSC-Exo-sourced miR-455-3p was crucially responsible for MSC-dependent immunosuppression in ALF[37]. MSC-derived miR-455-3p modulated activation of PI3K and attenuated synthesis of inflammatory cytokines IL-6, G-CSF, IL-17, IP-10, and MCP-1 in liver macrophages[37]. Significantly reduced necrosis of hepatocytes, down-regulated serum levels of aspartate aminotransferase, alanine transaminase and bilirubin were observed in CCL4 + miR-455-3p-treated mice. Additionally, miR-455-3p significantly reduced presence of CD68-expressing macrophages in the liver and decreased total number of circulating inflammatory CD14+ CD16+ and CCR2+ CD16+ monocytes in peripheral blood of CCL4-treated animals, indicating that beneficial effects of miR-455-3p was mainly relied on the suppression of inflammatory monocytes/macrophages[37] (Figure 2).

CONCLUSION

MSCs possess potent immunoregulatory, angiomodulatory and hepatoprotective properties[25,38]. Results obtained in experimental studies showed that MSCs and MSC-Exos were able to efficiently attenuate acute liver inflammation and to promote enhanced repair and regeneration of injured liver tissue, suggesting their potential clinical use in the therapy of ALF[25,38].

However, it should be noted that there are several issues that need to be addressed before MSCs and their exosomes could be offered as new remedy in regenerative hepatology. The optimal tissue source, dose, and frequency of MSCs/MSC-Exos should be defined. Up-coming studies should also precisely determine all MSC-sourced immunoregulatory and hepatoprotective factors which are responsible for their beneficial effects. Afterwards, bioengineering of MSCs which will massively produce these bioactive molecules should be performed. It is highly expected that these newly developed MSCs and their exosomes will have significantly enhanced efficacy in in the treatment of ALF.

FOOTNOTES

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REFERENCES

1 **Trefts E**, Gannon M, Wasserman DH. The liver. *Curr Biol* 2017; **27**: R1147-R1151 [PMID: 29112863 DOI: 10.1016/j.cub.2017.09.019]
2 **Stravitz RT**, Lee WM. Acute liver failure. *Lancet* 2019; **394**: 869-881 [PMID: 31498101 DOI: 10.1016/S0140-6736(19)31894-X]
3 **Bower WA**, Johns M, Margolis HS, Williams IT, Bell BP. Population-based surveillance for acute liver failure. *Am J Gastroenterol* 2007; **102**: 2459-2463 [PMID: 17608778 DOI: 10.1111/j.1572-0241.2007.01388.x]
4 **Lemmer P**, Pospiech JC, Canbay A. Liver failure-future challenges and remaining questions. *Ann Transl Med* 2021; **9**: 734 [PMID: 33987432 DOI: 10.21037/atm-20-4968]
5 **Dong V**, Nanchal R, Karvellas CJ. Pathophysiology of Acute Liver Failure. *Nutr Clin Pract* 2020; **35**: 24-29 [PMID: 31840297 DOI: 10.1002/ncp.10459]
6 **Zhang H**, Jiang Z, Zhang L. Dual effect of T helper cell 17 (Th17) and regulatory T cell (Treg) in liver pathological process: From occurrence to end stage of disease. *Int Immunopharmacol* 2019; **69**: 50-59 [PMID: 30669025 DOI: 10.1016/j.intimp.2019.01.005]
7 **Przybyl H**, Grindler J, Lauer D. Unfreezing What's Hot in Liver Transplantation: A Review of Current Trends. *AACN Adv Crit Care* 2022; **33**: 56-67 [PMID: 35259225 DOI: 10.4037/aacnacc2022728]
8 **Biolato M**, Bianco A, Lucchini M, Gasbarrini A, Mirabella M, Grieco A. The Disease-Modifying Therapies of Relapsing-Remitting Multiple Sclerosis and Liver Injury: A Narrative Review. *CNS Drugs* 2021; **35**: 861-880 [PMID: 34319570 DOI: 10.1007/s40263-021-00842-9]
9 **Hu C**, Li L. Improvement of mesenchymal stromal cells and their derivatives for treating acute liver failure. *J Mol Med (Berl)* 2019; **97**: 1065-1084 [PMID: 31197403 DOI: 10.1007/s00109-019-01804-x]
10 **Hu C**, Wu Z, Li L. Mesenchymal stromal cells promote liver regeneration through regulation of immune cells. *Int J Biol Sci* 2020; **16**: 893-903 [PMID: 32071558 DOI: 10.7150/ijbs.39725]
11 **Zhou JH**, Lu X, Yan CL, Sheng XY, Cao HC. Mesenchymal stromal cell-dependent immunoregulation in chemically-induced acute liver failure. *World J Stem Cells* 2021; **13**: 208-220 [PMID: 33815670 DOI: 10.4252/wjsc.v13.i3.208]
12 **Wu R**, Fan X, Wang Y, Shen M, Zheng Y, Zhao S, Yang L. Mesenchymal Stem Cell-Derived Extracellular Vesicles in Liver Immunity and Therapy. *Front Immunol* 2022; **13**: 833878 [PMID: 35309311 DOI: 10.3389/fimmu.2022.833878]
13 **Sang JF**, Shi XL, Han B, Huang T, Huang X, Ren HZ, Ding YT. Intraportal mesenchymal stem cell transplantation prevents acute liver failure through promoting cell proliferation and inhibiting apoptosis. *Hepatobiliary Pancreat Dis Int* 2016; **15**: 602-611 [PMID: 27919849 DOI: 10.1016/s1499-3872(16)60141-8]
14 **Cao H**, Yang J, Yu J, Pan Q, Li J, Zhou P, Li Y, Pan X, Wang Y, Li L. Therapeutic potential of transplanted placental mesenchymal stem cells in treating Chinese miniature pigs with acute liver failure. *BMC Med* 2012; **10**: 56 [PMID: 22673529 DOI: 10.1186/1741-7015-10-56]
15 **Teshima T**, Matsumoto H, Michishita M, Matsuoka A, Shiba M, Nagashima T, Koyama H. Allogenic Adipose Tissue-Derived Mesenchymal Stem Cells Ameliorate Acute Hepatic Injury in Dogs. *Stem Cells Int* 2017; **2017**: 3892514 [PMID: 29445402 DOI: 10.1155/2017/3892514]
16 **Sun L**, Fan X, Zhang L, Shi G, Aili M, Lu X, Jiang T, Zhang Y. Bone mesenchymal stem cell transplantation *via* four routes for the treatment of acute liver failure in rats. *Int J Mol Med* 2014; **34**: 987-996 [PMID: 25110277 DOI: 10.3892/ijmm.2014.1890]
17 **Wang J**, Liu Y, Ding H, Shi X, Ren H. Mesenchymal stem cell-secreted prostaglandin E_2 ameliorates acute liver failure *via* attenuation of cell death and regulation of macrophage polarization. *Stem Cell Res Ther* 2021; **12**: 15 [PMID: 33413632 DOI: 10.1186/s13287-020-02070-2]
18 **Miao CM**, Jiang XW, He K, Li PZ, Liu ZJ, Cao D, Ou ZB, Gong JP, Liu CA, Cheng Y. Bone marrow stromal cells attenuate LPS-induced mouse acute liver injury *via* the prostaglandin E 2-dependent repression of the NLRP3 inflammasome in Kupffer cells. *Immunol Lett* 2016; **179**: 102-113 [PMID: 27666012 DOI: 10.1016/j.imlet.2016.09.009]
19 **Liu J**, Feng B, Xu Y, Zhu J, Feng X, Chen W, Sheng X, Shi X, Pan Q, Yu J, Zeng X, Cao H, Li L. Immunomodulatory effect of mesenchymal stem cells in chemical-induced liver injury: a high-dimensional analysis. *Stem Cell Res Ther* 2019; **10**: 262 [PMID: 31443686 DOI: 10.1186/s13287-019-1379-6]
20 **Hua D**, Ju Z, Gan X, Wang Q, Luo C, Gu J, Yu Y. Human amniotic mesenchymal stromal cells alleviate acute liver injury

by inhibiting the pro-inflammatory response of liver resident macrophage through autophagy. *Ann Transl Med* 2019; **7**: 392 [PMID: 31555706 DOI: 10.21037/atm.2019.08.83]

21 **Zhang Y**, Cai W, Huang Q, Gu Y, Shi Y, Huang J, Zhao F, Liu Q, Wei X, Jin M, Wu C, Xie Q, Zhang Y, Wan B. Mesenchymal stem cells alleviate bacteria-induced liver injury in mice by inducing regulatory dendritic cells. *Hepatology* 2014; **59**: 671-682 [PMID: 23929707 DOI: 10.1002/hep.26670]

22 **Gazdic M**, Markovic BS, Arsenijevic A, Jovicic N, Acovic A, Harrell CR, Fellabaum C, Djonov V, Arsenijevic N, Lukic ML, Volarevic V. Crosstalk between mesenchymal stem cells and T regulatory cells is crucially important for the attenuation of acute liver injury. *Liver Transpl* 2018; **24**: 687-702 [PMID: 29500914 DOI: 10.1002/lt.25049]

23 **Gazdic M**, Simovic Markovic B, Vucicevic L, Nikolic T, Djonov V, Arsenijevic N, Trajkovic V, Lukic ML, Volarevic V. Mesenchymal stem cells protect from acute liver injury by attenuating hepatotoxicity of liver natural killer T cells in an inducible nitric oxide synthase- and indoleamine 2,3-dioxygenase-dependent manner. *J Tissue Eng Regen Med* 2018; **12**: e1173-e1185 [PMID: 28488390 DOI: 10.1002/term.2452]

24 **Milosavljevic N**, Gazdic M, Simovic Markovic B, Arsenijevic A, Nurkovic J, Dolicanin Z, Djonov V, Lukic ML, Volarevic V. Mesenchymal stem cells attenuate acute liver injury by altering ratio between interleukin 17 producing and regulatory natural killer T cells. *Liver Transpl* 2017; **23**: 1040-1050 [PMID: 28481005 DOI: 10.1002/lt.24784]

25 **Gazdic M**, Arsenijevic A, Markovic BS, Volarevic A, Dimova I, Djonov V, Arsenijevic N, Stojkovic M, Volarevic V. Mesenchymal Stem Cell-Dependent Modulation of Liver Diseases. *Int J Biol Sci* 2017; **13**: 1109-1117 [PMID: 29104502 DOI: 10.7150/ijbs.20240]

26 **Musina RA**, Bekchanova ES, Belyavskii AV, Sukhikh GT. Differentiation potential of mesenchymal stem cells of different origin. *Bull Exp Biol Med* 2006; **141**: 147-151 [PMID: 16929987 DOI: 10.1007/s10517-006-0115-2]

27 **Taléns-Visconti R**, Bonora A, Jover R, Mirabet V, Carbonell F, Castell JV, Gómez-Lechón MJ. Hepatogenic differentiation of human mesenchymal stem cells from adipose tissue in comparison with bone marrow mesenchymal stem cells. *World J Gastroenterol* 2006; **12**: 5834-5845 [PMID: 17007050 DOI: 10.3748/wjg.v12.i36.5834]

28 **Sawitza I**, Kordes C, Götze S, Herebian D, Häussinger D. Bile acids induce hepatic differentiation of mesenchymal stem cells. *Sci Rep* 2015; **5**: 13320 [PMID: 26304833 DOI: 10.1038/srep13320]

29 **Chivu M**, Dima SO, Stancu CI, Dobrea C, Uscatescu V, Necula LG, Bleotu C, Tanase C, Albulescu R, Ardeleanu C, Popescu I. In vitro hepatic differentiation of human bone marrow mesenchymal stem cells under differential exposure to liver-specific factors. *Transl Res* 2009; **154**: 122-132 [PMID: 19665688 DOI: 10.1016/j.trsl.2009.05.007]

30 **Manzini BM**, da Silva Santos Duarte A, Sankaramanivel S, Ramos AL, Latuf-Filho P, Escanhoela C, Kharmandayan P, Olalla Saad ST, Boin I, Malheiros Luzo ÂC. Useful properties of undifferentiated mesenchymal stromal cells and adipose tissue as the source in liver-regenerative therapy studied in an animal model of severe acute fulminant hepatitis. *Cytotherapy* 2015; **17**: 1052-1065 [PMID: 26139545 DOI: 10.1016/j.jcyt.2015.04.010]

31 **Volarevic V**, Markovic BS, Gazdic M, Volarevic A, Jovicic N, Arsenijevic N, Armstrong L, Djonov V, Lako M, Stojkovic M. Ethical and Safety Issues of Stem Cell-Based Therapy. *Int J Med Sci* 2018; **15**: 36-45 [PMID: 29333086 DOI: 10.7150/ijms.21666]

32 **Gazdic M**, Volarevic V, Arsenijevic N, Stojkovic M. Mesenchymal stem cells: a friend or foe in immune-mediated diseases. *Stem Cell Rev Rep* 2015; **11**: 280-287 [PMID: 25592610 DOI: 10.1007/s12015-014-9583-3]

33 **Rahman MS**, Akhtar N, Jamil HM, Banik RS, Asaduzzaman SM. TGF-β/BMP signaling and other molecular events: regulation of osteoblastogenesis and bone formation. *Bone Res* 2015; **3**: 15005 [PMID: 26273537 DOI: 10.1038/boneres.2015.5]

34 **Harrell CR**, Jovicic N, Djonov V, Arsenijevic N, Volarevic V. Mesenchymal Stem Cell-Derived Exosomes and Other Extracellular Vesicles as New Remedies in the Therapy of Inflammatory Diseases. *Cells* 2019; **8** [PMID: 31835680 DOI: 10.3390/cells8121605]

35 **Chen L**, Xiang B, Wang X, Xiang C. Exosomes derived from human menstrual blood-derived stem cells alleviate fulminant hepatic failure. *Stem Cell Res Ther* 2017; **8**: 9 [PMID: 28115012 DOI: 10.1186/s13287-016-0453-6]

36 **Jiang W**, Tan Y, Cai M, Zhao T, Mao F, Zhang X, Xu W, Yan Z, Qian H, Yan Y. Human Umbilical Cord MSC-Derived Exosomes Suppress the Development of CCl_4-Induced Liver Injury through Antioxidant Effect. *Stem Cells Int* 2018; **2018**: 6079642 [PMID: 29686713 DOI: 10.1155/2018/6079642]

37 **Shao M**, Xu Q, Wu Z, Chen Y, Shu Y, Cao X, Chen M, Zhang B, Zhou Y, Yao R, Shi Y, Bu H. Exosomes derived from human umbilical cord mesenchymal stem cells ameliorate IL-6-induced acute liver injury through miR-455-3p. *Stem Cell Res Ther* 2020; **11**: 37 [PMID: 31973730 DOI: 10.1186/s13287-020-1550-0]

38 **Harrell CR**, Djonov V, Volarevic V. The Cross-Talk between Mesenchymal Stem Cells and Immune Cells in Tissue Repair and Regeneration. *Int J Mol Sci* 2021; **22** [PMID: 33804369 DOI: 10.3390/ijms22052472]

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MINIREVIEWS

Effective combinations of anti-cancer and targeted drugs for pancreatic cancer treatment

Arata Nishimoto

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Abstract

Pancreatic cancer is highly aggressive and lethal. Due to the lack of effective methods for detecting the disease at an early stage, pancreatic cancer is frequently diagnosed late. Gemcitabine has been the standard chemotherapy drug for patients with pancreatic cancer for over 20 years, but its anti-tumor effect is limited. Therefore, FOLFIRINOX (leucovorin, fluorouracil, irinotecan, oxaliplatin) as well as combination therapies using gemcitabine and conventional agents, such as cisplatin and capecitabine, has also been administered; however, these have not resulted in complete remission. Therefore, there is a need to develop novel and effective therapies for pancreatic cancer. Recently, some studies have reported that combinations of gemcitabine and targeted drugs have had significant anti-tumor effects on pancreatic cancer cells. As gemcitabine induced DNA damage response, the proteins related to DNA damage response can be suitable additional targets for novel gemcitabine-based combination therapy. Furthermore, KRAS/RAF/MEK/ERK signaling triggered by oncogenic mutated *KRAS* and autophagy are frequently activated in pancreatic cancer. Therefore, these characteristics of pancreatic cancer are potential targets for developing effective novel therapies.

In this minireview, combinations of gemcitabine and targeted drugs to these characteristics, combinations of targeted drugs, combinations of natural products and anti-cancer agents, including gemcitabine, and combinations among natural products are discussed.

Key Words: Pancreatic cancer; Gemcitabine; Targeted drug; Combination therapy

Core Tip: Gemcitabine has been the standard chemotherapy drug for patients with pancreatic cancer; however, its effectiveness is limited. Therefore, various combination therapies involving gemcitabine and targeted drugs are being explored. A review of combination therapies based mainly on clinical studies has been published recently; therefore, this minireview focuses on the findings of basic studies and discusses combinations of gemcitabine and targeted drugs, combinations of targeted drugs, combinations of natural products and anti-cancer agents, including gemcitabine, and combinations among natural products.

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INTRODUCTION

Pancreatic cancer is fatal and has a 5-year survival rate of approximate 10%[1]. It is estimated that pancreatic cancer will become the second most common cause of cancer-related deaths in the United States by 2030[2]. Pancreatic cancer is frequently diagnosed at a late stage owing to the lack of effective methods for detecting it at earlier stages and non-specific symptoms. Therefore, there is an urgent need to develop both novel effective therapies for pancreatic cancer that has progressed to a late stage and effective methods for detecting pancreatic cancer at an early stage.

Gemcitabine is the standard treatment for patients with pancreatic cancer. However, as the anti-tumor effect of gemcitabine is limited, FOLFIRINOX (leucovorin, fluorouracil, irinotecan, oxaliplatin) as well as combination therapies of gemcitabine and conventional agents, such as cisplatin and capecitabine, has also been administered[3-5]. Although these combination therapies improved overall survival compared to gemcitabine alone, they did not achieve complete remission. In addition, the incidence of toxicity associated with these combination therapies has increased[3-6].

Recent studies have attempted to identify effective combinations of gemcitabine and targeted drugs for pancreatic cancer. As gemcitabine, a well-known DNA-damaging agent, induced DNA damage response, the proteins related to DNA damage response can be suitable additional targets for novel gemcitabine-based combination therapy. Furthermore, KRAS/RAF/MEK/ERK signaling triggered by oncogenic mutated *KRAS* and autophagy, which are described as the characteristics of pancreatic ductal adenocarcinoma (PDAC), are frequently activated. Therefore, these characteristics are promising targets for effective novel therapeutic strategies[7-12]. An excellent review on effective combination therapies for pancreatic cancer was published recently[13]. This review was based mainly on the findings of preclinical and clinical studies[13]; therefore, this minireview focuses on the findings of basic studies and discusses combinations of gemcitabine and targeted drugs, combinations of targeted drugs, combinations of natural products and anti-cancer agents, including gemcitabine, and combinations among natural products.

COMBINATIONS OF GEMCITABINE AND TARGETED DRUGS

Gemcitabine, a well-known DNA-damaging agent, has been the standard first-line drug for patients with pancreatic cancer. However, the efficacy of gemcitabine in pancreatic cancer is limited and a novel gemcitabine-based combination therapy is required. In this section and Figure 1, combinations of gemcitabine and targeted drugs to enhance the anti-tumor effect are summarized.

Gemcitabine and Chk1 inhibitor

Quinone-methide triterpenoid pristimerin induces lysosomal degradation of checkpoint kinase 1 (Chk1) and augments the expression of γ-H2AX, which is a biomarker of DNA damage following gemcitabine treatment[14]. Furthermore, the combination of gemcitabine and pristimerin was shown to increase apoptosis of pancreatic cancer cells[14]. The Chk1 inhibitor MK-8776 also enhances the sensitivity of multiple human cancer cell lines, including pancreatic cancer cells, to gemcitabine[15]. The DNA damage response mediated by Chk1 in pancreatic cancer stem cells was greater than that in non-pancreatic cancer stem cells, indicating that Chk1 inhibition selectively sensitizes pancreatic cancer stem cells to gemcitabine[16]. The combination of Chk1 inhibition and gemcitabine reduces the ability of tumor initiation in pancreatic cancer stem cells[16]. The anti-tumor effect of the combination of gemcitabine and doublecortin-like kinase 1 (Dclk1) inhibitor has also been reported[17]. The latter significantly decreased the expression of gemcitabine-induced phosphorylated Chk1 in pancreatic cancer cells. The combination of gemcitabine and Dclk1 inhibitor did not arrest the cell cycle at the S

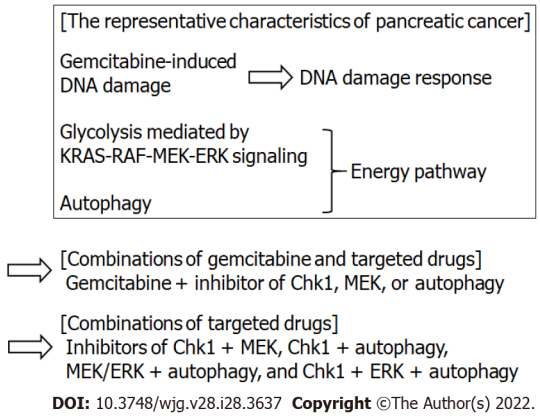

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Figure 1 The representative characteristics of pancreatic cancer as potential targets for effective combination therapy.

phase and allowed cell cycle progression[17]. In addition, the combination of gemcitabine and Dclk1 inhibitor increased the rate of γ-H2AX-positive cells compared to individual treatments. The combination of gemcitabine and Dclk1 inhibitor induced PARP1 cleavage as well as caspase-3 activation and significantly decreased the survival rate of pancreatic cancer cells compared to gemcitabine treatment alone[17].

Gemcitabine and KRAS antibody/MEK inhibitor

Oncogenic *KRAS* mutations are present in approximately 90% of pancreatic cancer cases. Consequently, KRAS and its downstream proteins, such as RAF, MEK, and ERK, are activated in pancreatic cancer cases and contribute to the progression of the disease. Therefore, inhibitors targeting these proteins may be effective in inhibiting this progression.

Antibodies that bind intracellularly to the activated GTP-bound form of oncogenic KRAS mutants have been developed and significantly sensitize pancreatic cancer cells to gemcitabine[18,19]. These antibodies synergistically increase the anti-tumor effect of gemcitabine by inhibiting the RAF/MEK/ERK signaling pathway downstream of KRAS[18,19]. The antibodies are internalized in the cytoplasm by endocytosis through the tumor-associated receptors of extracellular epithelial cell adhesion molecules[19]. These antibodies synergistically increase the anti-tumor effect of gemcitabine by inhibiting KRAS/RAF/MEK/ERK signaling in pancreatic cancer cells[18,19].

The MEK inhibitor, trametinib, both alone and in combination with gemcitabine, was shown to exhibit significantly enhanced anti-tumor effects compared to gemcitabine alone[20]. The combination of gemcitabine and trametinib also increased the inhibition of tumor growth in pancreatic cancer patient-derived orthotopic xenografts in nude mice compared to trametinib alone[20]. Moreover, the combination of the MEK inhibitors, trametinib and cobimetinib, prevented tumor growth in gemcitabine-resistant pancreatic cancer patient-derived orthotopic xenografts in nude mice[21]. These results suggest that such combinations have therapeutic potential against pancreatic cancer.

Gemcitabine and autophagy inhibitor

Gemcitabine has significantly been shown to increase autophagy induction in human pancreatic cancer cells, and combined treatment with gemcitabine and chloroquine, an autophagy inhibitor, triggered a marked boost in reactive oxygen species (ROS) levels and increased lysosomal membrane permeability [22]. Consequently, proteases, including cathepsins, are released from lysosomes into the cytoplasm, leading to apoptosis. Thus, the combination of gemcitabine and chloroquine has an anti-tumor effect on pancreatic cancer cells through the apoptotic pathway by lysosomal dysfunction *via* a marked boost of ROS[22]. Cancer stem cells are considered to be responsible for the recurrence and chemoresistance of cancer. The expression of the markers of cancer stem cells, aldehyde dehydrogenase 1, CD44, and CD133, was found to be positively correlated with the expression of LC3 type II, an autophagy marker, in pancreatic cancer tissues[23]; this suggests an association between autophagy and cancer stem cells. Indeed, autophagy inhibition decreased the activity of sphere formation of pancreatic cancer stem cells, and gemcitabine and autophagy inhibition markedly reduced the populations of cancer stem cells[23].

COMBINATIONS OF TARGETED DRUGS

Recent studies have demonstrated that combinations of targeted drugs have potential for developing novel and effective therapy for pancreatic cancer. In this section and Figure 1, combinations of targeted drugs for PDAC therapy are summarized.

ERK and autophagy inhibitors

KRAS suppression or ERK inhibition was shown to decrease both glycolytic and mitochondrial functions and to increase autophagic flux in PDAC, suggesting that ERK inhibition enhances dependence on autophagy[24]. The combination of ERK and autophagy inhibitors synergistically enhanced anti-tumor activity in KRAS-driven PDAC *via* the dysfunction of the energy pathways consisting of glycolysis and autophagy[24]. It has been reported that inhibition of the KRAS/RAF/MEK/ERK signaling pathway elicits autophagy, resulting in protection of PDAC cells from cytotoxic effects[25]. The combination of MEK1/2 and autophagy inhibitors showed synergistic anti-tumor effects against PDAC cells *in vitro* and promoted the regression of patient-derived xenografts of PDAC in mice[25]. Furthermore, the effect of the combination of trametinib and chloroquine was not limited to PDAC and resulted in similar responses in patient-derived xenografts of *BRAF*-mutated colorectal cancer and *NRAS*-mutated melanoma[25].

ERK, Chk1, and autophagy inhibitors

Screening of druggable genes by genetic loss-of-function using CRISPR-Cas9 and small interfering RNA revealed that components of the ATR-Chk1 DNA damage response pathway were modulators of sensitivity to ERK inhibitor treatment in *KRAS-mutant* PDAC[26]. Chk1 inhibition suppressed the growth of both PDAC cell lines and organoids and activated ERK signaling and autophagy, suggesting that Chk1 inhibition enhances dependence on ERK signaling and autophagy[26]. These findings provide a mechanistic basis for the effectiveness of the inhibition of Chk1, ERK, and autophagy. Indeed, this triple combination of inhibitors synergistically enhanced the anti-tumor effect in *KRAS-mutant* PDAC [26].

2-deoxyglucose and MEK inhibitor

Pooled shRNA library screening was used in an orthotopic xenograft model to identify multiple glycolysis genes as potential targets that may sensitize PDAC cells to MEK inhibition[27]. Apoptosis in KrasG12D-driven PDAC cells was synergistically induced, *in vitro*, *via* the combination of 2-deoxyglucose, a glycolysis inhibitor, and a MEK inhibitor; the same also inhibited tumor growth of PDAC xenografts, leading to prolonged overall survival in a genetically engineered PDAC mouse model[27]. Molecular and metabolic analyses revealed that the combined inhibition of glycolysis and ERK signaling synergistically caused apoptosis by inducing lethal stress in the endoplasmic reticulum. These results indicate that the combination of 2-deoxyglucose and a MEK inhibitor may be an effective approach for targeting KRAS-driven PDAC[27].

Replication stress response and autophagy inhibitors

PDAC exhibits high basal lysosomal activity and relies on lysosome-dependent recycling pathways, such as autophagy, to generate substrates for metabolism[28]. Kinase inhibitor screening revealed that the replication stress response inhibitor and chloroquine, an autophagy inhibitor that works *via* the functional inhibition of lysosomes, were synthetically lethal in PDAC cells[28]. Chloroquine induces replication stress due to aspartate depletion, and a replication stress response inhibitor and chloroquine synergistically inhibit tumor growth in PDAC[28].

Immune checkpoint and autophagy inhibitors

Major histocompatibility complex class I (MHC-I) molecules are selectively degraded *via* autophagy in PDAC[29]. Consequently, the expression of MHC-I at the cell surface is reduced, and MHC-I is localized predominantly within autophagosomes and lysosomes. Autophagy inhibition was shown to restore the expression of MHC-I at the cell surface and improve antigen presentation[29]. Furthermore, autophagy inhibition synergizes with dual immune checkpoint blockade therapy (anti-PD1 and anti-CTLA4 antibodies) to enhance the anti-tumor immune response and reduce tumor growth in syngeneic host mice[29].

COMBINATIONS OF NATURAL PRODUCTS AND ANTI-CANCER AGENTS, INCLUDING GEMCITABINE

The use of natural products as adjunctive therapies for pancreatic cancer has a great potential due to the anti-cancer efficacy and low toxicity. Yue *et al*[30] summarized combinations of natural products and anti-cancer agents, including gemcitabine, and combinations among natural products. They focused on the following: combinations of natural products and gemcitabine (for example, cucurbitacin B and gemcitabine[31], glaucarubinone and gemcitabine[32], escin and gemcitabine[33], and gum mastic and gemcitabine[34]); combinations of natural products and other agents, such as all-trans retinoic acid and sulindac (for example, 12-O-tetradecanoylphorbol-13-acetate and all-trans retinoic acid[35], parthenolide and sulindac[36], and triptolide and hydroxycamptothecin[37]); and combinations among natural products (for example, sulforaphane and quercetin[38], wogonin, apigenin, and chrysin[39], and

metformin and aspirin[40]).

While agents from purified chemical compounds generally target single molecules, natural products mostly consist of multiple components that concurrently act on various molecular targets. Therefore, natural products are expected to have various functions, including improvement of anti-cancer efficacy, enhancement of immune system, and reduction of side effects[41].

CONCLUSION

In general, it is widely accepted that combination therapy is more effective than monotherapy. In this minireview, combinations of gemcitabine and targeted drugs, combinations of targeted drugs, combinations of natural products and anti-cancer agents, including gemcitabine, and combinations among natural products are described. Hereafter, preclinical and clinical studies are needed to examine the possibility for clinical applications. Concurrently, additional basic studies that attempt to identify combinations that synergize anti-cancer effects are needed to find the better treatment options.

FOOTNOTES

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REFERENCES

1 **Siegel RL**, Miller KD, Fuchs HE, Jemal A. Cancer statistics, 2022. *CA Cancer J Clin* 2022; **72**: 7-33 [PMID: 35020204 DOI: 10.3322/caac.21708]

2 **Rahib L**, Smith BD, Aizenberg R, Rosenzweig AB, Fleshman JM, Matrisian LM. Projecting cancer incidence and deaths to 2030: the unexpected burden of thyroid, liver, and pancreas cancers in the United States. *Cancer Res* 2014; **74**: 2913-2921 [PMID: 24840647 DOI: 10.1158/0008-5472.CAN-14-0155]

3 **Neoptolemos JP**, Palmer DH, Ghaneh P, Psarelli EE, Valle JW, Halloran CM, Faluyi O, O'Reilly DA, Cunningham D, Wadsley J, Darby S, Meyer T, Gillmore R, Anthoney A, Lind P, Glimelius B, Falk S, Izbicki JR, Middleton GW, Cummins S, Ross PJ, Wasan H, McDonald A, Crosby T, Ma YT, Patel K, Sherriff D, Soomal R, Borg D, Sothi S, Hammel P, Hackert T, Jackson R, Büchler MW; European Study Group for Pancreatic Cancer. Comparison of adjuvant gemcitabine and capecitabine with gemcitabine monotherapy in patients with resected pancreatic cancer (ESPAC-4): a multicentre, open-label, randomised, phase 3 trial. *Lancet* 2017; **389**: 1011-1024 [PMID: 28129987 DOI: 10.1016/S0140-6736(16)32409-6]

4 **Ouyang G**, Liu Z, Huang S, Li Q, Xiong L, Miao X, Wen Y. Gemcitabine plus cisplatin versus gemcitabine alone in the treatment of pancreatic cancer: a meta-analysis. *World J Surg Oncol* 2016; **14**: 59 [PMID: 26927942 DOI: 10.1186/s12957-016-0813-9]

5 **Conroy T**, Desseigne F, Ychou M, Bouché O, Guimbaud R, Bécouarn Y, Adenis A, Raoul JL, Gourgou-Bourgade S, de la Fouchardière C, Bennouna J, Bachet JB, Khemissa-Akouz F, Péré-Vergé D, Delbaldo C, Assenat E, Chauffert B, Michel P, Montoto-Grillot C, Ducreux M; Groupe Tumeurs Digestives of Unicancer; PRODIGE Intergroup. FOLFIRINOX versus gemcitabine for metastatic pancreatic cancer. *N Engl J Med* 2011; **364**: 1817-1825 [PMID: 21561347 DOI: 10.1056/NEJMoa1011923]

6 **Peixoto RD**, Ho M, Renouf DJ, Lim HJ, Gill S, Ruan JY, Cheung WY. Eligibility of Metastatic Pancreatic Cancer Patients for First-Line Palliative Intent nab-Paclitaxel Plus Gemcitabine Versus FOLFIRINOX. *Am J Clin Oncol* 2017; **40**: 507-511 [PMID: 25844823 DOI: 10.1097/COC.0000000000000193]

7 **Waters AM**, Der CJ. KRAS: The Critical Driver and Therapeutic Target for Pancreatic Cancer. *Cold Spring Harb Perspect Med* 2018; **8** [PMID: 29229669 DOI: 10.1101/cshperspect.a031435]

8 **Bannoura SF**, Uddin MH, Nagasaka M, Fazili F, Al-Hallak MN, Philip PA, El-Rayes B, Azmi AS. Targeting KRAS in pancreatic cancer: new drugs on the horizon. *Cancer Metastasis Rev* 2021; **40**: 819-835 [PMID: 34499267 DOI:

10.1007/s10555-021-09990-2]
9 **Mann KM**, Ying H, Juan J, Jenkins NA, Copeland NG. KRAS-related proteins in pancreatic cancer. *Pharmacol Ther* 2016; **168**: 29-42 [PMID: 27595930 DOI: 10.1016/j.pharmthera.2016.09.003]
10 **Neuzillet C**, Tijeras-Raballand A, de Mestier L, Cros J, Faivre S, Raymond E. MEK in cancer and cancer therapy. *Pharmacol Ther* 2014; **141**: 160-171 [PMID: 24121058 DOI: 10.1016/j.pharmthera.2013.10.001]
11 **Raufi AG**, Liguori NR, Carlsen L, Parker C, Hernandez Borrero L, Zhang S, Tian X, Louie A, Zhou L, Seyhan AA, El-Deiry WS. Therapeutic Targeting of Autophagy in Pancreatic Ductal Adenocarcinoma. *Front Pharmacol* 2021; **12**: 751568 [PMID: 34916936 DOI: 10.3389/fphar.2021.751568]
12 **Piffoux M**, Eriau E, Cassier PA. Autophagy as a therapeutic target in pancreatic cancer. *Br J Cancer* 2021; **124**: 333-344 [PMID: 32929194 DOI: 10.1038/s41416-020-01039-5]
13 **Miller AL**, Garcia PL, Yoon KJ. Developing effective combination therapy for pancreatic cancer: An overview. *Pharmacol Res* 2020; **155**: 104740 [PMID: 32135247 DOI: 10.1016/j.phrs.2020.104740]
14 **Jiang Z**, Zhao Y, Liu Y, Tao L. Pristimerin synergizes with gemcitabine through abrogating Chk1/53BP1-mediated DNA repair in pancreatic cancer cells. *Food Chem Toxicol* 2021; **147**: 111919 [PMID: 33301843 DOI: 10.1016/j.fct.2020.111919]
15 **Engelke CG**, Parsels LA, Qian Y, Zhang Q, Karnak D, Robertson JR, Tanska DM, Wei D, Davis MA, Parsels JD, Zhao L, Greenson JK, Lawrence TS, Maybaum J, Morgan MA. Sensitization of pancreatic cancer to chemoradiation by the Chk1 inhibitor MK8776. *Clin Cancer Res* 2013; **19**: 4412-4421 [PMID: 23804422 DOI: 10.1158/1078-0432.CCR-12-3748]
16 **Venkatesha VA**, Parsels LA, Parsels JD, Zhao L, Zabludoff SD, Simeone DM, Maybaum J, Lawrence TS, Morgan MA. Sensitization of pancreatic cancer stem cells to gemcitabine by Chk1 inhibition. *Neoplasia* 2012; **14**: 519-525 [PMID: 22787433 DOI: 10.1593/neo.12538]
17 **Kawamura D**, Takemoto Y, Nishimoto A, Ueno K, Hosoyama T, Shirasawa B, Tanaka T, Kugimiya N, Harada E, Hamano K. Enhancement of cytotoxic effects of gemcitabine by Dclk1 inhibition through suppression of Chk1 phosphorylation in human pancreatic cancer cells. *Oncol Rep* 2017; **38**: 3238-3244 [PMID: 29048622 DOI: 10.3892/or.2017.5974]
18 **Kang YW**, Lee JE, Jung KH, Son MK, Shin SM, Kim SJ, Fang Z, Yan HH, Park JH, Han B, Cheon MJ, Woo MG, Lim JH, Kim YS, Hong SS. KRAS targeting antibody synergizes anti-cancer activity of gemcitabine against pancreatic cancer. *Cancer Lett* 2018; **438**: 174-186 [PMID: 30217561 DOI: 10.1016/j.canlet.2018.09.013]
19 **Lee JE**, Kang YW, Jung KH, Son MK, Shin SM, Kim JS, Kim SJ, Fang Z, Yan HH, Park JH, Yoon YC, Han B, Cheon MJ, Woo MG, Seo MS, Lim JH, Kim YS, Hong SS. Intracellular KRAS-specific antibody enhances the anti-tumor efficacy of gemcitabine in pancreatic cancer by inducing endosomal escape. *Cancer Lett* 2021; **507**: 97-111 [PMID: 33744388 DOI: 10.1016/j.canlet.2021.03.015]
20 **Kawaguchi K**, Igarashi K, Miyake K, Lwin TM, Miyake M, Kiyuna T, Hwang HK, Murakami T, Delong JC, Singh SR, Clary B, Bouvet M, Unno M, Hoffman RM. MEK inhibitor trametinib in combination with gemcitabine regresses a patient-derived orthotopic xenograft (PDOX) pancreatic cancer nude mouse model. *Tissue Cell* 2018; **52**: 124-128 [PMID: 29857821 DOI: 10.1016/j.tice.2018.05.003]
21 **Kawaguchi K**, Igarashi K, Murakami T, Kiyuna T, Lwin TM, Hwang HK, Delong JC, Clary BM, Bouvet M, Unno M, Hoffman RM. MEK inhibitors cobimetinib and trametinib, regressed a gemcitabine-resistant pancreatic-cancer patient-derived orthotopic xenograft (PDOX). *Oncotarget* 2017; **8**: 47490-47496 [PMID: 28537897 DOI: 10.18632/oncotarget.17667]
22 **Fu Z**, Cheng X, Kuang J, Feng H, Chen L, Liang J, Shen X, Yuen S, Peng C, Shen B, Jin Z, Qiu W. CQ sensitizes human pancreatic cancer cells to gemcitabine through the lysosomal apoptotic pathway *via* reactive oxygen species. *Mol Oncol* 2018; **12**: 529-544 [PMID: 29453806 DOI: 10.1002/1878-0261.12179]
23 **Yang MC**, Wang HC, Hou YC, Tung HL, Chiu TJ, Shan YS. Blockade of autophagy reduces pancreatic cancer stem cell activity and potentiates the tumoricidal effect of gemcitabine. *Mol Cancer* 2015; **14**: 179 [PMID: 26458814 DOI: 10.1186/s12943-015-0449-3]
24 **Bryant KL**, Stalnecker CA, Zeitouni D, Klomp JE, Peng S, Tikunov AP, Gunda V, Pierobon M, Waters AM, George SD, Tomar G, Papke B, Hobbs GA, Yan L, Hayes TK, Diehl JN, Goode GD, Chaika NV, Wang Y, Zhang GF, Witkiewicz AK, Knudsen ES, Petricoin EF 3rd, Singh PK, Macdonald JM, Tran NL, Lyssiotis CA, Ying H, Kimmelman AC, Cox AD, Der CJ. Combination of ERK and autophagy inhibition as a treatment approach for pancreatic cancer. *Nat Med* 2019; **25**: 628-640 [PMID: 30833752 DOI: 10.1038/s41591-019-0368-8]
25 **Kinsey CG**, Camolotto SA, Boespflug AM, Guillen KP, Foth M, Truong A, Schuman SS, Shea JE, Seipp MT, Yap JT, Burrell LD, Lum DH, Whisenant JR, Gilcrease GW 3rd, Cavalieri CC, Rehbein KM, Cutler SL, Affolter KE, Welm AL, Welm BE, Scaife CL, Snyder EL, McMahon M. Protective autophagy elicited by RAF→MEK→ERK inhibition suggests a treatment strategy for RAS-driven cancers. *Nat Med* 2019; **25**: 620-627 [PMID: 30833748 DOI: 10.1038/s41591-019-0367-9]
26 **Klomp JE**, Lee YS, Goodwin CM, Papke B, Klomp JA, Waters AM, Stalnecker CA, DeLiberty JM, Drizyte-Miller K, Yang R, Diehl JN, Yin HH, Pierobon M, Baldelli E, Ryan MB, Li S, Peterson J, Smith AR, Neal JT, McCormick AK, Kuo CJ, Counter CM, Petricoin EF 3rd, Cox AD, Bryant KL, Der CJ. CHK1 protects oncogenic KRAS-expressing cells from DNA damage and is a target for pancreatic cancer treatment. *Cell Rep* 2021; **37**: 110060 [PMID: 34852220 DOI: 10.1016/j.celrep.2021.110060]
27 **Yan L**, Tu B, Yao J, Gong J, Carugo A, Bristow CA, Wang Q, Zhu C, Dai B, Kang Y, Han L, Feng N, Jin Y, Fleming J, Heffernan TP, Yao W, Ying H. Targeting Glucose Metabolism Sensitizes Pancreatic Cancer to MEK Inhibition. *Cancer Res* 2021; **81**: 4054-4065 [PMID: 34117030 DOI: 10.1158/0008-5472.CAN-20-3792]
28 **Elliott IA**, Dann AM, Xu S, Kim SS, Abt ER, Kim W, Poddar S, Moore A, Zhou L, Williams JL, Capri JR, Ghukasyan R, Matsumura C, Tucker DA, Armstrong WR, Cabebe AE, Wu N, Li L, Le TM, Radu CG, Donahue TR. Lysosome inhibition sensitizes pancreatic cancer to replication stress by aspartate depletion. *Proc Natl Acad Sci U S A* 2019; **116**: 6842-6847 [PMID: 30894490 DOI: 10.1073/pnas.1812410116]
29 **Yamamoto K**, Venida A, Yano J, Biancur DE, Kakiuchi M, Gupta S, Sohn ASW, Mukhopadhyay S, Lin EY, Parker SJ,

Banh RS, Paulo JA, Wen KW, Debnath J, Kim GE, Mancias JD, Fearon DT, Perera RM, Kimmelman AC. Autophagy promotes immune evasion of pancreatic cancer by degrading MHC-I. *Nature* 2020; **581**: 100-105 [PMID: 32376951 DOI: 10.1038/s41586-020-2229-5]

30 **Yue Q**, Gao G, Zou G, Yu H, Zheng X. Natural Products as Adjunctive Treatment for Pancreatic Cancer: Recent Trends and Advancements. *Biomed Res Int* 2017; **2017**: 8412508 [PMID: 28232946 DOI: 10.1155/2017/8412508]

31 **Thoennissen NH**, Iwanski GB, Doan NB, Okamoto R, Lin P, Abbassi S, Song JH, Yin D, Toh M, Xie WD, Said JW, Koeffler HP. Cucurbitacin B induces apoptosis by inhibition of the JAK/STAT pathway and potentiates antiproliferative effects of gemcitabine on pancreatic cancer cells. *Cancer Res* 2009; **69**: 5876-5884 [PMID: 19605406 DOI: 10.1158/0008-5472.CAN-09-0536]

32 **Yeo D**, Huynh N, Beutler JA, Christophi C, Shulkes A, Baldwin GS, Nikfarjam M, He H. Glaucarubinone and gemcitabine synergistically reduce pancreatic cancer growth *via* down-regulation of P21-activated kinases. *Cancer Lett* 2014; **346**: 264-272 [PMID: 24491405 DOI: 10.1016/j.canlet.2014.01.001]

33 **Wang YW**, Wang SJ, Zhou YN, Pan SH, Sun B. Escin augments the efficacy of gemcitabine through down-regulation of nuclear factor-κB and nuclear factor-κB-regulated gene products in pancreatic cancer both in vitro and in vivo. *J Cancer Res Clin Oncol* 2012; **138**: 785-797 [PMID: 22270965 DOI: 10.1007/s00432-012-1152-z]

34 **Huang XY**, Wang HC, Yuan Z, Li A, He ML, Ai KX, Zheng Q, Qin HL. Gemcitabine combined with gum mastic causes potent growth inhibition and apoptosis of pancreatic cancer cells. *Acta Pharmacol Sin* 2010; **31**: 741-745 [PMID: 20523344 DOI: 10.1038/aps.2010.54]

35 **Avila GE**, Zheng X, Cui XX, Ryan AD, Hansson A, Suh J, Rabson AB, Chang RL, Shih WJ, Lin Y, Crowell P, Lu YP, Lou YR, Conney AH. Inhibitory effects of 12-O-tetradecanoylphorbol-13-acetate alone or in combination with all-trans retinoic acid on the growth of cultured human pancreas cancer cells and pancreas tumor xenografts in immunodeficient mice. *J Pharmacol Exp Ther* 2005; **315**: 170-187 [PMID: 15976015 DOI: 10.1124/jpet.105.087585]

36 **Yip-Schneider MT**, Nakshatri H, Sweeney CJ, Marshall MS, Wiebke EA, Schmidt CM. Parthenolide and sulindac cooperate to mediate growth suppression and inhibit the nuclear factor-kappa B pathway in pancreatic carcinoma cells. *Mol Cancer Ther* 2005; **4**: 587-594 [PMID: 15827332 DOI: 10.1158/1535-7163.MCT-04-0215]

37 **Yang SW**, Wang W, Xie XY, Zhu WP, Li FQ. In vitro synergistic cytotoxic effect of triptolide combined with hydroxycamptothecin on pancreatic cancer cells. *Am J Chin Med* 2011; **39**: 121-134 [PMID: 21213403 DOI: 10.1142/S0192415X11008695]

38 **Srivastava RK**, Tang SN, Zhu W, Meeker D, Shankar S. Sulforaphane synergizes with quercetin to inhibit self-renewal capacity of pancreatic cancer stem cells. *Front Biosci (Elite Ed)* 2011; **3**: 515-528 [PMID: 21196331 DOI: 10.2741/e266]

39 **Ding J**, Polier G, Köhler R, Giaisi M, Krammer PH, Li-Weber M. Wogonin and related natural flavones overcome tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) protein resistance of tumors by down-regulation of c-FLIP protein and up-regulation of TRAIL receptor 2 expression. *J Biol Chem* 2012; **287**: 641-649 [PMID: 22086925 DOI: 10.1074/jbc.M111.286526]

40 **Yue W**, Zheng X, Lin Y, Yang CS, Xu Q, Carpizo D, Huang H, DiPaola RS, Tan XL. Metformin combined with aspirin significantly inhibit pancreatic cancer cell growth in vitro and in vivo by suppressing anti-apoptotic proteins Mcl-1 and Bcl-2. *Oncotarget* 2015; **6**: 21208-21224 [PMID: 26056043 DOI: 10.18632/oncotarget.4126]

41 **Li L**, Leung PS. Use of herbal medicines and natural products: an alternative approach to overcoming the apoptotic resistance of pancreatic cancer. *Int J Biochem Cell Biol* 2014; **53**: 224-236 [PMID: 24875648 DOI: 10.1016/j.biocel.2014.05.021]

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ORIGINAL ARTICLE

Basic Study

Mechanism of electroacupuncture and herb-partitioned moxibustion on ulcerative colitis animal model: A study based on proteomics

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Abstract

BACKGROUND

Ulcerative colitis (UC) is a chronic, nonspecific intestinal inflammatory disease. Acupuncture and moxibustion is proved effective in treating UC, but the mechanism has not been clarified. Proteomic technology has revealed a variety of biological markers related to immunity and inflammation in UC, which provide new insights and directions for the study of mechanism of acupuncture and moxibustion treatment of UC.

AIM

To investigate the mechanism of electroacupuncture (EA) and herb-partitioned moxibustion (HM) on UC rats by using proteomics technology.

METHODS

Male Sprague-Dawley rats were randomly divided into the normal (N) group, the dextran sulfate sodium (DSS)-induced UC model (M) group, the HM group, and the EA group. UC rat model was prepared with 3% DSS, and HM and EA interventions at the bilateral Tianshu and Qihai acupoints were performed in HM or EA group. Haematoxylin and eosin staining was used for morphological evaluation of colon tissues. Isotope-labeled relative and absolute quantification (iTRAQ) and liquid chromatography-tandem mass spectrometry were performed for proteome analysis of the colon tissues, followed by bioinformatics analysis and protein-protein interaction networks establishment of differentially expressed

proteins (DEPs) between groups. Then western blot was used for verification of selected DEPs.

RESULTS

The macroscopic colon injury scores and histopathology scores in the HM and EA groups were significantly decreased compared to the rats in the M group ($P < 0.01$). Compared with the N group, a total of 202 DEPs were identified in the M group, including 111 up-regulated proteins and 91 down-regulated proteins, of which 25 and 15 proteins were reversed after HM and EA interventions, respectively. The DEPs were involved in various biological processes such as biological regulation, immune system progression and in multiple pathways including natural killer cell mediated cytotoxicity, intestinal immune network for immunoglobulin A (IgA) production, and FcγR-mediated phagocytosis. The Kyoto Encyclopedia of Genes and Genomes pathways of DEPs between HM and M groups, EA and M groups both included immune-associated and oxidative phosphorylation. Network analysis revealed that multiple pathways for the DEPs of each group were involved in protein-protein interactions, and the expression of oxidative phosphorylation pathway-related proteins, including ATP synthase subunit g (ATP5L), ATP synthase beta subunit precursor (Atp5f), cytochrome c oxidase subunit 4 isoform 1 (Cox4i1) were down-regulated after HM and EA interventions. Subsequent verification of selected DEPs (Synaptic vesicle glycoprotein 2A; nuclear cap binding protein subunit 1; carbamoyl phosphate synthetase 1; Cox4i1; ATP synthase subunit b, Atp5f1; doublecortin like kinase 3) by western blot confirmed the reliability of the iTRAQ data, HM and EA interventions can significantly down-regulate the expression of oxidative phosphorylation-associated proteins (Cox4i1, Atp5f1) ($P < 0.01$).

CONCLUSION

EA and HM could regulate the expression of ATP5L, Atp5f1, Cox4i1 that associated with oxidative phosphorylation, then might regulate immune-related pathways of intestinal immune network for IgA production, FcγR-mediated phagocytosis, thereby alleviating colonic inflammation of DSS-induced UC rats.

Key Words: Proteomics; Ulcerative colitis; Moxibustion; Electroacupuncture; Differential proteins

Core Tip: Ulcerative colitis (UC) is a nonspecific inflammatory bowel disease with unclear etiology. Acupuncture and moxibustion are benefit to UC by improving colonic mucosa damage, regulating inflammatory cytokines. In recent years, proteomic technology has been widely used in the study of UC, revealing a variety of biological markers related to immunity and inflammation in UC. We applied isotope-labeled relative and absolute quantification proteomics technology to identify UC-relevant protein targets and further explore the mechanism of acupuncture and moxibustion. It was found that electroacupuncture and herb-partitioned moxibustion could regulate the expression of multiple proteins, such as ATP synthase subunit g, ATP synthase beta subunit precursor 1, cytochrome c oxidase subunit 4 isoform 1 that associated with oxidative phosphorylation, that might regulate immune-related pathways, thereby alleviating colonic inflammation of dextran sulfate sodium-induced UC rats.

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INTRODUCTION

Ulcerative colitis (UC) is a chronic, nonspecific inflammatory disease, with lesions are mainly confined to the large intestinal mucosa and submucosal layer. Mucosal inflammation at the affected site is characterized by a diffuse distribution and its clinical manifestations mainly include abdominal pain, diarrhoea, mucus pus and blood in stool[1]. UC has a prolonged disease course, which can lead to the development of colorectal cancer, accounting for 10% to 15% of deaths among patients with UC, and is listed as one of the refractory diseases by the World Health Organization. Epidemiological data show that the prevalence of UC in Asia has increased exponentially in recent years, and the growth rate of UC

in some East Asian countries including China has increased in multiples over the past decade[2,3].

The pathogenesis of UC is not yet clear, but it is believed to be related to genetic susceptibility, epithelial barrier deficiency, immune disorders, and environmental factors[4]. The modern treatment methods for UC mainly include glucocorticoids, immunosuppressants, 5-aminosalicylic acid, and biological agents (monoclonal antibodies), but it is prone to relapse after drug withdrawal, and multiple drugs have adverse side effects. Therefore, active exploration of the pathogenesis of UC, identification of disease biomarkers, and screening of specific therapeutic targets are important for the diagnosis and treatment of UC. As one of the unique treatments of traditional Chinese medicine, acupuncture and moxibustion have significant efficacy and advantages in the treatment of UC[5-7], but the specific mechanism requires further clarification.

In recent years, proteomic technology has been widely used in the study of UC[8,9], revealing a variety of biological markers related to immunity and inflammation in UC[10]. The activity of mitochondrial oxidative phosphorylation complex in intestinal tissues of UC patients decreases, and the dysfunction of mitochondrial oxidative phosphorylation may be involved in the pathogenesis of UC, but it is not clear whether acupuncture and moxibustion have a regulatory effect on it. Therefore, our study used isotope-labeled relative and absolute quantification (iTRAQ) proteomics technology to quantitatively analyse proteins in the colon tissues of UC rats, to analyse the biological functions of differentially expressed proteins (DEPs) and observe changes of immune and oxidative phosphorylation-associated protein expression profiles in colon tissues of UC rats and regulatory effect of acupuncture and moxibustion, in order to identify UC-relevant protein targets and further explore the mechanism of effects of acupuncture and moxibustion on UC.

MATERIALS AND METHODS

Animals

Healthy clean grade naïve male Sprague-Dawley (SD) rats, aged 6 wk, with body weight of 180 ± 20 g were purchased from Shanghai Slac Laboratory Animal Co., Ltd. [Laboratory Animal Use Permit No. SYXK (Shanghai) 2014-0008] and housed in the Experimental Animal Center of Shanghai University of Traditional Chinese Medicine. The animal protocol was designed to minimize pain or discomfort to the animals. The housing environment was a 12 h circadian rhythm, with a room temperature of 20 ± 2 °C and 50%-70% indoor humidity, 4 rats per cage, and rats were free to standard food and pure water. This study was approved by the Ethics Committee of the Experimental Animal Center of Shanghai University of Traditional Chinese Medicine. We tried all efforts to minimize animal suffering.

Dextran sulfate sodium-induced UC model preparation

For preparing the UC rat model, the rats received 3% dextran sulfate sodium (DSS, molecular weight: 36000-50000, MP Biomedicals, United States) in drinking water by oral for 7 d, and then were switched to pure water for 7 d, and the same procedures were repeated once (drinking 3% DSS solution for 7 d, followed by drinking pure water for 7 d)[11]. After modeling, rats were anaesthetized *via* intraperitoneal injection with 2% pentobarbital sodium (30-40 mg/kg) (P3761, Sigma, United States) for tissue collection, the gross injury and hematoxylin and eosin (HE) staining were used to evaluate whether the model was successfully established.

Grouping and interventions

After one week of adaptive feeding, 32 rats were randomly divided into the normal (N) group, the DSS-induced UC model (M) group, the herb-partitioned moxibustion (HM) group, and the electroacupuncture (EA) group, with 8 rats in each group. UC rat model was prepared by DSS except for the N group. After model establishment, the rats in the HM and EA group were treated with corresponding treatment at Tianshu (ST25, bilateral) and Qihai (CV6) acupoints[12]. In the HM group, Chinese medicine powder containing Coptis chinensis, Radix aconiti lateralis, Cortex Cinnamomi, Radix aucklandiae, Flos carthami, Salvia miltiorrhiza, and Angelica sinensis was mixed and stirred with yellow wine to make herbal cake, then an herbal cake was prepared with a thickness of 0.5 cm and a diameter of 1 cm using a specific mould, and conical moxa cones weighing approximately 90 mg were made with moxa (Nanyang Hanyi Moxa Co., Ltd., Nanyang, China) by a mould. The rats were supine immobilized on a self-made fixator, the prepared moxa cone was placed on the top of herbal cake, and the herbal cake was placed at the ST25 and CV6 acupoints, then the moxa cone was ignited using line incense, 2 moxa cones per acupoint per time, once a day for 7 d (Figure 1)[13]. In the EA group, the rats were also supine immobilized on the self-made fixator, a 0.25 mm × 25 mm acupuncture needle was inserted into the ST25 and CV6 acupoints for 5 mm, then the needle handle was connected to a Han's-200 acupoint nerve stimulator, with a frequency of 2/100 Hz and a current of 1 mA. The needle was kept for 10 min per time, once a day for a total of 7 d[14]. The rats in the N group and the M group did not receive any treatment, but only the same grasping fixation as that in the treatment groups at 10 o'clock every day for a total of 7 d.

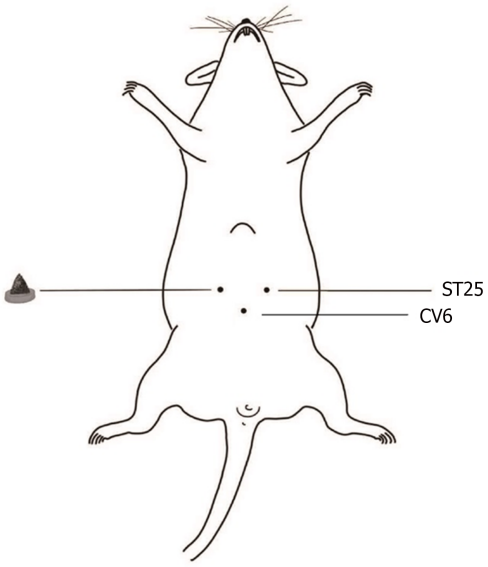


Figure 1 Schematic diagram of herb-partitioned moxibustion at the rat Tianshu (ST25, bilateral) and Qihai (CV6) acupoints.

Collection of colon tissue and macroscopic scoring of colon injury

After the intervention, rats were anaesthetized with intraperitoneal injection of 2% pentobarbital sodium. The abdominal cavity was exposed and the entire colon from the pubic symphysis to the distal cecum was collected, and the length of the colon was recorded. The distal colon with a length of 6-8 cm was collected and cut longitudinally along the mesentery, observed the gross morphology, the macroscopic scoring of the colon was performed according to the following parameters: Hyperemia, wall thickening, ulceration, inflammation extension, and damage no damage, score 0; hyperemia without ulcers, score 1; hyperemia and wall thickening without ulcers, score 2; one ulceration site without wall thickening, score 3; two or more ulceration sites, score 4; 0.5 cm extension of inflammation or major damage, score 5; 1 cm extension of inflammation or severe damage, score 6[15]. Then was cut into two parts transversely, one part was fixed in 4% paraformaldehyde for morphological observation, and the remaining part was placed in liquid nitrogen for 1 h and transferred to a -80 °C freezer for future detection.

Morphological observation of the colon

Colon tissues fixed in 4% paraformaldehyde fixative solution were dehydrated, embedded in paraffin, and sliced into 4 μm sections for HE staining with the following steps: Dewaxing in xylene I and II solutions for 20 min each; elution in 100%, 95%, 90%, 80%, and 70% ethanol for 5 min each; rinsing with double distilled water for 5 min × 2 times; haematoxylin staining for 2-3 min; rinsing with running water for 10 min; 1% hydrochloric acid ethanol differentiation solution for 1-2 s; rinsing with running water for 5 min; eosin dye staining for 2-3 min; dehydration in 70%, 80%, 90%, and 100% ethanol for 1-2 s each; xylene I and II solutions for 15 min each. After drying and sealing, the colon sections were observed under a light microscope and microscopic scoring of the colon was performed according to the following parameters: Damage/necrosis, inflammatory cell infiltration, submucosal edema, and hemorrhage of mucosa. Colonic gross damage scores were recorded according to the severity of changes: No change, score 0; mild, score 1; moderate, score 2; severe, score 3[16].

Proteomic analysis

Protein extraction and enzymatic digestion: Colon samples from 3 rats in each group were randomly collected and ground into powder, and an appropriate amount of radioimmunoprecipitation assay (RIPA) protein lysis buffer (R0010, Solarbio, United States) was added (150 μL RIPA was add to 20 mg colon tissue), high-abundance protein in the sample was removed, a 5-fold volume of 10% trichloroacetic acid/cold acetone was added to the sample and precipitated at -20 °C overnight. The precipitated proteins were centrifuged at 4 °C, 15000 g for 20 min, the supernatant was removed, and samples were air-dried and precipitated after repeating the above steps several times. After lysis, samples were centrifuged at 25000 g for 20 min at 4 °C, and the supernatant was the protein solution. A total of 100 μg of protein solution was collected from each sample, and trypsin (2.5 μg) was added to the protein solution at a ratio of 40: 1 of protein: Enzyme, enzymolysis at 37 °C for 4 h. Trypsin was then added according to the above ratio and continue enzymolysis at 37 °C for 8 h.

Peptide labelling and separation: The digested peptide fragments were desalted using a Strata X column and vacuum-dried. The peptide samples were dissolved in 0.5 M triethylammonium

bicarbonate, and then were were labeled with iTRAQ (iTRAQ8-plexreagent kit, Sigma, United States) and maintained at room temperature for 2 h. The Shimadzu LC-20AB liquid chromatography system (separation column: 5 μm, 4.6 mm × 250 mm Gemini C18) was used for liquid phase separation of samples. Dried peptide samples were redissolved in 2 mL of mobile phase A (5% ACN, pH 9.8), the solution was loaded, and gradient elution was performed at a flow rate of 1 mL/min as follows: 5% mobile phase B (95% ACN, pH 9.8) for 10 min, 5%-35% mobile phase B for 40 min, 35%-95% mobile phase B for 1 min, mobile phase B for 3 min, and equilibration with 5% mobile phase B for 10 min. The elution peak was monitored at a wavelength of 214 nm, and 1 fractionated peptide was collected every minute. The chromatographic elution peaks were combined with the samples to obtain 20 fractionated peptides, which were then freeze-dried.

High-performance liquid chromatography: The dried peptide samples were redissolved using mobile phase A (2% ACN, 0.1% FA) and centrifuged at 20000 g for 10 min. The supernatant was collected and loaded onto the column, and samples were separated by a Prominence nano LC (LC-20AD, Shimadzu, Japan). The sample was first enriched in a trap column and desalted, and the tandem self-assembled C18 column (75-μm inner diameter, 3.6-μm pore size, 15-cm length) was used. Separation was performed at a flow rate of 300 nL/min through an effective gradient: at 0-8 min, 5% mobile phase B (98% ACN, 0.1% FA); at 8-43 min, mobile phase B increased linearly from 8% to 35%; at 43-48 min, mobile phase B increased from 35% to 60%; at 48-50 min, mobile phase B increased from 60% to 80%; at 50-55 min, 80% mobile phase B; and at 55-65 min, 5% mobile phase B.

Mass spectrometric detection: The separated peptides were transferred into an electrospray ionization tandem mass spectrometer: TripleTOF 5600 (SCIEX, Framingham, United States) and theion source was Nanospray III source (SCIEX, Framingham, United States). During data collection, the parameters of the mass spectrometer were set as follows: An ion source spray voltage of 2300 V, a nitrogen pressure of 30 psi, atomizer 15, and a spray interface temperature of 150 °C. High-sensitivity mode was used for scanning. The cumulative time of primary mass spectrometry scanning was 250 ms, and the cumulative of secondary mass spectrometry scanning time was 100 ms.

iTRAQ quantification and bioinformatics analysis

ITRAQ quantification was performed using IQuant software. The selection of significantly DEPs in a single experiment were as follows: Fold change (FC) ≥ 1.2 was set as up-regulation and FC ≤ 0.83 as down-regulation, as well as Q-value < 0.05. The Gene Ontology (GO) database was used for functional annotation analysis of DEPs. The Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway database was used for pathway enrichment analysis of DEPs. The protein-protein interaction network was performed using STRING software with an interaction confidence of 0.7 (the maximum confidence was 1). Cytoscape (v.3.7.1) software was used to visualize the enrichment analysis results. Figure 2 showed the process of proteomics detection and analysis.

Western blot analysis

We selected DEPs in UC that were reversed by HM and EA for verification by using western blot method. Total proteins were extracted from colon tissues using RIPA buffer supplemented with protease and phosphatase inhibitors. Protein concentration was measured with the bicinchoninic acid protein concentration assay kit. The proteins were separated by polyacrylamide gelelectrophoresis method, with the voltage of concentrated glue was 90 V and that of separating glue was 120 V (BIO-RAD, 1645050), then the separated proteins were transferred to polyvinylidene fluoride membranes. The modified membranes were blocked with 5% bovine serum albumin in TBST, and then was incubated overnight at 4 °C with primary antibody. The antibody dilutions were prepared according to the instructions [Synaptic vesicle glycoprotein 2A (Sv2a), 1: 1000; nuclear cap binding protein subunit 1 (Ncbp1), 1: 800; Cps, 1: 5000; cytochrome c oxidase subunit 4 isoform 1 (Cox4i1), 1: 2000; ATP synthase beta subunit precursor 1 (Atp5f1), 1: 1000; doublecortin like kinase 3 (Dclk3), 1: 800)]. The membranes were washed with western detergent at room temperature for 6 times, 5 min each and then were incubated with secondary antibodies at room temperature for 1 h. Followed by washed with western detergent at room temperature for 6 times, 5 min each. The membranes were stained with enhanced chemiluminescence solution and visualized in a gel imager system (Tanon, 4600).

Statistical analysis

The western blot analysis data were analyzed with SPSS 20.0 statistical software. Data that consistent with the normal distribution were presented as mean ± SD, and one-way analysis of variance was used for comparison between groups, followed by the Fisher's Least significant difference method if data met the homogeneity of variance. For data that did not meet the normal distribution were presented as medians (P_{25}, P_{75}), and nonparametric test was used for comparison. $P < 0.05$ was considered statistically significance.

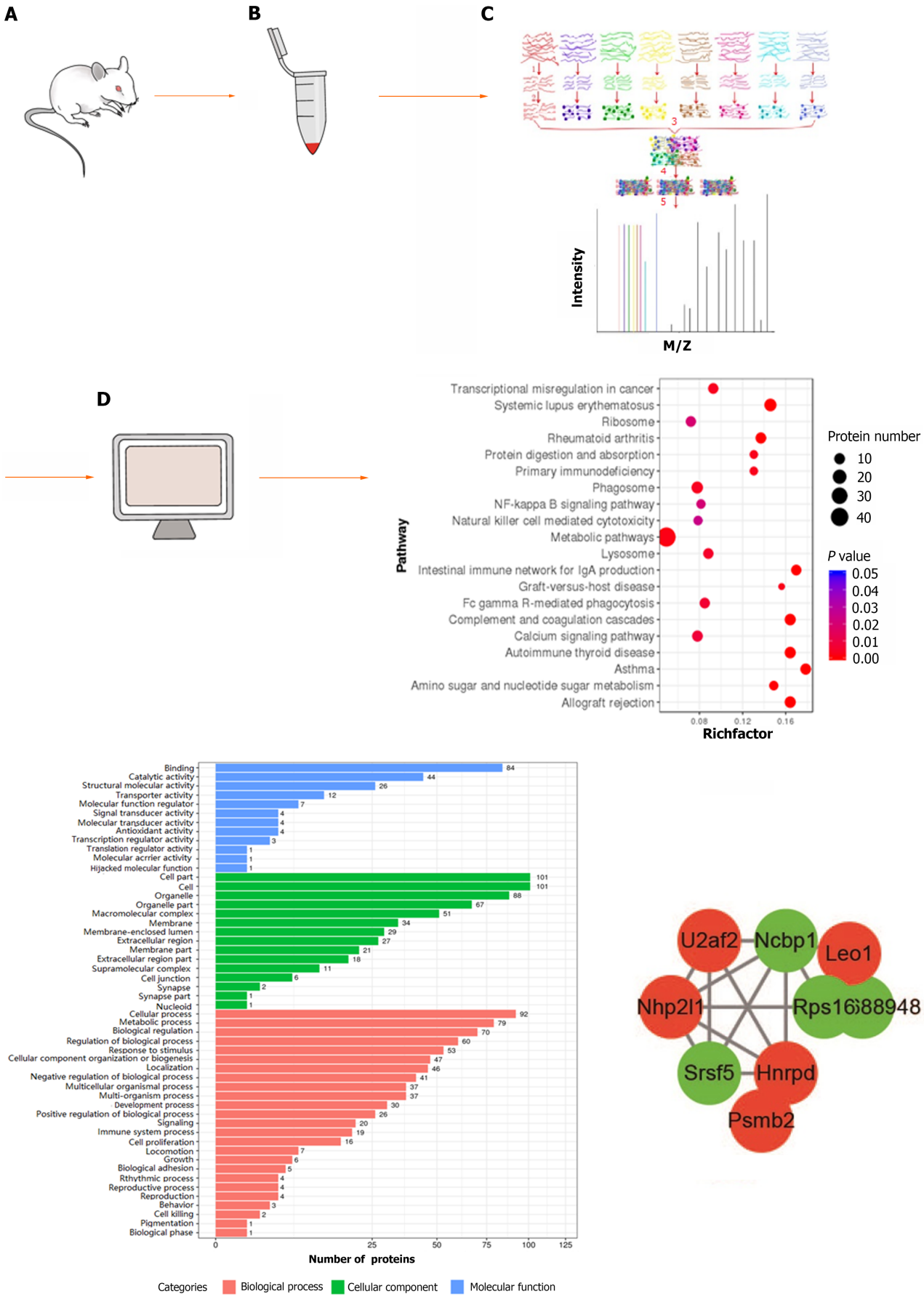


Figure 2 Flow chart of proteomics detection and analysis. A: Sprague-Dawley rat; B: Protein extraction of colon tissue; C: Protein enzymatic digestion, peptide labelling, separation, and high-performance liquid chromatography analysis; D: Bioinformatics analysis.

RESULTS

Effects of HM and EA on DSS induced UC rats

In the N group, rats were active and energetic, and their feces were moderately hard and soft. The rats in the M group had loose, bloody stools and decreased activities. Compared with the M group, the activity of rats in the HM and EA groups was obviously improved, and the stool gradually took shape without obvious mucus and blood. We found that the colon in the N group did not exhibit adhesion or bleeding points; the colonic mucosal surfaces were clean and smooth. In contracts, the colon in the M group exhibited significant adhesion and multiple bleeding points; the inner wall was not smooth, and partially visible scattered ulcers and thickened intestinal walls were observed. In the HM and EA groups, the adhesion and the bleeding points were decreased (Figure 3A).

The morphological changes of colon tissues were observed using HE staining. The colonic mucosal epithelium of the normal group was intact, with clear tissue structure, regular arranged glands, and no congestion, oedema or ulcers were evident. While the mucosal epithelium of UC rats was absent, ulcers had formed, with a loss or reduction of glands and goblet cells, and substantial inflammatory cells were infiltrated in mucosa and submucosa. After HM and EA interventions, the mucosal epithelium was restored, with healed ulcer, increased glands and goblet cells, decreased mucosal and submucosal inflammatory cell infiltration and congestion (Figure 3B).

The macroscopic colon injury scores and histopathology scores in the M group were significantly increased than that in the N group ($P < 0.01$). Conversely, the macroscopic colon injury scores and histopathology scores in the HM and EA groups were significantly decreased compared to the rats in the M group ($P < 0.01$) (Figures 3C and D). The colonic length of the rats in the M group were significantly decreased compared with the N group, the colonic length of the rats in the HM and EA groups were significantly increased compared with the M group ($P < 0.01$) (Figure 3E).

iTRAQ quantification of DEPs

The volcano plot in Figure 4 described the DEPs between the groups. The quantitative results showed that 202 DEPs were identified in DSS-induced UC rats compared with the N group, of which 111 were up-regulated and 91 were down-regulated; 117 DEPs were identified in HM group compared with the M group, of which 41 were up-regulated and 76 were down-regulated; 145 DEPs were identified in EA group compared with the M model group, of which 59 were up-regulated and 86 were down-regulated (Table 1, Figure 5).

Comparison of the M/N and HM/M groups showed that among these proteins, 25 DEPs were common to N, M and HM groups, with 17 [Sv2a, Igkv13-85, Ncbp1, Aldo-keto reductase family 1 member B8, Rathemoglobin beta-chain, Ribosomal protein S8 (RpS8), Fga, Rps2-ps6, carbamoyl phosphate synthetase 1 (Cps1), Txn, ATP synthase subunit g (ATP5L), Cox4i1, Atp5h, Necap2, Atp5f1, Papss2, Acly] up-regulated in UC but were down-regulated by HM, and 8 [Spout1, calcium-activated chloride channel regulator 1 (CLCA1), Sval1, Hspb7, Peptidyl-prolyl cis-trans isomerase, Aldh1a1, Lyz2, Dclk3] down-regulated in UC but were up-regulated by HM (Figure 5A, Table 2). Comparison of the M/N and EA/M groups showed that 15 DEPs were common to N, M and EA groups, with 9 (Sv2a, Ncbp1, Rat hemoglobin beta-chain, Fga, Cps1, ATP5L, Cox4i1, Atp5f1, Lmod1) up-regulated in UC but were down-regulated by EA, and 6 (Spout1, Sh3bgrl3, Peptidyl-prolyl cis-trans isomerase, MHC class I RT1.Aw3 protein, Tkt, Dclk3) down-regulated in UC but were up-regulated by EA (Figure 5B, Table 3).

GO enrichment analysis for DEPs

GO annotation of DEPs between groups describes the characteristics of genes and gene products in terms of molecular functions, cellular components and biological processes. 53 GO functions were significantly differentiated between the M and the N group, 52 GO functions are significantly differentiated between the M group and the HM group, and 51 GO functions are significantly differentiated between the M group and the EA group. The GO functional annotations of DEPs in the M/N, HM/M, and EA/M comparisons were basically similar. Biological process was the most favorable enrichment component, in which DEGs were significantly enriched in cellular process, metabolic process, biological regulation and so on. Cellular component analysis was enriched in cell part, cell, organelles, macromolecular complexes, *etc.* Molecular functions mainly included binding, synaptic activity, structural molecule activity, molecular function regulation, molecular sensor activity, and transcriptional regulation activity (Figure 6).

KEGG pathway enrichment analysis for DEPs

KEGG pathway enrichment analysis was performed for DEPs between the groups, we found that these DEPs were mainly enriched in inflammation responses and immune-related pathways. The pathways in which DEPs between the N and M groups mainly enriched included primary immunodeficiency, the noncanonical nuclear factor-kappaB (NF-κB) signalling pathway, natural killer cells mediated cytotoxicity, intestinal immune network for immunoglobulin A (IgA) production, FcγR-mediated phagocytosis, and complement and coagulation cascades (Figure 7A, Table 4). The pathways in which DEPs between the HM and the M groups mainly enriched included primary immunodeficiency,

Table 1 List of differential protein numbers

Compare group	Up-regulated	Down-regulated	All-regulated
M/N	111	91	202
HM/M	41	76	117
EA/M	59	86	145

N: Normal group; M: Dextran sulfate sodium-induced ulcerative colitis model group; HM: Herb-partitioned moxibustion group; EA: Electroacupuncture group.

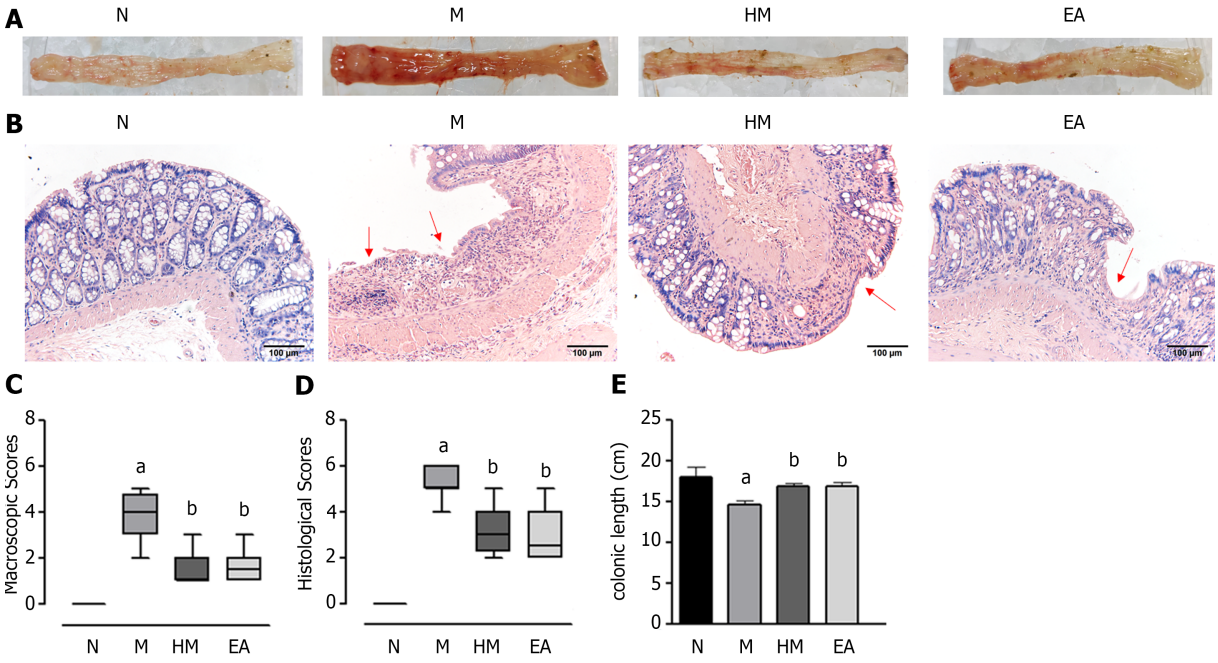


Figure 3 Colon tissue injuries in each group. A: Gross structure; B: Histopathological observation (hematoxylin and eosin, × 200); C: Macroscopic scores; D: Histopathological scores; E: Colonic length. $^{a}P < 0.01$ *vs* N group; $^{b}P < 0.01$ *vs* M group. N: Normal group; M: Dextran sulfate sodium-induced ulcerative colitis model group; HM: Herb-partitioned moxibustion group; EA: Electroacupuncture group.

oxidative phosphorylation, nitrogen metabolism, natural killer cells mediated cytotoxicity, the intestinal immune network for IgA production, FcγR-mediated phagocytosis, complement and coagulation cascades, and B cell receptor signalling pathway (Figure 7B, Table 5). The pathways in which DEPs between the EA and the M groups mainly enriched included the phosphoinositol-3 kinase (PI3K)-protein kinase B (Akt) signalling pathway, oxidative phosphorylation, intestinal immune network for IgA production, and the calcium signalling pathway (Figure 7C, Table 6).

Protein-protein interactions analysis

The STRING database (v.11.0) was used to construct the protein-protein interactions (PPIs) network for the transcription factors with an interaction score > 0.7 (high confidence) among the groups. Cytoscape (v.3.7.1) software was used to visualize the enrichment results. The statistical significance was set as $P < 0.05$. The analysis showed extensive interactions among the DEPs between groups (Figure 8). A total of 100 DEPs (63 up-regulated proteins and 37 down-regulated proteins) were included in the PPI network constructed by DEPs between the N and M groups, and Nme2 served as the junction point of two pathways, indicating that it was important for screening key candidate proteins. 38 DEPs (8 up-regulated proteins and 30 down-regulated proteins) were included in the PPI network constructed from DEPs between the M and HM groups, in which ribosomal pathway-related proteins were all down-regulated, and oxidative phosphorylation pathway-related proteins, including Atp5o, ATP5L, Atp5f, Atp5h, Cox4i1 were also down-regulated. 55 DEPs (19 up-regulated proteins and 36 down-regulated proteins) were included in the PPI network constructed from DEPs between the EA and M groups. In addition, proteins involved in inflammation regulation such as serpins were identified (serpinb6 in M/N comparisons, serpinc1 in HM/M and EA/M comparisons).

Table 2 Differentially expressed proteins in dextran sulfate sodium-induced ulcerative colitis model group rats that regulated by herb-partitioned moxibustion

Protein ID	Description	Symbol	M/N		HM/M	
			Mean ratio	Q value	Mean ratio	Q value
Q02563	Synaptic vesicle glycoprotein 2A	Sv2a	4.42	0.024	0.25	0.021
A0A0G2JZN1	Immunoglobulin kappa chain variable 13-85	Igkv13-85	4.07	0.020	0.37	0.028
Q56A27	Nuclear cap-binding protein subunit 1	Ncbp1	2.47	0.018	0.56	0.004
Q91W30	Aldose reductase-like protein	Akr1b8	1.85	0.006	0.63	0.017
Q63223	Rat hemoglobin beta-chain (Fragment)	Rat hemoglobin beta-chain (Fragment)	1.57	0.005	0.46	0.004
P62243	40S ribosomal protein S8	Rps8	1.56	0.006	0.83	0.023
P06399	Fibrinogen alpha chain	Fga	1.51	0.003	0.75	0.004
O55215	Ribosomal protein S2	Rps2-ps6	1.45	0.003	0.80	0.004
P07756	Carbamoyl-phosphate synthase (ammonia), mitochondrial	Cps1	1.33	0.009	0.70	0.004
P11232	Thioredoxin	Txn	1.28	0.002	0.74	0.004
Q6PDU7	ATP synthase subunit g, mitochondrial	ATP5L	1.27	0.028	0.76	0.004
P10888	Cytochrome c oxidase subunit 4 isoform 1, mitochondrial	Cox4i1	1.26	0.002	0.80	0.004
P31399	ATP synthase subunit d, mitochondrial	Atp5h	1.34	0.003	0.77	0.004
Q6P756	Adaptin ear-binding coat-associated protein 2	Necap2	1.48	0.011	0.83	0.023
P19511	ATP synthase F (0) complex subunit B1, mitochondrial	Atp5f1	1.24	0.003	0.83	0.004
A0A0G2K950	3′-phosphoadenosine 5′-phospho-sulfate synthase 2	Papss2	1.27	0.002	0.74	0.004
A0A0G2K5E7	ATP-citrate synthase	Acly	1.35	0.017	0.83	0.030
A0A0G2QC59	RCG45649, isoform CRA_a	Spout1	0.54	0.013	2.13	0.011
A0A0G2JWX9	Chloride channel accessory 1	Clca1	0.50	0.002	1.25	0.004
Q99N82	Colon SVA-like protein	Sval1	0.73	0.028	6.82	0.004
B5DFG4	Heat shock 27kD protein family, member 7 (cardiovascular)	Hspb7	0.76	0.016	1.24	0.050
D3ZSF3	Peptidyl-prolyl cis-trans isomerase	-	0.75	0.003	1.40	0.013
P51647	Retinal dehydrogenase 1	Aldh1a1	0.81	0.002	1.22	0.004
Q05820	Putative lysozyme C-2	Lyz2	0.67	0.013	1.48	0.050
F1LWF2	Doublecortin-like kinase 3	Dclk3	0.24	0.005	4.67	0.011

N: Normal group; M: Dextran sulfate sodium-induced ulcerative colitis model group; HM: Herb-partitioned moxibustion group; ATP5L: ATP synthase subunit g; Atp5f1: ATP synthase beta subunit precursor; Sv2a: Synaptic vesicle glycoprotein 2A; Ncbp1: Nuclear cap binding protein subunit 1; Cps1: Carbamoyl phosphate synthetase 1; Cox4i1: Cytochrome c oxidase subunit 4 isoform 1; Dclk3: Doublecortin like kinase 3; Akr1b8: Aldo-keto reductase family 1 member B8; RpS8: Ribosomal protein S8.

Western blot verification

We verified the expression of some DEPs that could be regulated by HM and EA. The western blot results showed that, compared with the N group, the expression of Sv2a, Ncbp1, Cps1, Cox4i1, Atp5f1 proteins were increased in the colon of UC rats, while the expression of Dclk3 protein was decreased ($P < 0.01$). HM and EA interventions can significantly down-regulate the expression of Sv2a, Ncbp1, Cps1, Cox4i1, Atp5f1 proteins and up-regulate Dclk3 protein ($P < 0.01$). It indicated that the western blot

Table 3 Differentially expressed proteins in dextran sulfate sodium-induced ulcerative colitis model group rats that regulated by electroacupuncture

Protein ID	Description	Symbol	M/N		EA/M	
			Mean ratio	Q value	Mean ratio	Q value
Q02563	Synaptic vesicle glycoprotein 2A	Sv2a	4.42	0.024	0.15	0.013
Q56A27	Nuclear cap-binding protein subunit 1	Ncbp1	2.47	0.018	0.55	0.003
Q63223	Rat hemoglobin beta-chain (Fragment)	-	1.57	0.005	0.38	0.002
P06399	Fibrinogen alpha chain	Fga	1.51	0.003	0.76	0.002
P07756	Carbamoyl-phosphate synthase (ammonia), mitochondrial	Cps1	1.33	0.009	0.69	0.004
Q6PDU7	ATP synthase subunit g, mitochondrial	ATP5L	1.27	0.028	0.82	0.002
P10888	Cytochrome c oxidase subunit 4 isoform 1, mitochondrial	Cox4i1	1.26	0.002	0.74	0.002
P19511	ATP synthase F (0) complex subunit B1, mitochondrial	Atp5f1	1.51	0.003	0.81	0.002
A0A0G2K0D3	Leiomodin-1	Lmod1	1.13	0.003	0.77	0.002
A0A0G2QC59	RCG45649, isoform CRA_a	Spout1	0.54	0.013	1.88	0.002
B2RZ27	SH3 domain binding glutamic acid-rich protein-like 3	Sh3bgrl3	0.74	0.027	1.31	0.027
D3ZSF3	Peptidyl-prolyl cis-trans isomerase	-	0.75	0.003	1.47	0.003
Q31266	MHC class I RT1.Aw3 protein	-	0.81	0.029	1.44	0.005
G3V826	Transketolase OS = rattus norvegicus	Tkt	0.71	0.003	1.23	0.002
F1LWF2	Doublecortin-like kinase 3	Dclk3	0.24	0.005	4.09	0.002

N: Normal group; M: Dextran sulfate sodium-induced ulcerative colitis model group; EA: Electroacupuncture group; ATP5L: ATP synthase subunit g; Atp5f1: ATP synthase beta subunit precursor; Sv2a: Synaptic vesicle glycoprotein 2A; Ncbp1: Nuclear cap binding protein subunit 1; Cps1: Carbamoyl phosphate synthetase 1; Cox4i1: Cytochrome c oxidase subunit 4 isoform 1; Dclk3: Doublecortin like kinase 3.

verification results were consistent with the iTRAQ results (Figure 9).

DISCUSSION

UC is a type of inflammatory bowel disease (IBD) characterized by chronic recurrent, and the specific aetiology and pathogenesis of UC are still not clear. Proteomics is a discipline that systematically quantifies all the proteins in cells or organisms and elucidates their biological functions, it has enormous potential for early diagnosis, prevention, and prognosis prediction of diseases[17,18]. In recent years, proteomics has gradually been applied to the study of UC, providing strong support for the research of pathogenesis, clinical diagnosis, and treatment of UC. A study has been found that 7 protein genes were differentially expressed in the intestinal tissues of UC patients using proteomics, including prohibitin, heat shock proteins, alpha-1 antitrypsin, ventralis intermedius, caspase-1, cytokeratin 20, Filamin Ainteracting protein 1, FLNa[19]. Another study analysed the transcriptome and proteome characteristics of the colon of UC patients, and found that the expression of genes and proteins related to immune and inflammatory responses in UC patients were dysregulated. At the same time, the complement cascade, metabolic processes, and peroxisome proliferator-activated receptor signal transduction were inhibited[20]. Therefore, iTRAQ proteomic technology can also be a feasible method to reveal potential targets for UC drug therapy[8].

In the present study, we performed proteomic analysis using iTRAQ labelling combined with mass spectrometry technology to identify DEPs in colon of DSS-induced UC rats, and selected DEPs that could be reversed by HM and EA. DSS has been widely used to prepare experimental colitis to study the new drug therapy, immunity and mechanism of action[21-23]. Our results showed that HM and EA improved the colonic mucosal injury of UC rats. At the same time, we have compared the normal and

Table 4 The Kyoto Encyclopedia of Genes and Genomes pathway related to immunity and inflammation with differentially expressed proteins between normal group and dextran sulfate sodium-induced ulcerative colitis model group

Pathway name	Protein ID	Number	*P* value
Primary immunodeficiency	M0RDF2, IGG2B, M0RA79, Q4QQW0, F1LXY6, Q569B3	6	0.003
Nuclear factor-kappa B signalling pathway	M0RDF2, IGG2B, M0RA79, Q4QQW0, F1LXY6, Q569B3, B2RZB2	7	0.021
Natural killer cell mediated cytotoxicity	GRZ2, M0RDF2, IGG2B, M0RA79, Q4QQW0, F1LXY6, Q569B3	7	0.025
Intestinal immune network for IgA production	M0RDF2, IGG2B, HB2B, M0RA79, Q8VI32, Q4QQW0, F1LXY6, Q569B3, B2RZB2	9	4.25E-05
Fc gamma R-mediated phagocytosis	M0RDF2, Q6AYB2, IGG2B, E9PU64, M0RA79, Q4QQW0, F1LXY6, B2GV73, Q569B3	9	0.007
Complement and coagulation cascades	CFAI, FIBB, M0RBJ7, PLMN, A1L114, CO9, A1M, FIBG, FIBA, Q99N82, A0A0G2JY31	11	8.29E-06

N: Normal group; M: Dextran sulfate sodium-induced ulcerative colitis model group; IgA: Immunoglobulin A.

Table 5 The Kyoto Encyclopedia of Genes and Genomes pathway related to immunity, inflammation and oxidative phosphorylation with differentially expressed proteins between herb-partitioned moxibustion group and dextran sulfate sodium-induced ulcerative colitis model group

Pathway name	Protein ID	Number	*P* value
Primary immunodeficiency	IGG2C, Q5BJZ2, F1LXY6, Q569B3	4	0.009
Oxidative phosphorylation	ATP5H, ATPO, COX41, AT5F1, Q5UAJ5, NMES1, ATP5L	7	0.002
Nitrogen metabolism	CAH1, CAH3, CPSM	3	0.001
Natural killer cell mediated cytotoxicity	GRZ2, IGG2C, Q5U1Y2, Q5BJZ2, F1LXY6, Q569B3	6	0.005
Intestinal immune network for IgA production	IGG2C, Q5BJZ2, Q6MG98, F1LXY6, Q569B3	5	0.002
Fc gamma R-mediated phagocytosis	IGG2C, Q5U1Y2, Q5BJZ2, F1LXY6, Q569B3	5	0.040
Complement and coagulation cascades	Q5M7T5, Q99N82, Q7TQ70, FIBA	4	0.031
B cell receptor signalling pathway	IGG2C, Q5U1Y2, Q5BJZ2, F1LXY6, Q569B3	5	0.020

M: Dextran sulfate sodium-induced ulcerative colitis model group; HM: Herb-partitioned moxibustion group; IgA: Immunoglobulin A; ATP5L: ATP synthase subunit g.

Table 6 The Kyoto Encyclopedia of Genes and Genomes pathway related to immunity, inflammation and oxidative phosphorylation with differentially expressed proteins between electroacupuncture group and dextran sulfate sodium-induced ulcerative colitis model group

Pathway name	Protein ID	Number	*P* value
PI3K-Akt signalling pathway	F1M6Q3, D4A3K7, P70570, Q5M7V3, A0A096P6L8, Q5BJZ2, F1LPR6, GBB4, PCKGC	9	0.042
Oxidative phosphorylation	B2RYT5, CX7A2, D4A565, CX6C2, COX41, M0RA24, AT5F1, B2RYS8, G3V8S4, D3ZFQ8, NMES1, ATP5L, ATPK	13	3.42E-07
Intestinal immune network for IgA production	Q5M7V3, Q5BJZ2, F1LPR6, Q6MG98	4	0.032
Calcium signalling pathway	GNAS2, Q5M7V3, A0A0G2K059, Q5BJZ2, F1LPR6, A0A0G2JSR0, ADT2	7	0.027

M: Dextran sulfate sodium-induced ulcerative colitis model group; EA: Electroacupuncture group; IgA: Immunoglobulin A; ATP5L: ATP synthase subunit g; PI3K-Akt: Phosphatidylinositol 3-kinase protein kinase B; COX41: Cytochrome c oxidase subunit IV isoform 1; PCKGC: Phosphoenolpyruvate carboxykinase 1; ATPK: ATP synthase membrane subunit F; ADT2: Arogenate dehydratase 2.

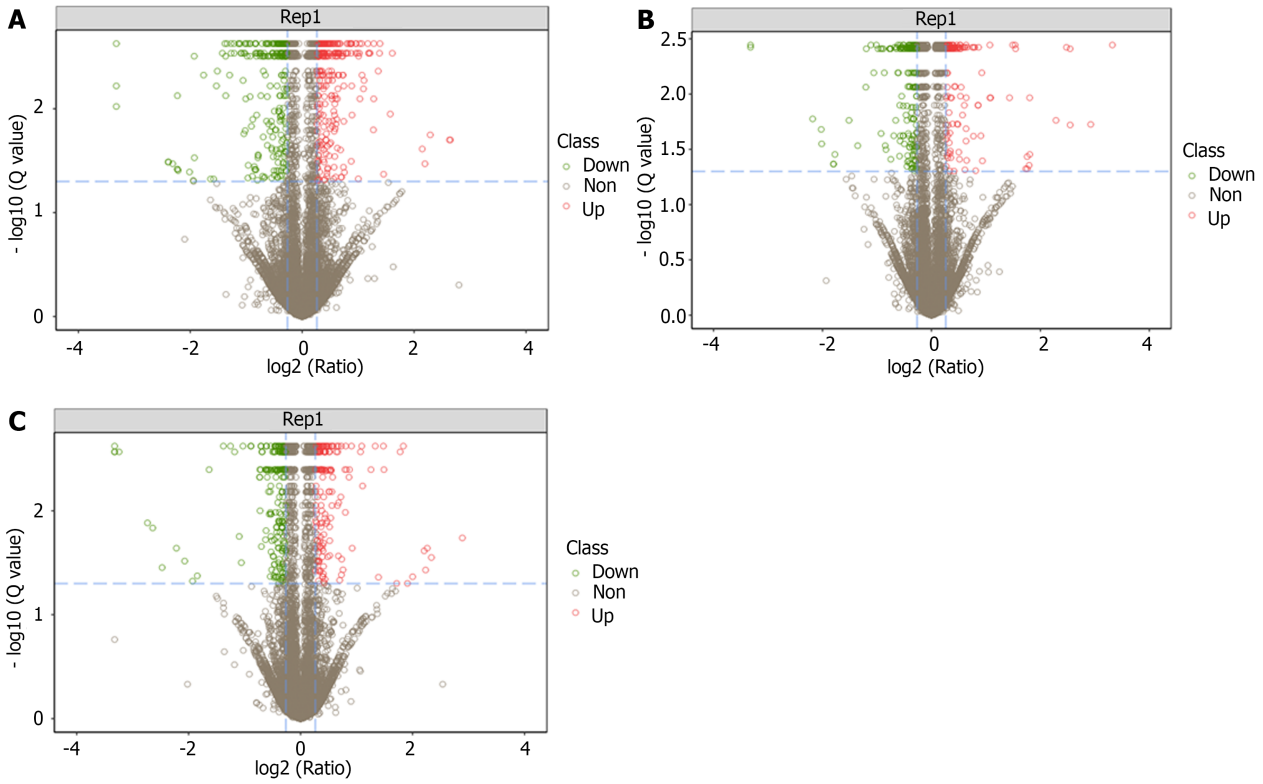


Figure 4 Volcano plot of differentially expressed proteins between groups. A: Volcano plot of differentially expressed proteins (DEPs) between the normal and dextran sulfate sodium-induced ulcerative colitis model group; B: Volcano plot of DEPs between the dextran sulfate sodium-induced ulcerative colitis model and herb-partitioned moxibustion group; C: Volcano plot of DEPs between the dextran sulfate sodium-induced ulcerative colitis model and electroacupuncture group.

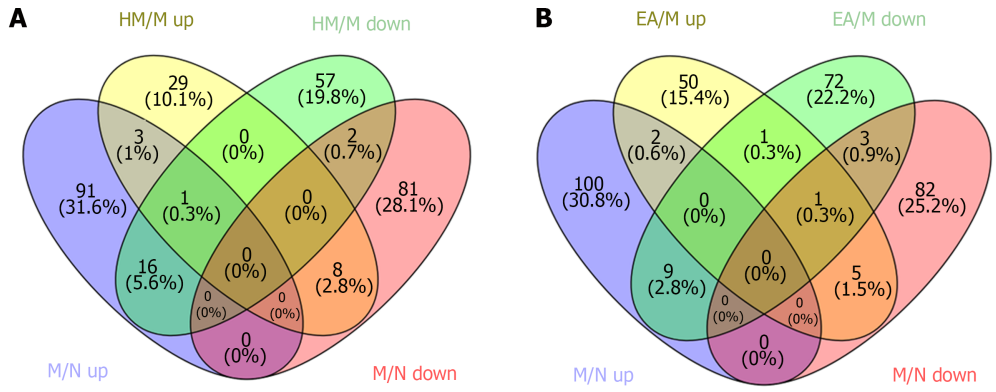
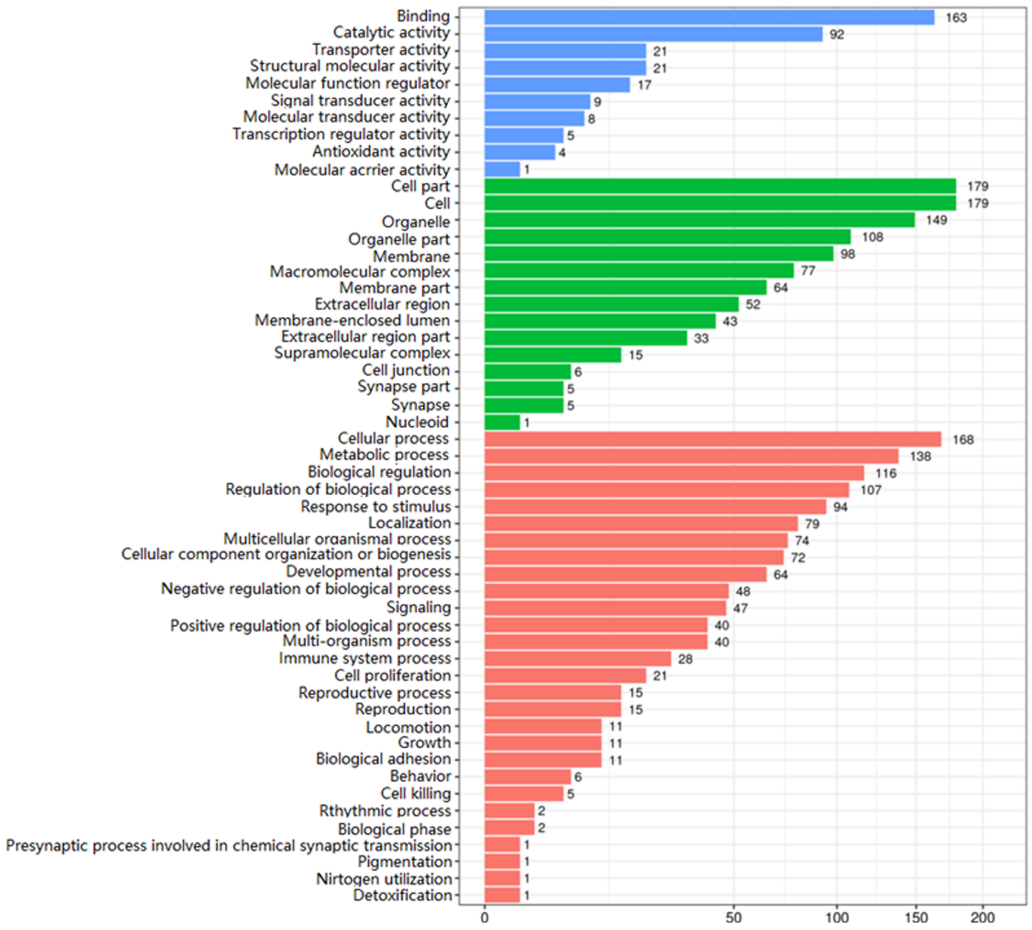


Figure 5 Venn diagram of differentially expressed proteins among groups. A: Venn diagram of differentially expressed proteins (DEPs) in dextran sulfate sodium-induced ulcerative colitis model group that can be regulated by herb-partitioned moxibustion; B: Venn diagram of DEPs in dextran sulfate sodium-induced ulcerative colitis model group that can be regulated by electroacupuncture. N: Normal group; M: Dextran sulfate sodium-induced ulcerative colitis model group; HM: Herb-partitioned moxibustion group; EA: Electroacupuncture group.

DSS-induced UC rats using iTRAQ labelling and found that 202 protein genes were differentially expressed between the two group. Among them, galectin-3, an up-regulated protein, is an endogenous lectin with extensive immunomodulatory functions, and can promote the inflammatory response in DSS-induced colitis by activating the NLR family pyrin domain containing 3 inflammasome[24]. Moreover, galectin-3 has also been shown to be a key regulator of inflammation and can be a potential biomarker for IBD[25]. s100a9 is a subunit of calprotectin with pro-inflammatory activity, and the level of s100a9 was significantly increased in the faeces of DSS-induced colitis mice compared with normal mice[26,27]. This is consistent with the upregulation of s100a9 in UC colon tissue in our study. Guanylate cyclase activator 2A (GUCA2A) is the ligand of guanylate cyclase C, which is mainly expressed in intestinal epithelial cells, and can regulate intestinal barrier function and intestinal homeostasis through the cyclic guanosine monophosphate-dependent signalling pathway[28]. The

A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Catalytic activity 92
Transporter activity 21
Structural molecular activity 21
Molecular function regulator 17
Signal transducer activity 9
Molecular transducer activity 8
Transcription regulator activity 5
Antioxidant activity 4
Molecular acrrier activity 1
Cell part 179
Cell 179
Organelle 149
Organelle part 108
Membrane 98
Macromolecular complex 77
Membrane part 64
Extracellular region 52
Membrane-enclosed lumen 43
Extracellular region part 33
Supramolecular complex 15
Cell junction 6
Synapse part 5
Synapse 5
Nucleoid 1
Cellular process 168
Metabolic process 138
Biological regulation 116
Regulation of biological process 107
Response to stimulus 94
Localization 79
Multicellular organismal process 74
Cellular component organization or biogenesis 72
Developmental process 64
Negative regulation of biological process 48
Signaling 47
Positive regulation of biological process 40
Multi-organism process 40
Immune system process 28
Cell proliferation 21
Reproductive process 15
Reproduction 15
Locomotion 11
Growth 11
Biological adhesion 11
Behavior 6
Cell killing 5
Rthythmic process 2
Biological phase 2
Presynaptic process involved in chemical synaptic transmission 1
Pigmentation 1
Nirtogen utilization 1
Detoxification 1
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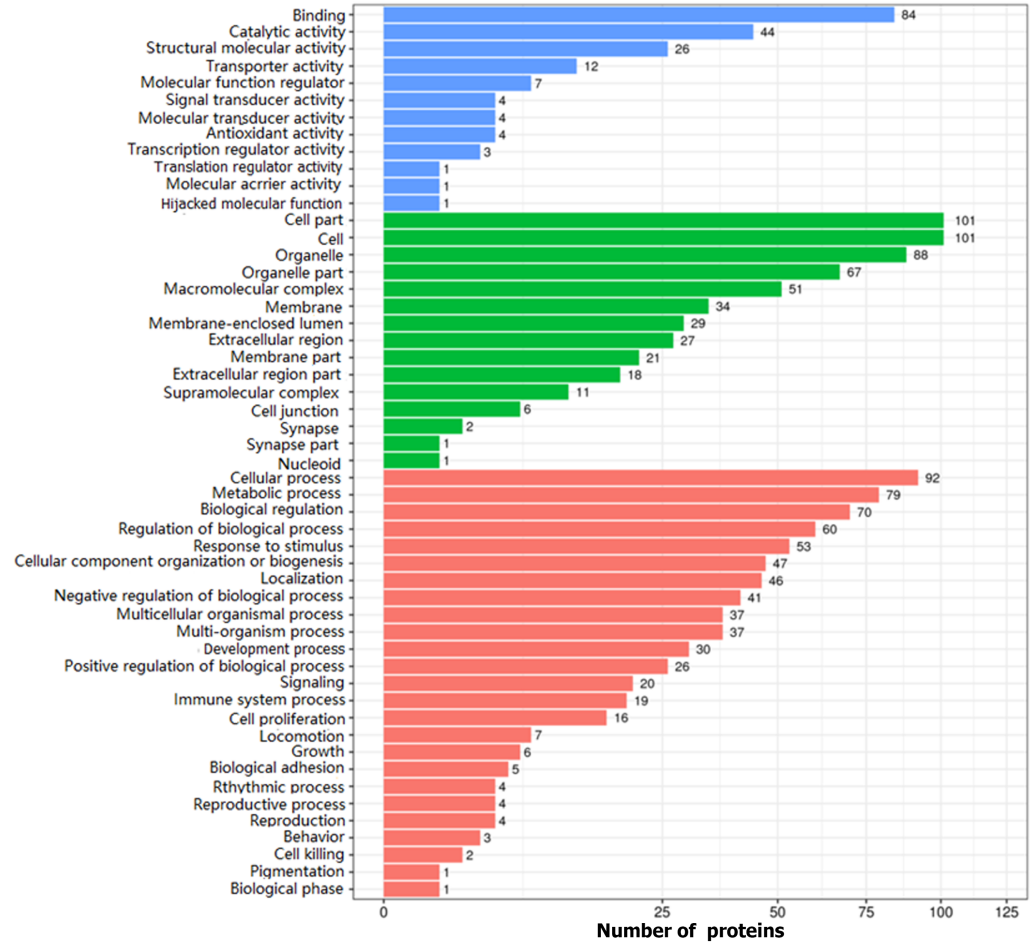
50
100
150
200

B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Catalytic activity 44
Structural molecular activity 26
Transporter activity 12
Molecular function regulator 7
Signal transducer activity 4
Molecular transducer activity 4
Antioxidant activity 4
Transcription regulator activity 3
Translation regulator activity 1
Molecular acrrier activity 1
Hijacked molecular function 1
Cell part 101
Cell 101
Organelle 88
Organelle part 67
Macromolecular complex 51
Membrane 34
Membrane-enclosed lumen 29
Extracellular region 27
Membrane part 21
Extracellular region part 18
Supramolecular complex 11
Cell junction 6
Synapse 2
Synapse part 1
Nucleoid 1
Cellular process 92
Metabolic process 79
Biological regulation 70
Regulation of biological process 60
Response to stimulus 53
Cellular component organization or biogenesis 47
Localization 46
Negative regulation of biological process 41
Multicellular organismal process 37
Multi-organism process 37
Development process 30
Positive regulation of biological process 26
Signaling 20
Immune system process 19
Cell proliferation 16
Locomotion 7
Growth 6
Biological adhesion 5
Rthythmic process 4
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Number of proteins

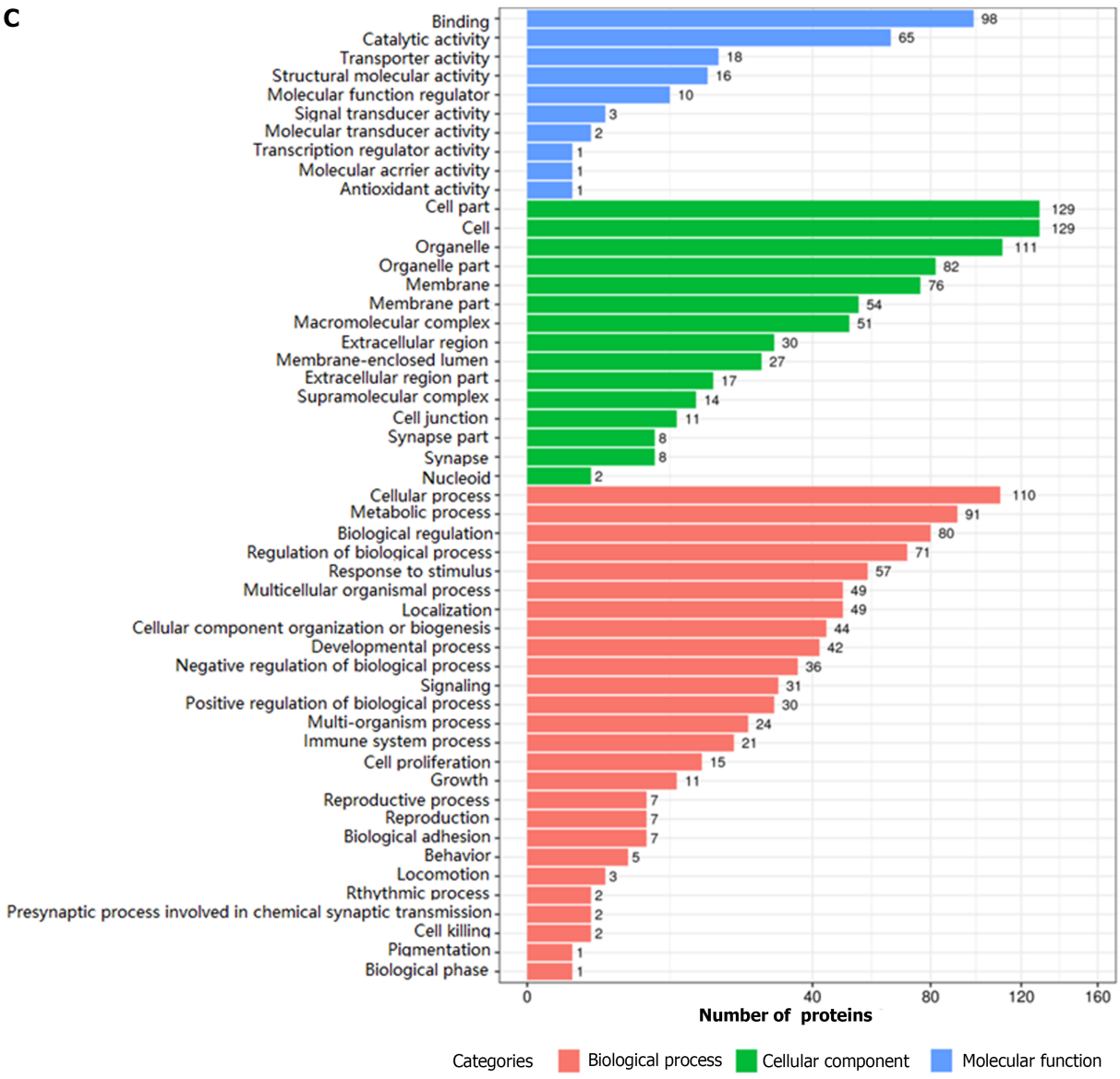
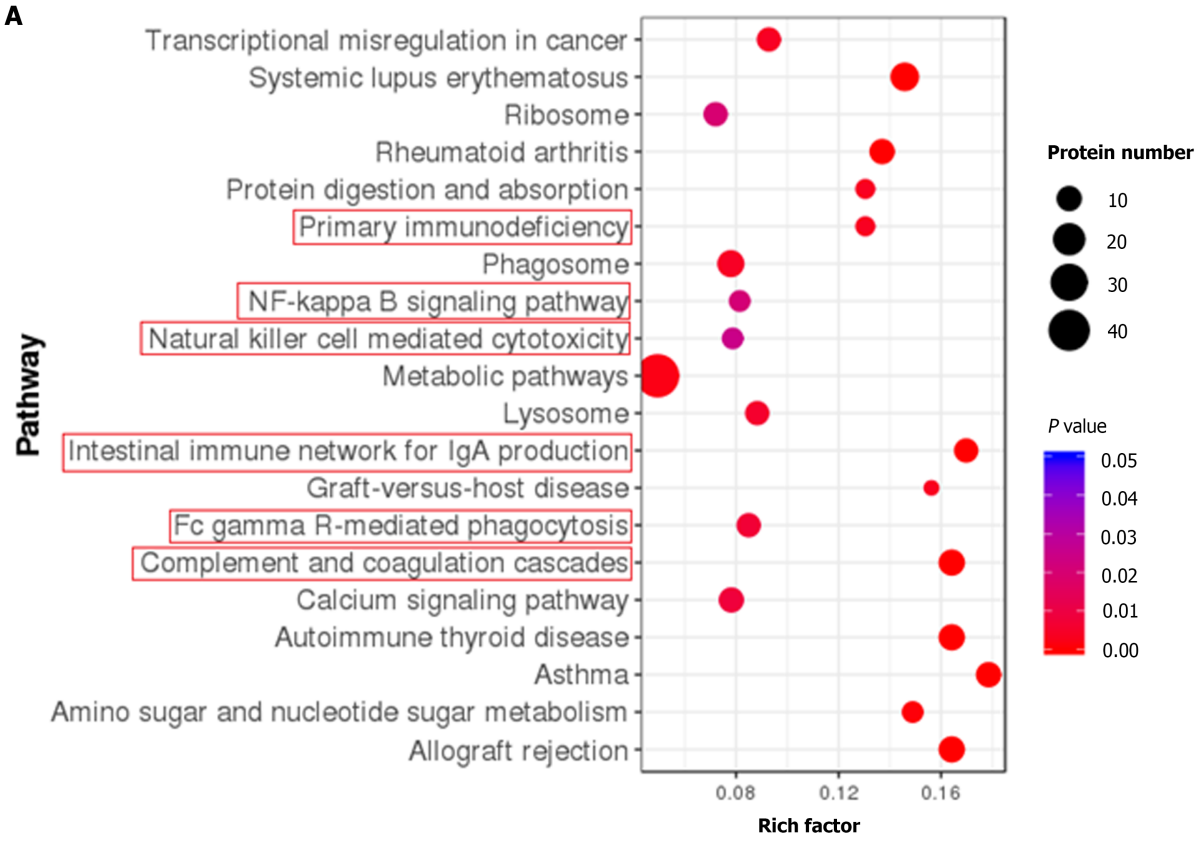


Figure 6 Biological function annotation of differentially expressed proteins between groups. A: Biological function annotation of differentially expressed proteins (DEPs) between the normal and dextran sulfate sodium-induced ulcerative colitis model group; B: Biological function annotation of DEPs between the dextran sulfate sodium-induced ulcerative colitis model and herb-partitioned moxibustion group; C: biological function annotation of DEPs between the dextran sulfate sodium-induced ulcerative colitis model and electroacupuncture group.

down-regulation of GUCA2A can injure the intestinal mucosa barrier and affect the growth and repair of intestinal epithelial cells in IBD patients[29]. Interestingly, we found that the expression of GUCA2A was down-regulated in UC colon tissue, which was consistent with previous findings. These results suggest that galectin-3, s100a9 and GUCA2A may be potential biomarkers of UC.

After receiving the HM intervention, a total of 117 DEPs were identified, of which 25 proteins were reversely regulated by HM, including 17 proteins up-regulated in UC but were down-regulated by HM, and 8 down-regulated in UC but were up-regulated by HM. Among the 17 proteins down-regulated by HM, ATP5L, Atp5f, and Atp5h are the subunits of ATP synthase that involved in energy metabolism and oxidative phosphorylation; Cox4i1 is an important regulatory subunit of cytochrome c oxidase and is mainly associated with mitochondrial oxidative phosphorylation; Cps1, the carbamyl phosphate synthetase 1, was over-expressed in the non-dysplastic tissue of UC progressors[30]; RpS8 is a small subunit protein of ribosomes that plays a critical role in regulation of protein translation. Although it is unclear whether these proteins play a role in UC, ATP-induced energy metabolism disorders and intestinal epithelial mitochondrial damage are important pathogenesis of UC[31]. Therefore, changes in the expression of these proteins may be related to the occurrence of UC and the therapeutic effect of HM. Further studies should be performed to clarify the exact mechanisms of these oxidative phosphorylation proteins. Among the 8 proteins up-regulated by HM, CLCA1 is one of the major non-mucinous proteins in intestinal mucus, which is normally expressed in the gastrointestinal tract and is most abundant in the intestinal mucosa. Studies have shown that CLCA1 regulates the structural


A
Transcriptional misregulation in cancer
Systemic lupus erythematosus
Ribosome
Rheumatoid arthritis
Protein digestion and absorption
Primary immunodeficiency
Phagosome
NF-kappa B signaling pathway
Natural killer cell mediated cytotoxicity
Metabolic pathways
Lysosome
Intestinal immune network for IgA production
Graft-versus-host disease
Fc gamma R-mediated phagocytosis
Complement and coagulation cascades
Calcium signaling pathway
Autoimmune thyroid disease
Asthma
Amino sugar and nucleotide sugar metabolism
Allograft rejection
Pathway
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0.12
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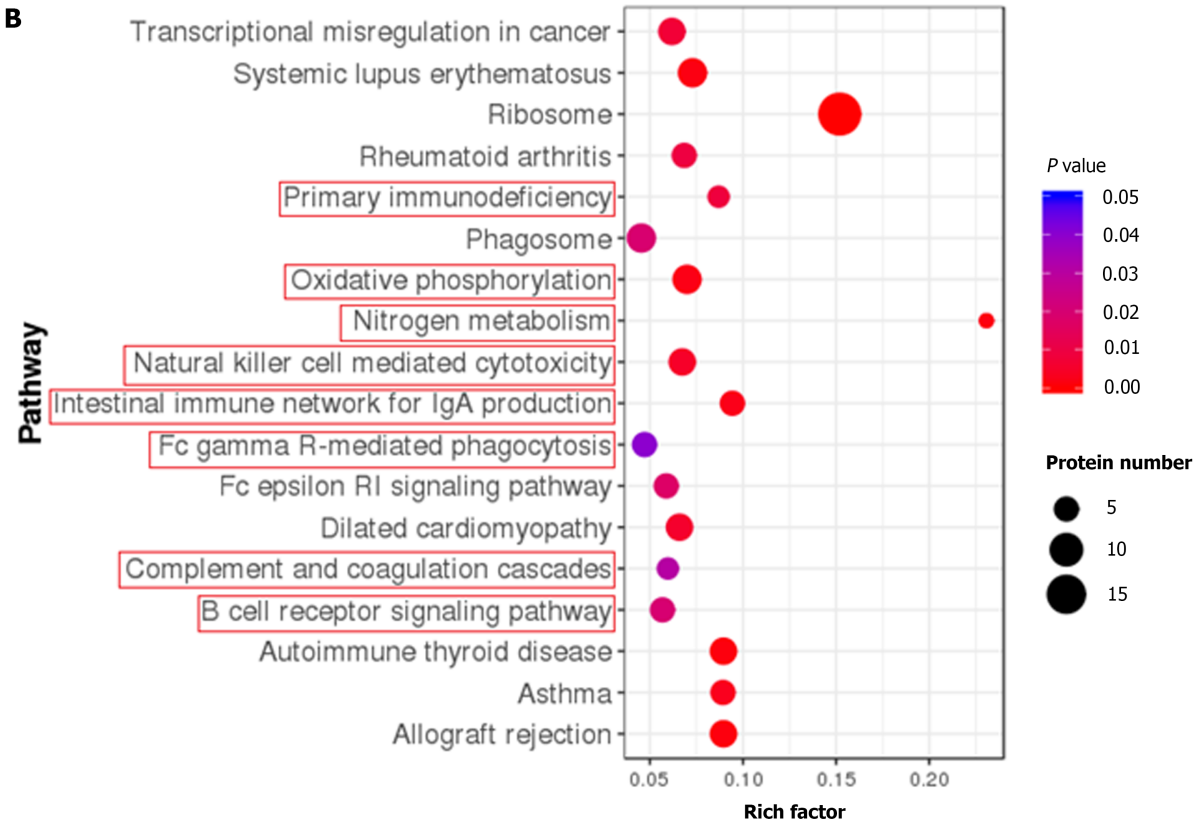
B
Transcriptional misregulation in cancer
Systemic lupus erythematosus
Ribosome
Rheumatoid arthritis
Primary immunodeficiency
Phagosome
Oxidative phosphorylation
Nitrogen metabolism
Natural killer cell mediated cytotoxicity
Intestinal immune network for IgA production
Fc gamma R-mediated phagocytosis
Fc epsilon RI signaling pathway
Dilated cardiomyopathy
Complement and coagulation cascades
B cell receptor signaling pathway
Autoimmune thyroid disease
Asthma
Allograft rejection
Pathway
0.05
0.10
0.15
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P value
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Protein number
5
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15

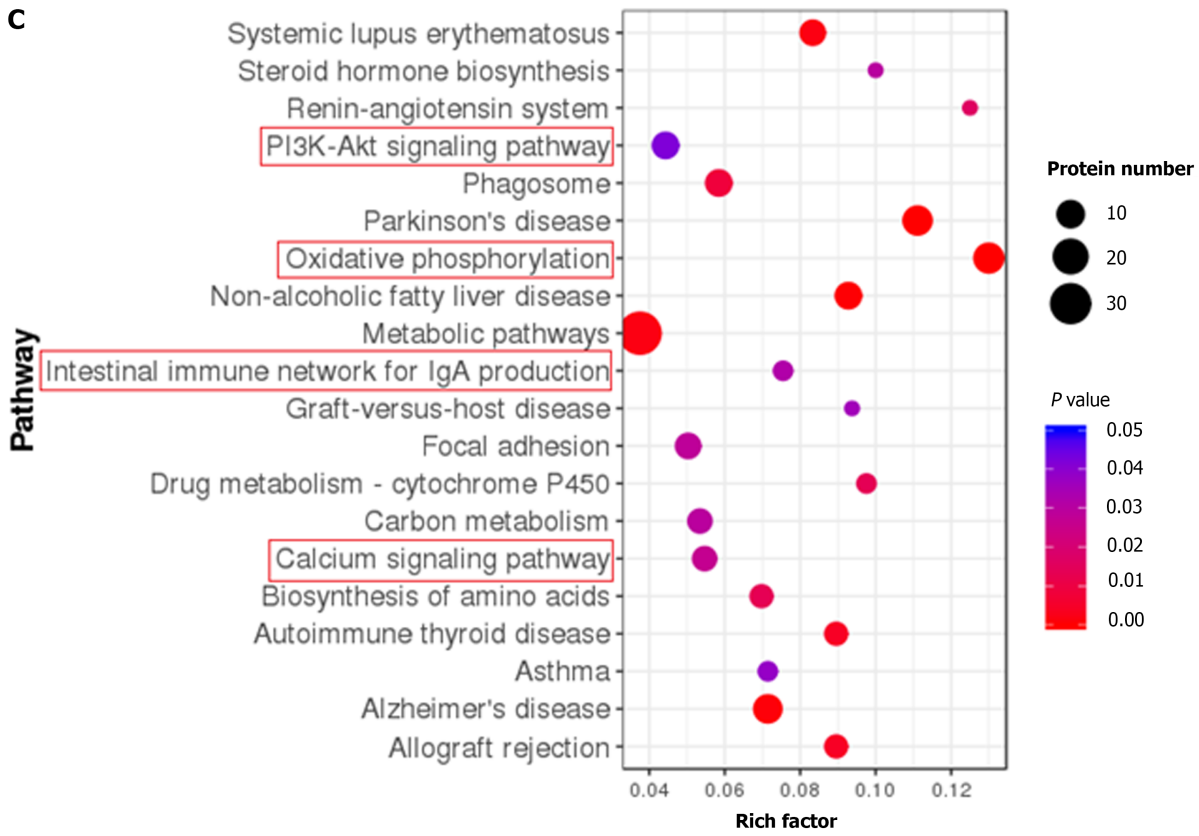

Figure 7 Kyoto Encyclopedia of Genes and Genomes pathway enrichment of differentially expressed proteins between groups. A: Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment of differentially expressed proteins (DEPs) between the normal and dextran sulfate sodium-induced ulcerative colitis model group; B: KEGG pathway enrichment of DEPs between the dextran sulfate sodium-induced ulcerative colitis model and herb-partitioned moxibustion group; C: KEGG pathway enrichment of DEPs between the dextran sulfate sodium-induced ulcerative colitis model and electroacupuncture group. Each point in the KEGG pathway enrichment plot represents a KEGG pathway, with the left axis showing the pathway name and the abscissa showing the enrichment factor, which represents the ratio of the number of DEPs annotated to that pathway to the number of proteins annotated to that pathway for that species' protein. A larger enrichment factor indicates more reliable enrichment of DEPs in the pathway.

arrangement of mucus, participate in the mucus processing, and plays an important role in regulating intestinal homeostasis and intestinal inflammation[32-34]. CLCA1 can also exert a tumor suppressor effect in colorectal cancer (CRC) by inhibiting the Wnt/β-catenin signalling pathway and epithelial-mesenchymal transition process[35]. The serum concentration and mRNA expression of CLCA1 in CRC tissues were negatively correlated with metastasis and tumor staging[36,37]. These findings suggest that CLCA1 may play an important role in moxibustion in inhibiting the development and carcinogenesis of UC. While 15 proteins were reversely regulated by EA, of which 9 up-regulated in UC but were down-regulated by EA, and 6 down-regulated in UC but were up-regulated by EA. We found EA could also regulate the expression of Atp5f, Cox4i1, Cps1 that explained above, but the functions of other reversely regulated proteins in UC and immune inflammation remain unknown and require further validation and study.

We further explored the potential functions of DEPs by GO and KEGG functional enrichment analyses. GO analysis revealed that the majority of the DEPs were primarily involved in cellular process, metabolic process, biological regulation in biological processes, cell part, cell, organelles, macromolecular complexes in cellular component, and binding, synaptic activity, structural molecule activity, molecular function regulation, molecular sensor activity, and transcriptional regulation activity in molecular function. The results indicated that most differentially abundant proteins were associated with cell structure and catalytic activity which play an important role in cell function. For KEGG analysis, we found that these DEPs were mainly enriched in inflammation responses and immune-related pathways, such as primary immunodeficiency, NF-κB signalling pathway, natural killer cells mediated cytotoxicity, intestinal immune network for IgA production and so on. It has been confirmed that inflammatory response and immune response play an important role in UC. Numerous literature evidence that NF-κB pathway plays an essential role in pathogenic development of UC[38]. In addition, PPI network analysis showed that DEPs related to ribosomal pathways and oxidative phosphorylation pathways were all down-regulated by HM. As we all know that classical signalling pathways, such as the NF-κB signalling pathway, PI3K-Akt signalling pathway, have been confirmed to be associated with UC[39-42], and IgG receptor FcγR also plays an important role in UC intestinal immunity and inflam-

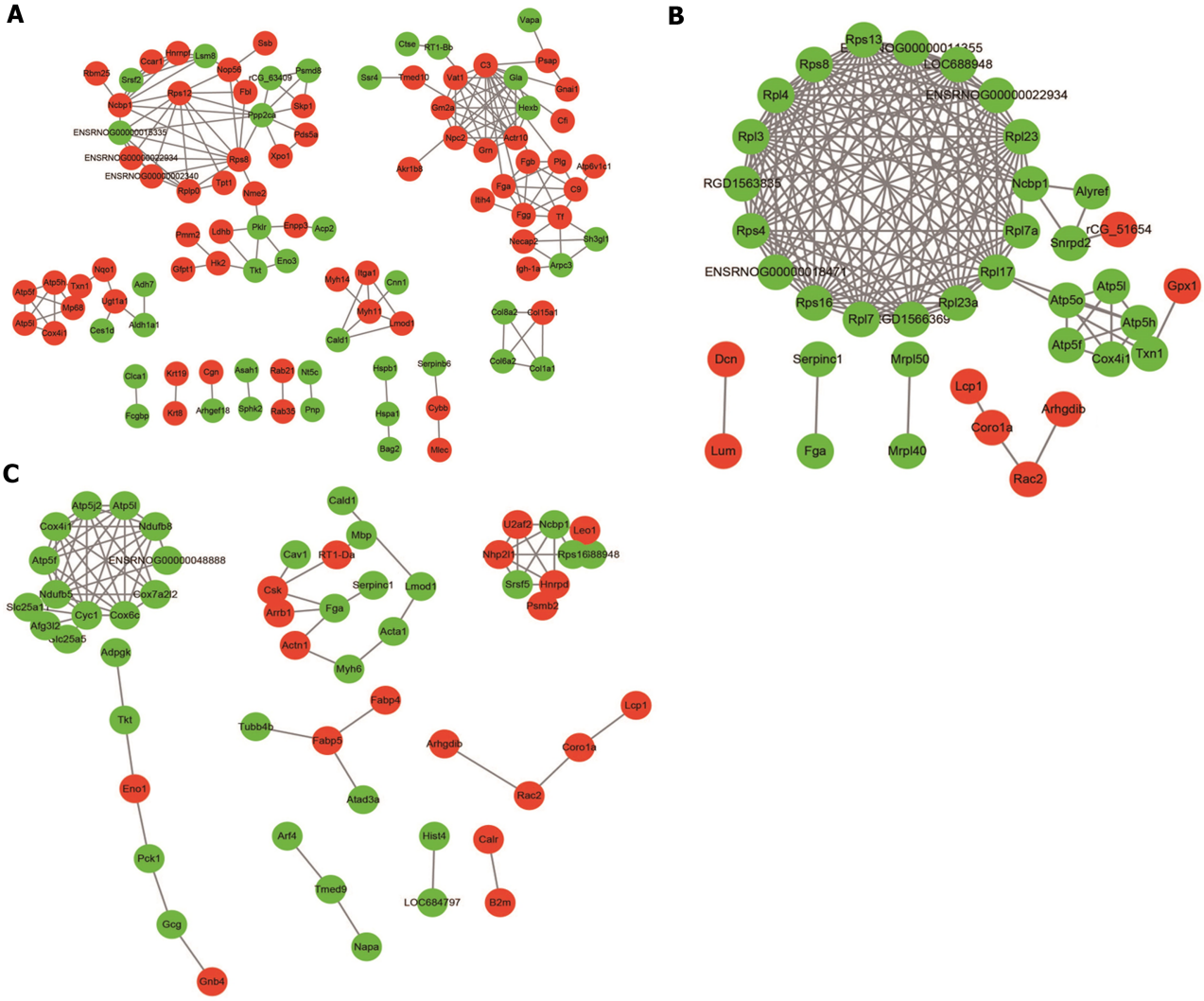

Figure 8 Protein-protein interaction network of differentially expressed proteins between groups. A: Protein-protein interaction (PPI) network of differentially expressed proteins (DEPs) between the normal and dextran sulfate sodium-induced ulcerative colitis model group; B: PPI network of DEPs between the dextran sulfate sodium-induced ulcerative colitis model and herb-partitioned moxibustion group; C: PPI network of DEPs between the dextran sulfate sodium-induced ulcerative colitis model and electroacupuncture group. Circle colours indicate changes in protein expression, with red indicating up-regulation and green indicating down-regulation, and line thickness indicates interaction intensity.

mation[43,44]. The intestinal immune network for IgA production and Fc epsilon RI signalling pathway may also the UC-specific signalling pathway. A study on pathway enrichment analysis of differentially expressed genes between IBD and healthy individuals revealed that primary immunodeficiency, complement and coagulation cascades, and nitrogen metabolism pathways may also play a key role in the progression of IBD[45]. Multiple algorithm analysis found that calcium signalling pathway was involved in the process of UC[46]. This is consistent with our findings at the protein level. Therefore, the abnormality of above pathways may be the key to the pathogenesis of UC, and HM and EA may play the therapeutic role in UC through the regulation of key molecules in these pathways. In addition, we found that proteins involved in inflammation regulation such as serpins were identified (serpinb6 in M/N comparisons, serpinc1 in HM/M and EA/M comparisons). Serpins are the largest known family of serine proteinase inhibitors, which regulate innate immunity by inhibiting the serine proteinase cascades that initiate immune responses such as melanization and antimicrobial peptide production [47]. Several human serpins have been shown to regulate serine proteases associated with processes such as inflammation and immune responses[48]. And increased activity of serine proteases is demonstrated in IBD patients and may contribute to the onset and the maintenance of the disease[49]. This appears to be a novel finding that will require additional research.

We also found that both HM and EA reversed the expression of Sv2a, Ncbp1, Rat hemoglobin beta-chain (Fragment), Fga, Cps1, ATP5L, Cox4i1, Atp5f1, Spout1, Peptidyl-prolyl cis-trans isomerase, Dclk3 proteins, while the rest is what they can reverse individually. The KEGG pathways of DEPs between HM and M groups, EA and M groups were also partly different. These results may reflect the commonness and characteristics of the therapeutic effects of HM and EA. So, the effects of HM and EA on UC may involve the differently regulating proteins and pathways. In addition, based on the results

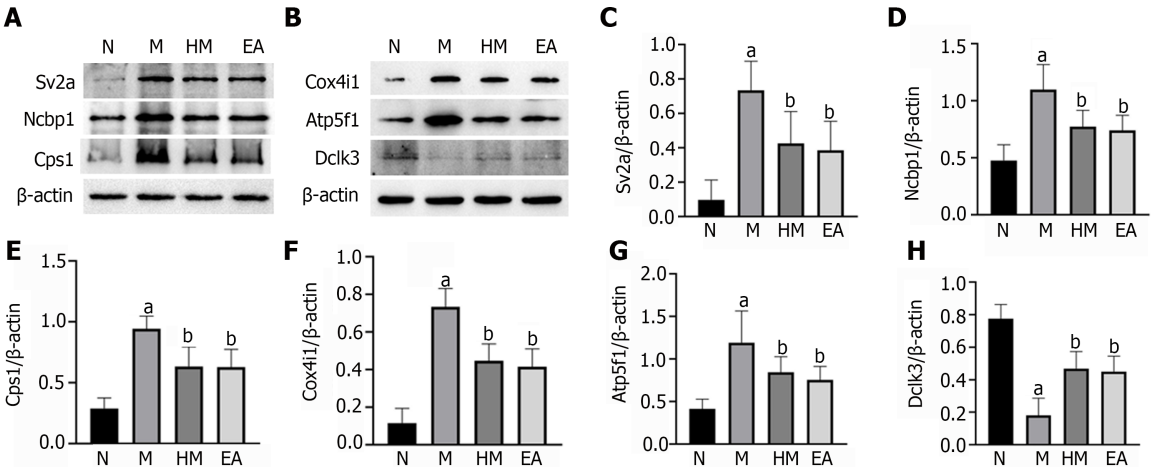

Figure 9 Verification of differentially expressed proteins expressions by western blot. A and B: Band diagrams of protein expressions. All data are expressed as mean ± SD; C: Synaptic vesicle glycoprotein 2A; D: Nuclear cap binding protein subunit 1; E: Carbamoyl phosphate synthetase 1; F: Cytochrome c oxidase subunit 4 isoform 1; G: ATP synthase beta subunit precursor 1; H: Doublecortin like kinase 3. [a]*P* < 0.01 *vs* normal group; [b]*P* < 0.01 *vs* dextran sulfate sodium-induced ulcerative colitis model group. N: Normal group; M: Dextran sulfate sodium-induced ulcerative colitis model group; HM: Herb-partitioned moxibustion group; EA: Electroacupuncture group; Sv2a: Synaptic vesicle glycoprotein 2A; Ncbp1: Nuclear cap binding protein subunit 1; Cps1: Carbamoyl phosphate synthetase 1; Cox4i1: Cytochrome c oxidase subunit 4 isoform 1; Atp5f1: ATP synthase beta subunit precursor; Dclk3: Doublecortin like kinase 3.

of proteomics, we selected 6 DEPs that could be reversed by HM and EA for verification. We found that both HM and EA decreased the expression of Sv2a, Ncbp1, Cps1, Cox4i1, Atp5f1 and increased the expression of Dclk3, which were consistent with the iTRAQ results, confirming the reliability of the iTRAQ results. Cox4i1 is the main subunit of cytochrome c oxidase, a key enzyme in energy metabolism, and plays an important role in mitochondrial oxidative phosphorylation. Defects in oxidative phosphorylation leads to a decrease in cellular ATP production. Studies have reported that the activity of mitochondrial respiratory chain complexes in UC patients is reduced, and mucosal ATP is absent[50, 51]. Atp5f1 is a nuclear gene responsible for encoding the F0 subunit of ATP synthase, which is closely related to energy metabolism. Studies have found that the high expression of Cps1 plays an important role in the formation stage from UC to colitis associated cancer[52]. The intestinal mucosa of UC patients has been under chronic stress for a long time. The dynamic balance between oxidation and antioxidant defense mechanisms in the body is broken, causing mitochondrial damage, weakened oxidative phosphorylation, and reduced energy supply, which in turn causes cell apoptosis and colonic mucosal barrier dysfunction. The above results showed that HM and EA play a therapeutic effect on UC for mitochondrial function and energy metabolism. However, the role of Sv2a, Ncbp1 and Dclk3 in UC or mitochondrial oxidative phosphorylation has not been reported yet, and further research is needed.

In summary, iTRAQ proteomics was used in this study to identify DEPs in the colon of DSS-induced UC rats, as well as proteins that could be regulated by EA and HM, and analysed the function of these DEPs. However, the specific mechanisms of these DEPs were still unclear. They may be involved in one or more regulatory pathways, thus participate in the pathogenesis of UC and the mechanism of EA and HM treatments. The underlying mechanisms may require further study through animal and cell experiments.

CONCLUSION

In conclusion, our study used proteomics to provide evidence that EA and HM might regulate immune-related pathways by regulating the expression of ATP5L, Atp5f1, Cox4i1 that associated with oxidative phosphorylation, thereby alleviating colonic inflammation of DSS-induced UC rats. Further investigations at the role of these proteins in UC will be helpful for revealing the pathogenesis and the mechanism of acupuncture and moxibustion on UC.

ARTICLE HIGHLIGHTS

Research background

Ulcerative colitis (UC) is a chronic, nonspecific intestinal inflammatory disease with unclear etiology. Our previous studies have confirmed that acupuncture and moxibustion is effective in treating UC, but the mechanisms of treatment is still not completely clarified. Proteomic technology has revealed a

variety of biological markers related to immunity and inflammation in UC, which provide new insights and directions for the study of mechanism of acupuncture and moxibustion treatment of UC.

Research motivation

The mechanisms of UC and the therapeutic targets of acupuncture and moxibustion treatment are complicated, and whether acupuncture and moxibustion play a therapeutic role in UC by regulating proteome changes remains unclear.

Research objectives

The present study aims to investigate the underlying mechanism of electroacupuncture (EA) and moxibustion on UC rats by using isotope-labeled relative and absolute quantification (iTRAQ) proteomics technology.

Research methods

Male Sprague-Dawley rats were randomly divided into the normal (N) group, the DSS-induced UC model (M) group, the herb-partitioned moxibustion (HM) group, and the EA group. 3% DSS was used to establish the UC rat model except for the N group, and HM and EA at the Tianshu (bilateral) and Qihai acupoints were performed respectively. Haematoxylin and eosin staining was used for morphological evaluation of colon tissues. iTRAQ and liquid chromatography-tandem mass spectrometry were performed for proteome analysis of the colon tissues, followed by bioinformatics analysis and protein-protein interaction networks establishment of differentially expressed proteins (DEPs) between groups. Then western blot was used for verification of selected DEPs.

Research results

Our study revealed that HM and EA could regulate the expression of multiple proteins in colon of DSS-induced UC model rat. The DEPs were involved in various biological processes such as biological regulation, immune system progression and in multiple pathways including natural killer cell mediated cytotoxicity, intestinal immune network for immunoglobulin A production, and FcγR-mediated phagocytosis. Network analysis revealed that multiple pathways for the DEPs of each group were involved in protein-protein interactions. Subsequent verification of selected DEPs [synaptic vesicle glycoprotein 2A, nuclear cap binding protein subunit 1, carbamoyl phosphate synthetase 1, cytochrome c oxidase subunit 4 isoform 1 (Cox4i1), ATP synthase beta subunit precursor (Atp5F1), doublecortin like kinase 3] by western blot confirmed the reliability of the iTRAQ data.

Research conclusions

HM and EA might regulate immune-related pathways by regulating the expression of ATP5L, Atp5f1, Cox4i1 that associated with oxidative phosphorylation pathways, thereby alleviating colonic inflammation of DSS-induced UC rats.

Research perspectives

The present study revealed the possible molecular mechanisms of acupuncture and moxibustion treatment on UC, it may provide new light on clinical therapy of acupuncture and moxibustion treatment of UC.

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FOOTNOTES

Author contributions: Wu LY, Huang Y and Qi Q conceived and designed this study; Zhong R, Liu YN, Ma Z and Zheng HD performed the animal experiments, acquired and analyzed the data; Qi Q wrote the main manuscript; Liu YN and Ma Z prepared the figures and tables; Lu Y gave guidance to the manuscript writing; Zhao C, Huang Y and Wu LY revised and improved the manuscript; and all authors reviewed and approved the final version of this manuscript.

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REFERENCES

1 **Ungaro R**, Mehandru S, Allen PB, Peyrin-Biroulet L, Colombel JF. Ulcerative colitis. *Lancet* 2017; **389**: 1756-1770 [PMID: 27914657 DOI: 10.1016/S0140-6736(16)32126-2]
2 **Ng WK**, Wong SH, Ng SC. Changing epidemiological trends of inflammatory bowel disease in Asia. *Intest Res* 2016; **14**: 111-119 [PMID: 27175111 DOI: 10.5217/ir.2016.14.2.111]
3 **Wei SC**, Sollano J, Hui YT, Yu W, Santos Estrella PV, Llamado LJQ, Koram N. Epidemiology, burden of disease, and unmet needs in the treatment of ulcerative colitis in Asia. *Expert Rev Gastroenterol Hepatol* 2021; **15**: 275-289 [PMID: 33107344 DOI: 10.1080/17474124.2021.1840976]
4 **Du L**, Ha C. Epidemiology and Pathogenesis of Ulcerative Colitis. *Gastroenterol Clin North Am* 2020; **49**: 643-654 [PMID: 33121686 DOI: 10.1016/j.gtc.2020.07.005]
5 **Ji J**, Huang Y, Wang XF, Ma Z, Wu HG, Im H, Liu HR, Wu LY, Li J. Review of Clinical Studies of the Treatment of Ulcerative Colitis Using Acupuncture and Moxibustion. *Gastroenterol Res Pract* 2016; **2016**: 9248589 [PMID: 27885326 DOI: 10.1155/2016/9248589]
6 **Yang L**, Zhao JM, Guan X, Wang XM, Zhao C, Liu HR, Wu LY, Ji J, Cheng F, Liu XR, Wu HG. Observation on the effects of different partitioned moxibustion in treating ulcerative colitis. *J Acupunct Tuina Sci* 2016; **14**: 231-241 [DOI: 10.1007/s11726-016-0931-5]
7 **Lin YY**, Zhao JM, Ji YJ, Ma Z, Zheng HD, Huang Y, Cui YH, Lu Y, Wu HG. Typical ulcerative colitis treated by herbs-partitioned moxibustion: A case report. *World J Clin Cases* 2020; **8**: 1515-1524 [PMID: 32368545 DOI: 10.12998/wjcc.v8.i8.1515]
8 **Li YH**, Sun W, Zhou BJ, Rosenstein A, Zhao J, Wang J, Bian ZX. iTRAQ-based pharmacoproteomics reveals potential targets of berberine, a promising therapy for ulcerative colitis. *Eur J Pharmacol* 2019; **850**: 167-179 [PMID: 30771347 DOI: 10.1016/j.ejphar.2019.02.021]
9 **Schniers A**, Goll R, Pasing Y, Sørbye SW, Florholmen J, Hansen T. Ulcerative colitis: functional analysis of the in-depth proteome. *Clin Proteomics* 2019; **16**: 4 [PMID: 30718987 DOI: 10.1186/s12014-019-9224-6]
10 **Pisani LF**, Moriggi M, Gelfi C, Vecchi M, Pastorelli L. Proteomic insights on the metabolism in inflammatory bowel disease. *World J Gastroenterol* 2020; **26**: 696-705 [PMID: 32116417 DOI: 10.3748/wjg.v26.i7.696]
11 **Liu W**, Chen Y, Golan MA, Annunziata ML, Du J, Dougherty U, Kong J, Musch M, Huang Y, Pekow J, Zheng C, Bissonnette M, Hanauer SB, Li YC. Intestinal epithelial vitamin D receptor signaling inhibits experimental colitis. *J Clin Invest* 2013; **123**: 3983-3996 [PMID: 23945234 DOI: 10.1172/JCI65842]
12 **Yu SG**. Experimental Acupuncture. Beijing: People's medical publishing house, 2012
13 **Huang Y**, Ma Z, Cui YH, Dong HS, Zhao JM, Dou CZ, Liu HR, Li J, Wu HG. Effects of Herb-Partitioned Moxibustion on the miRNA Expression Profiles in Colon from Rats with DSS-Induced Ulcerative Colitis. *Evid Based Complement Alternat Med* 2017; **2017**: 1767301 [PMID: 28246536 DOI: 10.1155/2017/1767301]
14 **Li H**, Li KS, Wu LY, Zhou ZG, Huang RJ, Wu HG, Liu YN, Huang Y, Ma XP, Liu HR, Lu Y. Study on the Regulatory Effects of Acupuncture on Vitamin D Receptor and p53 Signal Pathway in Ulcerative Colitis Rats. *World Chinese Med* 2020; **15**: 2259-63+70 [DOI: 10.3969/j.issn.1673-7202.2020.15.012]
15 **Wang L**, Xie H, Xu L, Liao Q, Wan S, Yu Z, Lin D, Zhang B, Lv Z, Wu Z, Sun X. rSj16 Protects against DSS-Induced

Colitis by Inhibiting the PPAR-α Signaling Pathway. *Theranostics* 2017; **7**: 3446-3460 [PMID: 28912887 DOI: 10.7150/thno.20359]

16 **Lu Y**, Ding G, Zheng H, Lü T, Ma Z, Wu H, Weng Z, Zhang F, Wu L, Liu H, Xu M, Feng H. Effect of herb-partitioned moxibustion on dopamine levels and dopamine receptor 1 expression in the colon and central nervous system in rats with Crohn's disease. *J Tradit Chin Med* 2019; **39**: 356-363 [PMID: 32186008]

17 **Li N**, Xu Z, Zhai L, Li Y, Fan F, Zheng J, Xu P, He F. Rapid development of proteomics in China: from the perspective of the Human Liver Proteome Project and technology development. *Sci China Life Sci* 2014; **57**: 1162-1171 [PMID: 25119674 DOI: 10.1007/s11427-014-4714-2]

18 **Lees T**, Nassif N, Simpson A, Shad-Kaneez F, Martiniello-Wilks R, Lin Y, Jones A, Qu X, Lal S. Recent advances in molecular biomarkers for diabetes mellitus: a systematic review. *Biomarkers* 2017; **22**: 604-613 [PMID: 28074664 DOI: 10.1080/1354750X.2017.1279216]

19 **Zhang YF**. Differential proteomics analysis of tissues from ulcerative colitis patients and the establishment of acute damaged models with ulcerative colitis. Medical College of the Chinese People's Liberation Army, 2012

20 **Jin L**, Li L, Hu C, Paez-Cortez J, Bi Y, Macoritto M, Cao S, Tian Y. Integrative Analysis of Transcriptomic and Proteomic Profiling in Inflammatory Bowel Disease Colon Biopsies. *Inflamm Bowel Dis* 2019; **25**: 1906-1918 [PMID: 31173627 DOI: 10.1093/ibd/izz111]

21 **Ahmad A**, Ansari MM, Mishra RK, Kumar A, Vyawahare A, Verma RK, Raza SS, Khan R. Enteric-coated gelatin nanoparticles mediated oral delivery of 5-aminosalicylic acid alleviates severity of DSS-induced ulcerative colitis. *Mater Sci Eng C Mater Biol Appl* 2021; **119**: 111582 [PMID: 33321628 DOI: 10.1016/j.msec.2020.111582]

22 **Mishra RK**, Ahmad A, Kumar A, Vyawahare A, Raza SS, Khan R. Lipid-based nanocarrier-mediated targeted delivery of celecoxib attenuate severity of ulcerative colitis. *Mater Sci Eng C Mater Biol Appl* 2020; **116**: 111103 [PMID: 32806257 DOI: 10.1016/j.msec.2020.111103]

23 **Lo Sasso G**, Phillips BW, Sewer A, Battey JND, Kondylis A, Talikka M, Titz B, Guedj E, Peric D, Bornand D, Dulize R, Merg C, Corciulo M, Ouadi S, Yanuar R, Tung CK, Ivanov NV, Peitsch MC, Hoeng J. The reduction of DSS-induced colitis severity in mice exposed to cigarette smoke is linked to immune modulation and microbial shifts. *Sci Rep* 2020; **10**: 3829 [PMID: 32123204 DOI: 10.1038/s41598-020-60175-3]

24 **Simovic Markovic B**, Nikolic A, Gazdic M, Bojic S, Vucicevic L, Kosic M, Mitrovic S, Milosavljevic M, Besra G, Trajkovic V, Arsenijevic N, Lukic ML, Volarevic V. Galectin-3 Plays an Important Pro-inflammatory Role in the Induction Phase of Acute Colitis by Promoting Activation of NLRP3 Inflammasome and Production of IL-1β in Macrophages. *J Crohns Colitis* 2016; **10**: 593-606 [PMID: 26786981 DOI: 10.1093/ecco-jcc/jjw013]

25 **Papa Gobbi R**, De Francesco N, Bondar C, Muglia C, Chirdo F, Rumbo M, Rocca A, Toscano MA, Sambuelli A, Rabinovich GA, Docena GH. A galectin-specific signature in the gut delineates Crohn's disease and ulcerative colitis from other human inflammatory intestinal disorders. *Biofactors* 2016; **42**: 93-105 [PMID: 26891020 DOI: 10.1002/biof.1252]

26 **Simard JC**, Cesaro A, Chapeton-Montes J, Tardif M, Antoine F, Girard D, Tessier PA. S100A8 and S100A9 induce cytokine expression and regulate the NLRP3 inflammasome *via* ROS-dependent activation of NF-κB(1.). *PLoS One* 2013; **8**: e72138 [PMID: 23977231 DOI: 10.1371/journal.pone.0072138]

27 **Sekiya S**, Murata M, Arai S, Murayama H, Kawasaki A, Ashida N, Okada K, Ikemoto M. Enzyme-linked immunosorbent assay for S100A9 in the stool of rats with dextran sulfate sodium-induced colitis. *J Immunol Methods* 2016; **439**: 44-49 [PMID: 27693389 DOI: 10.1016/j.jim.2016.09.009]

28 **Steinbrecher KA**, Harmel-Laws E, Garin-Laflam MP, Mann EA, Bezerra LD, Hogan SP, Cohen MB. Murine guanylate cyclase C regulates colonic injury and inflammation. *J Immunol* 2011; **186**: 7205-7214 [PMID: 21555532 DOI: 10.4049/jimmunol.1002469]

29 **Brenna Ø**, Furnes MW, Munkvold B, Kidd M, Sandvik AK, Gustafsson BI. Cellular localization of guanylin and uroguanylin mRNAs in human and rat duodenal and colonic mucosa. *Cell Tissue Res* 2016; **365**: 331-341 [PMID: 27044258 DOI: 10.1007/s00441-016-2393-y]

30 **Brentnall TA**, Pan S, Bronner MP, Crispin DA, Mirzaei H, Cooke K, Tamura Y, Nikolskaya T, Jebailey L, Goodlett DR, McIntosh M, Aebersold R, Rabinovitch PS, Chen R. Proteins That Underlie Neoplastic Progression of Ulcerative Colitis. *Proteomics Clin Appl* 2009; **3**: 1326 [PMID: 20098637 DOI: 10.1002/prca.200900061]

31 **Wang D**, Zhang Y, Yang S, Zhao D, Wang M. A polysaccharide from cultured mycelium of Hericium erinaceus relieves ulcerative colitis by counteracting oxidative stress and improving mitochondrial function. *Int J Biol Macromol* 2019; **125**: 572-579 [PMID: 30543884 DOI: 10.1016/j.ijbiomac.2018.12.092]

32 **Nyström EEL**, Arike L, Ehrencrona E, Hansson GC, Johansson MEV. Calcium-activated chloride channel regulator 1 (CLCA1) forms non-covalent oligomers in colonic mucus and has mucin 2-processing properties. *J Biol Chem* 2019; **294**: 17075-17089 [PMID: 31570526 DOI: 10.1074/jbc.RA119.009940]

33 **Nyström EEL**, Birchenough GMH, van der Post S, Arike L, Gruber AD, Hansson GC, Johansson MEV. Calcium-activated Chloride Channel Regulator 1 (CLCA1) Controls Mucus Expansion in Colon by Proteolytic Activity. *EBioMedicine* 2018; **33**: 134-143 [PMID: 29885864 DOI: 10.1016/j.ebiom.2018.05.031]

34 **Erickson NA**, Mundhenk L, Giovannini S, Glauben R, Heimesaat MM, Gruber AD. Role of goblet cell protein CLCA1 in murine DSS colitis. *J Inflamm (Lond)* 2016; **13**: 5 [PMID: 26855614 DOI: 10.1186/s12950-016-0113-8]

35 **Li X**, Hu W, Zhou J, Huang Y, Peng J, Yuan Y, Yu J, Zheng S. CLCA1 suppresses colorectal cancer aggressiveness *via* inhibition of the Wnt/beta-catenin signaling pathway. *Cell Commun Signal* 2017; **15**: 38 [PMID: 28974231 DOI: 10.1186/s12964-017-0192-z]

36 **Bian Q**, Chen J, Qiu W, Peng C, Song M, Sun X, Liu Y, Ding F, Zhang L. Four targeted genes for predicting the prognosis of colorectal cancer: A bioinformatics analysis case. *Oncol Lett* 2019; **18**: 5043-5054 [PMID: 31612015 DOI: 10.3892/ol.2019.10866]

37 **Hu D**, Ansari D, Bauden M, Zhou Q, Andersson R. The Emerging Role of Calcium-activated Chloride Channel Regulator 1 in Cancer. *Anticancer Res* 2019; **39**: 1661-1666 [PMID: 30952704 DOI: 10.21873/anticanres.13271]

38 **Lu PD**, Zhao YH. Targeting NF-κB pathway for treating ulcerative colitis: comprehensive regulatory characteristics of Chinese medicines. *Chin Med* 2020; **15**: 15 [PMID: 32063999 DOI: 10.1186/s13020-020-0296-z]

39 **Wang Y**, Tang Q, Duan P, Yang L. Curcumin as a therapeutic agent for blocking NF-κB activation in ulcerative colitis. *Immunopharmacol Immunotoxicol* 2018; **40**: 476-482 [PMID: 30111198 DOI: 10.1080/08923973.2018.1469145]

40 **Huang XL**, Xu J, Zhang XH, Qiu BY, Peng L, Zhang M, Gan HT. PI3K/Akt signaling pathway is involved in the pathogenesis of ulcerative colitis. *Inflamm Res* 2011; **60**: 727-734 [PMID: 21442372 DOI: 10.1007/s00011-011-0325-6]

41 **Wei J**, Feng J. Signaling pathways associated with inflammatory bowel disease. *Recent Pat Inflamm Allergy Drug Discov* 2010; **4**: 105-117 [PMID: 20001899 DOI: 10.2174/187221310791163071]

42 **Atreya I**, Atreya R, Neurath MF. NF-kappaB in inflammatory bowel disease. *J Intern Med* 2008; **263**: 591-596 [PMID: 18479258 DOI: 10.1111/j.1365-2796.2008.01953.x]

43 **Castro-Dopico T**, Clatworthy MR. IgG and Fcγ Receptors in Intestinal Immunity and Inflammation. *Front Immunol* 2019; **10**: 805 [PMID: 31031776 DOI: 10.3389/fimmu.2019.00805]

44 **Castro-Dopico T**, Dennison TW, Ferdinand JR, Mathews RJ, Fleming A, Clift D, Stewart BJ, Jing C, Strongili K, Labzin LI, Monk EJM, Saeb-Parsy K, Bryant CE, Clare S, Parkes M, Clatworthy MR. Anti-commensal IgG Drives Intestinal Inflammation and Type 17 Immunity in Ulcerative Colitis. *Immunity* 2019; **50**: 1099-1114.e10 [PMID: 30876876 DOI: 10.1016/j.immuni.2019.02.006]

45 **Xie D**, Zhang Y, Qu H. Crucial genes of inflammatory bowel diseases explored by gene expression profiling analysis. *Scand J Gastroenterol* 2018; **53**: 685-691 [PMID: 29909694 DOI: 10.1080/00365521.2018.1461923]

46 **Shi W**, Zou R, Yang M, Mai L, Ren J, Wen J, Liu Z, Lai R. Analysis of Genes Involved in Ulcerative Colitis Activity and Tumorigenesis Through Systematic Mining of Gene Co-expression Networks. *Front Physiol* 2019; **10**: 662 [PMID: 31214045 DOI: 10.3389/fphys.2019.00662]

47 **Wang F**, Song Z, Chen J, Wu Q, Zhou X, Ni X, Dai J. The immunosuppressive functions of two novel tick serpins, HlSerpin-a and HlSerpin-b, from Haemaphysalis longicornis. *Immunology* 2020; **159**: 109-120 [PMID: 31606893 DOI: 10.1111/imm.13130]

48 **Lucas A**, Yaron JR, Zhang L, Ambadapadi S. Overview of Serpins and Their Roles in Biological Systems. *Methods Mol Biol* 2018; **1826**: 1-7 [PMID: 30194590 DOI: 10.1007/978-1-4939-8645-3_1]

49 **Mkaouar H**, Mariaule V, Rhimi S, Hernandez J, Kriaa A, Jablaoui A, Akermi N, Maguin E, Lesner A, Korkmaz B, Rhimi M. Gut Serpinome: Emerging Evidence in IBD. *Int J Mol Sci* 2021; **22** [PMID: 34200095 DOI: 10.3390/ijms22116088]

50 **Sifroni KG**, Damiani CR, Stoffel C, Cardoso MR, Ferreira GK, Jeremias IC, Rezin GT, Scaini G, Schuck PF, Dal-Pizzol F, Streck EL. Mitochondrial respiratory chain in the colonic mucosal of patients with ulcerative colitis. *Mol Cell Biochem* 2010; **342**: 111-115 [PMID: 20440543 DOI: 10.1007/s11010-010-0474-x]

51 **Santhanam S**, Rajamanickam S, Motamarry A, Ramakrishna BS, Amirtharaj JG, Ramachandran A, Pulimood A, Venkatraman A. Mitochondrial electron transport chain complex dysfunction in the colonic mucosa in ulcerative colitis. *Inflamm Bowel Dis* 2012; **18**: 2158-2168 [PMID: 22374887 DOI: 10.1002/ibd.22926]

52 **Lee YY**, Li CF, Lin CY, Lee SW, Sheu MJ, Lin LC, Chen TJ, Wu TF, Hsing CH. Overexpression of CPS1 is an independent negative prognosticator in rectal cancers receiving concurrent chemoradiotherapy. *Tumour Biol* 2014; **35**: 11097-11105 [PMID: 25099619 DOI: 10.1007/s13277-014-2425-8]

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How has the disease course of pediatric ulcerative colitis changed throughout the biologics era? A comparison with the IBSEN study

Yiyoung Kwon, Eun Sil Kim, Yon Ho Choe, Mi Jin Kim

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Abstract

BACKGROUND

In Korea, infliximab was approved for use in children with ulcerative colitis (UC) in October 2012.

AIM

To compare the clinical course of UC before and after the introduction of biological agents, and to compare with the IBSEN study.

METHODS

Patients under 18 years of age, who were diagnosed with UC and followed from January 2003 to October 2020, were included in the study. Group A (n = 48) was followed for at least 2 years between January 2003 and October 2012, and Group B (n = 62) was followed for at least 2 years between November 2012 and October 2020. We compared endoscopic remission, drug composition, relapse rate, steroid-free period, and the quality of life of each group. We plotted the clinical course of the included patients using the pediatric UC activity index score, and compared our patients with those in the IBSEN study.

RESULTS

After 2 years of treatment, colonoscopy evaluation revealed different outcomes in the two treatment groups. Remission was confirmed in 14 patients (29.2%) of Group A, and in 31 patients (50.0%) of Group B (P < 0.012). The median cumulative corticosteroid-free period was 3.0 years in Group A and 4.4 years in Group B. Steroid-free period of Group B was significantly longer than that of Group A (P < 0.001). There was a statistically significant difference between the two groups in evaluation of the relapse rate during the observation period (P < 0.001). The plotted clinical course graphs of Group A showed similar proportions to the

graphs in the IBSEN study. However, in Group B, the proportion of patients corresponding to curve 1 (remission or mild severity after initial high activity) was high at 76% (47/62).

CONCLUSION

The incidence of relapse has decreased and the steroid-free period has increased after the introduction of the biological agent. The clinical course also showed a different pattern from that of IBSEN study. The active use of biological agents may change the long-term disease course in moderate to severe pediatric UC.

Key Words: Colitis; Ulcerative; Children; Infliximab; Relapse; Steroid

Core Tip: This was a retrospective study that assessed how the introduction of biological agents has altered the disease course over time in pediatric ulcerative colitis (UC). Endoscopic remission, relapse rate, steroid-free period, and the quality of life of each group were evaluated as outcomes. Clinical course was plotted with the pediatric UC activity index score, and compared to that of the IBSEN study. The incidence of relapse has decreased and the steroid-free period has increased after the introduction of biological agents. The clinical course also showed a different pattern from the IBSEN study.

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INTRODUCTION

Ulcerative colitis (UC) is a chronic inflammatory bowel disease (IBD) that was first described in 1875 by two English physicians. Although it has been the subject of many studies over the years, the etiology remains unknown[1]. The potential causes explained in the literature include immune system dysfunction, genetics, changes in normal intestinal bacteria, environmental factors, and a combination of any or all of these variables[2]. The incidence of UC is higher in developed countries than in less developed ones, which is thought to be the result of reduced exposure to intestinal infections and a Western-style diet and lifestyle[3]. As people in Asia adopt more Westernized dietary habits, the incidence rate is gradually increasing in that part of the world[4].

The age of onset of UC can be anywhere from 15 to 30 to > 60 years, and the incidence of UC is lower than that of Crohn's disease in children[5]. For this reason, the number of large-scale studies and long-term follow-up studies for pediatric patients with UC is insufficient, and more studies are needed to evaluate the clinical course of UC in such patients. The IBSEN study evaluated the long-term clinical course and outcomes of 423 adult patients with UC during the first 10 years. Mortality risk and cumulative colorectal resection were evaluated as clinical outcomes. The proportion of patients who relapsed and who remain in remission was evaluated as a clinical course. In addition, the authors divided the disease course of UC into four types and showed them in graphs, which became a representative figure of this paper[6]. However, this study was done before any biological agents were approved as treatments. No studies have compared the clinical course of UC before and after the introduction of biological agents in a single cohort.

Infliximab was first introduced in Europe in 1999 for the treatment of Crohn's disease[7]. Since the introduction of infliximab, several studies have reported improvements in clinical outcomes with infliximab treatment[8-11]. Nevertheless, relapse has been observed in patients treated with infliximab, and many studies have been conducted on factors that can predict relapse in these patients[12-14]. Therefore, we assessed what changes have occurred in the long-term clinical course of UC since the introduction of biological agents as treatment.

In Korea, infliximab was approved for use in adults with UC in May 2007, and in children in October 2012. Since long-term follow up is possible due to the characteristics of pediatric patients, the aim of this study was to compare the clinical course of UC before and after the introduction of biological agents. Another goal of this study was to compare the clinical course of our patients to that in the IBSEN study.

MATERIALS AND METHODS

Patients

Patients under 18 years of age, who were diagnosed with UC and had been followed from January 2003 to October 2020, were included in this study. Initially, the total number of patients was 138, but patients who had been observed for less than 2 years were eliminated. Finally, 110 patients were selected as the study group (Figure 1). All patients were children and adolescents under 18 years of age at the time of diagnosis, but some patients became adults during the follow-up period. UC was diagnosed in accordance with the guidelines of the European Society for Pediatric Gastroenterology, Hepatology and Nutrition (the Porto criteria)[15]. This study was approved by the institutional review board of Samsung Medical Center (IRB File No. SMC 2020-12-005).

Study design

This was a retrospective study. The first goal was to evaluate how the active use of biological agents causes a change in the clinical course of UC. This was done using data from patients who were treated after the approval of infliximab in October 2012. With the cut-off date of October 2012, the patients were divided into two groups, A and B. Group A had been followed for at least 2 years between January 2003 and October 2012, and Group B had been followed for at least 2 years between November 2012 and October 2020. Since the two groups were classified according to a time difference, we first checked whether there was any difference in the baseline characteristics of the patients, and then conducted a comparative analysis.

Both patient groups were evaluated by laboratory tests (including hemoglobin, albumin, erythrocyte sedimentation rate [ESR], and C-reactive protein [CRP]), colonoscopy, abdominal computed tomography (CT), and pediatric UC activity index (PUCAI) score at the time of diagnosis. Laboratory tests were performed and a PUCAI score questionnaire was submitted at each outpatient follow-up visit, which took place at intervals of 1-3 mo. We confirmed that there were no clinical differences between the two groups in terms of demographic characteristics, the extent and severity of disease according to the laboratory test and colonoscopy results, or the PUCAI score at the time of diagnosis. The fecal calprotectin test has only been available in Korea since 2017 and thus could not be included as a comparative test. Based on the colonoscopy findings, disease extent was classified into E1 (proctitis), E2 (left colitis), E3 (right colitis), and E4 (pancolitis) according to the Paris classification[16], and severity was classified from 0 to 3 using the Mayo endoscopic subscore (MES)[17].

Evaluation of clinical course including relapse and clinical outcome

Colonoscopy was performed at the time of initial diagnosis and at intervals of 1 to 2 years during the follow-up period. The colonoscopy was performed with a CF scope after sedation with midazolam and pethidine. All procedures reached the cecum and the entire colon was examined. Characteristic endoscopic findings of UC such as peri-appendiceal patches, demarcation line, mucosal edema, decreased vascularity of loss of vascularity, and superficial tiny ulcers were observed and described. We reviewed the colonoscopy findings at 2 and 5 years after the start of treatment. Remission was defined as endoscopic mucosal healing, which means that no lesion was observed and the MES was 0 with histological healing (Geboes grade 0-1). The reason for excluding MES 1 is that the authors aimed at deep remission because they experienced cases of MES 1 that relapsed easily. In addition, we investigated the drugs prescribed to both groups of patients during the follow-up period.

To compare the clinical course between the groups, the number of relapses that occurred during the follow-up period was investigated, along with the time interval at which the first relapse occurred (from the time of diagnosis) and the time interval of each relapse when multiple relapses occurred in the same patient. Since the follow-up period was different for each patient, the relapse rate was finally evaluated between the two groups. Clinical relapse was defined as a PUCAI score > 10 with modification of treatment (addition of steroids or biologic agents). To evaluate the clinical risk factors for relapse, age, PUCAI score, disease extent, MES, and initial use of corticosteroids were statistically analyzed. Situations in which symptoms temporarily worsened due to infections such as gastroenteritis were excluded.

In addition, the corticosteroid-free period, cumulative colectomy, and changes in PUCAI score during the follow-up period were evaluated as clinical outcomes of both groups. As another quality of life-related factor, patients who did not experience relapse but were hospitalized or admitted to the emergency room for antibiotic use or blood transfusions were also investigated and the rates of such patients were compared between the groups.

The second goal of this study was to actually draw a clinical course using PUCAI score in these two patient groups and compare these distributions to the four predefined curves, depicting different clinical courses of UC, as published in the IBSEN study[6].

Statistical analyses

The demographic and clinical characteristics of the study groups at initial diagnosis and the clinical outcomes of the study groups were compared using the χ^2 test and Mann-Whitney test. Steroid-free

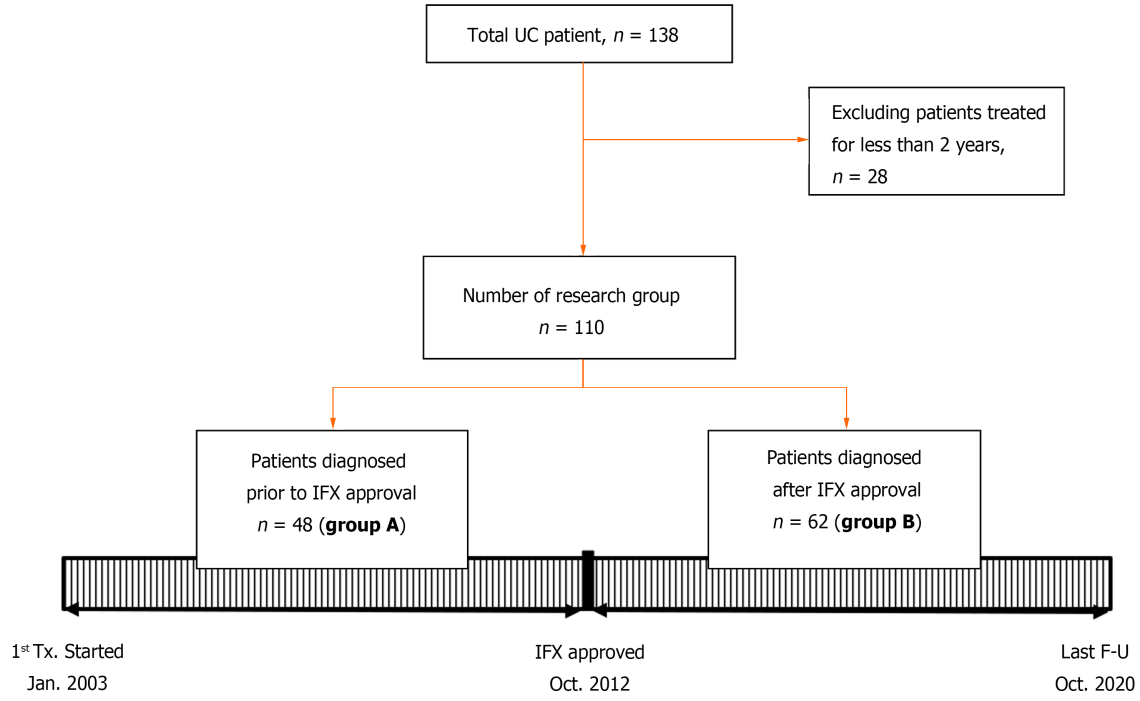


Figure 1 Patient group classification. F-U: Follow-up; IFX: Infliximab; Jan: January; *n*: Number; UC: Ulcerative colitis; Oct: October; Tx: Treatment.

survival curves were presented for the whole cohort using the Kaplan-Meier method. Differences in survival curves between the two groups were evaluated using the log-rank test. Repeated event data for relapses were exhibited by estimating mean cumulative function (MCF), which is the average number of cumulative events experienced by an individual in the study at each point in time since the start of follow-up[18,19]. Univariable/multivariable analyses of the associations between relapse rate and other factors were analyzed by Cox's proportional hazard regression using count process[20] because of multiple events for relapse. Multivariable analysis was performed by selecting variables with $P < 0.1$ in univariate analysis. The 95% confidence intervals (CIs) of the hazard ratio (HR) using robust sandwich covariance estimate to account the within subject correlation[21] were estimated. All of the above statistical analyses were conducted using SAS version 9.4. MCF was analyzed using reReg 1.4.0 package in R 4.0.4 (Vienna, Austria; http://www.R-project.org/. $P < 0.05$ was considered statistically significant.

RESULTS

Baseline characteristics

Table 1 shows the patient characteristics and disease severity information of Group A (n = 48) and Group B (n = 62) at the time of diagnosis, as well as information on the medications that were used for treatment. The median age at the time of diagnosis of UC was 14.4 years for Group A and 15.8 years for Group B. There was no significant difference between the two groups. The median value of the total follow-up period was 3.3 years in Group A and 4.5 years in Group B, and there was no significant difference between the two groups. Serum hemoglobin, albumin, ESR, and CRP levels confirmed by laboratory tests at the time of diagnosis also did not show any significant difference between the two groups.

Based on the results of endoscopy performed at the time of diagnosis, the disease extents of the two groups were classified according to the Paris classification, and severity was evaluated using the endoscopy subscore. There was no difference in disease severity between the two groups, but regarding disease extent, Group B patients had a higher rate of pancolitis and lower rate of proctitis. In Group A, 18 patients (37.5%) had proctitis and 16 (33.3%) had pancolitis, but in Group B, 13 (21%) had proctitis and 34 (54.8%) had pancolitis.

Within 3 mo of diagnosis, most patients in both groups were treated with 5-aminosalicylate and immunosuppressants, primarily azathioprine. In both groups, about 50% of patients used corticosteroids to reduce disease activity at the time of diagnosis. Among the 21 patients who used corticosteroids in Group A, 8 (38.1%) showed dependence and 1 (4.8%) showed refractory findings. In Group B, 9/30 (30.0%) of patients were corticosteroid-dependent and 1/30 (3.3%) was corticosteroid-refractory. There was no statistical difference between the two groups.

Table 1 Demographic and clinical features of the two groups before and after infliximab approval

	Group A (*n* = 48)	Group B (*n* = 62)	*P* value
Age at diagnosis, yr	14.4 (12.2-17.1)	15.8 (13.1-16.5)	0.574[c]
Total duration of follow up with treatment, yr	4.0 (4.0-5.0)	5.5 (3.0-6.9)	0.073[c]
PUCAI[a] at diagnosis	35 (30-65)	45 (35-55)	0.969[c]
Hemoglobin at diagnosis, g/dL	12.3 (10.5-14.1)	12.1 (9.6-13.5)	0.245[c]
Albumin at diagnosis, g/dL	4.3 (4.0-4.6)	4.2 (3.9-4.6)	0.702[c]
ESR at diagnosis, mm/h	14.5 (7-31.3)	20 (7.5-39)	0.249[c]
CRP at diagnosis, mg/dL	0.04 (0.03-0.13)	0.12 (0.03-0.56)	0.791[c]
Disease extent of Paris classification at diagnosis			0.018[d]
E1 proctitis	18 (37.5)	13 (21)	
E2 left colitis	8 (16.7)	8 (12.9)	
E3 right colitis	6 (12.5)	7 (11.3)	
E4 pancolitis	16 (33.3)	34 (54.8)	
Mayo endoscopic subscore at diagnosis[b]			0.310[d]
0 normal or inactive	0	0	
1 mild	12 (25.0)	7 (11.3)	
2 moderate	26 (54.2)	43 (69.4)	
3 severe	10 (20.8)	12 (19.4)	
Corticosteroid use at baseline	21 (43.8)	30 (48.4)	0.630[d]
Corticosteroid-dependent	8/21 (38.1)	9/30 (30.0)	0.550[d]
Corticosteroid-refractory	1/21 (4.8)	1/30 (3.3)	0.360[d]
Cumulative number receiving medication by			0.211[d]
3 mo after diagnosis			0.453[d]
5-aminosalicylate	48 (100)	60 (96.8)	
Azathioprine	42 (87.5)	51 (82.3)	
Methotrexate	0 (0)	1 (1.6)	
Cyclosporine	1 (2.1)	0 (0)	

[a]Pediatric Ulcerative Colitis Activity Index (PUCAI) is a 6-item disease activity index intended for use in pediatric UC clinical trials with a score ranging from 0-85.
[b]Mayo endoscopy subscores were as follows: 0: Normal or inactive disease; 1: Mild disease; 2: Moderate disease; and 3: Severe disease.
[c]χ^2 test.
[d]Mann-Whitney test.
Values are *n* (percentage) or median (interquartile range); *n*: Number of patients; PUCAI: Pediatric Ulcerative Colitis Activity Index.

Comparison of drug composition after 2 and 5 years of treatment

When the drugs that were used for treatment were investigated at the 2-year follow-up visits, 5 patients in Group B did not use any drugs, but no patient in Group A terminated drug treatment (Supplementary Figure 1). In Group A, most patients were still using 5-aminosalicylate (97.9%) and azathioprine (89.6%). In Group B, 96.8% of patients used 5-aminosalicylate as an initial treatment, but at 2 years, some patients had discontinued the use of drugs and 74% of patients were still using the drugs. Also, only 58.1% of the patients in Group B maintained azathioprine treatment at 2 years. In the case of infliximab, on the other hand, more than half of patients in Group B started infliximab treatment within 2 years of diagnosis, and 34 patients were maintaining infliximab. In line with the above results, a statistically significant difference was found between the two groups in the composition of the treatment drugs at the time of the 2-year follow-up visits.

After 5 years, drug composition was re-evaluated in patients who were treated for more than 5 years (Group A = 24 patients, Group B = 31 patients). The same 5 patients in Group B continued to not use any drugs, and 2 patients in Group A also terminated drug treatment. In Group A, mesalazine (87.5%)

and azathioprine (58.3%) use had been tapered and discontinued in many patients. Nevertheless, the proportion of patients using oral drugs was still high compared to Group B (mesalazine: 48.4%, azathioprine: 45.2%). In Group B, the percentage of patients taking infliximab and other biological agents increased (67.7%) after 5 years. In terms of drug composition, the rate of use of mesalazine and infliximab was significantly different, with *P* values of 0.003 and 0.001, respectively (Supplementary Figure 1).

Comparison of disease states after 2 and 5 years of treatment

The patients underwent colonoscopy to re-evaluate disease extent and severity after 2 years of treatment (Table 2). In Group A, remission was confirmed in 14 patients and the proportion of each category of disease extent decreased slightly in comparison to the time of diagnosis, but the differences were small and pancolitis patients still accounted for 27.1% of the total. On the other hand, although the incidence of pancolitis was significantly higher in Group B at the time of diagnosis, 50% of the patients reached remission, and the number of pancolitis patients was significantly reduced to 4 (6.5%). In addition, severity according to the MES also showed a significant difference between the two groups ($P = 0.037$). A large percentage of patients in both groups had moderately severe disease at the time of diagnosis, but after 2 years of treatment, more had mildly severe disease; this was especially true in Group B, in which the percentage of patients with subscores of 0 or 1 was high. In Group B, 53.2% of cases was evaluated as normal or inactive.

Colonoscopy findings were analyzed again after 5 years of treatment (Table 2). As with year 2, the proportion of patients who reached remission at 5 years was greater in Group B (41.9%) than in Group A (12.5%). Overall, a higher proportion of cases in Group B was evaluated as E1 or E2, so disease severity in Group B was still milder than that in Group A ($P = 0.016$). Regarding MES, 41.9% of patients in Group B maintained an inactive disease state, whereas 12.5% of patients in Group B remained low. In Group A, 58.3% of cases was evaluated as moderate, and evidence of the disease was often difficult to see as it was well controlled. In Group B, 51.6% of patients had mild disease. The difference in disease severity between the two groups was statistically significant ($P < 0.001$).

Comparison of clinical outcomes during the total follow-up period

Disease relapse occurred in 23 patients (47.9%) in Group A and 16 (25.8%) in Group B, which was a significant difference ($P = 0.027$) (Table 3). The total cumulative number of relapses was also significantly higher in Group A (40 in Group A *vs* 22 in Group B; $P = 0.006$). In addition, although the difference was not significant, the number of relapses that occurred per person per year was also higher in Group A, with a ratio of 0.44 (Group A) *vs* 0.25 (Group B). The first relapse occurred at a median of 1.2 years after diagnosis in Group A, but in Group B, relapse occurred after a median interval of 1.95 years; thus, first relapse seemed to be delayed in Group B. The time interval between each relapse was investigated in patients who experienced multiple relapses. In Group A, relapse occurred approximately every 1.3 years, whereas relapse took about 1.7 years in Group B, showing that the interval between relapses was relatively wide in Group B. Since each patient's follow-up period was different, we also evaluated the relapse rate by comparing the cumulative hazard rate up to a specific point in time. There were statistically significant differences ($P < 0.001$) between the two groups, as shown in Figure 2.

The PUCAI scores at each outpatient visit during maintenance treatment (excluding times at which the patients had relapsed) were significantly lower in Group B than in Group A ($P < 0.001$) (Table 3). The PUCAI score at the time of relapse was also compared, which showed that the PUCAI score was significantly lower in Group B than in Group A ($P < 0.001$).

Because Group B patients were treated with infliximab, the frequency of steroid use was lower in that group (Table 3). The corticosteroid-free period was compared between the two groups. The median value of the cumulative corticosteroid-free period was 3.0 years in Group A and 4.4 years in Group B. The steroid-free period of Group B was significantly longer than that of Group A ($P < 0.001$). Figure 3 shows the analysis of corticosteroid-free survival curve after initiation of treatment using the Kaplan-Meier method. Over the course of 2 years, the likelihood of remaining corticosteroid-free in Group A was 21%, while it was 88% in Group B ($P = 0.003$).

Only 1 patient underwent colectomy in Group A. At the time of colectomy, the patient used mesalazine, azathioprine, methylprednisolone, and antibiotics. The patient finally underwent total proctocolectomy due to persistent uncontrolled hematochezia at 3 years after diagnosis.

Evaluation of risk factors related to relapse

Since relapse was often observed in both Group A and Group B, the risk factors associated with an increase in relapse rate were investigated in all patients who experienced relapse using Cox's proportional hazard regression for counting processes (Table 4). Since all patients were not followed up for the same period of time, the relapse rate was evaluated but not simply the number of relapses. All factors that were analyzed reflected the status at the time of diagnosis. Young age (> 10 years), PUCAI score over 45, disease extent according to the Paris classification, MES, and initial corticosteroid use were investigated as potential risk factors. In univariable analysis, MES, and initial corticosteroid use were identified as clinical risk factors with statistical significance ($P = 0.010$ and < 0.001) In multivariable

Table 2 Comparison of composition of drugs for treatment and disease states according to colonoscopy findings 2 and 5 yr after diagnosis before (group A) and after (group B) infliximab approval

	Group A (*n* = 48)	Group B (*n* = 62)	*P* value
Maintenance treatment 2 year after diagnosis			
None	0	5 (8.1)	0.045
5-Aminosalicylate	47 (97.9)	46 (74.2)	0.001
Azathioprine	43 (89.6)	36 (58.1)	0.001
Infliximab	0	34 (54.8)	< 0.001
Adalimumab	0	0	
Vedolizumab	0	0	
Ustekinumab	0	0	
Tofacitinib	0	0	
Disease extent of Paris classification 2 years after diagnosis			0.012[b]
Remission	14 (29.2)	31 (50.0)	
E1 proctitis	13 (27.1)	11 (17.7)	
E2 left colitis	2 (4.2)	8 (12.9)	
E3 right colitis	6 (12.5)	8 (12.9)	
E4 pancolitis	13 (27.1)	4 (6.5)	
Mayo endoscopic subscore 2 years after diagnosis[a]			0.037[b]
0 normal or inactive	15 (24.2)	33 (53.2)	
1 mild	24 (50.0)	21 (33.9)	
2 moderate	8 (16.7)	8 (12.9)	
3 severe	1 (2.1)	0	
	Group A (*n* = 24)	**Group B (*n* = 31)**	***P* value**
Maintenance treatment 5 year after diagnosis			
None	2 (8.3)	5 (16.1)	0.394
5-aminosalicylate	21 (87.5)	15 (48.4)	0.003
Azathioprine	14 (58.3)	14 (45.2)	0.337
Infliximab	0	18 (58.1)	< 0.001
Adalimumab	0	2 (6.5)	
Vedolizumab	0	1 (3.2)	
Ustekinumab	0	0	
Tofacitinib	0	0	
Disease extent of Paris classification 5 years after diagnosis			0.016[b]
Remission	3 (12.5)	13 (41.9)	
E1 proctitis	9 (37.5)	9 (29.0)	
E2 left colitis	3 (12.5)	4 (12.9)	
E3 right colitis	2 (8.3)	2 (6.5)	
E4 pancolitis	7 (29.2)	3 (9.7)	
Mayo endoscopic subscore 5 years after diagnosis[a]			< 0.001[b]
0 normal or inactive	3 (12.5)	13 (41.9)	
1 mild	6 (25.0)	16 (51.6)	
2 moderate	14 (58.3)	2 (6.5)	

3 severe	1 (4.2)	0

[a]Mayo endoscopy subscores were as follows: 0: Normal or inactive disease; 1: Mild disease; 2: Moderate disease; and 3: Severe disease.
[b]Mann-Whitney test.
Values are *n* (percentage).

Table 3 Clinical outcomes during the total follow-up period before (group A) and after (group B) infliximab approval

	Group A (*n* = 48)	Group B (*n* = 62)	*P* value
Number of relapsed patients	23 (47.9)	16 (25.8)	0.027[b]
Cumulative total relapses	40	22	0.006[b]
Number of relapses per person	1.74	1.38	
Number of relapses per person per year	0.44	0.25	
First relapse interval from diagnosis, yr	1.20 (0.60-2.50)	1.95 (1.35-3.93)	0.194[b]
Each relapse interval, yr	1.30 (0.60-3.55)	1.70 (1.00-4.20)	0.943[b]
Initial disease extent of relapsed patients			0.080[c]
E1 proctitis	7 (30.4)	0	
E2 left colitis	2 (8.7)	0	
E3 right colitis	3 (13.0)	2 (12.5)	
E4 pancolitis	11 (47.8)	14 (87.5)	
Number of hospitalizations per person	0.13	0.18	0.964[b]
Median PUCAI[a] during treatment period	10 (5-20)	5 (3.75-15)	< 0.001[b]
Median PUCAI[a] at the time of relapse	65 (52.5-75)	45 (45-55)	< 0.001[b]
Median cumulative corticosteroid free period	3.0 (2.6-3.7)	4.4 (3.1-6.0)	< 0.001[b]
Number of cumulative colectomies[1]	1	0	

[a]Pediatric Ulcerative Colitis Activity Index (PUCAI) is a 6-item disease activity index intended for use in pediatric UC clinical trials with a score ranging from 0-85.
[b]χ^2 test.
[c]Mann-Whitney test.
[1]Statistical analysis was not performed as there was only one patient who underwent colectomy.
Values are *n* (percentage) or median (interquartile range); *n*: Number of patients; PUCAI: Pediatric Ulcerative Colitis Activity Index.

analysis, only severe status remained an independent risk factor with statistical significance (P = 0.015).

Supplementary Figure 2 shows a comparison of the timing of the start of infliximab treatment after diagnosis among non-relapsed and relapsed patients who used infliximab. In the group of 29 non-relapsed patients, the majority (22, 75.9%) of them began infliximab treatment within 3 mo. On the other hand, in the group that experienced relapse, there were many patients who first used oral drugs or steroids and then began to use infliximab after 6 mo as the disease progressed.

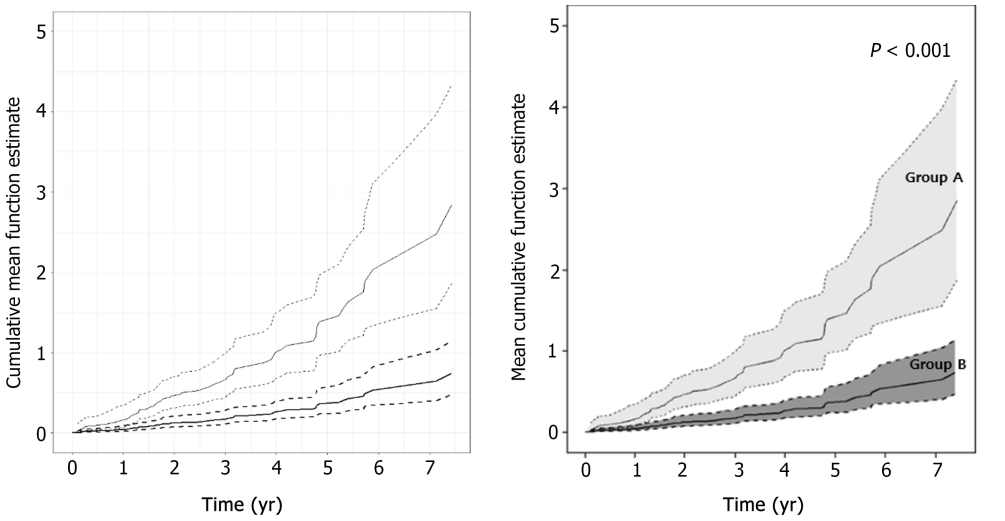
Clinical course curve: Comparison with the IBSEN study

In the IBSEN study, patients were asked to choose which of four predefined patterns best reflected their clinical course (Figure 4A). The predefined patterns were as follows: remission or mild severity of intestinal symptoms after initial high activity, increase in the severity of intestinal symptoms after an initial period of low activity, chronic continuous symptoms, or chronic intermittent symptoms[6]. These curves do not include the curve of the patients whose disease is consistently well controlled after showing mild disease activity at the time of diagnosis; they were probably included in curve 1 in the IBSEN study. In the current study, we plotted the clinical courses of all patients in both groups using the PUCAI score at each outpatient visit and the PUCAI score at relapse. We separated the patients with mild initial disease activity (PUCAI < 35) from the group with good disease control after treatment and drew a new curve (curve 5). Therefore, the proportion of patients corresponding to curve 1 was different from the IBSEN study. In this study, 27.1% of patients in Group A were included in curve 5, and 29.2% of patients showed moderate to severe initial disease activity and maintained well after treatment; they were included in curve 1 (Figure 4B). When these two were combined, it was 56%, which was similar to

Table 4 Univariable and multivariable analysis of the association between relapse rate and other factors

Parameter	Univariable analysis[b]				Multivariable analysis[b]			
	Pr > ChiSq	HR	95%CI[c] of HR		Pr > ChiSq	HR	95%CI[c] of HR	
Age at diagnosis, yr (< 10 yr)	0.063	0.434	0.642	3.714				
Disease extent at diagnosis	0.317							
E2	1	0.786	0.194	3.185[a]				
E3	1	1.097	0.555	2.166[a]				
E4	0.374	1.758	1.058	2.921[a]				
Corticosteroid uses at baseline (Yes)	**0.010**	2.111	1.201	3.712	0.109	1.682	0.890	3.176
Mayo endoscopic subscore at diagnosis (> 2 Moderate)	**< 0.001**	2.496	1.478	4.217	**0.015**	2.108	1.157	3.843
PUCAI[d] at diagnosis (> 45)	0.558	1.202	0.650	2.222				

[a]*P* value and 95%CI for hazard ratio were corrected by Bonferroni's method due to multiple testing.
[b]Cox's proportional hazard regression using counting process.
[c]The robust sandwich covariance estimate was used to account for the within-subject correlation.
[d]Pediatric Ulcerative Colitis Activity Index (PUCAI) is a 6-item disease activity index intended for use in pediatric UC clinical trials with a score ranging from 0-85.
CI: Confidence interval; HR: Hazard ratio.



Number of relapse events		Group A		Group B		Relapse Events Model	
		Number of events	Average time to event	Number of events	Average time to event	HR (95%CI[a])	P value[b]
1	41	24	1.89	17	3.13		
2	17	12	2.869	5	4.81	0.26 (0.16-0.43)	<0.01
3	3	3	3.619	0			
4	1	1	3.94				

Figure 2 Mean cumulative function for multiple relapses over time. The Y-axis is a cumulative relapse rate, which represents the cumulative hazard rate up to a specific point in time. The dotted line represents the 95% confidence interval for the cumulative risk rate. Group A: Thin black line; Group B: Thick black line.

the ratio of curve 1 in the IBSEN study. In the case of Group B, 27.4% of patients were included in curve 5 and the proportion was similar to Group A (Figure 4C). The patients of curve 1 in Group B were 48.4%, showing an increase in the proportion of patients compared to Group A. When combined with patients with mild disease initial activity, it was 76%, which was higher than that of curve 1 patients in the IBSEN study.

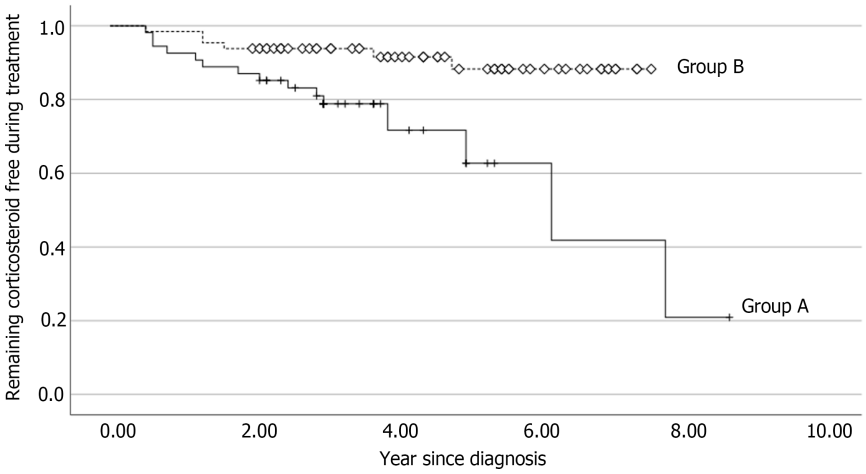


Figure 3 Analysis of corticosteroid-free survival curve after initiation of treatment using Kaplan-Meier method. The solid line represents the proportion of patients who were treated from January 2003 to September 2012 and did not receive corticosteroids. The dotted line represents the same proportion among patients who were treated from October 2012 to October 2020. In the former group, the 2-yr corticosteroid-free retention rate is 21%; in the latter group, the rate is 88% (log-rank test, $P = 0.003$).

The other clinical courses were also classified among patients with similar types and compared with the predefined curves of the IBSEN study. Patients in Group A were distributed among the four types in similar proportions as patients in the IBSEN study (Figure 4B). However, in Group B, the percentage of patients corresponding to curve 4 was relatively small (16%) (Figure 4C).

DISCUSSION

Many previous papers have cited young age at diagnosis as a risk factor for poor clinical outcomes such as relapse and colectomy in pediatric UC patients[22-25]. Thus, research on children with UC is vital. This study evaluated the ways in which the clinical course of children and adolescents with UC changed when the treatment options were diversified after the introduction of biological agents. Infliximab was approved in Korea in 2012 and is now widely used in patients with moderate to severe UC, but many patients who exhibit a good response to oral medication continue to be treated with oral medication only. Here, we compared the clinical course of patients before and after approval of the biological agent according to the passage of time, rather than directly comparing patients who used infliximab to those who did not, as was done in previous studies[8,26,27].

Table 1 shows the characteristics of the included patients. It is evident that the two groups had similar characteristics and differed only in terms of the initial disease extent. The difference in initial disease extent is thought to be due to the rise in incidence of IBD over time as dietary habits become more Westernized; accordingly, the severity of the disease is increasing in newly diagnosed patients[28-30]. Since all patients in this study were followed for at least 2 years, drug and endoscopic evaluation were performed in every patient at 2 years post-diagnosis; it was thus possible to assess changes in the disease extent after 2 years to follow up on this difference in initial disease extent. Only half of all included patients were followed for more than 5 years, but the two groups still exhibited a statistically significant difference in disease extent and severity at 5 years. Despite the high proportion of pancolitis at initial diagnosis, many patients in Group B reached remission. This finding indicates that, in patients with broad disease extent and severity, there is an active and ongoing need for biological agents such as infliximab.

The number of patients who relapsed, the total number of relapses, and the relapse rate were significantly lower in Group B than in Group A. Though both measures significantly differed between the two groups, because every patient had a different follow-up period, we assume that the relapse rate is a more relevant outcome than the total number of relapses (Figure 2). We believe that the higher relapse rate in Group A is attributable to the differences in treatment. Most of the patients with relapse in Group B initially showed pancolitis. Many previous studies have identified disease extent as a risk factor for relapse[23,31,32]. Our risk factor analysis also demonstrated that the initial disease severity (MES) was a significant risk factor for relapse, with P values of 0.006 in the univariate analysis and 0.0149 in the multivariate analysis (Table 4). The worse the initial disease state, the worse the prognosis; this relationship may be taken for granted, but it is important information to keep in mind when establishing a treatment strategy. We thought it was strange that disease extent was not significantly associated with relapse, so we delved a bit deeper into the issue. As a result, pancolitis at initial diagnosis was identified as a risk factor in the univariate analysis in Group A. In Group B, the relapse

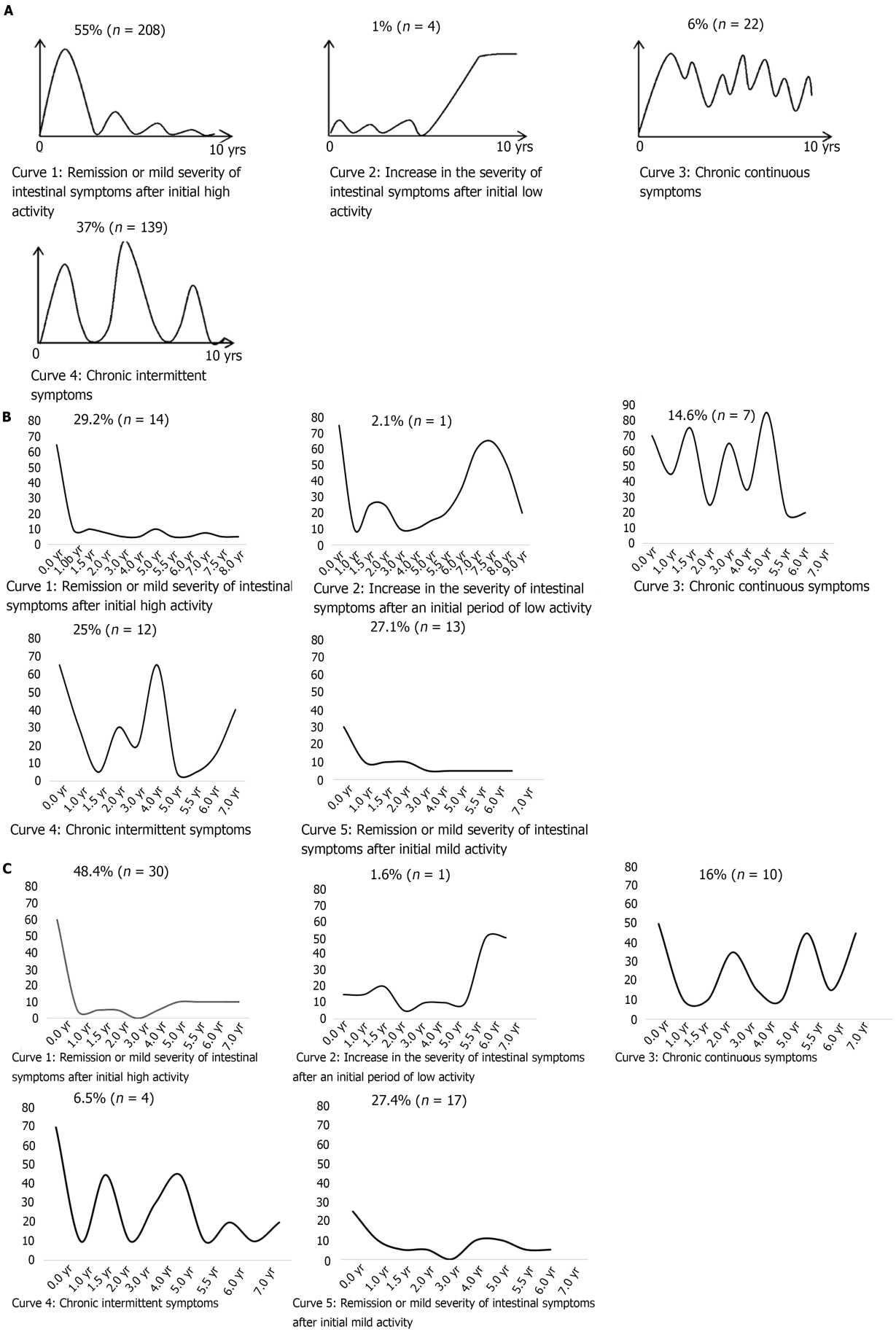


Figure 4 Comparison of clinical course curves of the IBSEN study. A: Graphs of self-identified clinical course in the IBSEN study[6]; B: Actual clinical

course according to pediatric ulcerative colitis activity index prior to the introduction of infliximab (Group A); C: Actual clinical course according to pediatric ulcerative colitis activity index after the introduction of infliximab (Group B). *n*: Number.

rate was relatively low, so statistical evaluation was not possible. In our opinion, disease extent can indicate how long a patient has been suffering from the disease without appropriate treatment. Therefore, it is less likely to be related to relapse rate than disease severity.

As with the endoscopic findings, a high PUCAI score and the use of corticosteroids, which indirectly indicate greater initial disease severity, were also identified as risk factors for relapse. We wondered whether the time at which the patients started infliximab treatment would change the clinical course in relapsed patients even in Group B. Rapid use of infliximab can be expected to reduce the risk of relapse in patients with severe disease, as shown in Supplementary Figure 2. In the non-relapsed group, there were 16 patients with pancolitis at the time of diagnosis, but no relapse occurred among those who received infliximab within 3 mo of diagnosis (Supplementary Figure 2). Since all of our patients were treated with mesalazine and azathioprine for severe disease before receiving additional treatments such as corticosteroids and infliximab, they received similar medications at similar times with the exception of infliximab. Therefore, we propose that the active use of infliximab for initial treatment of moderate to severe UC is important.

As the field of IBD research has expanded and more treatments have been developed, there have been many studies on the quality of life of IBD patients[33-35]. The average PUCAI score during maintenance treatment can be considered an important factor in terms of improving quality of life and preventing relapse. The PUCAI scores during maintenance treatment and at the time of relapse were significantly lower in Group B, indicating that the daily lives of patients in Group B may have been more enriching.

Perhaps the most important factors in evaluating the clinical outcome of treatment are how long the steroid-free period was maintained and whether colectomy was performed[31]. As children and adolescents are not yet finished growing, using steroids more sparingly can prevent osteoporosis and slow growth, which are the typical side effects of steroids[36]. It can also prevent hirsutism, moon face, and buffalo hump in adolescent patients, who tend to be sensitive to appearance. We confirmed that steroid dependency could be avoided through the use of biological agents in patients with high-severity UC (Figure 3). Since colectomy, like the length of the steroid-free period, is an important factor when evaluating long-term outcomes, other studies have also reported on colectomy rates according to treatment[37-39]. In this study, only 1 patient in Group A underwent colectomy due to uncontrolled hematochezia, so it was not possible to compare colectomy as a long-term outcome.

The IBSEN study has been cited in several papers because it is a representative paper that evaluated the clinical course of UC on a large scale[40,41]. The IBSEN study developed predefined curves to represent the clinical course of the disease (Figure 4A). Each patient was asked to choose which predefined graph was most similar to their clinical course. We wondered whether the actual clinical course is represented by these curves and whether the clinical course differs between adults and children. We also wondered whether the clinical course changed after the introduction of biological agents. Therefore, we plotted the clinical course using PUCAI scores. The PUCAI score was chosen because laboratory results such as ESR and CRP are only weakly correlated with symptoms in patients with UC. The shapes of our graphs are similar to the four curves of the IBSEN study. However, there are some differences. The biggest difference is that we separated the patients whose initial disease activity was mild and disease was well controlled from curve 1, which was not shown in the IBSEN study. We believe that patients with such clinical features were also included in curve 1 in the IBSEN study. What was newly confirmed in the process of dividing into curves 1 and 5 is that the proportion of patients in curve 5 was similar in Group A and Group B. It is an epidemiologically convincing finding that the proportion of mild cases is similar over time.

In Group A, if the patients in curve 1 and those in curve 5 were combined, the ratio was similar to that of curve 1 in the IBSEN study. Since the patients of Group A received treatments that were similar to those given in the IBSEN study, the proportion of patients corresponding to each curve was similar. In the case of curve 2, the relapse that occurred in the second half did not persist as relapses tended to do in the IBSEN study, and instead showed a trend towards improvement with treatment. In addition, curve 3 differed in that it showed a lower baseline than the IBSEN curve when disease was partially controlled.

When comparing the graphs of the two groups, fewer patients in Group B than Group A corresponded to curves 3 and 4, and more corresponded to curve 1, indicating that the disease course of patients in Group B was better controlled. Therefore, the proportion of Group B patients corresponding to each curve was also different from the IBSEN study. As mentioned above, the relapse rate dropped after the introduction of infliximab and patients showed improved clinical outcomes. Similarly, the disease course appeared to be changing due to the change in treatment. Taken together, this study suggests that the course of chronic diseases may change over time due to the expanded availability of different types of therapeutic drugs.

The main limitation of this study is that the time difference between the two groups may have introduced some degree of bias. The diagnostic environment and degree of training of the clinicians may have improved over time. To minimize this bias, we evaluated the patients' baseline characteristics using objective indicators and found that there were no significant differences between the two groups. Also, when assessing disease extent and Mayo subscore from the colonoscopy findings, the same experts evaluated all patients using the same criteria. Another limitation is that it was conducted at a single center. However, this hospital is one of the biggest pediatric IBD centers in Korea and has the advantage of having a steady patient cohort through long-term care.

CONCLUSION

As patients with moderate and severe UC have been treated with infliximab since its introduction almost a decade ago, the rate of relapse has decreased and the steroid-free period has increased. In addition, based on the PUCAI score and objective colonoscopy results, the disease is better controlled and patients' quality of life has improved. Before the introduction of the biological agent, the clinical course of pediatric patients showed patterns that resembled those in the IBSEN study, but since its introduction, the proportion of patients who have reached remission has significantly increased. The current goal of treatment for IBD is to change the disease course for a better quality of life, and appropriate treatment with biologic agents may be an option for achieving that goal.

ARTICLE HIGHLIGHTS

Research background

There are many articles comparing the clinical outcomes of patients treated with biological agents *vs* those who did not use biological agents, but no studies have compared the disease course of UC by era before and after the introduction of biological agents. The authors assessed how introduction of new treatment altered disease course over time.

Research motivation

The number of large-scale and long-term follow-up studies for pediatric patients with ulcerative colitis (UC) is insufficient. A representative paper dealing with the clinical course of adult UC is the IBSEN study. This paper dealt with the clinical course before the introduction of biological agents. If the use of biological agents in pediatric UC changed the clinical course of UC, it would be helpful in future treatment decisions.

Research objectives

The aim of this study was to compare the clinical course of pediatric UC by era before and after the introduction of biological agents, and to compare them with clinical course curve of the IBSEN study.

Research methods

Infliximab was approved for use in children in October 2012 in Korea. Group A (n = 48) was followed between January 2003 and October 2012, and Group B (n = 62) was followed between November 2012 and October 2020. Endoscopic remission, drug composition, relapse rate, steroid-free period, and the quality of life of the groups were evaluated as outcomes. Clinical course was plotted with the pediatric UC activity index score, and compared to the curve of the IBSEN study.

Research results

Despite a higher rate of pancolitis, patients in Group B had a higher rate of achieving endoscopic remission, longer steroid-free periods and reduced relapse rate. Unlike the clinical course curve of the IBSEN study, we drew one more independent curve (curve 5), and the proportion of the patients in Group B corresponding to curve 1 (remission or mild severity after initial high activity) was higher. In terms of quality of life, the number of hospitalizations and emergency room visits have improved after the introduction of biological agents. Comparison of treatment costs is also an important issue that needs future research.

Research conclusions

The active use of biological agents may change the long-term disease course in moderate to severe pediatric UC. Growth can also be achieved by reducing the use of steroids.

Research perspectives

Because biological agents are an expensive treatment option, whether there is a difference in economic

quality of life caused by treatment with biological agents is also an important topic for future research.

FOOTNOTES

Author contributions: Kwon Y designed and performed the research and wrote the paper; Kim MJ designed the research and supervised the report; Kim ES and Choe YH designed the research and contributed to the analysis.

Institutional review board statement: This study was reviewed and approved by the Ethics Committee of the Samsung Medical Center (IRB File No. SMC 2020-12-005).

Informed consent statement: Patients were not required to give informed consent to the study because the analysis used anonymous clinical data that were obtained after each patient agreed to treatment by written consent.

Conflict-of-interest statement: The authors have no conflict of interests to declare.

Data sharing statement: No additional data are available.

Country/Territory of origin: South Korea

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REFERENCES

1 **Wilks S**. Morbid appearances in the intestine of Miss Bankes. *Med Times Gazette* 1859; **2**: 264-265
2 **Akiho H**, Yokoyama A, Abe S, Nakazono Y, Murakami M, Otsuka Y, Fukawa K, Esaki M, Niina Y, Ogino H. Promising biological therapies for ulcerative colitis: A review of the literature. *World J Gastrointest Pathophysiol* 2015; **6**: 219-227 [PMID: 26600980 DOI: 10.4291/wjgp.v6.i4.219]
3 **Malesza IJ**, Malesza M, Walkowiak J, Mussin N, Walkowiak D, Aringazina R, Bartkowiak-Wieczorek J, Mądry E. High-Fat, Western-Style Diet, Systemic Inflammation, and Gut Microbiota: A Narrative Review. *Cells* 2021; **10** [PMID: 34831387 DOI: 10.3390/cells10113164]
4 **Rizzello F**, Spisni E, Giovanardi E, Imbesi V, Salice M, Alvisi P, Valerii MC, Gionchetti P. Implications of the Westernized Diet in the Onset and Progression of IBD. *Nutrients* 2019; **11** [PMID: 31072001 DOI: 10.3390/nu11051033]
5 **Kugathasan S**, Cohen S. Searching for new clues in inflammatory bowel disease: tell tales from pediatric IBD natural history studies. *Gastroenterology* 2008; **135**: 1038-1041 [PMID: 18786536 DOI: 10.1053/j.gastro.2008.08.030]
6 **Solberg IC**, Lygren I, Jahnsen J, Aadland E, Høie O, Cvancarova M, Bernklev T, Henriksen M, Sauar J, Vatn MH, Moum B; IBSEN Study Group. Clinical course during the first 10 years of ulcerative colitis: results from a population-based inception cohort (IBSEN Study). *Scand J Gastroenterol* 2009; **44**: 431-440 [PMID: 19101844 DOI: 10.1080/00365520802600961]
7 **Cornillie F**. Ten years of infliximab (remicade) in clinical practice: the story from bench to bedside. *Eur J Pharmacol* 2009; **623** Suppl 1: S1-S4 [PMID: 19837054 DOI: 10.1016/j.ejphar.2009.10.023]
8 **Colombel JF**, Rutgeerts P, Reinisch W, Esser D, Wang Y, Lang Y, Marano CW, Strauss R, Oddens BJ, Feagan BG, Hanauer SB, Lichtenstein GR, Present D, Sands BE, Sandborn WJ. Early mucosal healing with infliximab is associated with improved long-term clinical outcomes in ulcerative colitis. *Gastroenterology* 2011; **141**: 1194-1201 [PMID: 21723220 DOI: 10.1053/j.gastro.2011.06.054]
9 **Hyams JS**, Lerer T, Griffiths A, Pfefferkorn M, Stephens M, Evans J, Otley A, Carvalho R, Mack D, Bousvaros A, Rosh J, Grossman A, Tomer G, Kay M, Crandall W, Oliva-Hemker M, Keljo D, LeLeiko N, Markowitz J; Pediatric Inflammatory Bowel Disease Collaborative Research Group. Outcome following infliximab therapy in children with ulcerative colitis. *Am J Gastroenterol* 2010; **105**: 1430-1436 [PMID: 20104217 DOI: 10.1038/ajg.2009.759]
10 **Ferrante M**, Vermeire S, Fidder H, Schnitzler F, Noman M, Van Assche G, De Hertogh G, Hoffman I, D'Hoore A, Van Steen K, Geboes K, Penninckx F, Rutgeerts P. Long-term outcome after infliximab for refractory ulcerative colitis. *J Crohns Colitis* 2008; **2**: 219-225 [PMID: 21172214 DOI: 10.1016/j.crohns.2008.03.004]
11 **Oussalah A**, Evesque L, Laharie D, Roblin X, Boschetti G, Nancey S, Filippi J, Flourié B, Hebuterne X, Bigard MA, Peyrin-Biroulet L. A multicenter experience with infliximab for ulcerative colitis: outcomes and predictors of response, optimization, colectomy, and hospitalization. *Am J Gastroenterol* 2010; **105**: 2617-2625 [PMID: 20736936 DOI:

10.1038/ajg.2010.345]
12 **Farkas K**, Lakatos PL, Nagy F, Szepes Z, Miheller P, Papp M, Palatka K, Bálint A, Bor R, Wittmann T, Molnár T. Predictors of relapse in patients with ulcerative colitis in remission after one-year of infliximab therapy. *Scand J Gastroenterol* 2013; **48**: 1394-1398 [PMID: 24131338 DOI: 10.3109/00365521.2013.845906]
13 **Fiorino G**, Cortes PN, Ellul P, Felice C, Karatzas P, Silva M, Lakatos PL, Bossa F, Ungar B, Sebastian S, Furfaro F, Karmiris K, Katsanos KH, Muscat M, Christodoulou DK, Maconi G, Kopylov U, Magro F, Mantzaris GJ, Armuzzi A, Boscà-Watts MM, Ben-Horin S, Bonovas S, Danese S. Discontinuation of Infliximab in Patients With Ulcerative Colitis Is Associated With Increased Risk of Relapse: A Multinational Retrospective Cohort Study. *Clin Gastroenterol Hepatol* 2016; **14**: 1426-1432.e1 [PMID: 27317850 DOI: 10.1016/j.cgh.2016.05.044]
14 **De Vos M**, Louis EJ, Jahnsen J, Vandervoort JG, Noman M, Dewit O, D'haens GR, Franchimont D, Baert FJ, Torp RA, Henriksen M, Potvin PM, Van Hootegem PP, Hindryckx PM, Moreels TG, Collard A, Karlsen LN, Kittang E, Lambrecht G, Grimstad T, Koch J, Lygren I, Coche JC, Mana F, Van Gossum A, Belaiche J, Cool MR, Fontaine F, Maisin JM, Muls V, Neuville B, Staessen DA, Van Assche GA, de Lange T, Solberg IC, Vander Cruyssen BJ, Vermeire SA. Consecutive fecal calprotectin measurements to predict relapse in patients with ulcerative colitis receiving infliximab maintenance therapy. *Inflamm Bowel Dis* 2013; **19**: 2111-2117 [PMID: 23883959 DOI: 10.1097/MIB.0b013e31829b2a37]
15 **IBD Working Group of the European Society for Paediatric Gastroenterology Hepatology and Nutrition**. Inflammatory bowel disease in children and adolescents: recommendations for diagnosis--the Porto criteria. *J Pediatr Gastroenterol Nutr* 2005; **41**: 1-7 [PMID: 15990620 DOI: 10.1097/01.mpg.0000163736.30261.82]
16 **Levine A**, Griffiths A, Markowitz J, Wilson DC, Turner D, Russell RK, Fell J, Ruemmele FM, Walters T, Sherlock M, Dubinsky M, Hyams JS. Pediatric modification of the Montreal classification for inflammatory bowel disease: the Paris classification. *Inflamm Bowel Dis* 2011; **17**: 1314-1321 [PMID: 21560194 DOI: 10.1002/ibd.21493]
17 **Mazzuoli S**, Guglielmi FW, Antonelli E, Salemme M, Bassotti G, Villanacci V. Definition and evaluation of mucosal healing in clinical practice. *Dig Liver Dis* 2013; **45**: 969-977 [PMID: 23932331 DOI: 10.1016/j.dld.2013.06.010]
18 **Nelson WB**. Recurrent events data analysis for product repairs, disease recurrences, and other applications: SIAM, 2003 [DOI: 10.1137/1.9780898718454]
19 **Lawless JF**, Nadeau C. Some simple robust methods for the analysis of recurrent events. *Technometrics* 1995; **37**: 158-168 [DOI: 10.1080/00401706.1995.10484300]
20 **Andersen PK**, Borgan O, Gill RD, Keiding N. Statistical models based on counting processes: Springer Science & Business Media, 2012
21 **Lin DY**, Wei LJ. The robust inference for the Cox proportional hazards model. *J Am Stat Assoc* 1989; **84**: 1074-1078 [DOI: 10.1080/01621459.1989.10478874]
22 **Jang ES**, Lee DH, Kim J, Yang HJ, Lee SH, Park YS, Hwang JH, Kim JW, Jeong SH, Kim N, Jung HC, Song IS. Age as a clinical predictor of relapse after induction therapy in ulcerative colitis. *Hepatogastroenterology* 2009; **56**: 1304-1309
23 **Bitton A**, Peppercorn MA, Antonioli DA, Niles JL, Shah S, Bousvaros A, Ransil B, Wild G, Cohen A, Edwardes MD, Stevens AC. Clinical, biological, and histologic parameters as predictors of relapse in ulcerative colitis. *Gastroenterology* 2001; **120**: 13-20 [PMID: 11208709 DOI: 10.1053/gast.2001.20912]
24 **Lee JH**, Cheon JH, Moon CM, Park JJ, Hong SP, Kim TI, Kim WH. Do patients with ulcerative colitis diagnosed at a young age have more severe disease activity than patients diagnosed when older? *Digestion* 2010; **81**: 237-243 [PMID: 20110709 DOI: 10.1159/000253850]
25 **Leo S**, Leandro G, Di Matteo G, Caruso ML, Lorusso D. Ulcerative colitis in remission: it is possible to predict the risk of relapse? *Digestion* 1989; **44**: 217-221 [PMID: 2561106 DOI: 10.1159/000199914]
26 **Rutgeerts P**, Sandborn WJ, Feagan BG, Reinisch W, Olson A, Johanns J, Travers S, Rachmilewitz D, Hanauer SB, Lichtenstein GR, de Villiers WJ, Present D, Sands BE, Colombel JF. Infliximab for induction and maintenance therapy for ulcerative colitis. *N Engl J Med* 2005; **353**: 2462-2476 [PMID: 16339095 DOI: 10.1056/NEJMoa050516]
27 **Guo C**, Wu K, Liang X, Liang Y, Li R. Infliximab clinically treating ulcerative colitis: A systematic review and meta-analysis. *Pharmacol Res* 2019; **148**: 104455 [PMID: 31562896 DOI: 10.1016/j.phrs.2019.104455]
28 **Kim BJ**, Song SM, Kim KM, Lee YJ, Rhee KW, Jang JY, Park SJ, Yoon CH. Characteristics and trends in the incidence of inflammatory bowel disease in Korean children: a single-center experience. *Dig Dis Sci* 2010; **55**: 1989-1995 [PMID: 19763825 DOI: 10.1007/s10620-009-0963-5]
29 **Jung YS**, Han M, Kim WH, Park S, Cheon JH. Incidence and Clinical Outcomes of Inflammatory Bowel Disease in South Korea, 2011-2014: A Nationwide Population-Based Study. *Dig Dis Sci* 2017; **62**: 2102-2112 [PMID: 28593437 DOI: 10.1007/s10620-017-4640-9]
30 **Kim HJ**, Hann HJ, Hong SN, Kim KH, Ahn IM, Song JY, Lee SH, Ahn HS. Incidence and natural course of inflammatory bowel disease in Korea, 2006-2012: a nationwide population-based study. *Inflamm Bowel Dis* 2015; **21**: 623-630 [PMID: 25647154 DOI: 10.1097/MIB.0000000000000313]
31 **Farmer RG**, Easley KA, Rankin GB. Clinical patterns, natural history, and progression of ulcerative colitis. A long-term follow-up of 1116 patients. *Dig Dis Sci* 1993; **38**: 1137-1146 [PMID: 8508710 DOI: 10.1007/BF01295733]
32 **Qiu Y**, Chen B, Li Y, Xiong S, Zhang S, He Y, Zeng Z, Ben-Horin S, Chen M, Mao R. Risk factors and long-term outcome of disease extent progression in Asian patients with ulcerative colitis: a retrospective cohort study. *BMC Gastroenterol* 2019; **19**: 7 [PMID: 30630426 DOI: 10.1186/s12876-018-0928-2]
33 **Guthrie E**, Jackson J, Shaffer J, Thompson D, Tomenson B, Creed F. Psychological disorder and severity of inflammatory bowel disease predict health-related quality of life in ulcerative colitis and Crohn's disease. *Am J Gastroenterol* 2002; **97**: 1994-1999 [PMID: 12190166 DOI: 10.1111/j.1572-0241.2002.05842.x]
34 **Carcamo L**, Miranda P, Zúñiga A, Alexander E, Molina ME, Urrejola G, Larach T, Miguieles R, Bellolio F. Ileal pouch-anal anastomosis in ulcerative colitis: outcomes, functional results, and quality of life in patients with more than 10-year follow-up. *Int J Colorectal Dis* 2020; **35**: 747-753 [PMID: 32067061 DOI: 10.1007/s00384-020-03529-7]
35 **Hagelund LM**, Elkjær Stallknecht S, Jensen HH. Quality of life and patient preferences among Danish patients with ulcerative colitis - results from a survey study. *Curr Med Res Opin* 2020; **36**: 771-779 [PMID: 31944145 DOI: 10.1080/03007995.2020.1716704]

36 **Ali T**, Lam D, Bronze MS, Humphrey MB. Osteoporosis in inflammatory bowel disease. *Am J Med* 2009; **122**: 599-604 [PMID: 19559158 DOI: 10.1016/j.amjmed.2009.01.022]

37 **Abou Khalil M**, Boutros M, Nedjar H, Morin N, Ghitulescu G, Vasilevsky CA, Gordon P, Rahme E. Incidence Rates and Predictors of Colectomy for Ulcerative Colitis in the Era of Biologics: Results from a Provincial Database. *J Gastrointest Surg* 2018; **22**: 124-132 [PMID: 28808892 DOI: 10.1007/s11605-017-3530-y]

38 **Aratari A**, Papi C, Clemente V, Moretti A, Luchetti R, Koch M, Capurso L, Caprilli R. Colectomy rate in acute severe ulcerative colitis in the infliximab era. *Dig Liver Dis* 2008; **40**: 821-826 [PMID: 18472316 DOI: 10.1016/j.dld.2008.03.014]

39 **Worley G**, Almoudaris A, Bassett P, Segal J, Akbar A, Ghosh S, Aylin P, Faiz O. Colectomy rates for ulcerative colitis in England 2003-2016. *Aliment Pharmacol Ther* 2021; **53**: 484-498 [PMID: 33264468 DOI: 10.1111/apt.16202]

40 **Ossum AM**, Palm Ø, Lunder AK, Cvancarova M, Banitalebi H, Negård A, Høie O, Henriksen M, Moum BA, Høivik ML; IBSEN Study Group. Ankylosing Spondylitis and Axial Spondyloarthritis in Patients With Long-term Inflammatory Bowel Disease: Results From 20 Years of Follow-up in the IBSEN Study. *J Crohns Colitis* 2018; **12**: 96-104 [PMID: 28961700 DOI: 10.1093/ecco-jcc/jjx126]

41 **Monstad IL**, Solberg IC, Cvancarova M, Hovde O, Henriksen M, Huppertz-Hauss G, Gunther E, Moum BA, Stray N, Vatn M, Hoie O, Jahnsen J. Outcome of Ulcerative Colitis 20 Years after Diagnosis in a Prospective Population-based Inception Cohort from South-Eastern Norway, the IBSEN Study. *J Crohns Colitis* 2021; **15**: 969-979 [PMID: 33367569 DOI: 10.1093/ecco-jcc/jjaa232]

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ORIGINAL ARTICLE

Retrospective Study

Gastric mucosal precancerous lesions in *Helicobacter pylori*-infected pediatric patients in central China: A single-center, retrospective investigation

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Abstract

BACKGROUND

Helicobacter pylori (*H. pylori*) infects about 50% of the world population and is the major cause of chronic gastritis, peptic ulcers, and gastric cancer. Chronic *H. pylori* infection induces gastric mucosal precancerous lesions mostly in adulthood, and it is debatable whether these pathological conditions can occur in childhood and adolescents as well. Since this is a critical issue to determine if intervention should be offered for this population group, we investigated the gastric mucosal precancerous lesions in pediatric patients in an area in central China with a high prevalence of *H. pylori* and gastric cancer.

AIM

To investigate the relationship of *H. pylori* infection and gastric mucosal precancerous lesions in children and adolescents in central China.

METHODS

We screened 4258 ward-admitted children and adolescent patients with upper gastrointestinal symptoms, and finally enrolled 1015 pediatric patients with *H. pylori* infection and endoscopic and histological data. *H. pylori* infection status was determined by rapid urease test and histopathological examination. Both clinical and pathological data were collected and analyzed retrospectively. Occurrence of gastric mucosal precancerous lesions, inflammatory activity and degree of inflammatory cell infiltration between *H. pylori*-positive and -negative groups were compared.

RESULTS

Among the 1015 eligible children and adolescents, the overall *H. pylori* infection rate was 84.14% (854/1015). The infection rate increased with age. The incidence of gastric mucosal precancerous lesions in *H. pylori*-infected children was 4.33% (37/854), which included atrophic gastritis (17 cases), intestinal metaplasia (11 cases) and dysplasia (9 cases). In *H. pylori*-negative patients, only 1 atrophic gastritis case [0.62%, (1/161)] was found ($P < 0.05$). Active inflammation in *H. pylori*-infected patients was significantly higher than that in non-infected patients, and the *H. pylori*-infected group showed more severe lymphocyte and neutrophil granulocyte infiltration ($P < 0.001$). In addition, endoscopy revealed that the most common findings in *H. pylori*-positive patients were antral nodularity, but in *H. pylori*-negative patients only superficial gastritis was observed.

CONCLUSION

In children and adolescents, gastric mucosal precancerous lesions occurred in 4.33% of *H. pylori*-infected patients in central China. These cases included atrophic gastritis, intestinal metaplasia, and dysplasia. The data revealed an obvious critical issue requiring future investigation and intervention for this population group.

Key Words: *Helicobacter pylori*; Gastric cancer; Precancerous lesions; Inflammation; Children and adolescents

Core Tip: *Helicobacter pylori* (*H. pylori*) infection induces gastric mucosal precancerous lesions mostly in adulthood. Whether these lesions can also occur in children and adolescents remains controversial. Our study showed that in a region in central China with a high prevalence of *H. pylori* and gastric cancer, the incidence of gastric mucosal precancerous lesions was 4.33% among *H. pylori*-infected children and adolescents, which is significantly higher than the non-infected pediatric patients. The precancerous lesions included atrophic gastritis, intestinal metaplasia, and dysplasia. These data provide an alarming alert and call for further investigation and intervention for this population group.

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INTRODUCTION

Helicobacter pylori (*H. pylori*) has infected about 50% of the Chinese population and is responsible for 90% of non-cardia gastric cancer incidences. It is reported that one-third of asymptomatic or healthy children have serologically-confirmed *H. pylori* infection worldwide[1-3]. Although most *H. pylori*-infected children do not have obvious clinical symptoms, studies have shown that children with gastrointestinal symptoms have a higher rate of *H. pylori* infection, and the infection rates are affected by socioeconomic status, living habit, sanitary conditions, geographic region, *etc.*[4,5]. Despite the overall infection rate has slowly declined in China, the infection rate in rural areas is still much higher over that of city residents [6,7].

A recent meta-analysis in China in 2022 has indicated that *H. pylori* prevalence in children and adolescents is 28%[7]. Infection by *H. pylori* is closely associated with gastric mucosal inflammation and extra-gastrointestinal diseases[8-10]. A small portion of infected patients will follow Correa's cascade

and develop severe gastric mucosal lesions, such as atrophic gastritis, intestinal metaplasia (IM), and gastric cancer[11]. *H. pylori* CagA has been shown to be associated with gastric mucosal precancerous lesions in several epidemiological studies[5,12].

One 2006 review summarized the prevalence of gastric mucosal precancerous lesions in children and adolescents, ranging from 0% to 72% in *H. pylori*-infected patients[13]. Prevalence of gastric atrophy in different populations may also vary depending on different geographic/genetic origins or environmental factors in the pediatric population. For example, one Tunisian study in 2009 found that atrophic gastritis accounted for 9.3% (32/354) of enrolled children; of the 32 children with atrophic gastritis, 30 were infected with *H. pylori*[14]. In 2014, a Mexican study of 82 children with chronic gastritis found that 8.5% (7/82) children had antral atrophy and 6.1% (5/82) had IM; among the 7 children with atrophy, 6 had *H. pylori* infection[15]. Other studies carried out in different countries reported gastric atrophy was present in 4.4% to 10.7% of *H. pylori*-positive children[16,17]. However, large-scale investigation is lacking in this area.

H. pylori has the characteristics of family cluster infection and is transmissible among family members, including spouses and siblings[8,9,18]. The infected parents, especially mothers, play a major role in its transmission[19-21]. Most *H. pylori* infection is acquired during childhood and adolescents, and the infection will persist for decades unless proper treatment is offered. Because *H. pylori* infection usually does not cause or only causes mild gastric mucosal lesions in children and adolescents[22-24], the consequences were thought not to be as important as they were in adulthood. Hence, very few studies have focused on the relationships between *H. pylori* infection and gastric mucosa precancerous lesions in children and adolescents. Recent reports have begun to show that gastric mucosal atrophy and IM are also found in children and even in very young children, as mentioned above[13-15].

In this work, we retrospectively investigated a cohort of *H. pylori*-infected symptomatic pediatric inpatients on gastric mucosal inflammation status, incidence and pattern of gastric mucosal precancerous lesions in a major hospital in central China, which is an area with a high prevalence of *H. pylori* and gastric cancer (*H. pylori* infection rate is 49.6%[6], and gastric cancer incidence is 42.52/100000[25]). Studies in this area will be helpful to understand the pattern and prevalence of gastric mucosal precancerous lesions in pediatric patients and provide evidence for future *H. pylori* eradication recommendation and related disease prevention.

MATERIALS AND METHODS

Study population and patient enrollment

From August 1, 2018 to July 31, 2021, 4258 pediatric patients with an age less than 18-years-old who had upper gastrointestinal symptoms were admitted to the Department of Pediatrics, People's Hospital of Zhengzhou University; among them, 1852 underwent upper gastrointestinal endoscopy examination due to disease complaints, such as abdominal pain, abdominal distension, nausea, vomiting, hiccups, heartburn, *etc*. Patients who had taken biopsy specimens from the gastric mucosa for histopathological examination and rapid urease test were enrolled into the study. Exclusion criteria were as follows: (1) Use of antibiotics, bismuth salts, proton pump inhibitors, H2-receptor blockers, immunosuppressants and steroids over the past 1 mo; (2) History of gastrointestinal surgery; (3) Active upper gastrointestinal bleeding; and (4) Idiopathic inflammatory bowel disease.

The study finally enrolled 1015 eligible children and adolescents. Patient information was collected only for the purpose of disease analysis and were kept confidential. As this is a retrospective study, no informed consent was required. The research protocol was approved by the Ethics Committee of People's Hospital of Zhengzhou University (2019-KY-No. 10). The flow chart of study design and patients' enrollment is shown in Figure 1. Patients were classified into four groups according to age: (1) 1-4 years; (2) 5-8 years; (3) 9-12 years; and (4) 13-18 years.

Endoscopic and histological evaluation of patients

At least two biopsy specimens were collected from the antrum or angulus during endoscopic examination. One biopsy sample was used for immediate rapid urease test. The other was oriented, fixed in formalin and embedded in paraffin blocks. After samples were sectioned, hematoxylin and eosin staining was applied for histological analysis. *H. pylori* infection was detected by immunohistochemical staining. Due to technique limitations, all patients were not given ^{13}C-urea breath test, serological *H. pylori* antibody test or stool antigen test to determine *H. pylori* infection status at the time of their tests. Therefore, a patient was considered *H. pylori*-infected if either histological staining or rapid urease test was positive or if both were positive. Conversely, a patient was considered to be *H. pylori*-uninfected if either histological staining or rapid urease test was negative or if both were negative.

Histological variables (the activity of inflammation, lymphocyte and neutrophil granulocyte infiltration, glandular atrophy, IM and hyperplasia) were analyzed according to visual analog scale of the updated Sydney system[26]. Gastric mucosa changes were classified into absent, mild, moderate and severe levels according to the histological situation of each sample. Severity of lesions was determined by the most severe lesion in each patient. Atrophy of gastric mucosa was indicated by the destruction

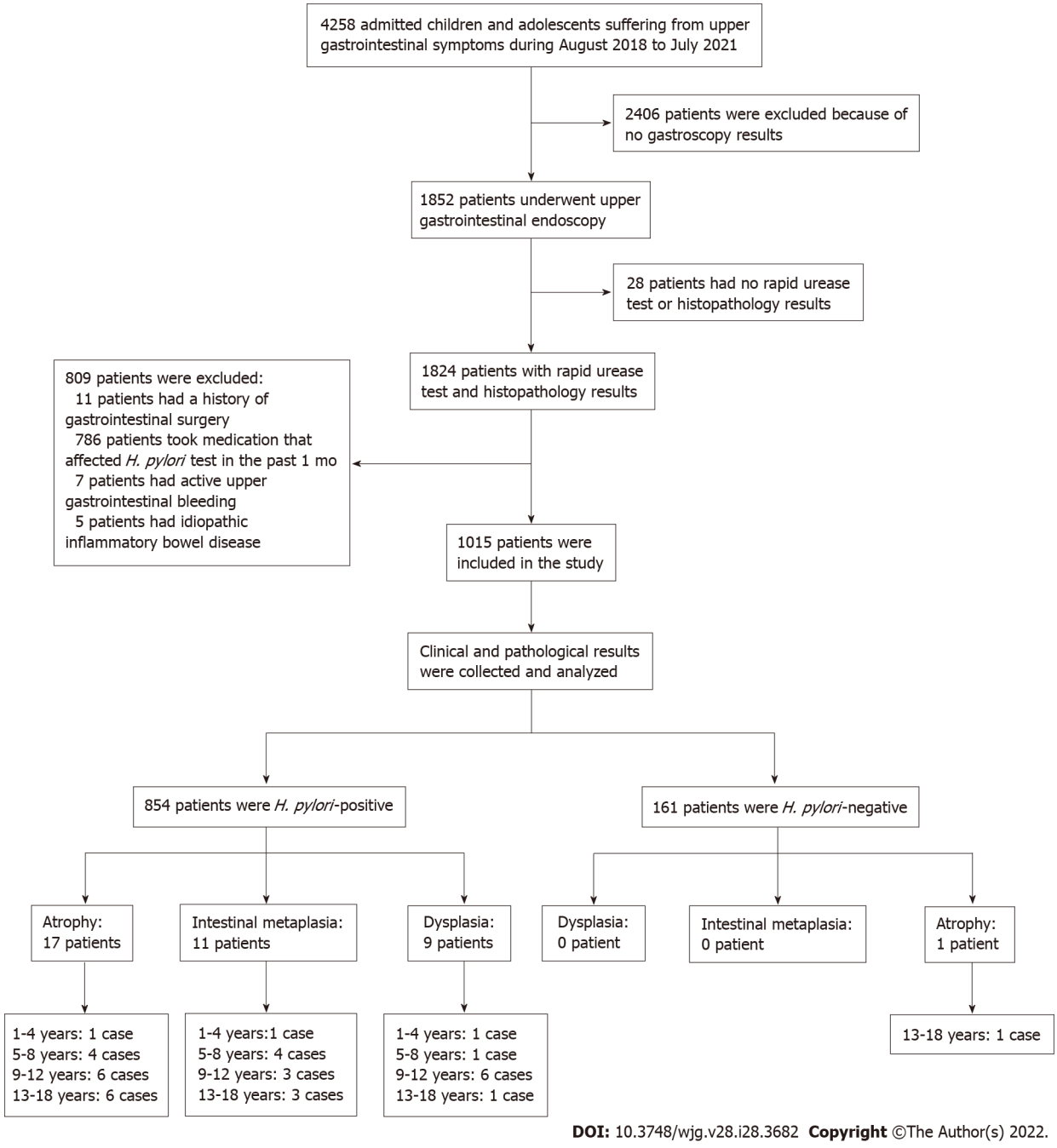

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Figure 1 Flow chart of patient enrollment on gastric mucosal precancerous lesions in children and adolescents in central China. There were 4258 patients with upper gastrointestinal symptoms under 18 year of age, 2406 patients were excluded for no gastroscopy examination, and the remaining 1852 children had underwent upper gastrointestinal endoscopy. Among these patients, 809 were excluded due to either surgery, medication, bleeding or idiopathic inflammatory bowel disease, and 28 patients were excluded due to no rapid urease test or histopathology test; the final enrolled patient number was 1015. In 854 *Helicobacter pylori* (*H. pylori*)-positive patients, 17 patients had atrophy, 11 patients had intestinal metaplasia and 9 patients had dysplasia, and only 1 of the 161 *H. pylori*-uninfected patients had atrophic gastritis. *H. pylori*: *Helicobacter pylori*.

and reduction in gastric glands of the stomach. IM is a condition in which cells that create the lining of the stomach are changed or replaced by intestinal cells. In many cases, when gastric atrophy and IM coexist, they were allocated into either the atrophy or IM category based on the major pathological presentations of the specific patients. Dysplasia refers to a proliferative lesion, abnormal hyperplasia of the gastric gland basement membrane epithelium and abnormal changes in morphology and structure. These specimens were evaluated by two pathologists in a blinded fashion.

Statistical analyses

Data were analyzed using SPSS for Windows version 25 (IBM Corp., Armonk, NY, United States). Continuous variables were described as mean ± SD or median (interquartile range), while categorical variables were described as percentages or frequencies. The χ^2 test or Fisher's exact test were used to

compare pathological changes of gastric mucosa, including activity of inflammation, presence of precancerous lesions and endoscopic pattern of gastric mucosa between patients with *H. pylori* infection and those without *H. pylori* infection. Grade data were analyzed using Wilcoxon signed-rank test, including degrees of inflammatory cell infiltration. A P value less than 0.05 (derived from two-tailed tests) was considered statistically significant.

RESULTS

Patients' demographic characteristics and clinical data

A total of 1852 selected patients were further examined from a pool of 4258 pediatric ward patients (Figure 1). Then, 809 patients were excluded due to surgery, medication, idiopathic inflammatory bowel disease or bleeding, and 28 more patients were excluded because of no rapid urease test or histopathological test. The remaining 1015 eligible patients were enrolled and analyzed. Patient demographic data were summarized in Table 1. Among the 1015 enrolled patients, 604 were males and 411 were females, with median age of 11-years-old, and 854 patients were infected by *H. pylori*, while 161 patients were not infected. The mean age of *H. pylori*-infected patients was significantly older than those of non-infected subjects (11 years *vs* 9 years, $P < 0.05$). There were no significant differences in sex distribution between the *H pylori*-infected and non-infected groups.

The clinical characteristics of patients are also presented in Table 1. For endoscopic manifestations, superficial gastritis was observed in 255 (29.86%) cases among the *H. pylori*-positive patients and 76 (47.20%) cases among the *H. pylori*-negative patients ($P < 0.05$). There was no significant difference in the proportion of superficial gastritis with erosion, superficial gastritis with bile reflux and esophagitis regardless of the status of *H. pylori* infection ($P > 0.05$). Antral nodularity was observed in 131 (15.34%) *H. pylori*-positive patients and 4 (2.48%) *H. pylori*-negative patients ($P < 0.05$). In both *H. pylori*-infected and non-infected patients, most patients were clinically diagnosed with non-atrophic gastritis, and only a small percentage of patients developed atrophic gastritis or peptic ulcers.

H. pylori infection status in different age groups

In the 1-4 years, 5-8 years, 9-12 years and 13-18 years age groups (Table 2), the *H. pylori* infection rates were 40.74%, 78.39%, 88.05% and 89.60%, respectively. The trend of infection rate increasing as age increased was noted. Patient infection rates of the 5-8 years, 9-12 years and 13-18 years age groups were significantly higher than that of the 1-4 years age group. Infection rates of the 9-12 years and 13-18 years age groups were also significantly higher than that of the 5-8 years age group, but the infection rates had no significant difference between the 9-12 and 13-18 years age groups.

Gastric mucosal precancerous lesions in different age groups

Patient gastric mucosal precancerous lesions are shown in Table 3. Among the 854 *H. pylori*-infected patients, 4.33% (37/854) had gastric mucosa precancerous lesions; among which, 17 patients had atrophy, 11 patients had IM, and 9 patients had dysplasia. Only 1 of the 161 *H. pylori*-uninfected patients (0.62%) had atrophic gastritis. The incidence of precancerous lesions of gastric mucosa in *H. pylori*-infected patients was significantly more than those uninfected patients ($\chi^2 = 5.178$, $P = 0.023$). Among the *H. pylori*-positive patients, there were gastric mucosal precancerous lesions in each age group, whereas among the *H. pylori*-negative patients, only 1 patient with atrophic gastritis was found in the 13-18 years age group. Representative pictures of normal, gastritis, and gastric mucosal precancerous lesions are shown in Figure 2.

Active inflammation in H. pylori-infected and -uninfected patients

As described in Table 4, active inflammation was observed in 16.04% (137/854) of the *H. pylori*-infected patients and only 3.73% (6/161) of the -uninfected patients. A significant difference was noticed between these two groups ($\chi^2 = 16.975$, $P < 0.001$). But in the 1-4 years age group, there was no significant difference in inflammatory activity between the *H. pylori*-infected and -uninfected patients (13.64% *vs* 6.25%, $\chi^2 = 0.196$, $P = 0.658$). Whereas in the 5-8 years, 9-12 years and 13-18 years age groups, the proportion of active inflammation in the *H. pylori*-positive patients was significantly higher than that in the *H. pylori*-negative patients ($\chi^2 = 4.901$, $P = 0.027$; $\chi^2 = 3.987$, $P = 0.046$; $\chi^2 = 6.012$, $P = 0.014$).

Characteristics of inflammatory cell infiltration in H. pylori-infected and -uninfected groups

The degree of neutrophil granulocyte infiltration with different *H. pylori* infection status is shown in Table 5. For the 854 infected patients, the proportions of absent, mild and moderate neutrophil granulocyte infiltration were 83.96%, 13.58% and 2.46%, respectively. While for the -uninfected patients, they were 96.27%, 3.73% and 0%, respectively. Compared with *H. pylori*-infected patients, the prevalence of neutrophil granulocyte infiltration in gastric mucosa was significantly higher than that in uninfected patients ($Z = 4.319$, $P < 0.001$). This difference between *H. pylori*-positive and -negative patients was also indicated in different age groups, with the exception of the 1-4 years age group.

Table 1 Demographic and clinical data of patients

Variables	Total	*H. pylori*+	*H. pylori*-	*P* value
Patients, *n*	1015	854	161	
Age in year, median (IQR)	11 (9-13)	11 (9-13)	9 (6-12)	< 0.001[a]
Sex, *n* (%)				
Female	411 (40.49)	346 (40.52)	65 (40.37)	
Male	604 (59.51)	508 (59.48)	96 (59.63)	0.973
Endoscopic pattern, *n* (%)				
Esophagitis	28 (2.76)	23 (2.69)	5 (3.11)	0.975
Superficial gastritis	331 (32.61)	255 (29.86)	76 (47.20)	< 0.001[b]
Superficial gastritis with erosion	236 (23.25)	200 (23.42)	36 (22.36)	0.77
Superficial gastritis with bile reflux	295 (29.06)	251 (29.39)	44 (27.33)	0.597
Antral nodularity	135 (13.30)	131 (15.34)	4 (2.48)	< 0.001[b]
Peptic ulcer	90 (8.87)	80 (9.37)	10 (6.21)	0.196
Atrophic gastritis	18 (1.77)	17 (1.99)	1 (0.62)	0.378
Clinical diagnosis, *n* (%)				
Esophagitis	28 (2.76)	23 (2.69)	5 (3.11)	0.975
Non atrophic gastritis	997 (98.23)	837 (98.01)	160 (99.38)	0.378
Atrophic gastritis	18 (1.77)	17 (1.99)	1 (0.62)	0.378
Gastric ulcer	18 (1.77)	16 (1.87)	2 (1.24)	0.817
Duodenal ulcer	72 (7.09)	64 (7.49)	8 (4.97)	0.252

[a]$P < 0.05$: Median age was compared between *Helicobacter pylori* (*H. pylori*)-positive and -negative groups.
[b]$P < 0.05$: When compared between *H. pylori*–positive and –negative within each disease group.
IQR: Interquartile range; *H. pylori*: *Helicobacter pylori*.

Table 2 *Helicobacter pylori* infection status in different age groups

Age group	Sex, *n* (male/female)	*H. pylori*+, *n* (%)	*H. pylori*-, *n* (%)
1-4	54 (29/25)	22 (40.74)	32 (59.26)
5-8	199 (111/88)	156 (78.39)[a]	43 (21.61)
9-12	435 (286/149)	383 (88.05)[a,b]	52 (11.95)
13-18	327 (178/149)	293 (89.60)[a,b]	34 (10.40)
Total	1015 (604/411)	854 (84.14)	161 (15.86)

[a]$P < 0.05$: When *Helicobacter pylori* (*H. pylori*) infection rates were compared with the 1-4 years age group.
[b]$P < 0.05$: When *H. pylori* infection rates were compared with the 5-8 years age group.
H. pylori: *Helicobacter pylori*.

As for lymphocyte infiltration, patients with *H. pylori* infection had more severe lymphocyte infiltration than uninfected patients ($Z = 3.997$, $P < 0.001$). In the *H. pylori*-positive group, 40.98%, 50.59% and 8.43% of mild, moderate and marked lymphocyte infiltration, respectively, were found, while in the *H. pylori*-negative group, the rates were 56.52%, 40.99% and 2.48%, respectively. In *H. pylori*-infected patients, the 9-12 years and 13-18 years age groups also showed more severe lymphocyte infiltration compared to the uninfected patients ($Z = 2.539$, $P = 0.011$; $Z = 2.164$, $P = 0.030$), but this difference was not significant between the 1-4 years and 5-8 years age groups ($Z = 0.570$, $P = 0.569$; $Z = 1.737$, $P = 0.082$) (Table 6). Representative pictures of gastric mucosal inflammatory cell infiltration are shown in Figure 2.

Table 3 Precancerous lesions in the gastric mucosa in children and adolescents

Age group	*H. pylori*+				Subtotal	*H. pylori*-				Subtotal
	n	Atrophy	IM	Dysplasia		*n*	Atrophy	IM	Dysplasia	
1-4	22	1	1	1	3	32	0	0	0	0
5-8	156	4	4	1	9	43	0	0	0	0
9-12	383	6	3	6	15	52	0	0	0	0
13-18	293	6	3	1	10	34	1	0	0	1
Total	854	37 (4.33%)[a]				161	1 (0.62%)			

[a]$P < 0.05$: The incidence of gastric mucosal precancerous lesions was compared between *Helicobacter pylori* (*H. pylori*)-positive and *H. pylori*-negative patients.
IM: Intestinal metaplasia; *H. pylori*: *Helicobacter pylori*.

Table 4 Comparison of active inflammation between *Helicobacter pylori*-positive and -negative patients

Age group	*H. pylori*+			*H. pylori*-		
	n	Active inflammation	Non-active inflammation	*n*	Active inflammation	Non-active inflammation
1-4	22	3 (13.64)	19 (86.36)	32	2 (6.25)	30 (93.75)
5-8	156	23 (14.74)[a]	133 (85.26)	43	1 (2.33)	42 (97.67)
9-12	383	52 (13.58)[b]	331 (86.42)	52	2 (3.85)	50 (96.15)
13-18	293	59 (20.14)[c]	234 (79.86)	34	1 (2.94)	33 (97.06)
Total	854	137 (16.04)[d]	717 (83.96)	161	6 (3.73)	155 (96.27)

[a]$P < 0.05$: Active inflammation in *Helicobacter pylori* (*H. pylori*)-positive patients were compared with -negative patients in the 5-8 years age group.
[b]$P < 0.05$: Active inflammation in *H. pylori*-positive patients was compared with -negative patients in the 9-12 years age group.
[c]$P < 0.05$: Active inflammation in *H. pylori*-positive patients was compared with -negative patients in the 13-18 years age group.
[d]$P < 0.05$: Active inflammation was compared between the *H. pylori*-positive and -negative groups.
Data are *n* (%), unless otherwise indicated. *H. pylori*: *Helicobacter pylori*.

Table 5 Degree of neutrophil granulocyte infiltration in *Helicobacter pylori*-positive and -negative patients

Age group	*H. pylori*+				*H. pylori*+				*Z*	*P* value
	n	Absent	Mild	Moderate	*n*	Absent	Mild	Moderate		
1-4	22	19 (86.36)	3 (13.64)	0 (0)	32	30 (93.75)	2 (6.25)	0 (0)	0.912	0.362
5-8	156	133 (85.26)	21 (13.46)	2 (1.28)	43	42 (97.67)	1 (2.33)	0 (0)	2.212	0.027[a]
9-12	383	331 (86.42)	44 (11.49)	8 (2.09)	52	50 (96.15)	2 (3.85)	0 (0)	2.009	0.045[b]
13-18	293	234 (79.86)	48 (16.38)	11 (3.76)	34	33 (97.06)	1 (2.94)	0 (0)	2.456	0.014[c]
Total	854	717 (83.96)	116 (13.58)	21 (2.46)	161	155 (96.27)	6 (3.73)	0 (0)	4.319	< 0.001[d]

[a]$P < 0.05$: Degree of neutrophil granulocyte infiltration in *Helicobacter pylori* (*H. pylori*)-positive patients were compared with -negative patients in the 5-8 years age group.
[b]$P < 0.05$: Degree of neutrophil granulocyte infiltration in *H. pylori*-positive patients was compared with -negative patients in the 9-12 years age group.
[c]$P < 0.05$: Degree of neutrophil granulocyte infiltration in *H. pylori*-positive patients was compared with -negative patients in the 13-18 years age group.
[d]$P < 0.05$: Degree of neutrophil granulocyte infiltration was compared between *H. pylori*-positive and -negative groups.
Data are *n* (%), unless otherwise indicated. *H. pylori*: *Helicobacter pylori*.

DISCUSSION

In the current study, we investigated gastric mucosal inflammation and precancerous lesions in pediatric patients who were hospitalized for gastrointestinal symptoms. Among the 1015 children in our study who underwent upper gastrointestinal endoscopy, gastric mucosa precancerous lesions occurred

Table 6 Degree of lymphocyte infiltration in *Helicobacter pylori*-positive and -negative patients

Age group	*H. pylori*+				*H. pylori*-				*Z*	*P* value
	n	Mild	Moderate	Marked	*n*	Mild	Moderate	Marked		
1-4	22	10 (45.45)	11 (50)	1 (4.55)	32	17 (53.12)	14 (43.75)	1 (3.13)	0.57	0.569
5-8	156	67 (42.95)	75 (48.08)	14 (8.97)	43	24 (55.81)	18 (41.86)	1 (2.33)	1.737	0.082
9-12	383	161 (42.04)	195 (50.91)	27 (7.05)	52	31 (59.62)	20 (38.46)	1 (1.92)	2.539	0.01[a]
13-18	293	112 (38.23)	151 (51.54)	30 (10.24)	34	19 (55.88)	14 (41.18)	1 (2.94)	2.164	0.03[b]
Total	854	350 (40.98)	432 (50.59)	72 (8.43)	161	91 (56.52)	66 (40.99)	4 (2.48)	3.997	< 0.00[c]

[a]$P < 0.05$: Degree of lymphocyte infiltration in *Helicobacter pylori* (*H. pylori*)-positive patients were compared with -negative patients in 9-12 years age group.
[b]$P < 0.05$: Degree of lymphocyte infiltration in *H. pylori*-positive patients were compared with -negative patients in 13-18 years age group.
[c]$P < 0.05$: Degree of lymphocyte infiltration was compared between *H. pylori*-positive and -negative groups.
Data are *n* (%), unless otherwise indicated. *H. pylori*: *Helicobacter pylori*.

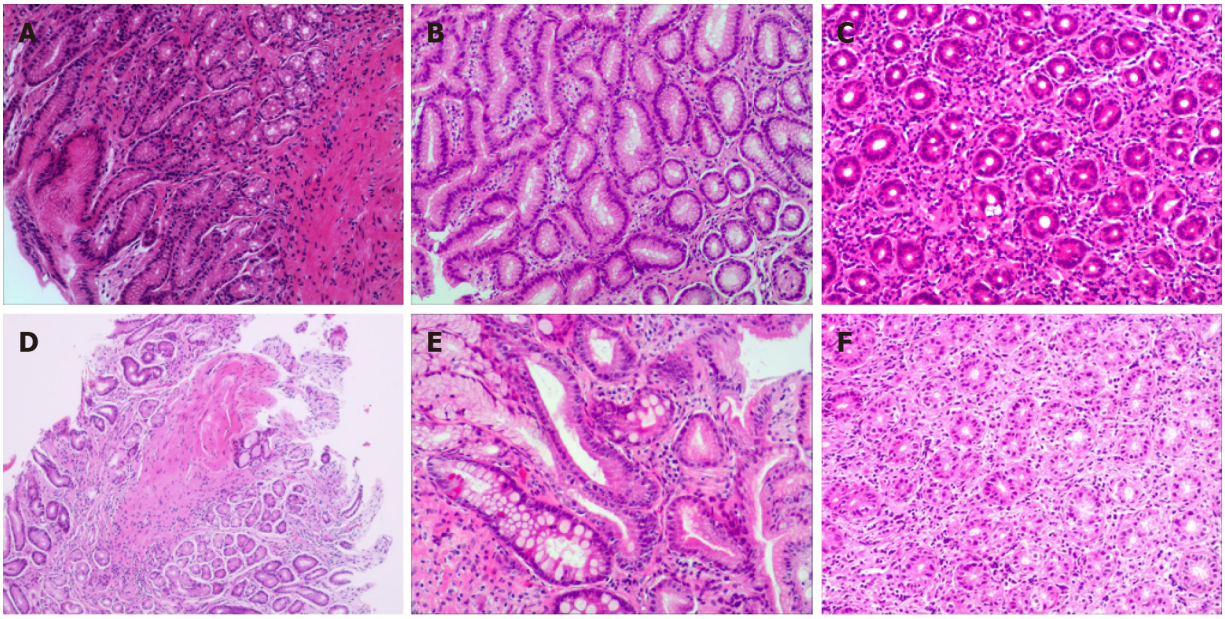

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Figure 2 Representative pictures of normal and pathological gastric mucosa manifestations in children and adolescents. A: Normal gastric mucosa; B: Chronic inflammation of the gastric mucosa; C: Active inflammation of the gastric mucosa; D: Gastric mucosal atrophy; E: Intestinal metaplasia; F: Mild dysplasia. Hematoxylin and eosin, × 400.

in 4.33% of *H. pylori*-infected children, in which the proportions of atrophy, IM and dysplasia were 1.99%, 1.29%, 1.05%, respectively, significantly higher than findings from *H. pylori*-negative patients.

Under endoscopy evaluation, *H. pylori*-positive children presented more nodular gastritis, which is an important feature of *H. pylori* infection in children. But, the proportions of esophagitis, chronic superficial gastritis with erosions, chronic superficial gastritis with bile reflux and esophagitis were not significantly different between the infected and non-infected groups. The results also showed that children with *H. pylori* infection had a significantly higher incidence of gastric mucosal inflammation, and the degrees of neutrophil and lymphocyte infiltration were also much more serious than in *H. pylori*-negative children. These results are in line with previous studies that showed various degrees of inflammation in *H. pylori*-infected pediatric patients. For example, in 2017, Broide *et al*[27] showed that monocyte and neutrophil infiltration was more severe in the infected children group with chronic gastritis in Israel. One retrospective study of 196 children in Japan in 2006 also reached a similar conclusion[17].

The above results indicate that *H. pylori* infection is closely related to gastric mucosal inflammation, which might be the basis for development of gastric precancerous lesions, as both basic experiments and clinical investigations have confirmed that certain bacterial components, such as CagA and VacA, have detrimental effects on gastric epithelial cells. In addition, CagA is a bacterial oncoprotein, which can cause epithelial cell oncogenic transformation *in vivo*[28-30]. Therefore, future experiments for more

detailed analysis of the *H. pylori* component on the pathogenesis of gastric precancerous lesions in children will be very helpful to understand the carcinogenesis and provide more rational for precise intervention and prevention.

The significance of this study is that it provides evidence to demonstrate that *H. pylori* infection is associated with the occurrence of precancerous lesions in pediatric patients, and these lesions increase with increase in age. Furthermore, the pediatric population might present in a similar way to adults with *H. pylori* infection, suggesting a healthcare threat to the pediatric patients. As eradication of *H. pylori* has been shown to prevent the development of atrophic gastritis, IM and gastric malignancy in adults[31,32], it is expected that proper intervention in pediatric patients could prevent the development of future diseases. Future investigation on the rationale and health economic evaluation would be required to provide more supporting evidence.

These results are also in line with previous reports. For example, in 2006, a study of 131 *H. pylori*-infected children in Japan showed that the proportion of gastric antrum atrophy was 10.7%[17]. In a 2020 study in Romania, the *H. pylori* infection rate was 33.06% (82/248); among 82 infected children, 9 (11%) had atrophic gastritis and 2 (2.4%) had IM[33]. A survey of 1634 children in China in 2014 found that atrophic gastritis accounted for 4.4% (23/524 cases) of the *H. pylori*-infected children[16]. However, one Austrian study in 2011 found gastric atrophy was rare (1/84) in children infected with *H. pylori*[34]. In addition, no gastric atrophy was found in 66 French children infected with *H. pylori* in 2009[35], and no precancerous lesions were found in 132 gastric biopsies from 22 symptomatic *H. pylori*-infected children in a 2009 study in Brazil[36].

As *H. pylori*-induced disease presentations are mostly present in adulthood, the presence of precancerous lesions in children unveiled a critical issue that was largely neglected previously[13,15,37]. Although there are still controversies on the significance and consequence of *H. pylori*-induced serious gastric mucosal lesions in children[13], current consensus reports from the Asia-Pacific region[38], China[8,39] and Japan[18] have indicated that *H. pylori* should be eradicated when such conditions are present, and their family members should also receive screening and treatment if confirmed positive. It is hypothesized that gastric mucosal atrophy and IM in children and adolescents might be present in a similar way to adult *H. pylori* infection and is probably more common in areas with high *H. pylori* infection rates than previously thought.

One of the major shortfalls of this retrospective study is that the rapid urease test was the primary test used to detect *H. pylori* infection because ^{13}C-urea breath test, serological *H. pylori* antibody tests and stool antigen tests were not available for more accurate diagnosis. This could result in the underestimation of *H. pylori* infection rates or have false negative results in the uninfected children group. Two perplexing results might be attributed to these drawbacks as there are 8 duodenal ulcer patients (Table 1) and few patients had active gastric inflammation (Tables 4 and 5) in the *H. pylori*-negative group. Due to the strong relationship of *H. pylori* in causing these diseases, it is unlikely these patients were not infected by *H. pylori*. Furthermore, it is likely because of the limitation of the rapid urease test, which is unable to detect patchy distribution of *H. pylori* in the stomach. Therefore, future studies will be required to confirm these discrepancies. Even with these drawbacks, the overall conclusions and significance of the investigation were not affected.

Although the current work provides novel points on *H. pylori*-induced gastric mucosal precancerous lesions in children, due to its retrospective nature, the investigation has limitations. First, this study only discussed the relationship between *H. pylori* infection and gastric mucosa precancerous lesions and was unable to analyze the effects of different genotypes of infected *H. pylori* strains. Therefore, more detailed information on *H. pylori* strains are not available. Nonetheless, our previous work demonstrated that type I *H. pylori* (CagA- and VacA-positive) is the primary type of *H. pylori* infection in this region[40]. It is expected that the pediatric population is similar to the adult population. Second, not all cases had biopsy specimens from both the gastric antrum and body. Some of the precancerous lesions and *H. pylori* infection may have been missed due to histopathological presentation of the stomach, and patchy *H. pylori* infection status in other places of the stomach were unable to be examined. In the future, multiple biopsies might be helpful to overcome these drawbacks. Third, this was a single-center, retrospective analysis, and all the children were hospitalized due to gastrointestinal symptoms, which may be the reason why the infection rate was much higher than among the general population. In the future, large-scale investigations in the general public and multi-center, prospective and randomized control analyses are needed to comprehensively assess the risk of *H. pylori* infection and precancerous lesions in children and adolescents in order to understand the overall precancerous lesions of *H. pylori*-infected pediatric patients.

CONCLUSION

Our results show that *H. pylori*-infected children have more active inflammation and inflammatory cell infiltration in the gastric mucosa, and infection rate increases with patient age. The incidence of precancerous lesions in *H. pylori*-infected patients is also significantly higher compared to that in -uninfected patients. Although the percentage is only 4.33%, it provides an alarming alert and call for further invest-

igation and intervention for this population group.

ARTICLE HIGHLIGHTS

Research background

Chronic *Helicobacter pylori* (*H. pylori*) infection causes gastric mucosal precancerous lesions and gastric cancer in adult patients. It remains to be determined, however, whether gastric mucosal precancerous lesions may also occur in children and adolescents, as this remains an important issue for clinical intervention.

Research motivation

Investigating the relationship between *H. pylori* infection and gastric mucosal precancerous lesions in pediatric patients will provide important evidence on whether intervention should be offered to prevent related disease development in this population group.

Research objectives

H. pylori infection status, gastric mucosal inflammation and gastric precancerous lesions in hospitalized pediatric patients were investigated in central China.

Research methods

We retrospectively enrolled 1015 symptomatic pediatric patients to analyze their clinical and pathological data. The endoscopic and histological findings, occurrence of gastric mucosal precancerous lesions, inflammatory activity and degree of inflammatory cell infiltration were analyzed between *H. pylori*-positive and -negative patient groups.

Research results

The overall *H. pylori* infection rate was 84.14% for the 1015 enrolled pediatric patients, and infection rate increased with age. The incidence of gastric mucosal precancerous lesions in *H. pylori*-infected children was 4.33%, which was significantly higher than that in *H. pylori*-negative children. Infected patients showed more active inflammation as well as more severe inflammatory cell infiltration compared to the non-infected patients. Additionally, endoscopy revealed that the most common presentation was antral nodularity in *H. pylori*-positive patients, whereas superficial gastritis was a marked feature for *H. pylori*-negative patients.

Research conclusions

In children and adolescents, gastric mucosal precancerous lesions occurred in 4.33% of *H. pylori*-infected patients in central China. The data revealed an obvious critical issue that requires future investigation and intervention for this population group.

Research perspectives

The results provide insights on *H. pylori* infection status and its relationship with gastric mucosal precancerous in symptomatic pediatric patients in central China. Further investigation and intervention for related disease prevention are required in this population group.

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FOOTNOTES

Author contributions: Yu M, Cheng YB, Jia BL, Kong LF, Chen CL and Ding SZ designed the research; Yu M, Song XX, Ma J, Shao QQ, Yu XC, Khan MN, Qi YB, Hu RB, Wei PR and Xiao W collected the clinical data and performed the experiments; Yu M analyzed the data; Yu M and Ding SZ wrote the paper; Ding SZ revised the article; all authors approved the final version of the manuscript.

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Institutional review board statement: This study was reviewed and approved by the Ethics Committee of People's Hospital of Zhengzhou University (2019-KY-No. 10).

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REFERENCES

1 **Yuan C**, Adeloye D, Luk TT, Huang L, He Y, Xu Y, Ye X, Yi Q, Song P, Rudan I; Global Health Epidemiology Research Group. The global prevalence of and factors associated with Helicobacter pylori infection in children: a systematic review and meta-analysis. *Lancet Child Adolesc Health* 2022; **6**: 185-194 [PMID: 35085494 DOI: 10.1016/S2352-4642(21)00400-4]
2 **Park JS**, Jun JS, Seo JH, Youn HS, Rhee KH. Changing prevalence of Helicobacter pylori infection in children and adolescents. *Clin Exp Pediatr* 2021; **64**: 21-25 [PMID: 32668822 DOI: 10.3345/cep.2019.01543]
3 **Li XC**, Wang HD, Zhang N, Wang YP, Zhou YN. Systematic review and meta-analysis of epidemiological investigation of Helicobacter pylori infection in children and adolescents in China. *Linchuang Erke Zazhi* 2017; **35**: 782-787 [DOI: 10.3969/j.issn.1000-3606.2017.10.016]
4 **Seo JH**, Bortolin K, Jones NL. Review: Helicobacter pylori infection in children. *Helicobacter* 2020; **25** Suppl 1: e12742 [PMID: 32918353 DOI: 10.1111/hel.12742]
5 **Subsomwong P**, Miftahussurur M, Uchida T, Vilaichone RK, Ratanachu-Ek T, Mahachai V, Yamaoka Y. Prevalence, risk factors, and virulence genes of Helicobacter pylori among dyspeptic patients in two different gastric cancer risk regions of Thailand. *PLoS One* 2017; **12**: e0187113 [PMID: 29084246 DOI: 10.1371/journal.pone.0187113]
6 **Li M**, Sun Y, Yang J, de Martel C, Charvat H, Clifford GM, Vaccarella S, Wang L. Time trends and other sources of variation in Helicobacter pylori infection in mainland China: A systematic review and meta-analysis. *Helicobacter* 2020; **25**: e12729 [PMID: 32686261 DOI: 10.1111/hel.12729]
7 **Ren S**, Cai P, Liu Y, Wang T, Zhang Y, Li Q, Gu Y, Wei L, Yan C, Jin G. Prevalence of Helicobacter pylori infection in China: A systematic review and meta-analysis. *J Gastroenterol Hepatol* 2022; **37**: 464-470 [PMID: 34862656 DOI: 10.1111/jgh.15751]
8 **Liu WZ**, Xie Y, Lu H, Cheng H, Zeng ZR, Zhou LY, Chen Y, Wang JB, Du YQ, Lu NH; Chinese Society of Gastroenterology, Chinese Study Group on Helicobacter pylori and Peptic Ulcer. Fifth Chinese National Consensus Report on the management of Helicobacter pylori infection. *Helicobacter* 2018; **23**: e12475 [PMID: 29512258 DOI: 10.1111/hel.12475]
9 **Malfertheiner P**, Megraud F, O'Morain CA, Gisbert JP, Kuipers EJ, Axon AT, Bazzoli F, Gasbarrini A, Atherton J, Graham DY, Hunt R, Moayyedi P, Rokkas T, Rugge M, Selgrad M, Suerbaum S, Sugano K, El-Omar EM; European Helicobacter and Microbiota Study Group and Consensus panel. Management of Helicobacter pylori infection-the Maastricht V/Florence Consensus Report. *Gut* 2017; **66**: 6-30 [PMID: 27707777 DOI: 10.1136/gutjnl-2016-312288]
10 **Jones NL**, Koletzko S, Goodman K, Bontems P, Cadranel S, Casswall T, Czinn S, Gold BD, Guarner J, Elitsur Y, Homan M, Kalach N, Kori M, Madrazo A, Megraud F, Papadopoulou A, Rowland M; ESPGHAN, NASPGHAN. Joint ESPGHAN/NASPGHAN Guidelines for the Management of Helicobacter pylori in Children and Adolescents (Update 2016). *J Pediatr Gastroenterol Nutr* 2017; **64**: 991-1003 [PMID: 28541262 DOI: 10.1097/MPG.0000000000001594]
11 **Correa P**. A human model of gastric carcinogenesis. *Cancer Res* 1988; **48**: 3554-3560 [PMID: 3288329]
12 **Miftahussurur M**, Sharma RP, Shrestha PK, Maharjan RK, Shiota S, Uchida T, Sato H, Yamaoka Y. Helicobacter pylori Infection and Gastric Mucosal Atrophy in Two Ethnic Groups in Nepal. *Asian Pac J Cancer Prev* 2015; **16**: 7911-7916

[PMID: 26625820 DOI: 10.7314/apjcp.2015.16.17.7911]
13 **Dimitrov G**, Gottrand F. Does gastric atrophy exist in children? *World J Gastroenterol* 2006; **12**: 6274-6279 [PMID: 17072948 DOI: 10.3748/wjg.v12.i39.6274]
14 **Boukthir S**, Mrad SM, Kalach N, Sammoud A. Gastric atrophy and Helicobacter pylori infection in children. *Trop Gastroenterol* 2009; **30**: 107-109 [PMID: 19760998]
15 **Villarreal-Calderon R**, Luévano-González A, Aragón-Flores M, Zhu H, Yuan Y, Xiang Q, Yan B, Stoll KA, Cross JV, Iczkowski KA, Mackinnon AC Jr. Antral atrophy, intestinal metaplasia, and preneoplastic markers in Mexican children with Helicobacter pylori-positive and Helicobacter pylori-negative gastritis. *Ann Diagn Pathol* 2014; **18**: 129-135 [PMID: 24656654 DOI: 10.1016/j.anndiagpath.2014.02.003]
16 **Yu Y**, Su L, Wang X, Xu C. Association between Helicobacter pylori infection and pathological changes in the gastric mucosa in Chinese children. *Intern Med* 2014; **53**: 83-88 [PMID: 24429445 DOI: 10.2169/internalmedicine.53.0918]
17 **Kato S**, Nakajima S, Nishino Y, Ozawa K, Minoura T, Konno M, Maisawa S, Toyoda S, Yoshimura N, Vaid A, Genta RM. Association between gastric atrophy and Helicobacter pylori infection in Japanese children: a retrospective multicenter study. *Dig Dis Sci* 2006; **51**: 99-104 [PMID: 16416219 DOI: 10.1007/s10620-006-3091-5]
18 **Sugano K**, Tack J, Kuipers EJ, Graham DY, El-Omar EM, Miura S, Haruma K, Asaka M, Uemura N, Malfertheiner P; faculty members of Kyoto Global Consensus Conference. Kyoto global consensus report on Helicobacter pylori gastritis. *Gut* 2015; **64**: 1353-1367 [PMID: 26187502 DOI: 10.1136/gutjnl-2015-309252]
19 **Osaki T**, Konno M, Yonezawa H, Hojo F, Zaman C, Takahashi M, Fujiwara S, Kamiya S. Analysis of intra-familial transmission of Helicobacter pylori in Japanese families. *J Med Microbiol* 2015; **64**: 67-73 [PMID: 25351712 DOI: 10.1099/jmm.0.080507-0]
20 **Rothenbacher D**, Winkler M, Gonser T, Adler G, Brenner H. Role of infected parents in transmission of Helicobacter pylori to their children. *Pediatr Infect Dis J* 2002; **21**: 674-679 [PMID: 12237602 DOI: 10.1097/00006454-200207000-00014]
21 **Kienesberger S**, Perez-Perez GI, Olivares AZ, Bardhan P, Sarker SA, Hasan KZ, Sack RB, Blaser MJ. When is Helicobacter pylori acquired in populations in developing countries? *Gut Microbes* 2018; **9**: 252-263 [PMID: 29494270 DOI: 10.1080/19490976.2017.1421887]
22 **Jaramillo-Rodríguez Y**, Nares-Cisneros J, Martínez-Ordaz VA, Velasco-Rodríguez VM, Márquez FC, Manríquez-Covarrubias LE. Chronic gastritis associated with Helicobacter pylori in Mexican children: histopathological patterns. *Pediatr Dev Pathol* 2011; **14**: 93-98 [PMID: 20658934 DOI: 10.2350/09-12-0754-OA.1]
23 **Okuda M**, Lin Y, Kikuchi S. Helicobacter pylori Infection in Children and Adolescents. *Adv Exp Med Biol* 2019; **1149**: 107-120 [PMID: 31037557 DOI: 10.1007/5584_2019_361]
24 **Whitney AE**, Guarner J, Hutwagner L, Gold BD. Helicobacter pylori gastritis in children and adults: comparative histopathologic study. *Ann Diagn Pathol* 2000; **4**: 279-285 [PMID: 11073332 DOI: 10.1053/adpa.2000.17871]
25 **Liu S**, Chen Q, Quan P, Zhang M, Zhang S, Guo L, Sun X, Wang C. Cancer incidence and mortality in Henan province, 2012. *Chin J Cancer Res* 2016; **28**: 275-285 [PMID: 27478313 DOI: 10.21147/j.issn.1000-9604.2016.03.02]
26 **Dixon MF**, Genta RM, Yardley JH, Correa P. Classification and grading of gastritis. The updated Sydney System. International Workshop on the Histopathology of Gastritis, Houston 1994. *Am J Surg Pathol* 1996; **20**: 1161-1181 [PMID: 8827022 DOI: 10.1097/00000478-199610000-00001]
27 **Broide E**, Richter V, Mendlovic S, Shalem T, Eindor-Abarbanel A, Moss SF, Shirin H. Lymphoid follicles in children with *Helicobacter pylori*-negative gastritis. *Clin Exp Gastroenterol* 2017; **10**: 195-201 [PMID: 28860835 DOI: 10.2147/CEG.S133421]
28 **Hatakeyama M**. Helicobacter pylori and gastric carcinogenesis. *J Gastroenterol* 2009; **44**: 239-248 [PMID: 19271114 DOI: 10.1007/s00535-009-0014-1]
29 **Myint T**, Miftahussurur M, Vilaichone RK, Ni N, Aye TT, Subsomwong P, Uchida T, Mahachai V, Yamaoka Y. Characterizing *Helicobacter pylori cagA* in Myanmar. *Gut Liver* 2018; **12**: 51-57 [PMID: 29069889 DOI: 10.5009/gnl17053]
30 **Hatakeyama M**. Linking epithelial polarity and carcinogenesis by multitasking Helicobacter pylori virulence factor CagA. *Oncogene* 2008; **27**: 7047-7054 [PMID: 19029944 DOI: 10.1038/onc.2008.353]
31 **Kong YJ**, Yi HG, Dai JC, Wei MX. Histological changes of gastric mucosa after Helicobacter pylori eradication: a systematic review and meta-analysis. *World J Gastroenterol* 2014; **20**: 5903-5911 [PMID: 24914352 DOI: 10.3748/wjg.v20.i19.5903]
32 **Ford AC**, Forman D, Hunt RH, Yuan Y, Moayyedi P. Helicobacter pylori eradication therapy to prevent gastric cancer in healthy asymptomatic infected individuals: systematic review and meta-analysis of randomised controlled trials. *BMJ* 2014; **348**: g3174 [PMID: 24846275 DOI: 10.1136/bmj.g3174]
33 **Domșa AT**, Lupușoru R, Gheban D, Șerban R, Borzan CM. *Helicobacter pylori* Gastritis in Children-The Link between Endoscopy and Histology. *J Clin Med* 2020; **9** [PMID: 32183130 DOI: 10.3390/jcm9030784]
34 **Hoepler W**, Hammer K, Hammer J. Gastric phenotype in children with Helicobacter pylori infection undergoing upper endoscopy. *Scand J Gastroenterol* 2011; **46**: 293-298 [PMID: 21073375 DOI: 10.3109/00365521.2010.533383]
35 **Kalach N**, Papadopoulos S, Asmar E, Spyckerelle C, Gosset P, Raymond J, Dehecq E, Decoster A, Creusy C, Dupont C. In French children, primary gastritis is more frequent than Helicobacter pylori gastritis. *Dig Dis Sci* 2009; **54**: 1958-1965 [PMID: 19003529 DOI: 10.1007/s10620-008-0553-y]
36 **Langner M**, Machado RS, Patrício FR, Kawakami E. Evaluation of gastric histology in children and adolescents with Helicobacter pylori gastritis using the Update Sydney System. *Arq Gastroenterol* 2009; **46**: 328-332 [PMID: 20232015 DOI: 10.1590/s0004-28032009000400015]
37 **Kara N**, Urganci N, Kalyoncu D, Yilmaz B. The association between Helicobacter pylori gastritis and lymphoid aggregates, lymphoid follicles and intestinal metaplasia in gastric mucosa of children. *J Paediatr Child Health* 2014; **50**: 605-609 [PMID: 24925694 DOI: 10.1111/jpc.12609]
38 **Mahachai V**, Vilaichone RK, Pittayanon R, Rojborwonwitaya J, Leelakusolvong S, Maneerattanaporn M, Chotivitayatarakorn P, Treeprasertsuk S, Kositchaiwat C, Pisespongsa P, Mairiang P, Rani A, Leow A, Mya SM, Lee YC,

Vannarath S, Rasachak B, Chakravuth O, Aung MM, Ang TL, Sollano JD, Trong Quach D, Sansak I, Wiwattanachang O, Harnsomburana P, Syam AF, Yamaoka Y, Fock KM, Goh KL, Sugano K, Graham D. Helicobacter pylori management in ASEAN: The Bangkok consensus report. *J Gastroenterol Hepatol* 2018; **33**: 37-56 [PMID: 28762251 DOI: 10.1111/jgh.13911]

39 **Ding SZ**, Du YQ, Lu H, Wang WH, Cheng H, Chen SY, Chen MH, Chen WC, Chen Y, Fang JY, Gao HJ, Guo MZ, Han Y, Hou XH, Hu FL, Jiang B, Jiang HX, Lan CH, Li JN, Li Y, Li YQ, Liu J, Li YM, Lyu B, Lu YY, Miao YL, Nie YZ, Qian JM, Sheng JQ, Tang CW, Wang F, Wang HH, Wang JB, Wang JT, Wang JP, Wang XH, Wu KC, Xia XZ, Xie WF, Xie Y, Xu JM, Yang CQ, Yang GB, Yuan Y, Zeng ZR, Zhang BY, Zhang GY, Zhang GX, Zhang JZ, Zhang ZY, Zheng PY, Zhu Y, Zuo XL, Zhou LY, Lyu NH, Yang YS, Li ZS; National Clinical Research Center for Digestive Diseases (Shanghai), Gastrointestinal Early Cancer Prevention & Treatment Alliance of China (GECA), Helicobacter pylori Study Group of Chinese Society of Gastroenterology, and Chinese Alliance for Helicobacter pylori Study. Chinese Consensus Report on Family-Based *Helicobacter pylori* Infection Control and Management (2021 Edition). *Gut* 2022; **71**: 238-253 [PMID: 34836916 DOI: 10.1136/gutjnl-2021-325630]

40 **Yuan L**, Zhao JB, Zhou YL, Qi YB, Guo QY, Zhang HH, Khan MN, Lan L, Jia CH, Zhang YR, Ding SZ. Type I and type II *Helicobacter pylori* infection status and their impact on gastrin and pepsinogen level in a gastric cancer prevalent area. *World J Gastroenterol* 2020; **26**: 3673-3685 [PMID: 32742135 DOI: 10.3748/wjg.v26.i25.3673]

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ORIGINAL ARTICLE

Observational Study

Secular trends of intrahepatic cholangiocarcinoma in a high endemic area: A population-based study

Chun-Ru Lin, Yu-Kwang Lee, Chun-Ju Chiang, Ya-Wen Yang, Hung-Chuen Chang, San-Lin You

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Abstract

BACKGROUND

Intrahepatic cholangiocarcinoma (ICC) is one of the most aggressive malignancies. However, because of its scarcity there are limited population-based data available for investigations into its epidemiologic characteristics. In Taiwan, we have a national cancer registry database that can be used to evaluate the secular trends of ICC.

AIM

To evaluate secular trends of ICC according to age, sex, and risk factors in Taiwan.

METHODS

In this population-based study, we used the national Taiwan Cancer Registry database. Age-standardized and relative percent changes in incidence rates were used to describe secular trends in incidence rates and sex ratios of ICC in Taiwan.

RESULTS

The age-standardized ICC incidence rate among males increased from 1.51 per 100000 in 1993-1997 to 4.07 per 100000 in 2013-2017 and among female from 1.73

per 100000 to 2.95 per 100000. The incidence in females tended to plateau after 2008-2012. For males, the ICC incidence increased as age increased. In the long-term incidence trend of ICC in females, the incidence of the four age groups (40-44, 45-49, 50-54 and 55-59 years) remained stable in different years; although, the incidence of the 60-64 group had a peak in 2003-2007, and the peak incidence of the 65-69 and 70-74 groups occurred in 2008-2012. Among males, beginning at the age of 65, there were increases in the incidence of ICC for the period of 2003-2017 as compared with females in the period of 2003-2017.

CONCLUSION

Increased incidence of ICC occurred in Taiwan over the past two decades. The increased incidence has progressively shifted toward younger people for both males and females.

Key Words: Intrahepatic cholangiocarcinoma; Incidence; Secular trend; Sex ratio; Risk factor

Core Tip: It is important to evaluate the secular trends of intrahepatic cholangiocarcinoma (ICC) incidence and to determine insightful etiological clues in a population with a high incidence of liver cancer. Using the national Taiwan Cancer Registry, we observed an increased incidence of ICC for both males and females. Our observations should be taken in the context of other studies conducted on secular trends of ICC.

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INTRODUCTION

Intrahepatic cholangiocarcinoma (ICC) is one of the most aggressive malignancies, and its mortality rates are 1–2 per 100000[1]. After surgery, patients with ICC have 5-year survival rates of approximately 60%, and the rates of recurrence are 60%-65%[2]. In the United States, there were 1.18 new cases per 100000 in 2012[3]. ICC is the second most commonly diagnosed liver cancer[4], and the incidence of ICC continues to increase[5]. Florio *et al*[5] reported that the incidence rate of ICC has regional differences. For example, the incidence rate of ICC in 2008-2012 was 2.80 per 100000 in South Korea [95% confidence interval (CI): 2.68 to 2.93], 2.19 per 100000 in Thailand (95%CI: 2.01-2.36), 0.58 per 100000 in Italy (95%CI: 0.48-0.69), 0.78 per 100000 in the United States (95%CI: 0.74-0.82), and 1.15 per 100000 in the United Kingdom (95%CI: 1.12-1.18)[5]. The average annual percent change (AAPC) of ICC in 2008-2012 also exhibits regional differences. In particular, the AAPC of ICC in 2008-2012 was 4.5% in South Korea (95%CI: 3.0-5.9), -1.0% in Thailand (95%CI: -3.8-1.9), 3.3% in Italy (95%CI: 1.5-5.2), 2.0% in the United States (95%CI: -2.3-6.5), and 4.6% in the United Kingdom (95%CI: 3.2-6.1)[5]. Although these previous studies show that the threat of ICC is rising in many parts of the world[4,5], few studies have used nationwide incidence data to study the epidemiology of ICC.

Moreover, this population-based data facilitates an unbiased estimate of the burden of ICC into the near future. We aimed to evaluate the current trends of ICC in Taiwan. We investigated secular trends in the incidence of ICC to provide insightful etiological analyses, particularly for the variations by age and sex.

MATERIALS AND METHODS

Incidence data from 1993–2017 were obtained from the Taiwan Cancer Registry (TCR). We extracted incidence data on ICC [topography code of International Classification of Diseases for Oncology (ICD-O-FT: T-155.1 before 2002 and ICD-O-3: C22.1, M code 9590-9993 was excluded)]. TCR is organized and funded by the Health Promotion Administration, Ministry of Health and Welfare, Taiwan. In 1979, TCR began to register all cancers nationwide[6,7].

Statistical analysis

In 1995, Taiwan launched the National Health Insurance system[8]. Since that time, the health status of the Taiwanese population has been fully registered[7]. The incidence data were grouped into 17 5-year age groups (0-4, 5-9, 10-14, 15-19, … to 80+ years) and 5 periods (1993–1997, 1998–2002, …, and 2013–2017). Therefore, the data design comprised 14 birth cohorts (the oldest: 1908-1912 to the youngest: 1973-1977). In this study, we reasoned the choice of 1993-1997 as the beginning of the analytical year to avoid incomplete records of incidence data, and the quality of diagnosis in 1993 was similar to that after 1995.

We calculated the incidence rates by sex for each age group by dividing the number of cases by the corresponding population size[9]. Age-standardized incidence rates by sex were calculated using the direct method with the 2000 world standard population as reference[10]. The male-to-female incidence ratios were calculated by dividing the rate in males by that in females for each age group.

All statistical analyses were performed using SAS version 9.4 (SAS Institute Inc., Cary, NC, United States). The research protocol was approved by the Institutional Review Board of Fu-Jen Catholic University (No. C107099).

RESULTS

Age-standardized incidence rates

Figure 1 shows the trend of age-standardized rate (ASR) of ICC in men and women in Taiwan from 1993-1997 to 2013-2017. The incidence of ICC in men increased linearly. The ASR increased from 1.51 per 100000 in 1993-1997 to 4.07 per 100000 in 2013-2017, and its relative percent change was 169%. The incidence of ICC in women tended to plateau after 2008-2012. The ASR increased from 1.73 per 100000 population in 1993-1997 to 2.95 per 100000 in 2008-2012. The relative percentage change was 70%. From 1993-1997 to 2013-2017, the ASR incidence of ICC in men increased more rapidly than that in women.

Age-specific incidence rates

Figure 2A showed the age-specific rates of ICC per 100000 men in Taiwan. For males, the incidence of ICC increased as age increased. The age-specific rate of ICC in seven age groups (40-44, 45-49, 50-54, 55-59, 60-64, 65-69, and 70-74) increased steadily between 1993-1997 and 2013-2017. The respective relative percent changes were 372%, 265%, 156%, 124%, 153%, 231%, and 139%. The 70-74 age group had a larger growth between 1993-1997 and 2003-2007, and its relative percent change was 114%. Then, it increased more slowly between 2003-2007 and 2013-2017, and its relative percent change was only 5%. The 80+ age group showed a trend from rising to declining, with peaks occurring in 2008-2012. Compared to 1993-1997, its relative percent change was 175%. From 2008-2007 to 2013-2017, the incidence decreased 4%.

Figure 2B shows the age-specific rates of ICC per 100000 women in Taiwan. The age-specific rates of the four age groups of 40-44, 45-49, 50-54, and 55-59 remained stable in different years. The incidence of the 60-64 age group had a peak in 2003-2007, and the incidence increased by 69% compared with 1993-1997. The peak incidence of the 65-69 and 70-74 age groups occurred in 2008-2012 compared with the incidence of 1993-1997. The respective relative percent change was 111% and 136%. The incidence of the 75-79 age group increased steadily from 1993-1997 to 2013-2017, with an increase of 121%. The incidence of the 80+ age group peaked in 2008-2012 compared with that in 1993-1997, and the relative percentage change was 227%. Then, the incidence decreased by 11% between 2008-2012 and 2013-2017.

Figure 3 showed the age-specific rates of men and women in different birth cohorts. The incidence of ICC increased as the birth year increased for both males and females. For example, in the 70-74 age group, the incidence of males born in 1943-1947 was 8.41 times of those born in 1913-1917, and the incidence of women born in 1943-1947 was 16.11 times of those born in 1913-1917.

Sex ratios of age-standardized incidence rates

Figure 4 shows the sex ratio of the ASR of ICC in Taiwan from 1993 to 2017. A turning point was apparent at 1998-2002. Before that period, the incidence of ICC in women was higher than that in men. In 1998-2002, the sex ratio of the incidence of ICC was 1.02, which means that the incidence of ICC in men and women was almost the same. After 2002, the incidence of ICC in men was higher than that of women. From 1993-1997 to 2013-2017, the sex ratio of the incidence of ICC increased from 0.87 to 1.52, and the relative percent change was 75%, which indicates a trend of an increasing gap in the incidence of ICC between men and women.

Sex ratios of age-specific incidence rates

Figure 5 shows the sex ratio of the age-specific rates of ICC in Taiwan by diagnostic period. For ICC patients younger than 70-year-old, the sex ratio of age-specific rates increased with year. For example, in the 40-44 age group, the sex ratio was 0.72 in 1993-1997 and 2.98 in 2013-2017. Its relative percent change was 314% from 1993-1997 to 2013-2017. In the age group over 70-years-old, the sex ratio showed a stable

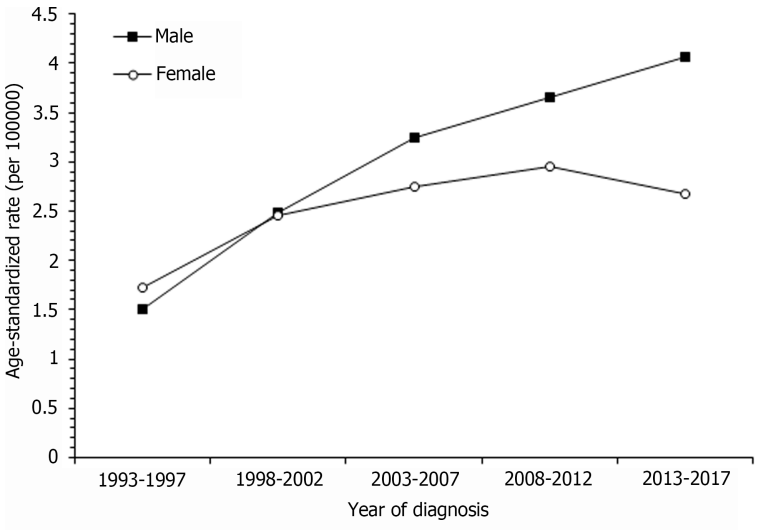

Figure 1 Secular trend in age-standardized incidence rates of intrahepatic cholangiocarcinoma in Taiwan, 1993–2017.

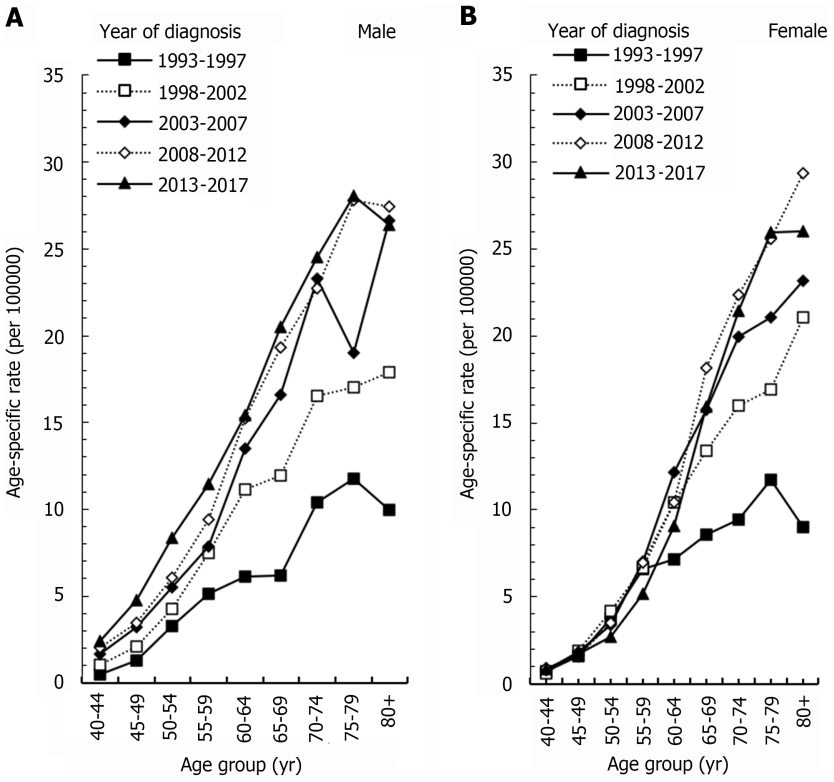

Figure 2 Age-specific incidence rates of intrahepatic cholangiocarcinoma by year of diagnosis and sex. A: Male age-specific rate; B: Female age-specific rate.

baseline in different years, which meant that the incidence of ICC was similar in men and women.

DISCUSSION

This population-based study showed that the incidence of ICC has rapidly increased in Taiwan. This increase remained after stratification by age, sex, diagnosis period, and birth cohort. These results, which highlight the current and projected incidence of ICC, suggest that there is a need for more research to prevent the burden of this cancer in Asian countries.

The incidence rate of ICC varies among different regions of the world[4]. Globally, the ASR is 0.85 per 100000[11]. In Asia, the highest ASR was 2.80 per 100000 in South Korea, and the lowest was 0.26 per 100000 in Israel during 2008-2012[5]. According to our result, the ASR in Taiwan was about 3.3 per

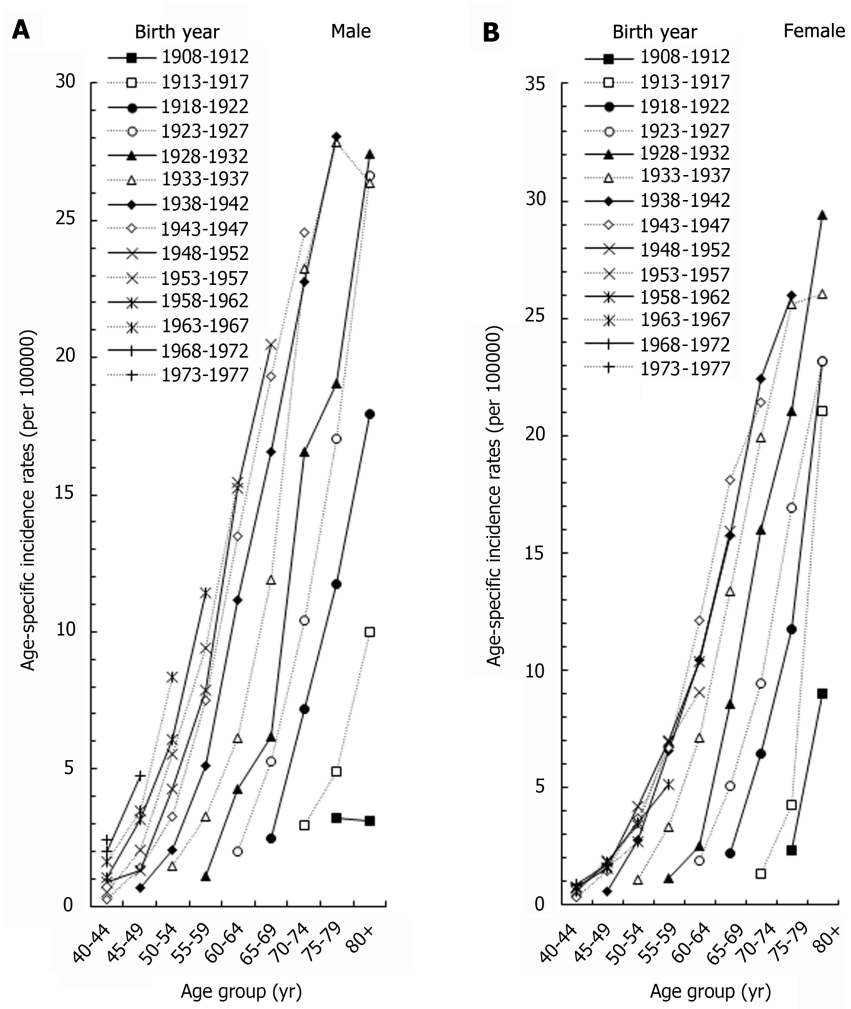

Figure 3 Age-specific incidence rates of intrahepatic cholangiocarcinoma by birth year and sex.

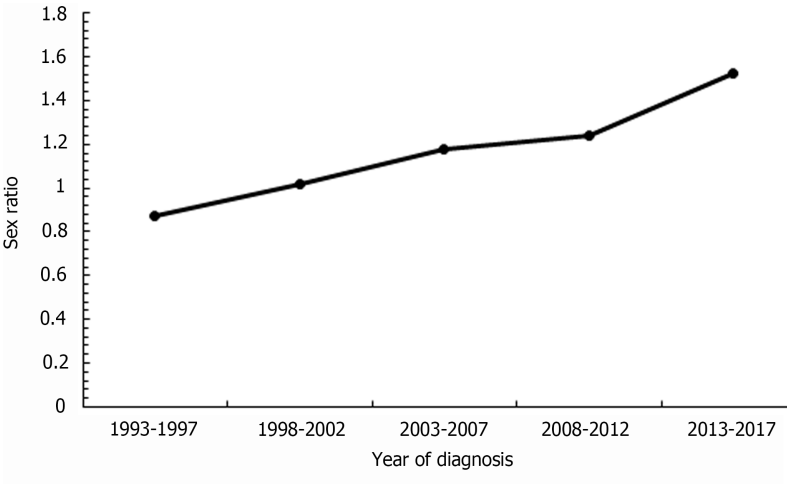

Figure 4 Sex ratio for age-standardized incidence rate of intrahepatic cholangiocarcinoma by year of diagnosis.

100000 during 2008-2012, which means that Taiwan is a high-risk region of ICC. Higher incidence of ICC in Asian countries is likely due to the presence of more risk factors[4,12].

Changes in the incidence of ICC also differs among different countries[5]. Overall, the incidence of ICC has increased in most of the countries in the world[5]. However, the incidence of ICC was higher in Asia, and the increase-rate was higher in Europe and North America[13]. The AAPC of ICC incidence from 1993 to 2012 was 5.0% in Canada, 6.1% in Costa Rica, 6.5% in France, 7.5% in Germany, 8.7% in Poland, 10.5% in Ireland, and 20.1% in Latvia[5]. In Asian countries, the AAPC of ICC incidence was -

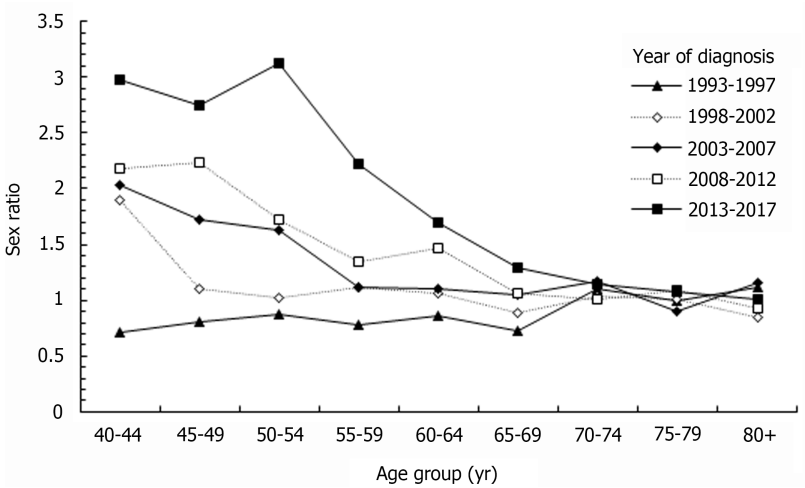

Figure 5 Age-specific sex ratio of intrahepatic cholangiocarcinoma by year of diagnosis and age groups.

0.7% in Japan, -1.0% in Thailand, -2.4% in Philippines, 4.5% in South Korea, and 11.1% in China[5]. In some Asian countries, such as Philippines, Thailand, and Japan, the incidence of ICC showed a nonsignificant decline[5]. In contrast to other studies, our results indicate a rising burden of ICC in the Asia-Pacific area.

A previous study attempted to report the population-based incidence rate of ICC[14]. A study conducted in the United States found that ICC incidence increased from 0.44 per 100000 in 1973 to 1.8 per 100000 in 2012. The AAPC from 2001 to 2012 was 2.3%, and AAPC was 4.36% from 2003 to 2012[3]. In this study, we used the national registry system, which provides comprehensive coverage of the entire population, to make an unbiased estimation of ICC incidence. We found that the incidence of ICC in Taiwan increased from 1.04 per 100000 to 3.36 per 100000 from 1988-1992 to 2013-2017.

An expert consensus document reported that ICC has several risk factors, such as aging of the population, smoking, obesity, diabetes mellitus (DM), hepatitis B virus (HBV) infection, and hepatitis C virus (HCV) infection[4], which may account for this projected increasing incidence. However, due to the number of risk factors of ICC, the phenomenon of the increasing trend of the ICC incidence in Taiwan cannot be explained by a single risk factor.

Aging is an established risk factor for ICC[4]. A study published in 2016 found that the median age at diagnosis among patients in the United States diagnosed with ICC was 67 years between 2008 and 2012 [3]. More than 73% of those patients with ICC were older than 60 years[15], which was similar to our results. We found that the incidence rate was low before age 60 and increased dramatically thereafter. The proportion of people in the population aged 60 and above increased from 13.6% in 2007 to 23.2% in 2020 in Taiwan[9]. A previous study found that the aging of populations in developed countries contributes to the increasing incidence of ICC[15]. Nevertheless, we found the age-adjusted incidence increased gradually over time, and the trend remained consistently elevated, even after stratification by age. This implies that population aging has contributed to the increasing ICC incidence in Taiwan.

A previous meta-analysis showed that DM significantly increased the risk of ICC independent of various confounding factors (relative risk: 1.97, 95%CI: 1.57-2.46; P = 0.025 for heterogeneity)[16]. A previous study reported an increasing prevalence of DM for both sexes in Taiwan from 1992 to 1996 [17], while the incidence remained stable at 6.9–7.7 per 1000 person-years from 1999 to 2004[18]. From 2000 to 2007, the ASR of type 2 DM remained high, at 8.7–9.8 per 1000[19]. In the elderly age group (≥ 65 years), the incidence rate in women (873.2/100000) was higher than that in men (721.4/100000)[20].

Smoking is the most comprehensive environmental factor responsible for ICC. A study based on the National Cancer Institute's SEER 18 database reported that smoking increases the risk of ICC by 46% (95%CI: 1.28-1.66)[20]. A cohort study that included 1518741 individuals showed that current smokers have a 47% greater risk of ICC compared to non-smokers (95%CI: 1.07-2.02)[21]. Taiwan has a high prevalence of smoking. The smoking rate among adult men increased from 59% in 1986 to 63% in 1990, which resulted from the cigarette market opening[22]. A study based on the National Health Interview Survey of Taiwan was conducted in 2001. The prevalence of smokers was 46.8% and the prevalence of ex-smokers was 6.8% in Taiwanese adult males in 2001. The prevalence of smoking was 4.3% and the prevalence of ex-smokers was 0.5% in Taiwanese adult females in 2001[23]. In 2009, a new anti-smoking law was established in Taiwan. Despite the efforts of the Taiwan government, a cross-sectional study that included 961 adults found that up to 42% of sampled Taiwanese adults had smoked cigarettes after that new law had been implemented[24]. The high prevalence of smoking among the early birth cohort in Taiwan may partly explain its high incidence of ICC and seemed to have a cohort effect on ICC.

Obesity was found to increase the risk of ICC by a previous meta-analysis [odds ratio (OR): 1.56, 95%CI: 1.26-1.94][25]. Recently, the prevalence of obesity in Taiwan has increased rapidly. More specifically, a study based on the Nutrition and Health Surveys in Taiwan reported that the prevalence of obesity in men increased from 10.5% in 1993–1996 to 17.0% in 2005. The prevalence of obesity tripled for elementary school boys from 1993 to 2002. Due to the consumption of high-fat foods and lack of physical activity, the prevalence of obesity is likely to increase further in Taiwan[26]. The aforementioned phenomenon may contribute to the increasing prevalence of ICC.

A previous study reported that ICC incidence rates were higher in males than in females in most countries in the world[5]. The sex ratio of the incidence of ICC was 2.9 in Malta, 1.9 in South Korea, 1.9 in Thailand, 1.9 in Japan, 1.9 in Spain, 1.6 in Slovakia, and 1.6 in France[5]. A study based on the SEER database reported that the incidence of ICC was higher in males than in females, and the sex ratio of the age-specific rates of ICC decreased in the aging group[27]. Our results also showed similar outcomes, and this phenomenon may be due to the different hormone profiles of males and females[28]. A study reported that estrogen may modulate cholangiocyte proliferation in nude mice[29]. A previous study used chromatography-tandem mass spectrometry and competitive electrochemiluminescence immunoassay to analyze the relationship between sex steroid hormones and ICC. In that study, a high level of estradiol was found to increase the risk of ICC (OR: 1.40, 95%CI: 1.05-1.89)[30]. A previous study in Taiwan reported that women born in younger cohorts had a later age at natural menopause (hazard ratio: 0.87 per 10-year difference, 95%CI: 0.81-0.95)[31]. The lower ICC incidence of females in our study may be attributed to decreasing estrogen levels in elderly and postmenopausal women. The lower prevalence of smoking among women (4%-8%) than men (47% in 2001)[22] may also contribute to their lower incidence of ICC.

A previous study found that hepatitis B surface antigen (HBsAg) and HCV antibodies had a high association with ICC[32]. A meta-analysis reported that HBV and HCV infection increased the risk of ICC (OR: 3.17, 95%CI: 1.88-5.34 and OR: 3.42, 95%CI: 1.96-5.99, respectively)[33]. Previously, Taiwan was classified as a high-risk region for HBsAg[34]. A study found that HBV DNA could interrupt seven genes (*TERT*, *CEACAM20*, *SPATA18*, *TRERF1*, *ZNF23*, *LINC01449*, and *LINC00486*) in ICC tissue, which may indicate a potential mechanism for the increased risk of ICC in HBV carriers[35].

A retrospective multicenter study in Taiwan reported that the HBsAg prevalence in hepatocellular carcinoma from 1981 to 2001 decreased from 81.5% to 61.2% in males and decreased from 66.7% to 41.4% in females[36]. The sex ratio of HBsAg prevalence of hepatocellular carcinoma increased from 1.22 to 1.61[36], which was consistent with the increasing trend of the sex ratio of the ICC incidence in our study. A previous review hypothesized that HCV could induce the transformation of hepatocytes into cholangiocyte precursors[37]. The HCV antibody prevalence in hepatocellular carcinoma was 31.5% (95%CI: 30.4%-32.6%) in Taiwanese males and 56.7% (95%CI: 54.4%-59.0%) in Taiwanese females[36]. A retrospective cross-sectional study in Taiwan reported that in patients with HCV infection, visceral obesity was significantly associated with waist-to-height ratio, body fat percentage, fat-free mass/body weight, and muscle mass/body weight[38]. Thus, we assumed that HBV and/or HCV infection may be at least partially explain why the incidence of ICC in Taiwan was high.

We found that the sex ratio of the incidence of ICC increased as the diagnostic year increased. In the group over the age of 70 years, the sex ratio of the incidence of ICC between men and women was close to 1, which meant that there was almost no difference in the incidence of ICC between men and women. The proportion of women with ICC increases with age. Another population-based study in the United States showed the proportion of females with ICC was 38.9% for those aged 40-59 years, 45.8% for those aged 60-79 years, and 56.3% for those aged ≥ 80 years[27]. The aforementioned research results are consistent with our study outcome. However, another study based on the Italian National Institute of Statistics A in Italy, another country with a high risk of ICC, reported that the incidence of ICC in males was always higher than that in females[39], which was different from our study outcome. Due to the variety of risk factors of ICC, it is difficult to identify the cause of this difference. A study based on the Netherlands Cancer Registry reported that the incidence of ICC in a low endemic area significantly increased in older populations, especially in in the 45–59 years age group[40]. Interestingly, we also found a similar pattern.

Assessing the temporal trends in the sex difference of ICC incidence has useful implications. Any temporal changes in the sex difference reflect the contributions from environmental and extrinsic risk factors, while a stable sex difference indicates the role of intrinsic exposures or environmental risk. The increase of the sex difference in the incidence of ICC may be explained by the decreasing prevalence of smoking, particularly in men with a historically higher prevalence[23,24]. The decline in smoking prevalence in Taiwan, which has been more rapid for men than for women, may have also contributed to the decreased sex difference in ICC[23,24]. In addition, we noted an increased sex difference in the incidence of ICC since 2008, which warrants confirmation through continued monitoring and investigations.

We found that the gap in the sex ratio between the 40-44 age group and the 80+ age group increased as the diagnostic year increased. In age groups younger than 70 years, the sex ratio increased as the diagnostic year increased. However, in the age groups over 70 years, the sex ratio was similar during each diagnostic period. In our cohort, we found that the incidence of ICC in males and females was higher in the elder birth cohort, which resulted in the aforementioned phenomenon.

Surgery is the only curative treatment for ICC[4]. A previous study found the incidence of sarcopenia among ICC patients following receipt of liver resection to be 50%. Preoperative nutritional evaluation is important for ICC[41]. In addition, nutritional factors may affect the disease incidence. Indeed, a population-based cohort study found that regular use of oil supplement lowered the risk of total liver cancer to 44% (95%CI: 25%-59%) and risk of ICC to 40% (95%CI: 7%-61%)[42]. A meta-analysis suggested that vegetable and fruit consumption may reduce the risk of ICC; specifically, the reported ORs of mixed vegetables, mixed fruits, and combined fruits and vegetables were 0.61 (95%CI: 0.50-0.75), 0.79 (95%CI: 0.65-0.96), and 0.68 (95%CI: 0.57-0.80), respectively[43]. A multi-center study determined that high dietary fiber intake could be associated with a lower risk of intrahepatic bile duct cancer[44]. Finally, an animal model-based study in Taiwan provided further evidence of a relationship between ICC and nutrition; vitamin D supplementation lead to significant suppression of ICC initiation and progression in the rat model *via* regulation of gene expression (*e.g.*, of lipocalin 2)[45].

This study has several limitations. As seen in other studies based on cancer registry databases[46], a substantial portion of cases did not receive histological verification. Old age and advanced tumor stage at presentation likely prohibited this verification. Nevertheless, integrity of the TCR database was 98.4% [47]. The morphological verification percentage of the TCR database is 93.0%[47], which minimizes the misdiagnosis of cancer incidence. In addition, quality and accuracy of clinical diagnosis in our databases has been validated, and the percentage of death certificate-only data is 1%[47]. In addition, we extracted the ICD-O-FT: T-155.1 before 2002 and ICD-O-3: C22.1 from 2003–2017, which may misclassify some diagnostic cases. However, due to the rarity of ICC cases in Taiwan, this deviation did not affect the statistical trend outcomes in our study. In addition, restriction to cases with histological proof would have introduced selection bias. Ultimately, although our study found that there are various risks associated with ICC, including sex, age, DM, HBV and/or HCV infection, obesity, nutritional factors, and smoking, the results need to be validated in larger cohorts, exploring the risk of ICC development in a high incidence area in particular.

CONCLUSION

The incidence of ICC continues to rise in Asia. The etiological role of ICC is still unclear. However, future prospective evaluations are warranted to explore potential risk factors of ICC. The assessment of their independent and/or interactive effects are suggested, which could lead to prevention of ICC, development of early detection methods for early curative surgery, and identification of potential prognostic factors related to improved therapy and outcomes.

ARTICLE HIGHLIGHTS

Research background

Intrahepatic cholangiocarcinoma (ICC) is one of the most aggressive malignancies. However, because of its scarcity few population-based studies have explored its epidemiology. In Taiwan, we have a national cancer registry database, which can be used to evaluate the epidemiology of ICC.

Research motivation

To discover the secular incidence trends and associated risk factors of ICC in Taiwan.

Research objectives

To observe secular trends in ICC incidence according to age, sex, and risk factors in Taiwan.

Research methods

In this population-based study, we used the national Taiwan Cancer Registry database. Relative percent change in incidence rates were used to describe secular trends in incidence rates and sex ratios of ICC in Taiwan.

Research results

The age-standardized ICC incidence rate among males increased from 1.51 per 100000 in 1993-1997 to 4.07 per 100000 in 2013-2017 and among females from 1.73 per 100000 to 2.95 per 100000. ICC incidence rates in females tended to plateau after 2008-2012. For males, the incidence of ICC increased as age increased. In the long-term incidence trend of ICC in females, the incidence of the four age groups of 40-44, 45-49, 50-54, and 55-59 years remained stable in different years, the incidence of the 60-64 age group had a peak in 2003-2007, and the peak incidence in the 65-69 and 70-74 age groups occurred in 2008-2012.

Research conclusions

An increased incidence of ICC has occurred in Taiwan over the past two decades. The increased sex ratios has progressively shifted toward younger people.

Research perspectives

Further long-term cohort studies are needed to investigate the relationship between ICC and its risk factors.

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FOOTNOTES

Author contributions: Lin CR, Lee YK, Chang HC and You SL conceived of and designed the study; Chiang CJ and You SL acquired the data; Lin CR, Lee YK and Yang YW performed the data analyses; Lin CR and You SL drafted the manuscript; Lin RC, Lee YK and You SL assisted in interpretation of the data; Lin RC, Lee YK, Chiang CJ, Yang YW and You SL critically revised the manuscript for important intellectual content.

Institutional review board statement: The study was reviewed and approved by Fu Jen Catholic University Institutional Review Board (No. C107099).

Informed consent statement: The data used in this study is from Taiwan Cancer Registry, a governmental database established by the Health Promotion Administration, Ministry of Health and Welfare, Taiwan. This study was approved by Taiwan Ministry of Health and Welfare, so the informed consent statement was not needed. The related authorization documents would be supplied in the supplement.

Conflict-of-interest statement: All authors report no relevant conflicts of interest for this article.

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REFERENCES

1 **Bertuccio P**, Malvezzi M, Carioli G, Hashim D, Boffetta P, El-Serag HB, La Vecchia C, Negri E. Global trends in mortality from intrahepatic and extrahepatic cholangiocarcinoma. *J Hepatol* 2019; **71**: 104-114 [PMID: 30910538 DOI: 10.1016/j.jhep.2019.03.013]
2 **Rizvi S**, Gores GJ. Pathogenesis, diagnosis, and management of cholangiocarcinoma. *Gastroenterology* 2013; **145**: 1215-1229 [PMID: 24140396 DOI: 10.1053/j.gastro.2013.10.013]
3 **Saha SK**, Zhu AX, Fuchs CS, Brooks GA. Forty-Year Trends in Cholangiocarcinoma Incidence in the U.S.: Intrahepatic Disease on the Rise. *Oncologist* 2016; **21**: 594-599 [PMID: 27000463 DOI: 10.1634/theoncologist.2015-0446]
4 **Banales JM**, Cardinale V, Carpino G, Marzioni M, Andersen JB, Invernizzi P, Lind GE, Folseraas T, Forbes SJ, Fouassier L, Geier A, Calvisi DF, Mertens JC, Trauner M, Benedetti A, Maroni L, Vaquero J, Macias RI, Raggi C, Perugorria MJ, Gaudio E, Boberg KM, Marin JJ, Alvaro D. Expert consensus document: Cholangiocarcinoma: current knowledge and future perspectives consensus statement from the European Network for the Study of Cholangiocarcinoma (ENS-CCA).

Nat Rev Gastroenterol Hepatol 2016; **13**: 261-280 [PMID: 27095655 DOI: 10.1038/nrgastro.2016.51]

5 **Florio AA**, Ferlay J, Znaor A, Ruggieri D, Alvarez CS, Laversanne M, Bray F, McGlynn KA, Petrick JL. Global trends in intrahepatic and extrahepatic cholangiocarcinoma incidence from 1993 to 2012. *Cancer* 2020; **126**: 2666-2678 [PMID: 32129902 DOI: 10.1002/cncr.32803]

6 **Taiwan Cancer Registry**, Health Promotion Administration, Ministry of Health and Welfare, Taiwan. Available: http://tcr.cph.ntu.edu.tw/main.php?Page=A1. Accessed 5 April 2013 [DOI: 10.1038/s41598-021-87770-2]

7 **Chiang CJ**, You SL, Chen CJ, Yang YW, Lo WC, Lai MS. Quality assessment and improvement of nationwide cancer registration system in Taiwan: a review. *Jpn J Clin Oncol* 2015; **45**: 291-296 [PMID: 25601947 DOI: 10.1093/jjco/hyu211]

8 **Cheng TM**. Taiwan's new national health insurance program: genesis and experience so far. *Health Aff (Millwood)* 2003; **22**: 61-76 [PMID: 12757273 DOI: 10.1377/hlthaff.22.3.61]

9 **Department of Household Registration**, MOI. Table-3. Population by Age, Sex and Marital Status and Marriage Type. 2021 [DOI: 10.4324/9781315829821-23]

10 **Ahmad OB**, Boschi-Pinto C, Lopez AD, Murray CJ, Inoue M. Age Standardization of Rates: A New WHO Standard. GPE Discussion Paper Series: No.31. EIP/GPE/EBD. World Health Organization 2001 [DOI: 10.1037/e571982006-001]

11 **Dodson RM**, Weiss MJ, Cosgrove D, Herman JM, Kamel I, Anders R, Geschwind JF, Pawlik TM. Intrahepatic cholangiocarcinoma: management options and emerging therapies. *J Am Coll Surg* 2013; **217**: 736-750.e4 [PMID: 23890842 DOI: 10.1016/j.jamcollsurg.2013.05.021]

12 **Khan SA**, Tavolari S, Brandi G. Cholangiocarcinoma: Epidemiology and risk factors. *Liver Int* 2019; **39** Suppl 1: 19-31 [PMID: 30851228 DOI: 10.1111/liv.14095]

13 **Blechacz B**. Cholangiocarcinoma: Current Knowledge and New Developments. *Gut Liver* 2017; **11**: 13-26 [PMID: 27928095 DOI: 10.5009/gnl15568]

14 **Al Mahjoub A**, Bouvier V, Menahem B, Bazille C, Fohlen A, Alves A, Mulliri A, Launoy G, Lubrano J. Epidemiology of intrahepatic, perihilar, and distal cholangiocarcinoma in the French population. *Eur J Gastroenterol Hepatol* 2019; **31**: 678-684 [PMID: 30633038 DOI: 10.1097/MEG.0000000000001337]

15 **Welzel TM**, Mellemkjaer L, Gloria G, Sakoda LC, Hsing AW, El Ghormli L, Olsen JH, McGlynn KA. Risk factors for intrahepatic cholangiocarcinoma in a low-risk population: a nationwide case-control study. *Int J Cancer* 2007; **120**: 638-641 [PMID: 17109384 DOI: 10.1002/ijc.22283]

16 **Jing W**, Jin G, Zhou X, Zhou Y, Zhang Y, Shao C, Liu R, Hu X. Diabetes mellitus and increased risk of cholangiocarcinoma: a meta-analysis. *Eur J Cancer Prev* 2012; **21**: 24-31 [PMID: 21857525 DOI: 10.1097/CEJ.0b013e3283481d89]

17 **Tseng CH**, Tseng CP, Chong CK, Huang TP, Song YM, Chou CW, Lai SM, Tai TY, Cheng JC. Increasing incidence of diagnosed type 2 diabetes in Taiwan: analysis of data from a national cohort. *Diabetologia* 2006; **49**: 1755-1760 [PMID: 16788802 DOI: 10.1007/s00125-006-0314-4]

18 **Chang CH**, Shau WY, Jiang YD, Li HY, Chang TJ, Sheu WH, Kwok CF, Ho LT, Chuang LM. Type 2 diabetes prevalence and incidence among adults in Taiwan during 1999-2004: a national health insurance data set study. *Diabet Med* 2010; **27**: 636-643 [PMID: 20546280 DOI: 10.1111/j.1464-5491.2010.03007.x]

19 **Lin CC**, Li CI, Hsiao CY, Liu CS, Yang SY, Lee CC, Li TC. Time trend analysis of the prevalence and incidence of diagnosed type 2 diabetes among adults in Taiwan from 2000 to 2007: a population-based study. *BMC Public Health* 2013; **13**: 318 [PMID: 23570503 DOI: 10.1186/1471-2458-13-318]

20 **Petrick JL**, Yang B, Altekruse SF, Van Dyke AL, Koshiol J, Graubard BI, McGlynn KA. Risk factors for intrahepatic and extrahepatic cholangiocarcinoma in the United States: A population-based study in SEER-Medicare. *PLoS One* 2017; **12**: e0186643 [PMID: 29049401 DOI: 10.1371/journal.pone.0186643]

21 **Petrick JL**, Campbell PT, Koshiol J, Thistle JE, Andreotti G, Beane-Freeman LE, Buring JE, Chan AT, Chong DQ, Doody MM, Gapstur SM, Gaziano JM, Giovannucci E, Graubard BI, Lee IM, Liao LM, Linet MS, Palmer JR, Poynter JN, Purdue MP, Robien K, Rosenberg L, Schairer C, Sesso HD, Sinha R, Stampfer MJ, Stefanick M, Wactawski-Wende J, Zhang X, Zeleniuch-Jacquotte A, Freedman ND, McGlynn KA. Tobacco, alcohol use and risk of hepatocellular carcinoma and intrahepatic cholangiocarcinoma: The Liver Cancer Pooling Project. *Br J Cancer* 2018; **118**: 1005-1012 [PMID: 29520041 DOI: 10.1038/s41416-018-0007-z]

22 **Wen CP**, Tsai SP, Chen CJ, Cheng TY, Tsai MC, Levy DT. Smoking attributable mortality for Taiwan and its projection to 2020 under different smoking scenarios. *Tob Control* 2005; **14** Suppl 1: i76-i80 [PMID: 15923454 DOI: 10.1136/tc.2004.007955]

23 **Wen CP**, Levy DT, Cheng TY, Hsu CC, Tsai SP. Smoking behaviour in Taiwan, 2001. *Tob Control* 2005; **14** Suppl 1: i51-i55 [PMID: 15923450 DOI: 10.1136/tc.2004.008011]

24 **Wu IH**, Essien EJ, Sansgiry SS, Peters RJ, Yang M, Abughosh SM. Cigarette Smoking among Taiwanese Adults. *Epidemiol* 2011; **1**: 107 [DOI: 10.4172/2161-1165.1000107]

25 **Palmer WC**, Patel T. Are common factors involved in the pathogenesis of primary liver cancers? *J Hepatol* 2012; **57**: 69-76 [PMID: 22420979 DOI: 10.1016/j.jhep.2012.02.022]

26 **Pan WH**, Lee MS, Chuang SY, Lin YC, Fu ML. Obesity pandemic, correlated factors and guidelines to define, screen and manage obesity in Taiwan. *Obes Rev* 2008; **9** Suppl 1: 22-31 [PMID: 18307695 DOI: 10.1111/j.1467-789X.2007.00434.x]

27 **Mosadeghi S**, Liu B, Bhuket T, Wong RJ. Sex-specific and race/ethnicity-specific disparities in cholangiocarcinoma incidence and prevalence in the USA: An updated analysis of the 2000-2011 Surveillance, Epidemiology and End Results registry. *Hepatol Res* 2016; **46**: 669-677 [PMID: 26508039 DOI: 10.1111/hepr.12605]

28 **Antwi SO**, Patel T. Increasing mortality of intrahepatic cholangiocarcinoma in the US: are gender-specific risk factors important? *Hepatobiliary Surg Nutr* 2019; **8**: 635-636 [PMID: 31929993 DOI: 10.21037/hbsn.2019.10.10]

29 **Singsuksawat E**, Thuwajit C, Charngkaew K, Thuwajit P. Increased ETV4 expression correlates with estrogen-enhanced proliferation and invasiveness of cholangiocarcinoma cells. *Cancer Cell Int* 2018; **18**: 25 [PMID: 29467595 DOI: 10.1186/s12935-018-0525-z]

30 **Petrick JL**, Florio AA, Zhang X, Zeleniuch-Jacquotte A, Wactawski-Wende J, Van Den Eeden SK, Stanczyk FZ, Simon

TG, Sinha R, Sesso HD, Schairer C, Rosenberg L, Rohan TE, Purdue MP, Palmer JR, Linet MS, Liao LM, Lee IM, Koshiol J, Kitahara CM, Kirsh VA, Hofmann JN, Guillemette C, Graubard BI, Giovannucci E, Gaziano JM, Gapster SM, Freedman ND, Engel LS, Chong DQ, Chen Y, Chan AT, Caron P, Buring JE, Bradwin G, Beane Freeman LE, Campbell PT, McGlynn KA. Associations Between Prediagnostic Concentrations of Circulating Sex Steroid Hormones and Liver Cancer Among Postmenopausal Women. *Hepatology* 2020; **72**: 535-547 [PMID: 31808181 DOI: 10.1002/hep.31057]

31 **Shen TY**, Chen HJ, Pan WH, Yu T. Secular trends and associated factors of age at natural menopause in Taiwanese women. *Menopause* 2019; **26**: 499-505 [PMID: 30672884 DOI: 10.1097/GME.0000000000001307]

32 **Matsumoto K**, Onoyama T, Kawata S, Takeda Y, Harada K, Ikebuchi Y, Ueki M, Miura N, Yashima K, Koda M, Sakamoto T, Endo M, Horie Y, Murawaki Y. Hepatitis B and C virus infection is a risk factor for the development of cholangiocarcinoma. *Intern Med* 2014; **53**: 651-654 [PMID: 24694471 DOI: 10.2169/internalmedicine.53.1410]

33 **Zhou Y**, Zhao Y, Li B, Huang J, Wu L, Xu D, Yang J, He J. Hepatitis viruses infection and risk of intrahepatic cholangiocarcinoma: evidence from a meta-analysis. *BMC Cancer* 2012; **12**: 289 [PMID: 22799744 DOI: 10.1186/1471-2407-12-289]

34 **Beasley RP**. Rocks along the road to the control of HBV and HCC. *Ann Epidemiol* 2009; **19**: 231-234 [PMID: 19344859 DOI: 10.1016/j.annepidem.2009.01.017]

35 **Li M**, Du M, Cong H, Gu Y, Fang Y, Li J, Gan Y, Tu H, Gu J, Xia Q. Characterization of hepatitis B virus DNA integration patterns in intrahepatic cholangiocarcinoma. *Hepatol Res* 2021; **51**: 102-115 [PMID: 33037855 DOI: 10.1111/hepr.13580]

36 **Lu SN**, Su WW, Yang SS, Chang TT, Cheng KS, Wu JC, Lin HH, Wu SS, Lee CM, Changchien CS, Chen CJ, Sheu JC, Chen DS, Chen CH. Secular trends and geographic variations of hepatitis B virus and hepatitis C virus-associated hepatocellular carcinoma in Taiwan. *Int J Cancer* 2006; **119**: 1946-1952 [PMID: 16708389 DOI: 10.1002/ijc.22045]

37 **Navas MC**, Glaser S, Dhruv H, Celinski S, Alpini G, Meng F. Hepatitis C Virus Infection and Cholangiocarcinoma: An Insight into Epidemiologic Evidences and Hypothetical Mechanisms of Oncogenesis. *Am J Pathol* 2019; **189**: 1122-1132 [PMID: 30953604 DOI: 10.1016/j.ajpath.2019.01.018]

38 **Tsao YC**, Chen JY, Yeh WC, Peng YS, Li WC. Association between visceral obesity and hepatitis C infection stratified by gender: a cross-sectional study in Taiwan. *BMJ Open* 2017; **7**: e017117 [PMID: 29133317 DOI: 10.1136/bmjopen-2017-017117]

39 **Alvaro D**, Crocetti E, Ferretti S, Bragazzi MC, Capocaccia R; AISF Cholangiocarcinoma committee. Descriptive epidemiology of cholangiocarcinoma in Italy. *Dig Liver Dis* 2010; **42**: 490-495 [PMID: 20022823 DOI: 10.1016/j.dld.2009.10.009]

40 **Witjes CD**, Karim-Kos HE, Visser O, de Vries E, IJzermans JN, de Man RA, Coebergh JW, Verhoef C. Intrahepatic cholangiocarcinoma in a low endemic area: rising incidence and improved survival. *HPB (Oxford)* 2012; **14**: 777-781 [PMID: 23043667 DOI: 10.1111/j.1477-2574.2012.00536.x]

41 **Ardito F**, Coppola A, Rinninella E, Razionale F, Pulcini G, Carano D, Cintoni M, Mele MC, Barbaro B, Giuliante F. Preoperative Assessment of Skeletal Muscle Mass and Muscle Quality Using Computed Tomography: Incidence of Sarcopenia in Patients with Intrahepatic Cholangiocarcinoma Selected for Liver Resection. *J Clin Med* 2022; **11** [PMID: 35329856 DOI: 10.3390/jcm11061530]

42 **Jiang W**, Li FR, Yang HH, Chen GC, Hua YF. Relationship Between Fish Oil Use and Incidence of Primary Liver Cancer: Findings From a Population-Based Prospective Cohort Study. *Front Nutr* 2021; **8**: 771984 [PMID: 35036409 DOI: 10.3389/fnut.2021.771984]

43 **Songserm N**, Woradet S, Charoenbut P. Fruit and Vegetables Consumption: A Pointer for Cholangiocarcinoma Prevention in Northeast Thailand, the Highest Incidence Area in the World. *Nutr Cancer* 2016; **68**: 1289-1294 [PMID: 27682423 DOI: 10.1080/01635581.2016.1225101]

44 **Fedirko V**, Lukanova A, Bamia C, Trichopolou A, Trepo E, Nöthlings U, Schlesinger S, Aleksandrova K, Boffetta P, Tjønneland A, Johnsen NF, Overvad K, Fagherazzi G, Racine A, Boutron-Ruault MC, Grote V, Kaaks R, Boeing H, Naska A, Adarakis G, Valanou E, Palli D, Sieri S, Tumino R, Vineis P, Panico S, Bueno-de-Mesquita HBA, Siersema PD, Peeters PH, Weiderpass E, Skeie G, Engeset D, Quirós JR, Zamora-Ros R, Sánchez MJ, Amiano P, Huerta JM, Barricarte A, Johansen D, Lindkvist B, Sund M, Werner M, Crowe F, Khaw KT, Ferrari P, Romieu I, Chuang SC, Riboli E, Jenab M. Glycemic index, glycemic load, dietary carbohydrate, and dietary fiber intake and risk of liver and biliary tract cancers in Western Europeans. *Ann Oncol* 2013; **24**: 543-553 [PMID: 23123507 DOI: 10.1093/annonc/mds434]

45 **Chiang KC**, Yeh CN, Lin KJ, Su LJ, Yen TC, Pang JH, Kittaka A, Sun CC, Chen MF, Jan YY, Chen TC, Juang HH, Yeh TS. Chemopreventive and chemotherapeutic effect of dietary supplementation of vitamin D on cholangiocarcinoma in a Chemical-Induced animal model. *Oncotarget* 2014; **5**: 3849-3861 [PMID: 24939880 DOI: 10.18632/oncotarget.2000]

46 **Kreng VB**, Yang CT. The equality of resource allocation in health care under the National Health Insurance System in Taiwan. *Health Policy* 2011; **100**: 203-210 [PMID: 21112116 DOI: 10.1016/j.healthpol.2010.08.003]

47 **Chiang CJ**, Wang YW, Lee WC. Taiwan's Nationwide Cancer Registry System of 40 years: Past, present, and future. *J Formos Med Assoc* 2019; **118**: 856-858 [PMID: 30773272 DOI: 10.1016/j.jfma.2019.01.012]

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Family-based *Helicobacter pylori* infection status and transmission pattern in central China, and its clinical implications for related disease prevention

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Abstract

BACKGROUND

Helicobacter pylori (*H. pylori*) has characteristics of family cluster infection; however, its family-based infection status, related factors, and transmission pattern in central China, a high-risk area for *H. pylori* infection and gastric cancer, have not been evaluated. We investigated family-based *H. pylori* infection in healthy households to understand its infection status, related factors, and patterns of transmission for related disease prevention.

AIM

To investigate family-based *H. pylori* infection status, related factors, and patterns of transmission in healthy households for related disease prevention.

METHODS

Blood samples and survey questionnaires were collected from 282 families

including 772 individuals. The recruited families were from 10 selected communities in the greater Zhengzhou area with different living standards, and the family members' general data, *H. pylori* infection status, related factors, and transmission pattern were analyzed. *H. pylori* infection was confirmed primarily by serum *H. pylori* antibody arrays; if patients previously underwent *H. pylori* eradication therapy, an additional ^{13}C-urea breath test was performed to obtain their current infection status. Serum gastrin and pepsinogens (PGs) were also analyzed.

RESULTS

Among the 772 individuals examined, *H. pylori* infection rate was 54.27%. These infected individuals were from 246 families, accounting for 87.23% of all 282 families examined, and 34.55% of these families were infected by the same strains. In 27.24% of infected families, all members were infected, and 68.66% of them were infected with type I strains. Among the 244 families that included both husband and wife, spouse co-infection rate was 34.84%, and in only 17.21% of these spouses, none were infected. The infection rate increased with duration of marriage, but annual household income, history of smoking, history of alcohol consumption, dining location, presence of gastrointestinal symptoms, and family history of gastric disease or GC did not affect infection rates; however, individuals who had a higher education level showed lower infection rates. The levels of gastrin-17, PGI, and PGII were significantly higher, and PGI/II ratio was significantly lower in *H. pylori*-infected groups than in *H. pylori*-negative groups.

CONCLUSION

In our study sample from the general public of central China, *H. pylori* infection rate was 54.27%, but in 87.23% of healthy households, there was at least 1 *H. pylori*-infected person; in 27.24% of these infected families, all members were infected. Type I *H. pylori* was the dominant strain in this area. Individuals with a higher education level showed significantly lower infection rates; no other variables affected infection rates.

Key Words: *Helicobacter pylori*; Atrophic gastritis; Family clustering infection; Gastric cancer; Gastrin; Pepsinogen

Core Tip: *Helicobacter pylori* (*H. pylori*) has characteristics of family cluster infection. However, few studies have investigated family-based infection status and pattern of intrafamilial transmission in the general public of central China. In our study, *H. pylori* infection rate was 54.27%, but in 87.23% of healthy households, there was at least 1 *H. pylori*-infected person; in 27.24% of these infected families, all members were infected. Type I *H. pylori* was the dominant strain in this area. Intrafamilial infection status and patterns of transmission represent one important source of *H. pylori* spread, indicating the urgent need for family-based infection control and related disease prevention.

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INTRODUCTION

Chronic *Helicobacter pylori* (*H. pylori*) infection is the major cause of chronic gastritis, peptic ulcers, and gastric cancer (GC), and is also closely associated with a number of extra-gastrointestinal (GI) diseases [1-3]. *H. pylori* infection rate in China is about 50%, but the infection rate varies widely in different regions due to economic development, age, lifestyle habit, and sanitary conditions[4-6]. *H. pylori* has characteristics of family cluster infection[7]. Most *H. pylori* infections are acquired during childhood and adolescence, and infection will persist for decades unless proper treatment is offered.

Mounting evidence has demonstrated that transmission of *H. pylori* is mainly by oral-oral and fecal-oral routes, and water sources[8,9], and intra-familial spread is the major source of *H. pylori* transmission[10-12]. The infected parent, especially the mother, is thought to play an important role in its transmission[9,11]. When parents are infected with *H. pylori*, the infection rates of their children

markedly increase; spread has also been demonstrated between spouses and among siblings[13-17]. Therefore, diagnosis and treatment of the whole family have important clinical implications for preventing related diseases[18]. Recently, the notion of "family-based *H. pylori* infection control and management" has been introduced to China as a practical strategy to curb *H. pylori* intra-familial transmission[3]. However, relatively few studies have been performed to investigate the family-based infection status, related factors, and pattern of intrafamilial transmission in the general population.

Type I [cytotoxin-associated protein-positive (CagA+), vacuolating cytotoxin-positive (VacA+)] *H. pylori* infection causes severe gastric inflammation and is prone to induce carcinogenesis[19,20]. Our previous study of 3572 patients admitted to the hospital showed that the *H. pylori* infection rate was 75.9% in this area of central China. The infection rate was further confirmed by investigation of 523 endoscopy-confirmed patients (76.9%), of whom 72.4% (291/402) had type I *H. pylori* infection and 27.5% had type II *H. pylori* infection. Importantly, 88.4% of GC patients were *H. pylori*-positive, of whom 84.2% had type I infection; only 11.6% of GC patients were *H. pylori*-negative[21]. At present, the genotype of family based-*H. pylori* infection in the healthy household is unclear, as well as its relationship with GC epidemiological markers such as gastrin-17 (G-17), pepsinogen (PG) level, and PG I/II ratio (PGR).

Henan province in central China is one of the high-risk areas for *H. pylori* and GC, with an *H. pylori* infection rate of 49.6%[22] and GC incidence of 42.52/100000[23]. The capital city, Zhengzhou, has a population of 12 million, but there has been no large-scale family-based *H. pylori* intrafamilial transmission survey, and the factors that affect *H. pylori* spread and cause disease are also unclear.

Therefore, we investigated family-based *H. pylori* infection status, factors related to bacteria spread, bacteria genotype, and patterns of transmission for the residents in this area, and analyzed their impact on GC epidemiological markers including G-17, PGI, PGII, and PGR. The results of this study will provide information on *H. pylori* infection status in the household and help to refine eradication strategies for the prevention of related diseases.

MATERIALS AND METHODS

Study population and data collection

From September 2020 to April 2021, blood samples and questionnaires were collected from family members of 10 selected communities in greater Zhengzhou area; each community enrolled 20-30 families, with all members participating. The 10 communities were selected based on high, middle, and low living standards to prevent biased selection of the population; these included two high-income communities, six middle-class communities, and two communities originating from rural areas. Specifically, the study included two communities located in Guancheng District, namely Lufu Pavilion and Houjiadong Street communities; three communities located in Jinshui District, namely Chengbei Road, Jiagang, and Huilong Digital City communities; four communities in New East Zhengzhou District, namely Hanhai Qingyu, Zhengzhou Academy of Aviation Administration, Henan University of Finance, Economics and Law, and Nanxi Fudi communities; and one community in Central Zhengzhou District, namely Kowloon City community.

A total of 282 families (family size ≥ 2 persons) including 772 individuals participated in the survey. Inclusion criteria were: Family members being long-term residents living in Zhengzhou area, with no age limit; all family members being willing to participate by providing blood samples and filling out the questionnaire; at least 2 people composing the family unit, but with no limitation on how many people are living in the same household; and all family members being willing to provide written informed consent. An infected family was defined as a household with various family members infected with *H. pylori*, ranging from only 1 person to all family members being infected, and a family could be composed of only a couple, with or without children. Exclusion criteria were: Pregnant and breast-feeding females; people with mental illness; or people who refused to fill out the questionnaire or sign the consent form.

This study was approved by the Ethics Committee of People's Hospital of Zhengzhou University (No. 53, 2021). All subjects provided written informed consent; for minor subjects, written informed consent was given by their legal guardian. This study was registered in the China Clinical Trial Registry (www.chictr.org.cn; No. ChiCTR2100052950), and the protocol is freely available from the website after registration.

Subject enrollment and questionnaire

Before and during enrollment, an introduction brochure or information booklet for the study was distributed to the community center staff, who were responsible for distributing and helping recruit community family members for onsite registration. A registration website was also open to community members for whole family-based registration; registration for only a single individual was declined. A questionnaire was filled out either online or onsite by each of the participating family members. Blood samples were collected from each participating member for *H. pylori*, gastrin, and PG analyses; if necessary, ^{13}C-urea breath test (UBT) was subsequently performed by appointment.

Based on the purpose of this study, questionnaire included the following 17 items: Age; sex; family ethic; number of family members; professions; marriage status; socioeconomic data; dining history; living habits; lifestyle; disease history; medication history; presence of GI symptoms; *H. pylori* eradication history; history of gastroscopy; infection history of other family members; and treatment history (Table 1).

H. pylori infection status, gastrin, and PG analyses

Three milliliters of fasting venous blood were collected from all subjects in the morning. Blood samples were centrifuged at 1000 × *g* for 10 min, (80-2 centrifuge; Jiangsu Zhongda Instrument Technology Co., Ltd., Jiangsu, China), and samples were either analyzed on ice on the same day or stored at -80 °C for subsequent analyses. Serum anti-*H. pylori* antibodies [detecting CagA, VacA, UreA, UreB *via H. pylori* enzyme-linked immunosorbent assay (ELISA) kit (Blot Biotech Co., Ltd., Shenzhen, Guangdong, China)] and G-17, PGI, PGII levels, as well as PGR (*via* PGI, PGII, G-17 ELISA kits; Biohit Biotechnology, Helsinki, Finland) were measured by an ELISA kit following manufacturer's instructions as previously reported[22]. If patients had previously undergone *H. pylori* eradication therapy, an additional ^{13}C-UBT was performed to obtain their current infection status (^{13}C-UBT Diagnostic Kit; Beijing Boran Pharmaceutical Co., Ltd., Beijing, China).

Statistical analyses

Data were analyzed using SPSS for Windows version 25 (IBM Corp, Armonk, NY, United States). Continuous variables are expressed as mean ± SD, whereas categorical variables are described as percentages or frequencies. The measurement data were compared by *t*-test, and the enumeration data were compared by χ^2 test or Fisher's exact test. $P < 0.05$ was considered statistically significant.

RESULTS

Demographic information of the enrolled families

As shown in Table 1, a total of 282 families including 772 members participated in this study. Among them, 419 had *H. pylori* infection, giving an overall infection rate of 54.27% (419/772); among the infected individuals, 328 (42.49%, 328/772) were infected with type I strains and 91 (11.79%, 91/772) were infected with type II strains. Type I strains accounted for 78.28% (328/419) of cases, and type II strains accounted for 21.7% (91/419) of infected individuals (Figure 1A).

In total, 330 (42.75%) of the study participants were male, with an average age of 44.56 ± 20.19 years, and 442 (57.25%) were female, with an average age of 45.95 ± 18.74 years ($P > 0.05$). The age range of the enrolled individuals was 3 years to 90 years, with the youngest and oldest infected individuals aged 5 years and 87 years, respectively.

As shown in Figure 2, stratified age and *H. pylori* genotype infection were further analyzed, type I strains was the dominant strains for all age groups. The infection rates of individuals under the age of 18 were 23.26% (20/86), and the age groups of 51-60 and 61-70 years had the highest infection rates of 63.01% (92/146) and 65.95% (93/141), respectively. Compared with age groups under 18-years-old, the infection rate was significantly higher in groups above 18-years-old ($P < 0.05$), but there was no difference in infection rates among groups above 18-years-old ($P > 0.05$).

Among questionnaire variables (Table 1), annual household income, history of smoking, history of alcohol consumption, dining location, presence of GI symptoms, and family history of gastric disease and GC did not affect infection rates ($P > 0.05$), but individuals with a higher education level showed significantly lower infection rates ($P < 0.05$).

H. pylori infection status of the enrolled families

The average family size of the study cohort was 2.74 persons *per* households, and family size ranged from as few as 2 persons *per* family to as many as 6 *per* family (Figure 1B). In this survey, 2- and 3-person households accounted for 80.85% (228/282) of the families enrolled.

As shown in Figure 1C-F, *H. pylori*-infected individuals were distributed in 246 of the 282 families with varying numbers of members infected, ranging from only 1 person to all family members infected. The family infection rate was 87.23% with at least 1 person infected in a family unit (246/282), in 12.77% of the 282 households, no family members were infected (36/282) (Figure 1C). In 67 of the 246 infected families, all members were infected (27.24%, 67/246), among these 67 all member-infected households, 46 households were infected with the same type I strains (68.66%, 46/67), 1 household was infected with type II strains (1.49%, 1/67), and 20 households had mixed type I and II strain infection (29.85%, 20/67) (Figure 1D). The data of the stratified family member infection rate of 282 households were shown in Figure 1E. In 53.66% (132/246) of families with at least 2 members infected, 59.09% (78/132) of these families were infected with type I strains, 5.30% (7/132) were infected with type II strains, and 35.61% (47/132) were infected with mixed type I and II strains (Figure 1F).

Table 1 Demography information and *Helicobacter pylori* infection status of 772 subjects

Variables items, *n*	*H. pylori* positive			*H. pylori* negative, *n*	Infection rate (%)	*P* value
	Sub total, *n*	Type I, *n*	Type II, *n*			
Total (772)	419	328	91	353	54.27	
Gender						
Male (330)	173	130	43	157	52.42	
Female (442)	246	198	48	196	55.66	0.373
Age (yr) (mean ± SD)[1]						
Total (45.36 ± 19.38)	49.38 ± 16.92	49.59 ± 17.04	48.65 ± 16.47	40.58 ± 20.97		0.000[a]
Male (44.56 ± 20.19)	49.62 ± 18.06	49.21 ± 18.53	50.86 ± 16.50	38.98 ± 20.93		
Female (45.95 ± 18.74)	49.22 ± 16.06	49.83 ± 15.97	46.67 ± 16.20	41.86 ± 20.92		0.240[b]
Family annual income[2] **(10000 RMB)**						
< 10 (287)	171	142	29	116	59.58	
10-20 (209)	119	86	33	90	56.94	0.555[c]
20-30 (84)	47	36	11	37	55.95	0.552[c]
> 30 (59)	34	27	7	25	57.63	0.781[c]
Unknown (47)	28	20	8	19	59.57	
Cigarette smoking[2]						
Yes (158)	91	64	27	67	57.59	
No (520)	302	242	60	218	58.08	0.914
Unknown (8)	6	5	1	2	75.00	
Alcohol drinking[2]						
Yes (149)	86	64	22	63	57.72	
No (511)	297	233	64	214	58.12	0.930
Unknown (26)	16	14	2	10	61.54	
Gastrointestinal symptoms[2]						
Yes (311)	183	145	38	128	58.84	
No (339)	193	148	45	146	56.93	0.622
Unknown (36)	23	18	5	13	63.89	
Dining location[2]						
Home (472)	276	215	61	196	58.47	
Restaurant (186)	106	83	23	80	56.99	0.728
Unknown (28)	17	13	4	11	60.71	
Family history of stomach disease[3]						
Yes (167)	92	65	27	75	55.09	
No (430)	256	205	51	174	59.53	0.323
Unknown (89)	51	41	10	38	57.30	
Family history of gastric cancer[3]						
Yes (41)	23	20	3	18	56.10	
No (576)	335	260	75	241	58.16	0.796
Unknown (69)	41	31	10	28	59.42	
Education level[2]						
Senior and below (286)	180	142	38	106	62.94	

University or above (376)	199	155	44	177	52.93	0.010[a]
Unknown (24)	20	14	6	4	83.33	

[a]$P < 0.05$, when *Helicobacter pylori* (*H. pylori*)-positive groups were compared with *H. pylori*-negative groups.
[b]$P > 0.05$, when male *H. pylori*-positive groups were compared with female *H. pylori*-positive groups.
[c]$P > 0.05$, when *H. pylori* infection rate in the annual income < 100000 RMB group was compared with other income groups.
[1]Data are presented as the mean ± SD.
[2]Data only included adults, and children and adolescents age ≤ 18 yr were not included.
[3]Data only included history of gastric disease or gastric cancer in families across three consecutive generations.
H. pylori: *Helicobacter pylori*.

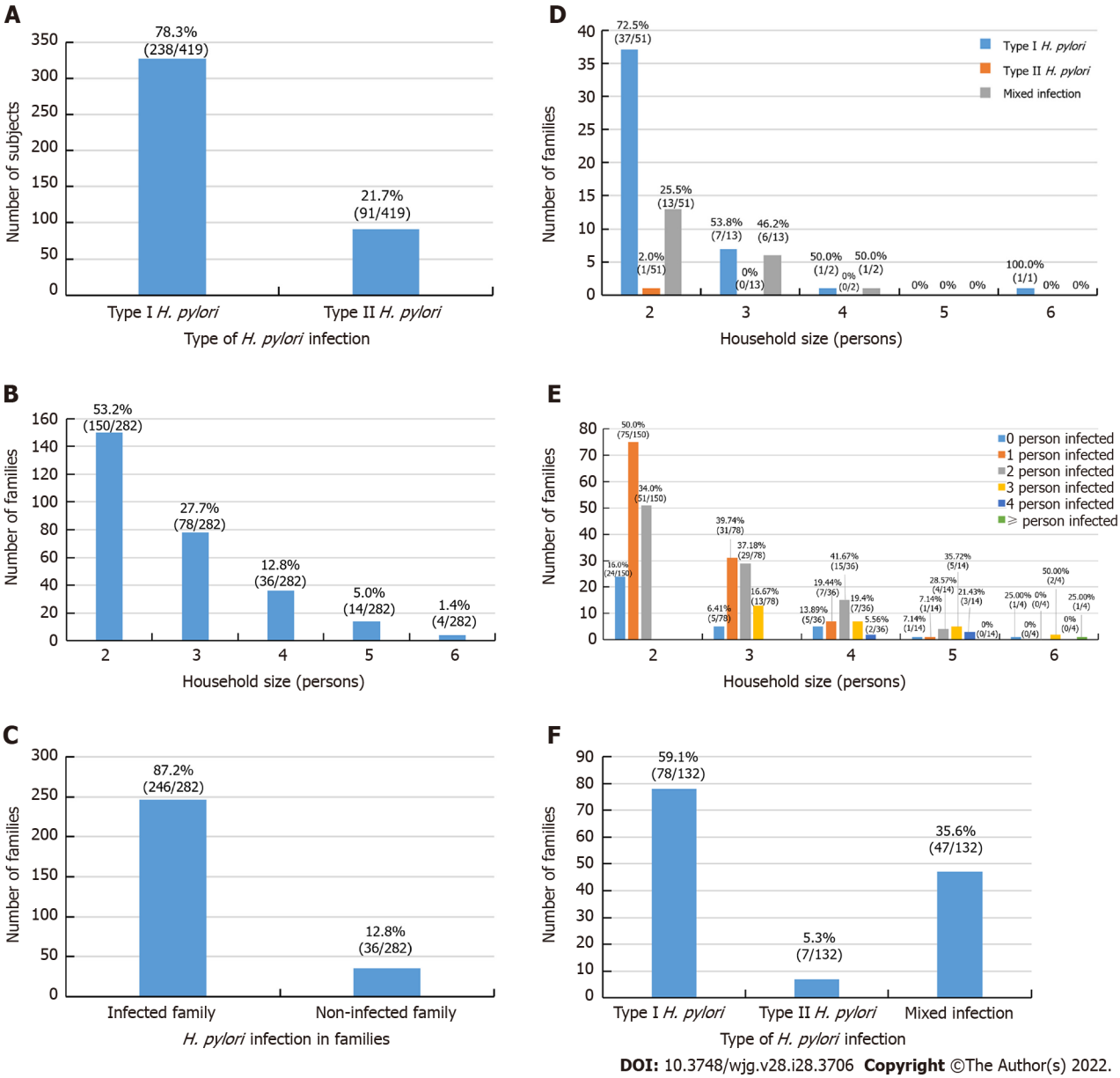

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Figure 1 *Helicobacter pylori* infection status of the 282 enrolled families. Values above each column are the case number and percentages of each group. A: Genotype pattern of the 419 infected subjects in the enrolled families; B: Distribution pattern of the 282 enrolled families ranged from 2-6 people; C: General *Helicobacter pylori* (*H. pylori*) infection status of 282 families; D: Genotype pattern of the 67 all member-infected families from the enrolled 282 families; E: Distribution pattern of the *H. pylori* infection status of the 282 enrolled families; F: Genotype pattern of the 132 *H. pylori*-infected families with ≥ 2 persons infected from the 282 enrolled families. Infected family: At least 1 person in a family was infected; Non-infected family: All members in a family were not infected; Type I *H. pylori*: *H. pylori* infection with type I strains; Type II *H. pylori*: *H. pylori* infection with type II strains; Mixed infection: *H. pylori* infection with type I and type II strains.

H. pylori infection status between couples

H. pylori infection status between couples is shown in Figure 3. In all, 244 of the 282 families had both spouses, and infection rate of both spouses was 34.84% (85/244); further, 17.21% (42/244) couples were not infected, and 47.95% (117/244) had only a single spouse infection (Figure 3A). Among 117 families with infection of only 1 spouse, 75.21% (88/117) were infected with type I strains and 24.79% (29/117) were infected with type II strains (Figure 3B). Of these spouses, the husband was infected in 49.57% (58/117) of cases and the wife was infected in 50.53% (59/117) of cases ($P > 0.05$), they were further stratified into type I and type II strains infections (Figure 3C). Furthermore, among the 85 families with both husband and wife co-infected with *H. pylori*, 68.24% (58/85) were infected with the same type of strain, of whom 63.53% (54/85) were infected with type I strains, 4.71% (4/85) were infected with type II strains, and 31.76% (27/85) had mixed type I and type II infection (Figure 3D). Significantly more couples were infected with the same type of strain than with mixed strains ($P < 0.05$). In addition, with the increase in marriage duration, infection rate of both husband and wife was significantly increased ($r = 0.98$, $P < 0.05$; Figure 3E).

Parental infection and infection status of children and adolescents

In 51 families with both parents and children younger than 18 years of age, as shown in Table 2, the infection rate of children was 23.08% (6/26) when both parents were *H. pylori*-infected; however, when both parents were not infected, the infection rate of children was 18.18% (2/11) ($P > 0.05$). When only mother was infected, the infection rate of children was 45.45% (5/11); no child was infected (0/9) when only father was infected ($P > 0.05$).

Table 3 shows infection status of the 51 families comprising both parents and children, for a total of 190 individuals. Among these family members, the infection rates were as follows: Father, 62.75% (32/51); mother, 62.75% (32/51); grandfather, 50.00% (2/4); grandmother, 66.67% (8/12); maternal grandfather, 25.00% (1/4); maternal grandmother, 37.50% (3/8); and other relatives, 66.67% (2/3).

Comparison of G-17, PGI, and PGII levels, and PGR with different types of *H. pylori* infection

To determine the impact of *H. pylori* infection on common GC epidemiological markers (*e.g.*, G-17, PGI, PGII, and PGR) in healthy households, we assayed their levels during *H. pylori* infection. As shown in Table 4, compared to *H. pylori*-negative groups, PGI levels were significantly higher and PGR was significantly lower in *H. pylori* type I infections compared with *H. pylori*-negative groups ($P < 0.05$). However, there were no differences in G-17, PGII level, and PGR between type II *H. pylori* infection and *H. pylori*-negative groups ($P > 0.05$). The levels of G-17, PGI, and PGII were significantly higher, and PGR was significantly lower in *H. pylori*-infected groups than in *H. pylori*-negative groups ($P < 0.05$).

DISCUSSION

H. pylori infection rates vary greatly among different countries and regions[24]. Although numerous studies have demonstrated that intrafamilial transmission is one of the most important sources of *H. pylori* spread[2,18,24], there are few studies on the characteristics and pattern of family-based *H. pylori* infection for disease prevention and control[9,25,26]. Therefore, focusing on family-based *H. pylori* infection control and management would be a novel approach to reduce the related diseases and GC burden in a society.

In this work, we analyzed *H. pylori* infection status in a total of 772 individuals from 282 families in the Zhengzhou area. The results showed that despite an overall infection rate of only 54.27%, in as high as 87.23% of the surveyed families (246/282), there was at least 1 person infected, and in 27.07% (67/246) of these infected families, all family members were infected; further, 34.55% (85/246) of these families were infected with the same type of strain. Therefore, this study provides new evidence showing the importance of family-based *H. pylori* infection control, which has substantive public health implications, and suggests that intrafamilial infection is a major source of *H. pylori* transmission. Thus, preventing intrafamilial spread is critical to eliminate the source of infection in order to prevent the related diseases.

Over the past several decades, the social and family structure in China has changed dramatically. The latest national statistics[27] (2020) revealed that the nation has a population of 1.41 billion and 492 million families with an average family size of 2.62 persons/family, which is much smaller than it was in 1990, when China had 1.13 billion citizens and 278.6 million families, with an average family size of 4.05 persons/family[27]. Due to the previous nationwide "one-children-per-family policy" (between 1982 and 2016), most families in China only have 1 child and two generations. As these children do not have siblings, transmission among siblings within a family unit did not appear to be the major route of transmission in the current analysis. *H. pylori* spread from parent or grandparent to children is probably more important for bacteria transmission. Although the current results provide a snapshot of *H. pylori* infection status in the general public, a nationwide large-scale investigation is needed to explore the nationwide infection status and develop policies for related disease prevention.

Table 2 Parental infection and infection status of children and adolescents in 51 families

Parental infection (Household numbers)	Children infection (57)		Infection rate (%)	*P* value
	H. pylori+ (*n*)	*H. pylori*- (*n*)		
Neither parent infected (11)	2	9	18.18	
Both parent infected (21)	6	20	23.08	0.109[a]
Only father infected (9)	0	9	0	
Only mother infected (10)	5	6	45.45	
Total (51)	13	44	22.81	

[a]*P* > 0.05 when both parents infected group were compared with neither parent infected group.
n: Person *per* group; Infection rate (%): Infection rate of children in different groups; *H. pylori*+: *H. pylori*-positive; *H. pylori*-: *H. pylori*-negative.

Table 3 *Helicobacter pylori* infection status in 51 families with children and other relatives

Family members (N)	*H. pylori* positive			*H. pylori* negative, *n*	Infection rate (%)
	Sub total, *n*	Type I *H. pylori*, *n*	Type II *H. pylori*, *n*		
Total (190)	93	66	27	97	48.95
Father (51)	32	23	9	19	62.75
Mother (51)	32	22	10	19	62.75
Grandfather (4)	2	1	1	2	50.00
Grandmother (12)	8	6	2	4	66.67
Maternal grandfather (4)	1	0	1	3	25.00
Maternal grandmother (8)	3	2	1	5	37.50
Other relatives (3)	2	2	0	1	66.67
Child (57)	13	10	3	45	22.81

N: Number of family members in 51 families; *n*: Person *per* group; Type I *H. pylori*: *H. pylori* infection with type I strains; Type II *H. pylori*: *H. pylori* infection with type II strains; *H. pylori*: *Helicobacter pylori*; Infection rate: Number of *H. pylori*-positive family members/number of family members.

Table 4 Serum gastrin-17, pepsinogen I, pepsinogen II and pepsinogen I/II ratio levels in *Helicobacter pylori*-infected population

	H. pylori+	Type I *H. pylori*	Type II *H. pylori*	*H. pylori*-
G-17 (pmol/L)	8.95 ± 14.53	9.87 ± 15.60	5.62 ± 9.10[c]	4.90 ± 9.21[a,d]
PGI (μg/L)	117.90 ± 55.99	123.12 ± 57.11	99.10 ± 47.45	91.13 ± 38.29[a,b,d]
PGII (μg/L)	12.66 ± 10.41	13.83 ± 11.07	8.43 ± 5.91[c]	7.39 ± 6.82[a,d]
PGR	12.16 ± 6.39	11.64 ± 6.40	14.05 ± 6.04[c]	15.58 ± 7.97[a,d]

[a]*P* < 0.05 when type I *Helicobacter pylori* (*H. pylori*)-infected groups were compared with *H. pylori*-negative groups.
[b]*P* < 0.05 when type II *H. pylori*-infected groups were compared with *H. pylori*-negative groups.
[c]*P* < 0.05 when type I *H. pylori*-infected groups were compared with type II *H. pylori*-infected groups.
[d]*P* < 0.05 when *H. pylori*-infected groups were compared with *H. pylori*-negative groups.
Data are presented as mean ± SD. G-17: Gastrin-17; PG: Pepsinogen; PGR: PG I/II ratio; Type I *H. pylori*: *H. pylori* infection with type I strains; Type II *H. pylori*: *H. pylori* infection with type II strains; *H. pylori*: *Helicobacter pylori*; *H. pylori*+: *H. pylori*-positive; *H. pylori*-: *H. pylori*-negative.

In single factor analysis, we noted that the highly infected age groups were between 31 years and 70 years, and infection rates increased along with age and duration of marriage. Annual household income, history of smoking, history of alcohol consumption, dining location, and family history of gastric disease or GC were not different between the infected and non-infected groups, but individuals with a higher education level showed a lower infection rate. A 2020 all-ages population-based cross-sectional study[28] in Wuwei county in northwestern China showed that the prevalence of *H. pylori*

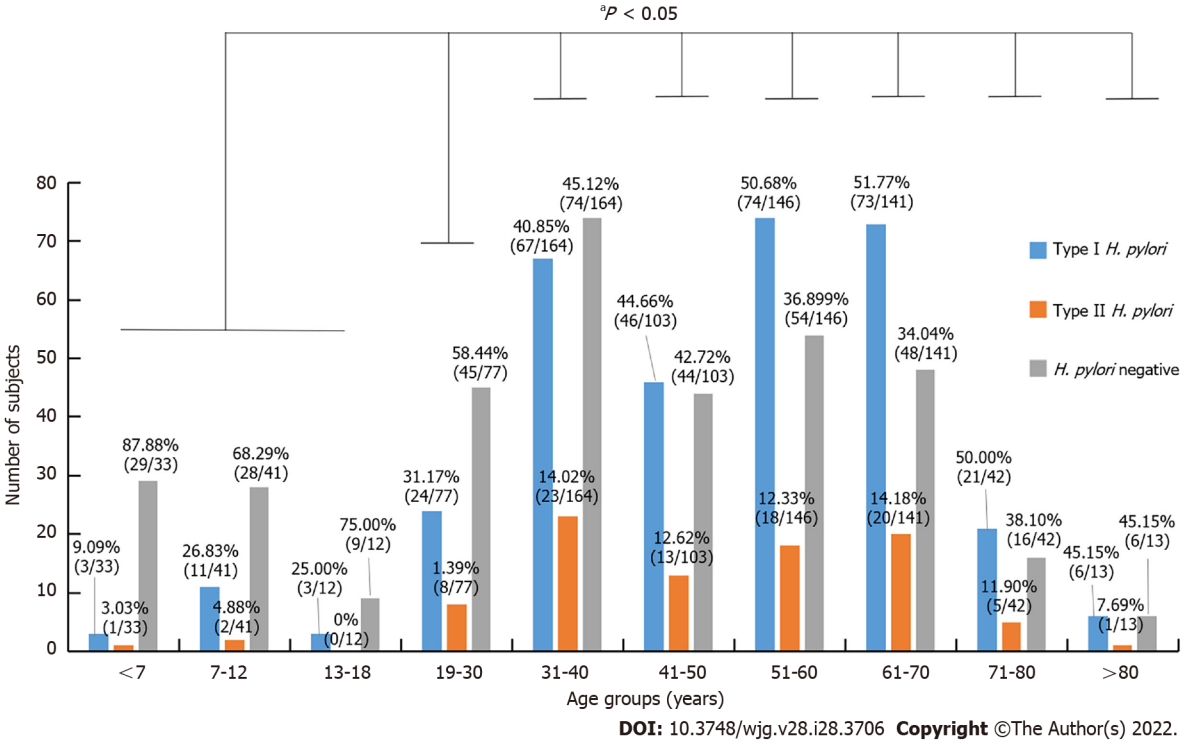

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Figure 2 *Helicobacter pylori* infection status of 772 subjects in different age groups. Values above each column are the case number and its percentages in each specific age group. $^aP < 0.05$ when *Helicobacter pylori* (*H. pylori*) infection rate in the age group of ≤ 18 yr was compared with other age groups. Type I *H. pylori*: *H. pylori* infection with type I strains; Type II *H. pylori*: *H. pylori* infection with type II strains.

infection was closely associated with socioeconomic conditions, sanitary situations, dietary habits of the participants in the city. Boarding, eating at school, and drinking untreated water were main factors explaining the rising infection rate in junior-senior high school students. The results indicated that close contact is associated with increased infection risk. In addition, differences in geographic location, study population, lifestyle habit, and sanitary conditions are important factors that greatly contribute to *H. pylori* infection[22,29,30].

Similar results were obtained from other regions, such as one community-based study[31] in Vietnam in 2017 on familial clustering in a multiple-generation population. The study showed that high monthly income, not regularly being fed chewed food, and being breastfed were protective factors against *H. pylori* infection. Risk factors for *H. pylori* infection in children were not regularly handwashing after defecation, *H. pylori*-infected mother and grandfather, father's occupation, mother's education, and household size. Other factors such as number of siblings, infected fathers, regularly sharing a bed, group living, and antibiotic use were not found to be significant risk factors for infection.

H. pylori-infected family members are a possible source of continued transmission, which is an important health threat for uninfected family members[7,32,33]. A 2003 study[9] in the Stockholm area of Sweden using bacterial isolates for family-based DNA fingerprinting technique demonstrated a high proportion of shared strains among siblings, and between spouses, but also showed different strains in a portion of subjects (8%). Similar results were also reported by a 2009 study in Bangladesh[26]. In the current analysis, we found that among the 67 all member-infected households (Figure 1F), 68.66% (46/67) were infected with *H. pylori* type I strains, 1.49% (1/67) were infected with type II strains, and 29.85% (20/67) had mixed type I and II strain infection. These data support the notion that intrafamilial transmission is the primary transmission route, but exogenous infection outside the family can occur, indicating that there may be multiple sources of transmission. In 244 couples comprising both husband and wife, 34.84% (85/244) of them were co-infected, and 68.24% (58/85) of households were infected with the same strain. With an increase of marriage duration, the infection rate of both husband and wife was significantly increased, suggesting that there was also cross-infection between husband and wife.

One unexpected result was that when both parents were infected, the infection rate of children was 23.08% (6/21), whereas when both parents were not infected, the infection rate of children was 18.18% (2/11), and the difference did not reach statistical significance. This result was slightly different from our previous concept that parental *H. pylori* infection is an independent factor for infection in young children, and that mothers play an important role in *H. pylori* transmission to their descendent[13-15,26]. However, the current results likely reflect the current infection status in this region, as the gradually improved living standard, sanitary condition, use of tap water, and avoiding chewing food to feed children over the past several decades in China's urban family have resulted in a reduced infection rate. This is in line with the fact that the overall *H. pylori* infection rate is declining in most of China's urban

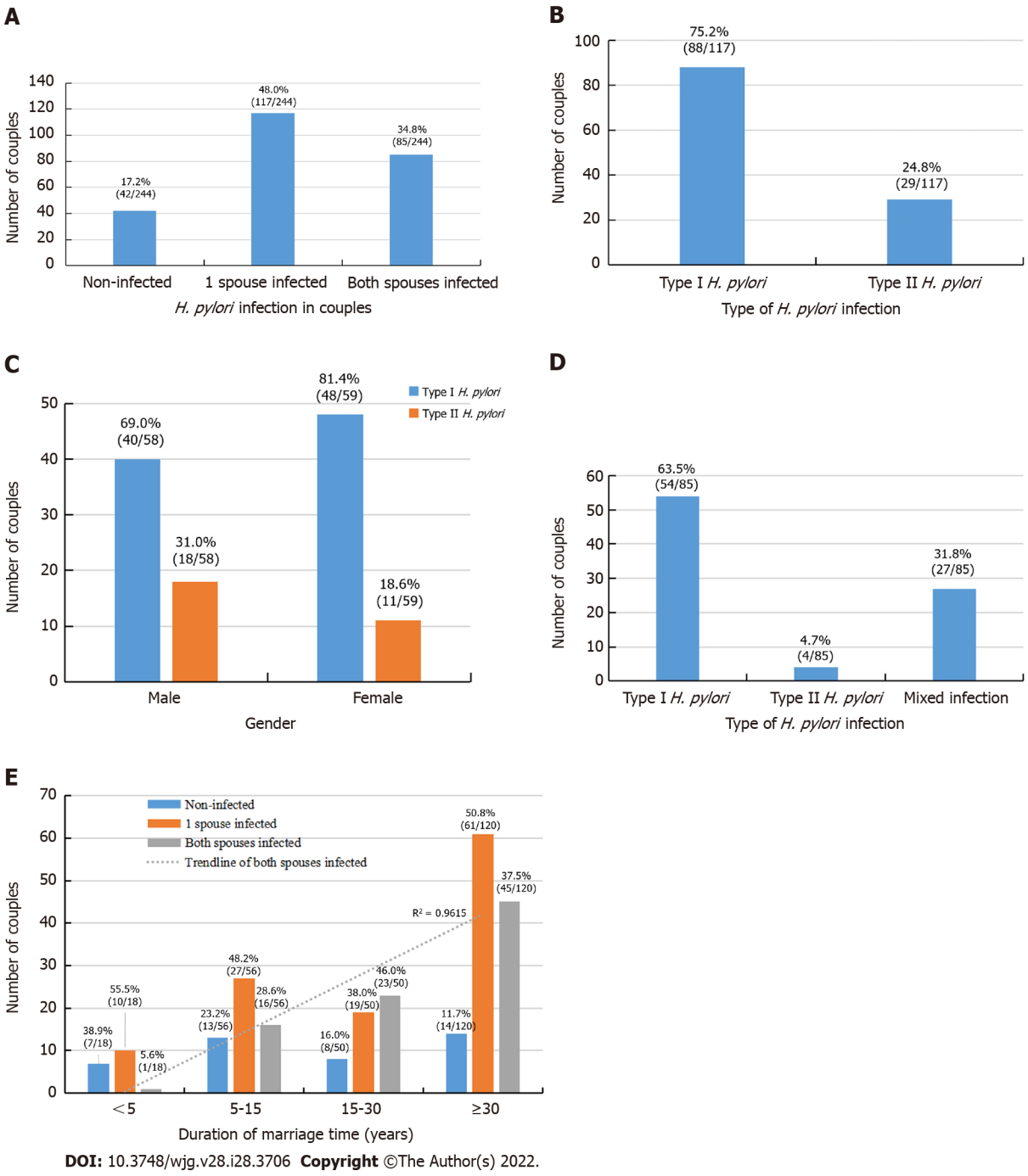

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Figure 3 *Helicobacter pylori* infection status between 244 couples. Values above each column are the case number and percentages of each group. A: *Helicobacter pylori* (*H. pylori*) infection status of 244 couples; B: Type I and II *H. pylori* genotype status of the 117 couples with 1 spouse infected; C: *H. pylori* genotype and sex of 117 couples with 1 spouse infected; D: *H. pylori* genotype status in 85 couples with both spouses infected; E: Relationship between infection and marriage duration in 244 couples. The dashed line across the figure is the trendline of both spouses infected, $r = 0.98$. Non-infected: All members in a couple were not infected; one spouse infected: Only 1 in a couple was infected; Type I *H. pylori*: *H. pylori* infection with type I strains; Type II *H. pylori*: *H. pylori* infection with type II strains; Mixed infection: *H. pylori* infection with type I and type II strains.

areas[3,22]. Another possibility is that the current cohort had relatively small numbers of children and adolescents, which may not have generated enough power for statistical significance. In addition, we were unable to perform bacterial DNA fingerprinting to confirm if the strains were identical, so the genotype that precise bacterial strains transmit within a family unit has yet to be determined. Future large-scale investigations are needed to confirm the current conclusion.

Type I *H. pylori* strain accounted for 78.28% of the infected population in this survey, similar to the results of our previous study in patients admitted to the hospital, which showed that type I *H. pylori* infection accounted for 72.4% (291/402) of the infected patients, and type II was 27.6%[21]. When compared to *H. pylori*-negative groups, G-17, PGI, and PGII levels were higher in *H. pylori*-infected groups. G-17, PGI, and PGII levels were significantly higher and PGR was significantly lower in the

type I *H. pylori*-infected groups than in *H. pylori*-negative groups. The levels of G-17 and PG II were significantly higher, and PGR was significantly lower in type I *H. pylori*-infected groups than in type II-infected groups. These results are in line with our previous endoscopy results from inpatients, and indicate that both type I and type II *H. pylori* strains increase G-17 Level, whereas only type I *H. pylori* infection affects PGI and PGII levels and the PGR in this geographic area[21].

Type I infection and reduced PGR are risk factors for gastric mucosal precancerous lesions and GC [19,21]. Therefore, these results have important clinical implications, as the abnormal expression of gastric markers was noted in a portion of individuals in healthy households infected with *H. pylori* before they sought medical examinations. It was unclear if this group of individuals had gastric mucosal precancerous lesions; thus, further examinations by endoscopy may be required for confirmation. The results of this study also provide another line of support showing that family-based *H. pylori* infection control and management would be an important strategy for infection control and related disease prevention[3,18,34,35].

Although this pilot work provides novel information regarding family-based *H. pylori* infection status, it had some limitations. First, the investigation was performed with a relatively small number of families, and in some groups, especially children and adolescents, the number of samples was not large enough to reach statistical significance; thus, future large-scale, multiple region sampling would provide more convincing data. Second, this was a cross-sectional study without data from endoscopy to confirm *H. pylori* infection-related disease status and pathological changes in gastric mucosa; therefore, some in-depth information was missing, and the work was performed in a Chinese setting, which may not be suitable to other areas. Third, type I and II *H. pylori* genotype concordance through antibody array analysis only provided a very general evaluation. As we did not obtain bacteria strain culture and DNA fingerprinting data, *H. pylori* intrafamilial transmission was unable to be evaluated precisely to assess the heterogeneity of *H. pylori* strains within families. Thus, future studies are needed to evaluate the *H. pylori* DNA fingerprinting pattern for more precise evaluation. Even with these limitations, this study provides novel points and information on family-based *H. pylori* infection characteristics, which merit further large-scale exploration.

CONCLUSION

The current results provide snapshots of family-based *H. pylori* infection status in central China. The high infection rate and coincidence of people infected with *H. pylori* within a family unit indicate the status and pattern of intrafamilial transmission, which provide a novel option for family-based *H. pylori* infection control and related disease prevention. The concept is applicable not only to Chinese residents but also to other communities with high infection rates.

ARTICLE HIGHLIGHTS

Research background

Helicobacter pylori (*H. pylori*) has characteristics of family cluster infection; however, its family-based infection status, related factors, and transmission pattern in central China have not been evaluated.

Research motivation

We evaluated family-based *H. pylori* infection status, related factors, and interfamilial transmission pattern in healthy households in central China, a high-risk area for *H. pylori* and gastric cancer (GC).

Research objectives

To investigate family-based *H. pylori* infection and identify a better approach for *H. pylori* infection control and related disease prevention.

Research methods

H. pylori infection was confirmed primarily by serum antibody arrays in 282 enrolled families, including a total of 772 family members. If patients previously underwent *H. pylori* eradication therapy, an additional ^{13}C-urea breath test was performed to obtain their current infection status. Serum levels of gastrin and pepsinogens (PGs) were also analyzed.

Research results

In our study sample from the general public of central China, *H. pylori* infection rate was 54.27%. In 87.23% of healthy households, there was at least 1 *H. pylori*-infected person, and in 27.24% of these infected families, all members were infected. Type I *H. pylori* was the dominant strain in this geographic area. Among many variables, only individuals with a higher education level showed lower infection

rates. *H. pylori* infection was also correlated with abnormal gastrin-17, PGI, and PGII levels and PGI/PGII ratio.

Research conclusions

H. pylori infection in healthy households is very common in central China, and poses an important health threat to uninfected family members. The intrafamilial infection status and patterns of transmission represent one important source of *H. pylori* spread, and indicate the urgent need for family-based infection control and related disease prevention.

Research perspectives

The results of this study provide new information on family-based *H. pylori* infection status in central China, and support the novel concept of family-based *H. pylori* infection control and management. This notion is also likely to benefit other *H. pylori* and GC prevalent areas.

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FOOTNOTES

Author contributions: Ding SZ, Wu G, Wang XM, Han SY and Sun PC conceived and designed the study; Yu XC, Shao QQ, Zhang C and Yu M searched and screened related literature; Yu XC, Shao QQ, Ma J, Lei L and Zhou Y performed the data extraction and quality assessment; Yu XC, Zhou Y, Chen WC, Zhang W, Fang XH and Zhu YZ analyzed the data; Yu XC and Ding SZ wrote the manuscript; all authors critically revised and approved the final version of the manuscript.

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REFERENCES

1 **Malfertheiner P**, Megraud F, O'Morain CA, Gisbert JP, Kuipers EJ, Axon AT, Bazzoli F, Gasbarrini A, Atherton J, Graham DY, Hunt R, Moayyedi P, Rokkas T, Rugge M, Selgrad M, Suerbaum S, Sugano K, El-Omar EM; European Helicobacter and Microbiota Study Group and Consensus panel. Management of Helicobacter pylori infection-the Maastricht V/Florence Consensus Report. *Gut* 2017; **66**: 6-30 [PMID: 27707777 DOI: 10.1136/gutjnl-2016-312288]

2 **Fallone CA**, Chiba N, van Zanten SV, Fischbach L, Gisbert JP, Hunt RH, Jones NL, Render C, Leontiadis GI, Moayyedi P, Marshall JK. The Toronto Consensus for the Treatment of Helicobacter pylori Infection in Adults. *Gastroenterology* 2016; **151**: 51-69.e14 [PMID: 27102658 DOI: 10.1053/j.gastro.2016.04.006]

3 **Ding SZ**, Du YQ, Lu H, Wang WH, Cheng H, Chen SY, Chen MH, Chen WC, Chen Y, Fang JY, Gao HJ, Guo MZ, Han Y, Hou XH, Hu FL, Jiang B, Jiang HX, Lan CH, Li JN, Li Y, Li YQ, Liu J, Li YM, Lyu B, Lu YY, Miao YL, Nie YZ, Qian JM, Sheng JQ, Tang CW, Wang F, Wang HH, Wang JB, Wang JT, Wang JP, Wang XH, Wu KC, Xia XZ, Xie WF, Xie Y, Xu JM, Yang CQ, Yang GB, Yuan Y, Zeng ZR, Zhang BY, Zhang GY, Zhang GX, Zhang JZ, Zhang ZY, Zheng PY, Zhu Y, Zuo XL, Zhou LY, Lyu NH, Yang YS, Li ZS; National Clinical Research Center for Digestive Diseases (Shanghai), Gastrointestinal Early Cancer Prevention & Treatment Alliance of China (GECA), Helicobacter pylori Study Group of Chinese Society of Gastroenterology, and Chinese Alliance for Helicobacter pylori Study. Chinese Consensus Report on Family-Based *Helicobacter pylori* Infection Control and Management (2021 Edition). *Gut* 2022; **71**: 238-253 [PMID: 34836916 DOI: 10.1136/gutjnl-2021-325630]

4 **Liu WZ**, Xie Y, Lu H, Cheng H, Zeng ZR, Zhou LY, Chen Y, Wang JB, Du YQ, Lu NH; Chinese Society of Gastroenterology, Chinese Study Group on Helicobacter pylori and Peptic Ulcer. Fifth Chinese National Consensus Report on the management of Helicobacter pylori infection. *Helicobacter* 2018; **23**: e12475 [PMID: 29512258 DOI: 10.1111/hel.12475]

5 **Sugano K**, Tack J, Kuipers EJ, Graham DY, El-Omar EM, Miura S, Haruma K, Asaka M, Uemura N, Malfertheiner P; faculty members of Kyoto Global Consensus Conference. Kyoto global consensus report on Helicobacter pylori gastritis. *Gut* 2015; **64**: 1353-1367 [PMID: 26187502 DOI: 10.1136/gutjnl-2015-309252]

6 **Brown LM**, Thomas TL, Ma JL, Chang YS, You WC, Liu WD, Zhang L, Pee D, Gail MH. Helicobacter pylori infection in rural China: demographic, lifestyle and environmental factors. *Int J Epidemiol* 2002; **31**: 638-645 [PMID: 12055167 DOI: 10.1093/ije/31.3.638]

7 **Drumm B**, Perez-Perez GI, Blaser MJ, Sherman PM. Intrafamilial clustering of Helicobacter pylori infection. *N Engl J Med* 1990; **322**: 359-363 [PMID: 2300088 DOI: 10.1056/NEJM199002083220603]

8 **Kato M**, Ota H, Okuda M, Kikuchi S, Satoh K, Shimoyama T, Suzuki H, Handa O, Furuta T, Mabe K, Murakami K, Sugiyama T, Uemura N, Takahashi S. Guidelines for the management of Helicobacter pylori infection in Japan: 2016 Revised Edition. *Helicobacter* 2019; **24**: e12597 [PMID: 31111585 DOI: 10.1111/hel.12597]

9 **Kivi M**, Tindberg Y, Sörberg M, Casswall TH, Befrits R, Hellström PM, Bengtsson C, Engstrand L, Granström M. Concordance of Helicobacter pylori strains within families. *J Clin Microbiol* 2003; **41**: 5604-5608 [PMID: 14662948 DOI: 10.1128/JCM.41.12.5604-5608.2003]

10 **Georgopoulos SD**, Mentis AF, Spiliadis CA, Tzouvelekis LS, Tzelepi E, Moshopoulos A, Skandalis N. Helicobacter pylori infection in spouses of patients with duodenal ulcers and comparison of ribosomal RNA gene patterns. *Gut* 1996; **39**: 634-638 [PMID: 9026475 DOI: 10.1136/gut.39.5.634]

11 **Rothenbacher D**, Winkler M, Gonser T, Adler G, Brenner H. Role of infected parents in transmission of helicobacter pylori to their children. *Pediatr Infect Dis J* 2002; **21**: 674-679 [PMID: 12237602 DOI: 10.1097/00006454-200207000-00014]

12 **Perry S**, de la Luz Sanchez M, Yang S, Haggerty TD, Hurst P, Perez-Perez G, Parsonnet J. Gastroenteritis and transmission of Helicobacter pylori infection in households. *Emerg Infect Dis* 2006; **12**: 1701-1708 [PMID: 17283620 DOI: 10.3201/eid1211.060086]

13 **Rocha GA**, Rocha AM, Silva LD, Santos A, Bocewicz AC, Queiroz Rd Rde M, Bethony J, Gazzinelli A, Corrêa-Oliveira R, Queiroz DM. Transmission of Helicobacter pylori infection in families of preschool-aged children from Minas Gerais, Brazil. *Trop Med Int Health* 2003; **8**: 987-991 [PMID: 14629764 DOI: 10.1046/j.1360-2276.2003.01121.x]

14 **Yang YJ**, Sheu BS, Lee SC, Yang HB, Wu JJ. Children of Helicobacter pylori-infected dyspeptic mothers are predisposed to H. pylori acquisition with subsequent iron deficiency and growth retardation. *Helicobacter* 2005; **10**: 249-255 [PMID: 15904483 DOI: 10.1111/j.1523-5378.2005.00317.x]

15 **Konno M**, Yokota S, Suga T, Takahashi M, Sato K, Fujii N. Predominance of mother-to-child transmission of Helicobacter pylori infection detected by random amplified polymorphic DNA fingerprinting analysis in Japanese families. *Pediatr Infect Dis J* 2008; **27**: 999-1003 [PMID: 18845980 DOI: 10.1097/INF.0b013e31817d756e]

16 **Nguyen VB**, Nguyen GK, Phung DC, Okrainec K, Raymond J, Dupond C, Kremp O, Kalach N, Vidal-Trecan G. Intra-familial transmission of Helicobacter pylori infection in children of households with multiple generations in Vietnam. *Eur J Epidemiol* 2006; **21**: 459-463 [PMID: 16826451 DOI: 10.1007/s10654-006-9016-y]

17 **Chen XX**, Ou BY, Shang SQ, Wu XY, Zhang XP, Chen LQ, Qu YP. The correlation between family aggregation and eradication therapy in children with Helicobacter pylori infection. *Zhongguo Shiyong Erke* 2003; **18**: 475-477 [DOI: 10.3969/j.issn.1005-2224.2003.08.011]

18 **Sari YS**, Can D, Tunali V, Sahin O, Koc O, Bender O. H pylori: Treatment for the patient only or the whole family? *World J Gastroenterol* 2008; **14**: 1244-1247 [PMID: 18300351 DOI: 10.3748/wjg.14.1244]

19 **Matos JI**, de Sousa HA, Marcos-Pinto R, Dinis-Ribeiro M. Helicobacter pylori CagA and VacA genotypes and gastric phenotype: a meta-analysis. *Eur J Gastroenterol Hepatol* 2013; **25**: 1431-1441 [PMID: 23929249 DOI: 10.1097/MEG.0b013e328364b53e]

20 **Ding SZ**, Goldberg JB, Hatakeyama M. Helicobacter pylori infection, oncogenic pathways and epigenetic mechanisms in gastric carcinogenesis. *Future Oncol* 2010; **6**: 851-862 [PMID: 20465395 DOI: 10.2217/fon.10.37]

21 **Yuan L**, Zhao JB, Zhou YL, Qi YB, Guo QY, Zhang HH, Khan MN, Lan L, Jia CH, Zhang YR, Ding SZ. Type I and type II *Helicobacter pylori* infection status and their impact on gastrin and pepsinogen level in a gastric cancer prevalent area.

World J Gastroenterol 2020; **26**: 3673-3685 [PMID: 32742135 DOI: 10.3748/wjg.v26.i25.3673]

22 **Li M**, Sun Y, Yang J, de Martel C, Charvat H, Clifford GM, Vaccarella S, Wang L. Time trends and other sources of variation in Helicobacter pylori infection in mainland China: A systematic review and meta-analysis. *Helicobacter* 2020; **25**: e12729 [PMID: 32686261 DOI: 10.1111/hel.12729]

23 **Liu S**, Chen Q, Quan P, Zhang M, Zhang S, Guo L, Sun X, Wang C. Cancer incidence and mortality in Henan province, 2012. *Chin J Cancer Res* 2016; **28**: 275-285 [PMID: 27478313 DOI: 10.21147/j.issn.1000-9604.2016.03.02]

24 **Zamani M**, Ebrahimtabar F, Zamani V, Miller WH, Alizadeh-Navaei R, Shokri-Shirvani J, Derakhshan MH. Systematic review with meta-analysis: the worldwide prevalence of Helicobacter pylori infection. *Aliment Pharmacol Ther* 2018; **47**: 868-876 [PMID: 29430669 DOI: 10.1111/apt.14561]

25 **Nahar S**, Kibria KM, Hossain ME, Sultana J, Sarker SA, Engstrand L, Bardhan PK, Rahman M, Endtz HP. Evidence of intra-familial transmission of Helicobacter pylori by PCR-based RAPD fingerprinting in Bangladesh. *Eur J Clin Microbiol Infect Dis* 2009; **28**: 767-773 [PMID: 19190943 DOI: 10.1007/s10096-008-0699-8]

26 **Kivi M**, Johansson AL, Reilly M, Tindberg Y. Helicobacter pylori status in family members as risk factors for infection in children. *Epidemiol Infect* 2005; **133**: 645-652 [PMID: 16050509 DOI: 10.1017/s0950268805003900]

27 **National Bureau of Statistics of China**. China statistical Yearbook 2020. China Statistical Press, 2020

28 **Wang X**, Shu X, Li Q, Li Y, Chen Z, Wang Y, Pu K, Zheng Y, Ye Y, Liu M, Ma L, Zhang Z, Wu Z, Zhang F, Guo Q, Ji R, Zhou Y. Prevalence and risk factors of *Helicobacter pylori* infection in Wuwei, a high-risk area for gastric cancer in northwest China: An all-ages population-based cross-sectional study. *Helicobacter* 2021; **26**: e12810 [PMID: 33904635 DOI: 10.1111/hel.12810]

29 **Ding Z**, Zhao S, Gong S, Li Z, Mao M, Xu X, Zhou L. Prevalence and risk factors of Helicobacter pylori infection in asymptomatic Chinese children: a prospective, cross-sectional, population-based study. *Aliment Pharmacol Ther* 2015; **42**: 1019-1026 [PMID: 26271484 DOI: 10.1111/apt.13364]

30 **Ito LS**, Oba-Shinjo SM, Shinjo SK, Uno M, Marie SK, Hamajima N. Community-based familial study of Helicobacter pylori infection among healthy Japanese Brazilians. *Gastric Cancer* 2006; **9**: 208-216 [PMID: 16952040 DOI: 10.1007/s10120-006-0384-5]

31 **Nguyen TV**, Phan TT, Nguyen VB, Hoang TT, Le TL, Nguyen TT, Vu SN. Prevalence and risk factors of Helicobacter pylori infection in Muong children in Vietnam. *Ann Clin Lab Res* 2017; **5**: 1-9 [DOI: 10.21767/2386-5180.1000159]

32 **Garg PK**, Perry S, Sanchez L, Parsonnet J. Concordance of Helicobacter pylori infection among children in extended-family homes. *Epidemiol Infect* 2006; **134**: 450-459 [PMID: 16283949 DOI: 10.1017/S0950268805005352]

33 **Osaki T**, Konno M, Yonezawa H, Hojo F, Zaman C, Takahashi M, Fujiwara S, Kamiya S. Analysis of intra-familial transmission of Helicobacter pylori in Japanese families. *J Med Microbiol* 2015; **64**: 67-73 [PMID: 25351712 DOI: 10.1099/jmm.0.080507-0]

34 **Zhao JB**, Yuan L, Yu XC, Shao QQ, Ma J, Yu M, Wu Y, Qi YB, Hu RB, Wei PR, Jia BL, Zhang LZ, Zhang YR, Ding SZ. Whole family-based Helicobacter pylori eradication is a superior strategy to single-infected patient treatment approach: A systematic review and meta-analysis. *Helicobacter* 2021; **26**: e12793 [PMID: 33675089 DOI: 10.1111/hel.12793]

35 **Zhou G**. *Helicobacter pylori* Recurrence after Eradication Therapy in Jiangjin District, Chongqing, China. *Gastroenterol Res Pract* 2020; **2020**: 7510872 [PMID: 32328098 DOI: 10.1155/2020/7510872]

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SCIENTOMETRICS

Global research on *Clostridium difficile*-associated diarrhoea: A visualized study

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Abstract

BACKGROUND

Clostridioides (Clostridium) difficile (*C. difficile*) is still the most common cause of healthcare-associated diarrhoea and is increasing in prevalence as a community-acquired infection. In addition, the emergence of antibiotic resistance in *C. difficile* can increase the likelihood of the disease developing and/or spreading.

AIM

To provide an up-to-date picture of the trends in publications related to *C. difficile* infection, together with specific insights into hot-button issues in this field.

METHODS

Publications on *C. difficile* infections in the field of microbiology between 2001 and 2020 were identified from the Scopus database and Reference Citation Analysis. Bibliometric indicators were determined, including the number and type of publications, countries, affiliations, funding agencies, journals and citation patterns. VOSviewer was used to determine research areas and hot-button issues by identifying recurring terms with a high relative occurrence in the title and abstract.

RESULTS

A total of 8127 documents on '*C. difficile*-associated diarrhoea' published between 2001 and 2020 were retrieved from the Scopus database. In the last decade, there has been a significant almost fourfold increase in the number of published papers on this topic. The United States was among the countries (44.11%) with the most publications, and the most involved institution was the *University of Leeds* in the

United Kingdom (2.50%). Three clusters of research were identified and included 'illness spectrum and severity, as well as the signs, symptoms and clinical pathogenesis of *C. difficile*'; 'laboratory diagnosis and characterization of *C. difficile*' and 'risk factors for *C. difficile* infection'.

CONCLUSION

This study contains the most up-to-date and comprehensive data ever compiled in this field. More international research and cross-institutional collaborations are needed to address more global *C. difficile* concerns and to benefit from greater sharing of expertise, which will result in higher quality or more effective studies in the future. Promising research avenues in the near future may draw the attention of relevant scientists and funding organizations and open up novel *C. difficile* infection–based diagnosis and treatment approaches.

Key Words: *Clostridioides*; *Clostridium difficile*; Bibliometric; Scopus; VOSviewer; Diarrhoea

Core Tip: The significance of this study lies in the fact that, to our best knowledge, there are no previous bibliometric studies on *Clostridioides (Clostridium) difficile* (*C. difficile*) infection research. This study presents the evolution of *C. difficile* infection-related publications over time. This bibliometric study will provide clinicians and researchers in gastroenterology and microbiology with a quantitative and timely summary of *C. difficile* infection-related publications. Promising research avenues in the near future may draw the attention of relevant scientists and funding organizations and open up novel *C. difficile* infection–based diagnosis and treatment approaches.

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INTRODUCTION

Clostridium difficile (*C. difficile*) has been reclassified as *Clostridioides difficile*, although the preferred term remains *C. difficile*. *C. difficile* infections are increasing in prevalence and are among the most common healthcare-associated illnesses globally[1-3]. *C. difficile* infections, also known as *C. difficile*-associated diarrhoea, are the most common signs of clinical infection and can range from mild diarrhoea to fulminant colitis[4]. *C. difficile* is frequently linked to the use of antibiotics. *C. difficile* was once thought to be predominantly a nosocomial illness; however, community-acquired *C. difficile* has already been identified[3].

Metronidazole and vancomycin have been the primary treatments for *C. difficile* infections for more than three decades. However, the low number of sustained cures and the rising incidence of *C. difficile* infections, as well as the accompanying morbidity and death, have necessitated the development and investigation of novel treatment approaches[1,5]. Despite ongoing attempts to enhance *C. difficile* prevention and treatment, *C. difficile* continues to be a major public health concern. In both hospitals and the community, *C. difficile* infection is still a prevalent and dangerous problem. In recent years, faecal microbial transplantation has been developed as a safe and successful method of treatment for recurrent infections[6-11]. Therefore, faecal microbial transplantation will most likely become the standard therapy for recurrent infection as a novel technique[6-11].

Bibliometrics and research performance assessments have been performed in a broad range of health areas[12,13], particularly to address environmental[14-17], and toxicological[18] issues. Yet, to our knowledge, a large number of bibliometric studies noticeably focused on microbiology[19-23] have been conducted by using different databases for data analysis. Because of these studies[19-23], microbiology research has recently been given increased scientific attention worldwide. Still, more research efforts are needed to thoroughly review and identify the existing literature related to *C. difficile* infection from different aspects, including authorships, country, affiliation, journals, citation patterns, and content analysis, to determine the research areas that are hot-button issues in this field.

C. difficile infection is considered one of the most debated topics in this era. Using the bibliometric approach to *C. difficile* infection would affect how scientists design and conduct studies and the selection of models that estimate risk. Using a bibliometric analysis of publications in Scopus, this study provides an up-to-date picture of the trends in publications related to *C. difficile* infection, together with specific insights into hot-button issues in this field. The significance of this study lies in the fact that, to our best

knowledge, there are no previous bibliometric studies on *C. difficile* infection research. Therefore, this study presents the evolution of *C. difficile* infection-related publications over time. This bibliometric study will provide clinicians and researchers in gastroenterology and microbiology with a quantitative and timely summary of *C. difficile* infection-related publications. Furthermore, it aims to provide clinicians and researchers with a resource for principles and current evidence. A detailed understanding of the historical trends in this field of research and its obstacles may help to establish a framework for future gastroenterology scholarship.

MATERIALS AND METHODS

Data acquisition

The research data were taken from the Scopus bibliographic database and Reference Citation Analysis (RCA) (https://www.referencecitationanalysis.com/). Scopus was chosen because it has a larger number of indexed journals than other databases (*e.g.* PubMed or Web of Science) and is completely inclusive of all journals in Medline[24-26]. Scopus is the most popular set of scientific publications used in bibliometric and scientometric studies, together with PubMed or Web of Science[27]. In addition, Scopus contains indexed journals in the health, social, physical and life sciences. This enhances the likelihood of retrieving as many relevant publications as is feasible. Baishideng Publishing Group Inc. owns RCA, which is an open transdisciplinary citation analysis database (Pleasanton, CA 94566, United States)[28].

Search strategy

To identify studies related to *C. difficile*-associated diarrhoea, we took the following steps.

Step 1: Data extraction was performed on July 25, 2021 and the results obtained within one day to avoid potential bias due to the regular updating of the database. The terms used in the search engines were applied in Title ((TITLE (Clostrid* difficile) OR TITLE('C. diff*') OR TITLE('Cl. diff*')))) AND Title/Abstract (TITLE-ABS(diarrh*) OR TITLE-ABS(Antibiotic) OR TITLE-ABS(infection) OR TITLE-ABS(AAD)). More precisely, in the results, the search strategy for research related to *Clostridium difficile* terms was limited to the title only to eliminate false-negative results. Search terms with different suffixes were truncated using an asterisk (*). The keywords used were chosen because they are commonly used in the literature related to *C. difficile*-associated diarrhoea[3,29-31].

Step 2: The year 2021 was omitted because the database records for this year would not have been completed at the time of the search.

Step 3: All retrieved documents were reviewed and analysed with respect to the following different bibliometric indicators, as in previous bibliometric studies[13,32-34]: (1) The annual number of publications on *C. difficile*-associated diarrhoea indexed in Scopus and published from 2001 to 2020; (2) Prolific countries, journals, and authors in this field in relation to the number of publications; (3) Research collaboration among the most productive countries; (4) The most frequently cited publications. It is likely that certain articles were cited more frequently than others due to the considerable period that had passed since their publication. Therefore, a citation index was generated for each article to overcome the bias caused by the period that had passed since publication. The citation index is derived by dividing the average number of citations by the number of years since the article was first published; (5) Hot-button issues in this field; and (6) RCA was used to determine the impact index *per* article for the top ten most-cited publications.

Step 4: A network visualization map based on the publications retrieved from the Scopus database was created using VOSviewer (version 1.6.16) software (www.vosviewer.com). The output results from VOSviewer are displayed in clusters. The existing connections between the bibliometric data can be clearly visualized to analyse collaboration between countries. Furthermore, it illustrated the terms widely used in the titles and abstracts of the publications collected, showing the hot research topics.

RESULTS

General description of the retrieved publications

A total of 8127 documents on '*C. difficile*-associated diarrhoea' published between 2001 and 2020 were retrieved from the Scopus database. From these publications, articles (n = 6062) were the most often published documents, comprising 74.59% of the total, followed by reviews (n = 1016; 12.50%) and letters (n = 384; 4.72%).

The trend of global publications

As shown in Figure 1, there was a growing trend in the number of publications on *C. difficile*-associated diarrhoea in the Scopus database between 2001 and 2020. It is obvious that there was an increasing number of publications mostly during two periods: From 2006 to 2013 and from 2014 to 2020. Since 2006, the number of relevant articles grew significantly, which is notable. Papers published during the last seven years (2014 to 2020) accounted for 60.16% of the total publications. As a result of these findings, the number of yearly publications grew progressively from 2014 to 2020, showing that the amount of research output increased steadily over that period.

Contributions by country

Table 1 shows that the United States was the most prolific country, whose authors published the most documents (n = 3585), followed by the United Kingdom (n = 1013), Canada (n = 556), and Germany (n = 434). The first 10 countries in Table 1 produced 89.84% of the documents published related to *C. difficile*-associated diarrhoea. Analysis of international collaboration was conducted on the downloaded data based on co-authorship relationships between countries (Figure 2).

Contributions by institution

The top 10 most productive institutes in terms of total papers are listed in Table 2. The major academic contributions mainly originated from *University of Leeds* (2.50%), *Leiden University Medical Center* (2.35%) and *Harvard Medical School* (2.25%).

Contributions by funding agency

Table 3 lists the top 10 global funding agencies that sponsored research output on *C. difficile*-associated diarrhoea. Among them, eight agencies were from the United States, and two were from the United Kingdom. The *National Institutes of Health* ranked first, supporting the highest number of studies at 884. The *U.S. Department of Health and Human Services* ranked second (n = 841), and the *National Institute of Allergy and Infectious Diseases* ranked third (n = 539).

Most active journals

The 10 most prolific journals are presented in Table 4. The most productive journal was *Infection Control and Hospital Epidemiology* (n = 304), followed by *Anaerobe* (n = 276), *Clinical Infectious Diseases* (n = 251) and *Journal of Hospital Infection* (n = 212). Thus, the first 10 journals in Table 4 produced 23.97% of the documents published related to *C. difficile*-associated diarrhoea.

Most cited documents

Table 5 presents the 10 most often cited articles published on *C. difficile*-associated diarrhoea[35-44]. Furthermore, the ten most cited articles have an impact index *per* article of 45.6 to 313.9 (Table 5).

Most frequent topics

We studied the distribution of co-occurrence terms using VOSviewer software (the minimum number of occurrences of a term in all publications is 100 times in titles and abstracts) to detect directions and topics in *C. difficile*-associated diarrhoea research and understand the growth of this discipline. The size of the circle or node of a term equals that particular term's number of occurrences. For example, in Figure 3, of the 84961 terms, 385 terms occurred at least 100 times, distributed in three clusters: Cluster 1, shown by green dots, includes those terms commonly found in studies related to clinical features of *C. difficile*, including the illness spectrum and severity, as well as the signs, symptoms and clinical pathogenesis of *C. difficile*. Cluster 2, shown by blue dots, includes those terms commonly found in laboratory diagnosis and characterization studies of *C. difficile*. Cluster 3, indicated by red dots, includes terms commonly found in studies related to risk factors for *C. difficile* infections. To investigate the changes in hotspots over time, a network visualization map of the most frequent terms in the titles/abstracts of the retrieved documents was generated using VOSviewer software, and the results revealed that the topic 'risk factors for *C. difficile* infection' began to appear more frequently in the last five years (Figure 4).

DISCUSSION

The current study was a descriptive study on global research output of publications related to *C. difficile* infection. It is important to examine the quantity and quality of research in this field, given the changing epidemiology of *C. difficile* morbidity and mortality, worldwide escalation of antibiotic resistance and limited alternative preventive strategies for *C. difficile* infection. This bibliometric analysis will aid in revealing key milestones and progressions in this field, detecting current shortages and developing trends and directing the field's future research path. The current study showed a fourfold increase in publications in the last decade. These results reflect those of Balsells *et al*[45] and Ofosu[46], who also

Table 1 Top 10 countries published *Clostridium difficile*-associated diarrhea between 2001 and 2020

Position	Country	No. of publication	%
1st	United States	3585	44.11
2nd	United Kingdom	1013	12.46
3rd	Canada	556	6.84
4th	Germany	434	5.34
5th	France	383	4.71
6th	China	315	3.88
7th	Netherlands	273	3.36
8th	Australia	261	3.21
9th	Italy	244	3.00
10th	Spain	238	2.93

Table 2 Ten most productive and influential institutions in *Clostridium difficile*-associated diarrhea between 2001 and 2020

Position	Institution	Country	No. of publication	%
1st	*University of Leeds*	United Kingdom	203	2.50
2nd	*Leiden University Medical Center-LUMC*	Netherlands	191	2.35
3rd	*Harvard Medical School*	United States	183	2.25
4th	*VA Medical Center*	United States	173	2.13
5th	*Leeds Teaching Hospitals NHS Trust*	United Kingdom	160	1.97
6th	*Beth Israel Deaconess Medical Center*	Israel	135	1.66
7th	*Washington University School of Medicine in St. Louis*	United States	118	1.45
8th	*Edward Hines Jr. VA Hospital*	United States	115	1.42
9th	*The University of Western Australia*	Australia	109	1.34
10th	*Baylor College of Medicine*	United States	108	1.33

Table 3 Top 10 related funding agencies in *Clostridium difficile*-associated diarrhea between 2001 and 2020

Position	Funding agencies	Country	No. of publication	%
1st	*National Institutes of Health*	United States	884	10.88
2nd	*U.S. Department of Health and Human Services*	United States	841	10.35
3rd	*National Institute of Allergy and Infectious Diseases*	United States	539	6.63
4th	*National Institute of Diabetes and Digestive and Kidney Diseases*	United States	239	2.94
5th	*Merck*	United States	171	2.10
6th	*National Center for Advancing Translational Sciences*	United States	154	1.89
7th	*Medical Research Council*	United Kingdom	150	1.85
8th	*National Institute of General Medical Sciences*	United States	146	1.80
9th	*Pfizer*	United States	137	1.69
10th	*United Kingdom Research and Innovation*	United Kingdom	132	1.62

stated that in recent years, there had been a growing understanding of the principle of *C. difficile* infection, the risk factors associated *with C. difficile* infection, the pathogenesis and clinical manifestation, prevention, diagnosis and *C. difficile* infection treatment, including new emerging therapies and faecal microbiota transplantation.

Table 4 Ten most productive and influential journals in *Clostridium difficile*-associated diarrhea between 2001 and 2020

Position	Journal	*n*	%	IF[1]
1st	*Infection Control and Hospital Epidemiology*	304	3.74	3.254
2nd	*Anaerobe*	276	3.4	3.331
3rd	*Clinical Infectious Diseases*	251	3.09	9.097
4th	*Journal of Hospital Infection*	212	2.61	3.926
5th	*Journal of Clinical Microbiology*	183	2.25	5.948
6th	*Plos One*	181	2.23	3.240
7th	*American Journal of Infection Control*	169	2.08	2.918
8th	*Antimicrobial Agents and Chemotherapy*	130	1.6	5.191
9th	*Journal of Antimicrobial Chemotherapy*	124	1.53	5.790
10th	*Clinical Microbiology and Infection*	117	1.44	8.067

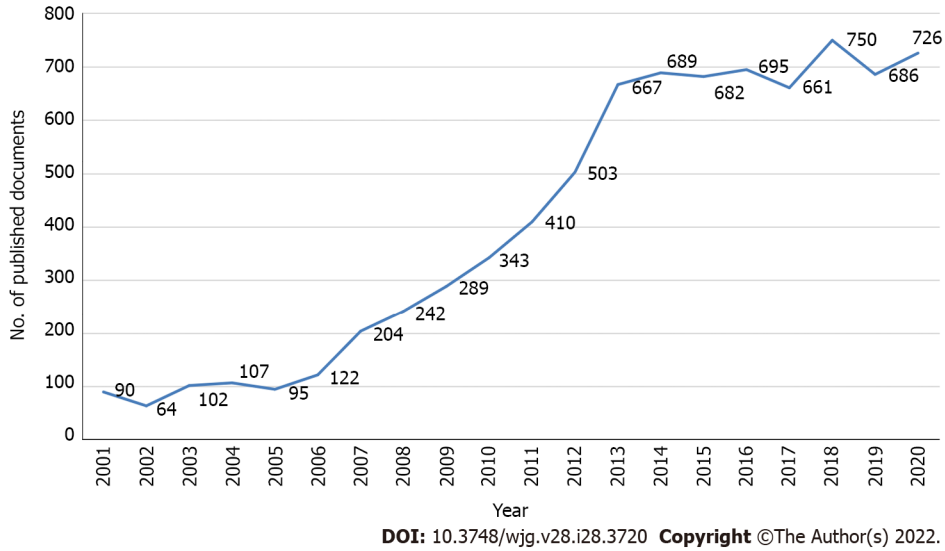
[1]Impact factor (IF) based on Journal Citation Reports (JCR) 2020 from Clarivate Analytics.

Table 5 Ten most cited publications and authors between 2001 and 2020 in in *Clostridium difficile*-associated diarrhea

Ref.	Title	Year	Source title	Cited by	Citation index	Impact index *per* article[1]
Cohen *et al* [35]	"Clinical practice guidelines for Clostridium difficile infection in adults: 2010 update by the Society for Healthcare Epidemiology of America (SHEA) and the Infectious Diseases Society of America (IDSA)"	2010	*Infection Control and Hospital Epidemiology*	2370	237.0	313.9
van Nood *et al*[42]	"Duodenal infusion of donor feces for recurrent clostridium difficile"	2013	*New England Journal of Medicine*	2140	305.7	241.3
Loo *et al*[37]	"A predominantly clonal multi-institutional outbreak of Clostridium difficile - Associated diarrhea with high morbidity and mortality"	2005	*New England Journal of Medicine*	1601	106.7	88.6
Lessa *et al* [44]	"Burden of Clostridium difficile infection in the United States"	2015	*New England Journal of Medicine*	1430	286.0	234.3
Surawicz *et al*[41]	"Guidelines for diagnosis, treatment, and prevention of clostridium difficile infections"	2013	*American Journal of Gastroenterology*	1087	155.3	116.8
Louie *et al* [38]	"Fidaxomicin versus vancomycin for Clostridium difficile infection"	2011	*New England Journal of Medicine*	1074	119.3	91.4
Kelly and LaMont[36]	"Clostridium difficile - More difficult than ever"	2008	*New England Journal of Medicine*	1019	84.9	68.1
Rupnik *et al* [40]	"Clostridium difficile infection: New developments in epidemiology and pathogenesis"	2009	*Nature Reviews Microbiology*	985	89.5	73.4
Zar *et al*[43]	"A comparison of vancomycin and metronidazole for the treatment of Clostridium difficile-associated diarrhea, stratified by disease severity"	2007	*Clinical Infectious Diseases*	935	71.9	58.9
Pépin *et al* [39]	"Clostridium difficile-associated diarrhea in a region of Quebec from 1991 to 2003: A changing pattern of disease severity"	2004	*CMAJ*	916	57.3	45.6

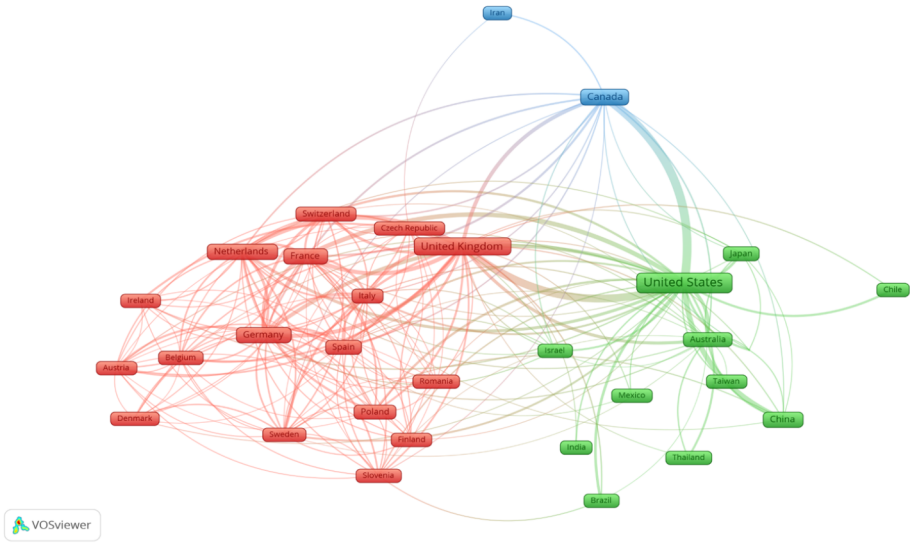
[1]The impact index *per* article is presented based on Reference Citation Analysis [Source: Baishideng Publishing Group Inc. (Pleasanton, CA 94566, United States)].

The United States was the leading country in *C. difficile* infection-related publications, contributing about half of all Scopus publications in this field. This is presumably due to economic prosperity and population growth, and the large number of microbiology researchers[47,48]. The economic basis plays an essential part in supporting scientific research in the current study. The majority of the top 10 funding agencies were based in the United States. High-income countries have published most *C. difficile* infection-related publications, with limited input from low- and middle-income countries. An analysis of the countries that generated the most *C. difficile* infection-related publications indicates that



DOI: 10.3748/wjg.v28.i28.3720

Figure 1 Annual number of publications on *Clostridium difficile*-associated diarrhea indexed in Scopus and published from 2001 to 2020.



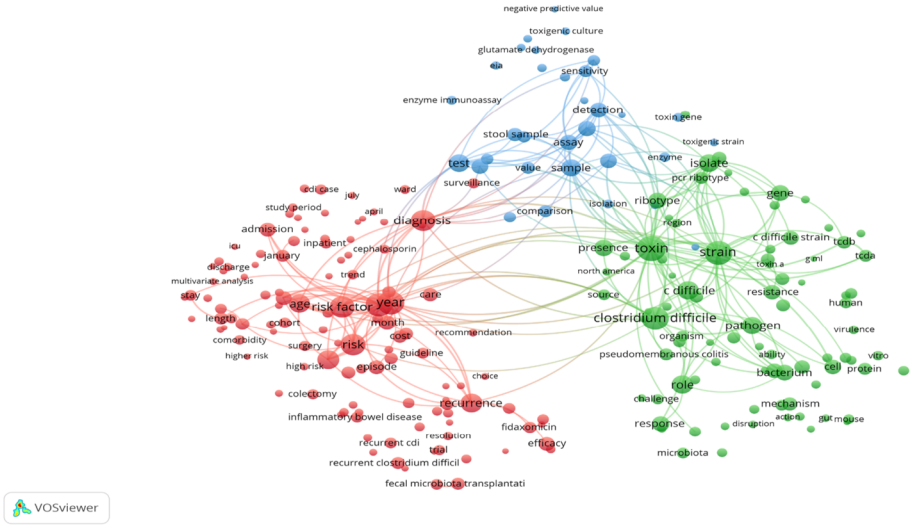
DOI: 10.3748/wjg.v28.i28.3720

Figure 2 Network visualization map of country co-authorships. Of the 103 countries, 31 had at least 50 publications.

countries with economic power indicators have the greatest say in this field. This finding broadly supports the work of other studies in different areas linking scientific research output with geographical location and financial growth[12,49,50]. Various bibliometric analysis studies have also shown that the United States is the most prolific country in microbiology research output[19,20,23,51].

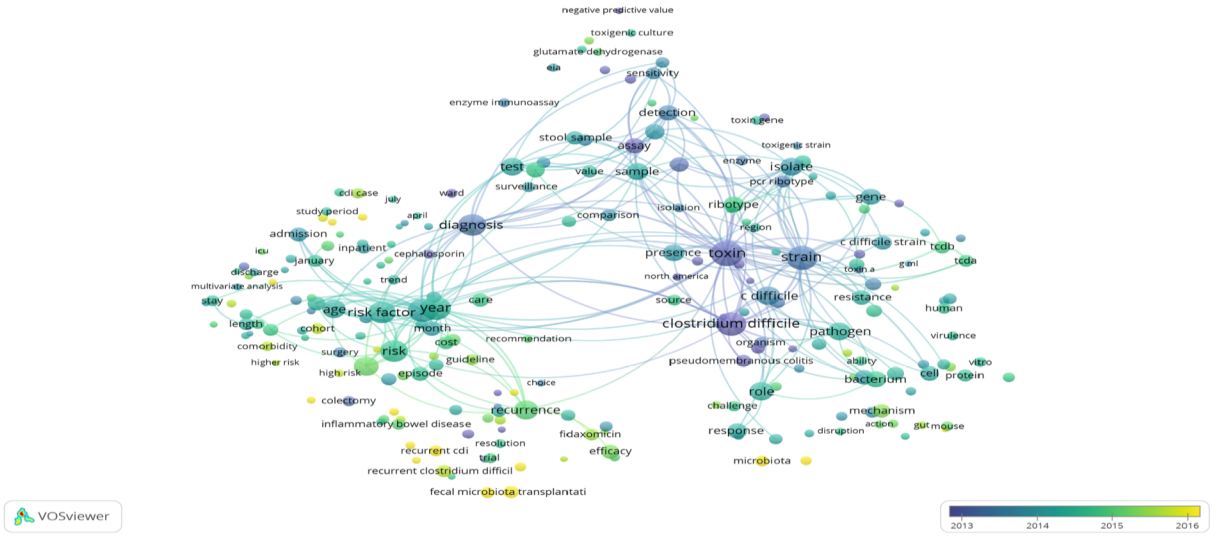
The current study showed that the most frequently cited article on *C. difficile*-associated diarrhoea, written by Cohen *et al*[35] and published in 2010, with 2370 citations, is a guideline that updates the recommendations for epidemiology, diagnostics, therapeutics, infection control, and environmental management. The second most frequently cited paper has 2140 citations and addresses the effect of duodenal infusion of donor faeces in patients with recurrent *C. difficile* infection; this article, published in 2013, was written by van Nood *et al*[42]. These two papers receive approximately 237 and 305.7 citations *per* year on average, respectively. However, the article with the second-highest number of citations *per* year, placed fourth in the ranking, was published in 2015 and was written by Lessa *et al* [44]. This paper aimed to produce more accurate national estimates the burden of illness, incidence, recurrence and death by collecting data from a variety of health care delivery and community contexts. Note that five papers published after 2010 appear in the top 10 most cited publications between 2001 and 2020 in *C. difficile*-associated diarrhoea.

Although it is challenging to reveal the quality or impact of publications through bibliometric analysis, to some degree, citations are considered an indirect measure of an article's contribution to the knowledge generated in the field, *i.e.* the connection between the research finding and its significance



DOI: 10.3748/wjg.v28.i28.3720

Figure 3 Network visualization map of the most frequent terms in the titles/abstracts of the retrieved documents. Of the 84961 terms, 385 had at least 100 publications. Terms with the same color represent a separate cluster (research theme).



DOI: 10.3748/wjg.v28.i28.3720

Figure 4 Overlay network visualization map of the most frequent terms in the titles/abstracts of the retrieved documents. The colors on the map reflected the period of emergence in the literature, with yellow representing terms that were relatively recent in the literature.

for science[52,53]. However, these analyses of the top 10 most cited publications will guide microbiologists interested in further studies by updating knowledge of current developments in *C. difficile* infection-related publications and potential future directions for study.

Analysis of the frequencies of occurrence of terms in publications can offer insights into certain fields' main and hot topics[54]. The current study found that highly cited literature focused on the signs, symptoms and clinical pathogenesis of *C. difficile* concepts and risk factors for *C. difficile* infections. A clear theme to emerge from the results is that the most frequently cited publications on *C. difficile* infections highlighted a range of subtopics similar to the hot research topics. A recent bibliometric study [55] was defined to assess global research activity on antimicrobial stewardship as one measure for efforts dedicated to containing antimicrobial resistance. This study found that *C. difficile* was frequently encountered as author keywords in the retrieved literature on antimicrobial stewardship. The United States Centers for Disease Control and Prevention has considered *C. difficile* infection an urgent danger in its 2019 Antibiotic Resistance Threats Report[56]. In a European point prevalence study, *C. difficile* was rated sixth among bacteria responsible for healthcare-associated illnesses[57]. The majority of *C. difficile* infections in the United States are considered hospital acquired[58].

Strengths and limitations

This study offered the first bibliometric analysis of *C. difficile* infections from the unique perspective of its research hotspots to determine the influential scientific areas and global trends. *C. difficile* infection-based publications in microbiology were collected in the online Scopus database and analysed comprehensively, thoroughly and objectively. As with all previous bibliometric studies[13,31,32,59], the current study has some limitations. First, we preferentially selected English articles from the database but lost some articles that were not in English. Second, we chose Scopus alone as the data source for *C. difficile* infection research because it presented the most reliable and credible information. Inevitably, any useful information from other medical sources such as PubMed and Web of Science would be overlooked. On the other hand, Scopus remains the best database available for analysing research activity and identifying research hotspots on a certain topic. Given these limitations, we believe that this study offers a qualified global view of *C. difficile* infection-based publications in the field of microbiology from 2001 to 2020.

CONCLUSION

The current study used a bibliometric analysis of *C. difficile* infection-based publications in the fields of microbiology and gastroenterology during the period 2001–2020 to determine research hotspots for possible future directions. The results showed that *C. difficile*-based publications have grown rapidly since 2006. Research activity on *C. difficile* infections has been an emerging topic during the last two decades and has been developed predominantly by scientists from the United States of America, the United Kingdom, Canada, Germany, France and China. Risk factors for *C. difficile* infection, laboratory diagnosis and characterization of *C. difficile*, signs, symptoms and clinical pathogenesis of *C. difficile* concepts were the main research hotspots in *C. difficile* infection, and related studies should pioneer these fields in the future. Promising research avenues in the near future may draw the attention of relevant scientists and funding organizations and open up novel *C. difficile* infection–based diagnosis and treatment approaches.

ARTICLE HIGHLIGHTS

Research background

Clostridioides (Clostridium) difficile (*C. difficile*) infections are growing more prevalent and are now one of the most often encountered healthcare-associated infections worldwide.

Research motivation

To our best knowledge, however, a large number of bibliometric studies notably focused on microbiology have been undertaken by using various databases for data analysis. More research efforts are still required to thoroughly analyse and identify the existing literature related to *C. difficile* infection from many perspectives in order to identify study area hot issues in this field.

Research objectives

This study gives an up-to-date picture of the trends in publications linked to *C. difficile* infection, as well as unique insights into hot topics in this field.

Research methods

This study was based on a bibliometric analysis of Scopus and Reference Citation Analysis publications.

Research results

Three clusters of research were highlighted as hot topics: 'illness spectrum and severity, as well as signs, symptoms and clinical pathogenesis of *C. difficile*'; 'laboratory diagnosis and characterization of *C. difficile*' and 'risk factors for *C. difficile* infection'.

Research conclusions

The current study conducted a bibliometric analysis of *C. difficile*-related publications in the disciplines of microbiology and gastroenterology from 2001 to 2020 to identify research hotspots for potential future directions. Results revealed that the topic 'risk factors for *C. difficile* infection' began to appear more frequently in the last five years.

Research perspectives

This bibliometric study will provide clinicians and researchers in gastroenterology and microbiology with a quantitative and timely summary of publications linked to *C. difficile* infection. It also intends to

be a resource for clinicians and researchers on principles and current evidence.

FOOTNOTES

Author contributions: Zyoud S developed the concept for the manuscript, reviewed the literature, formulated research questions, collected the data, conducted analyses and interpreted the data, and wrote the manuscript, the author read and approved the final manuscript.

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REFERENCES

1 **Beinortas T**, Burr NE, Wilcox MH, Subramanian V. Comparative efficacy of treatments for Clostridium difficile infection: a systematic review and network meta-analysis. *Lancet Infect Dis* 2018; **18**: 1035-1044 [PMID: 30025913 DOI: 10.1016/S1473-3099(18)30285-8]
2 **Burke KE**, Lamont JT. Clostridium difficile infection: a worldwide disease. *Gut Liver* 2014; **8**: 1-6 [PMID: 24516694 DOI: 10.5009/gnl.2014.8.1.1]
3 **Curcio D**, Cané A, Fernández FA, Correa J. Clostridium difficile-associated Diarrhea in Developing Countries: A Systematic Review and Meta-Analysis. *Infect Dis Ther* 2019; **8**: 87-103 [PMID: 30659481 DOI: 10.1007/s40121-019-0231-8]
4 **Leffler DA**, Lamont JT. Clostridium difficile infection. *N Engl J Med* 2015; **372**: 1539-1548 [PMID: 25875259 DOI: 10.1056/NEJMra1403772]
5 **DuPont HL**. Search for the optimal antimicrobial therapy of Clostridium difficile infection. *Lancet Infect Dis* 2018; **18**: 936-937 [PMID: 30025912 DOI: 10.1016/S1473-3099(18)30308-6]
6 **Haifer C**, Paramsothy S, Borody TJ, Clancy A, Leong RW, Kaakoush NO. Long-Term Bacterial and Fungal Dynamics following Oral Lyophilized Fecal Microbiota Transplantation in Clostridioides difficile Infection. *mSystems* 2021; **6** [PMID: 33531405 DOI: 10.1128/mSystems.00905-20]
7 **Hourigan SK**, Nicholson MR, Kahn SA, Kellermayer R. Updates and Challenges in Fecal Microbiota Transplantation for Clostridioides difficile Infection in Children. *J Pediatr Gastroenterol Nutr* 2021; **73**: 430-432 [PMID: 34238831 DOI: 10.1097/MPG.0000000000003229]
8 **Khoruts A**, Staley C, Sadowsky MJ. Faecal microbiota transplantation for Clostridioides difficile: mechanisms and pharmacology. *Nat Rev Gastroenterol Hepatol* 2021; **18**: 67-80 [PMID: 32843743 DOI: 10.1038/s41575-020-0350-4]
9 **Li Y**, Honda K. Toward the development of defined microbial therapeutics. *Int Immunol* 2021; **33**: 761-766 [PMID: 34232990 DOI: 10.1093/intimm/dxab038]
10 **D Goldenberg S**, Merrick B. The role of faecal microbiota transplantation: looking beyond *Clostridioides difficile* infection. *Ther Adv Infect Dis* 2021; **8**: 2049936120981526 [PMID: 33614028 DOI: 10.1177/2049936120981526]
11 **Sehgal K**, Khanna S. Gut microbiome and *Clostridioides difficile* infection: a closer look at the microscopic interface. *Therap Adv Gastroenterol* 2021; **14**: 1756284821994736 [PMID: 33747125 DOI: 10.1177/1756284821994736]
12 **Liu H**, Chen H, Hong R, Liu H, You W. Mapping knowledge structure and research trends of emergency evacuation studies. *Safety Sci* 2020; **121**: 348-361 [DOI: 10.1016/j.ssci.2019.09.020]
13 **Al-Jabi SW**. Arab world's growing contribution to global leishmaniasis research (1998-2017): a bibliometric study. *BMC Public Health* 2019; **19**: 625 [PMID: 31118003 DOI: 10.1186/s12889-019-6969-9]
14 **Briganti M**, Delnevo CD, Brown L, Hastings SE, Steinberg MB. Bibliometric Analysis of Electronic Cigarette Publications: 2003-2018. *Int J Environ Res Public Health* 2019; **16**: E320 [DOI: 10.3390/ijerph16030320]
15 **Olisah C**, Okoh OO, Okoh AI. Global evolution of organochlorine pesticides research in biological and environmental matrices from 1992 to 2018: A bibliometric approach. *Emerg Contam* 2019; **5**: 157-167 [DOI: 10.1016/j.emcon.2019.05.001]
16 **Yang B**, Huang K, Sun D, Zhang Y. Mapping the scientific research on non-point source pollution: a bibliometric analysis.

Environ Sci Pollut Res Int 2017; **24**: 4352-4366 [PMID: 27928755 DOI: 10.1007/s11356-016-8130-y]
17 **Zheng T**, Li P, Shi Z, Liu J. Benchmarking the scientific research on wastewater-energy nexus by using bibliometric analysis. *Environ Sci Pollut Res Int* 2017; **24**: 27613-27630 [PMID: 29134520 DOI: 10.1007/s11356-017-0696-5]
18 **Zyoud SH**, Waring WS, Al-Jabi SW, Sweileh WM, Rahhal B, Awang R. Intravenous Lipid Emulsion as an Antidote for the Treatment of Acute Poisoning: A Bibliometric Analysis of Human and Animal Studies. *Basic Clin Pharmacol Toxicol* 2016; **119**: 512-519 [PMID: 27098056 DOI: 10.1111/bcpt.12609]
19 **Sweileh WM**. Global research activity on antimicrobial resistance in food-producing animals. *Arch Public Health* 2021; **79**: 49 [PMID: 33849636 DOI: 10.1186/s13690-021-00572-w]
20 **Ahmad T**, Haroon, Khan M, Murad MA, Baig M, Murtaza BN, Khan MM, Harapan H, Hui J. Research trends in rabies vaccine in the last three decades: a bibliometric analysis of global perspective. *Hum Vaccin Immunother* 2021; **17**: 3169-3177 [PMID: 33945433 DOI: 10.1080/21645515.2021.1910000]
21 **Dehghanbanadaki H**, Aazami H, Keshavarz Azizi Raftar S, Ashrafian F, Ejtahed HS, Hashemi E, Hoseini Tavassol Z, Ahmadi Badi S, Siadat SD. Global scientific output trend for Akkermansia muciniphila research: a bibliometric and scientometric analysis. *BMC Med Inform Decis Mak* 2020; **20**: 291 [PMID: 33167984 DOI: 10.1186/s12911-020-01312-w]
22 **Zhong H**, Chen F, Li YJ, Zhao XY, Zhang ZL, Gu ZC, Yu YT. Global trends and hotspots in research of carbapenem-resistant Enterobacteriaceae (CRE): a bibliometric analysis from 2010 to 2020. *Ann Palliat Med* 2021; **10**: 6079-6091 [PMID: 34237952 DOI: 10.21037/apm-21-87]
23 **Zhu Y**, Li JJ, Reng J, Wang S, Zhang R, Wang B. Global trends of Pseudomonas aeruginosa biofilm research in the past two decades: A bibliometric study. *Microbiologyopen* 2020; **9**: 1102-1112 [PMID: 32120451 DOI: 10.1002/mbo3.1021]
24 **Falagas ME**, Pitsouni EI, Malietzis GA, Pappas G. Comparison of PubMed, Scopus, Web of Science, and Google Scholar: strengths and weaknesses. *FASEB J* 2008; **22**: 338-342 [PMID: 17884971 DOI: 10.1096/fj.07-9492LSF]
25 **Kokol P**, Vošner HB. Discrepancies among Scopus, Web of Science, and PubMed coverage of funding information in medical journal articles. *J Med Libr Assoc* 2018; **106**: 81-86 [PMID: 29339937 DOI: 10.5195/jmla.2018.181]
26 **Barqawi A**, Abushamma FA, Akkawi M, Al-Jabi SW, Shahwan MJ, Jairoun AA, Zyoud SH. Global trends in research related to sleeve gastrectomy: A bibliometric and visualized study. *World J Gastrointest Surg* 2021; **13**: 1509-1522 [PMID: 34950437 DOI: 10.4240/wjgs.v13.i11.1509]
27 **Mongeon P**, Paul-Hus A. The journal coverage of Web of Science and Scopus: a comparative analysis. *Scientometrics* 2016; **106**: 213-228 [DOI: 10.1007/s11192-015-1765-5]
28 **Baishideng Publishing Group Inc**. Reference Citation Analysis. 2022. [cited 10 June 2022]. Available from: https://www.referencecitationanalysis.com/
29 **Nasiri MJ**, Goudarzi M, Hajikhani B, Ghazi M, Goudarzi H, Pouriran R. Clostridioides (Clostridium) difficile infection in hospitalized patients with antibiotic-associated diarrhea: A systematic review and meta-analysis. *Anaerobe* 2018; **50**: 32-37 [PMID: 29408016 DOI: 10.1016/j.anaerobe.2018.01.011]
30 **Lau CS**, Chamberlain RS. Probiotics are effective at preventing Clostridium difficile-associated diarrhea: a systematic review and meta-analysis. *Int J Gen Med* 2016; **9**: 27-37 [PMID: 26955289 DOI: 10.2147/IJGM.S98280]
31 **Sholeh M**, Krutova M, Forouzesh M, Mironov S, Sadeghifard N, Molaeipour L, Maleki A, Kouhsari E. Antimicrobial resistance in Clostridioides (Clostridium) difficile derived from humans: a systematic review and meta-analysis. *Antimicrob Resist Infect Control* 2020; **9**: 158 [PMID: 32977835 DOI: 10.1186/s13756-020-00815-5]
32 **Al-Jabi SW**. Global Trends in Aspirin Resistance-Related Research from 1990 to 2015: A Bibliometric Analysis. *Basic Clin Pharmacol Toxicol* 2017; **121**: 512-519 [PMID: 28667787 DOI: 10.1111/bcpt.12840]
33 **Al-Jabi SW**. Global research trends in West Nile virus from 1943 to 2016: a bibliometric analysis. *Global Health* 2017; **13**: 55 [PMID: 28774315 DOI: 10.1186/s12992-017-0284-y]
34 **Zyoud SH**, Smale S, Waring WS, Sweileh WM, Al-Jabi SW. Global research trends in microbiome-gut-brain axis during 2009-2018: a bibliometric and visualized study. *BMC Gastroenterol* 2019; **19**: 158 [PMID: 31470803 DOI: 10.1186/s12876-019-1076-z]
35 **Cohen SH**, Gerding DN, Johnson S, Kelly CP, Loo VG, McDonald LC, Pepin J, Wilcox MH; Society for Healthcare Epidemiology of America; Infectious Diseases Society of America. Clinical practice guidelines for Clostridium difficile infection in adults: 2010 update by the society for healthcare epidemiology of America (SHEA) and the infectious diseases society of America (IDSA). *Infect Control Hosp Epidemiol* 2010; **31**: 431-455 [PMID: 20307191 DOI: 10.1086/651706]
36 **Kelly CP**, LaMont JT. Clostridium difficile--more difficult than ever. *N Engl J Med* 2008; **359**: 1932-1940 [PMID: 18971494 DOI: 10.1056/NEJMra0707500]
37 **Loo VG**, Poirier L, Miller MA, Oughton M, Libman MD, Michaud S, Bourgault AM, Nguyen T, Frenette C, Kelly M, Vibien A, Brassard P, Fenn S, Dewar K, Hudson TJ, Horn R, René P, Monczak Y, Dascal A. A predominantly clonal multi-institutional outbreak of Clostridium difficile-associated diarrhea with high morbidity and mortality. *N Engl J Med* 2005; **353**: 2442-2449 [PMID: 16322602 DOI: 10.1056/NEJMoa051639]
38 **Louie TJ**, Miller MA, Mullane KM, Weiss K, Lentnek A, Golan Y, Gorbach S, Sears P, Shue YK; OPT-80-003 Clinical Study Group. Fidaxomicin *vs* vancomycin for Clostridium difficile infection. *N Engl J Med* 2011; **364**: 422-431 [PMID: 21288078 DOI: 10.1056/NEJMoa0910812]
39 **Pépin J**, Valiquette L, Alary ME, Villemure P, Pelletier A, Forget K, Pépin K, Chouinard D. Clostridium difficile-associated diarrhea in a region of Quebec from 1991 to 2003: a changing pattern of disease severity. *CMAJ* 2004; **171**: 466-472 [PMID: 15337727 DOI: 10.1503/cmaj.1041104]
40 **Rupnik M**, Wilcox MH, Gerding DN. Clostridium difficile infection: new developments in epidemiology and pathogenesis. *Nat Rev Microbiol* 2009; **7**: 526-536 [PMID: 19528959 DOI: 10.1038/nrmicro2164]
41 **Surawicz CM**, Brandt LJ, Binion DG, Ananthakrishnan AN, Curry SR, Gilligan PH, McFarland LV, Mellow M, Zuckerbraun BS. Guidelines for diagnosis, treatment, and prevention of Clostridium difficile infections. *Am J Gastroenterol* 2013; **108**: 478-98; quiz 499 [PMID: 23439232 DOI: 10.1038/ajg.2013.4]
42 **van Nood E**, Vrieze A, Nieuwdorp M, Fuentes S, Zoetendal EG, de Vos WM, Visser CE, Kuijper EJ, Bartelsman JF, Tijssen JG, Speelman P, Dijkgraaf MG, Keller JJ. Duodenal infusion of donor feces for recurrent Clostridium difficile. *N*

Engl J Med 2013; **368**: 407-415 [PMID: 23323867 DOI: 10.1056/NEJMoa1205037]

43 **Zar FA**, Bakkanagari SR, Moorthi KM, Davis MB. A comparison of vancomycin and metronidazole for the treatment of Clostridium difficile-associated diarrhea, stratified by disease severity. *Clin Infect Dis* 2007; **45**: 302-307 [PMID: 17599306 DOI: 10.1086/519265]

44 **Lessa FC**, Mu Y, Bamberg WM, Beldavs ZG, Dumyati GK, Dunn JR, Farley MM, Holzbauer SM, Meek JI, Phipps EC, Wilson LE, Winston LG, Cohen JA, Limbago BM, Fridkin SK, Gerding DN, McDonald LC. Burden of Clostridium difficile infection in the United States. *N Engl J Med* 2015; **372**: 825-834 [PMID: 25714160 DOI: 10.1056/NEJMoa1408913]

45 **Balsells E**, Shi T, Leese C, Lyell I, Burrows J, Wiuff C, Campbell H, Kyaw MH, Nair H. Global burden of *Clostridium difficile* infections: a systematic review and meta-analysis. *J Glob Health* 2019; **9**: 010407 [PMID: 30603078 DOI: 10.7189/jogh.09.010407]

46 **Ofosu A**. Clostridium difficile infection: a review of current and emerging therapies. *Ann Gastroenterol* 2016; **29**: 147-154 [PMID: 27065726 DOI: 10.20524/aog.2016.0006]

47 **Elsevier**. Gender in the Global Research Landscape: Analysis of Research Performance Through a Gender Lens Across 20 Years, 12 Geographies, and 27 Subject Ares. Amsterdam, the Netherlands: Elsevier B.V, 2017

48 **Dehdarirad T**, Sotudeh H, Freer J. Bibliometric mapping of microbiology research topics (2012-16): a comparison by socioeconomic development and infectious disease vulnerability values. *FEMS Microbiol Lett* 2019; **366** [PMID: 30629167 DOI: 10.1093/femsle/fnz004]

49 **Demir E**, Yaşar E, Özkoçak V, Yıldırım E. The evolution of the field of legal medicine: A holistic investigation of global outputs with bibliometric analysis. *J Forensic Leg Med* 2020; **69**: 101885 [PMID: 31733463 DOI: 10.1016/j.jflm.2019.101885]

50 **Clarke A**, Gatineau M, Grimaud O, Royer-Devaux S, Wyn-Roberts N, Le Bis I, Lewison G. A bibliometric overview of public health research in Europe. *Eur J Public Health* 2007; **17** Suppl 1: 43-49 [PMID: 17666422 DOI: 10.1093/eurpub/ckm063]

51 **Akintunde TY**, Musa TH, Musa HH, Chen S, Ibrahim E, Muhideen S, Kawuki J. Mapping the global research output on Ebola vaccine from research indexed in web of science and scopus: a comprehensive bibliometric analysis. *Hum Vaccin Immunother* 2021; **17**: 4246-4258 [PMID: 34270380 DOI: 10.1080/21645515.2021.1948785]

52 **Durieux V**, Gevenois PA. Bibliometric indicators: quality measurements of scientific publication. *Radiology* 2010; **255**: 342-351 [PMID: 20413749 DOI: 10.1148/radiol.09090626]

53 **Joshi MA**. Bibliometric indicators for evaluating the quality of scientifc publications. *J Contemp Dent Pract* 2014; **15**: 258-262 [PMID: 25095854 DOI: 10.5005/jp-journals-10024-1525]

54 **van Eck NJ**, Waltman L. Software survey: VOSviewer, a computer program for bibliometric mapping. *Scientometrics* 2010; **84**: 523-538 [PMID: 20585380 DOI: 10.1007/s11192-009-0146-3]

55 **Sweileh WM**. Bibliometric analysis of peer-reviewed literature on antimicrobial stewardship from 1990 to 2019. *Global Health* 2021; **17**: 1 [PMID: 33397377 DOI: 10.1186/s12992-020-00651-7]

56 **Centers for Disease Control and Prevention**. Antibiotic/Antimicrobial Resistance (AR/AMR). 2020. [cited 9 August 2021]. Available from: https://www.cdc.gov/DrugResistance/biggest-threats.html

57 **Suetens C**, Latour K, Kärki T, Ricchizzi E, Kinross P, Moro ML, Jans B, Hopkins S, Hansen S, Lyytikäinen O, Reilly J, Deptula A, Zingg W, Plachouras D, Monnet DL; The Healthcare-Associated Infections Prevalence Study Group. Prevalence of healthcare-associated infections, estimated incidence and composite antimicrobial resistance index in acute care hospitals and long-term care facilities: results from two European point prevalence surveys, 2016 to 2017. *Euro Surveill* 2018; **23** [PMID: 30458912 DOI: 10.2807/1560-7917.ES.2018.23.46.1800516]

58 **Lessa FC**, Winston LG, McDonald LC; Emerging Infections Program C. difficile Surveillance Team. Burden of Clostridium difficile infection in the United States. *N Engl J Med* 2015; **372**: 2369-2370 [PMID: 26061850 DOI: 10.1056/NEJMc1505190]

59 **Al-Jabi SW**. Current global research landscape on COVID-19 and depressive disorders: Bibliometric and visualization analysis. *World J Psychiatry* 2021; **11**: 253-264 [PMID: 34168972 DOI: 10.5498/wjp.v11.i6.253]

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CASE REPORT

Delayed immune-related sclerosing cholangitis after discontinuation of pembrolizumab: A case report

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Abstract

BACKGROUND

Secondary sclerosing cholangitis, characterized by biliary obstruction, can be caused by drugs such as immune checkpoint inhibitors (ICIs). While there a few reports of sclerosing cholangitis after immune checkpoint inhibitor administration, no case has been reported after discontinuation of such drugs.

CASE SUMMARY

A 68-year-old man who underwent chemotherapy for lung adenocarcinoma with bone metastasis presented with abdominal pain and fever 4 mo after the final administration of pembrolizumab. Computed tomography revealed thickening of the gallbladder wall and dilatation of the common bile duct. Endoscopic retrograde cholangiopancreatography revealed an irregularly narrowed intrahepatic bile duct. Biopsy of the bile duct demonstrated that $CD8^+$ T cells were predominant over $CD4^+$T cells. Liver biopsy showed dominant infiltration of $CD8^+$T in the portal tract, but onion-skin lesions were not observed. The patient was diagnosed with immune-related sclerosing cholangitis induced by pembrolizumab. Administration of methylprednisolone and endoscopic nasobiliary drainage were performed, but the cholangiography and laboratory test findings did not improve. No further treatment was administered due to disease progression, and the patient was referred for palliative care.

CONCLUSION

Immune-related sclerosing cholangitis may have a late onset, and such cases occurring after discontinuation of ICIs should be carefully managed.

Key Words: Immune-related adverse events; Sclerosing cholangitis; Delayed immune-related events; Case report

Core Tip: Immune checkpoint inhibitors have become a new standard in cancer treatment, but have often been reported to induce adverse events, called immune-related adverse events (irAEs). Biliary system complications, such as irAEs, remain rare, and the management strategy remains unclear. We present herein, a rare case of delayed immune-related sclerosing cholangitis (SC) after discontinuation of pembrolizumab. Our case emphasizes that immune-related SC can occur later, but the mechanisms have not yet been elucidated. As such, our case contributes to new knowledge in the hopes of being able to establish proper diagnostic criteria and management strategies for similar patients.

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INTRODUCTION

Secondary sclerosing cholangitis (SC) is a chronic disease characterized by biliary obstruction and can be caused by a variety of specific etiologies such as infections, immune-related factors, toxicity, obstruction, ischemic injury, or drugs, including immune checkpoint inhibitors (ICIs)[1]. ICIs have transformed the treatment landscape for patients with many advanced malignancies but have often been reported to induce adverse events, called immune-related adverse events (irAEs)[2]. The irAEs differ from toxicities caused by cytotoxic or molecularly targeted agents, and the time to toxicity may be delayed and not follow a cyclical pattern, as observed with conventional cytotoxic agents[3]. Biliary system complications, such as irAEs, remain rare, and the management strategy remains unclear. There are a few reports of SC induced by ICIs; however, no cases developed immune-related SC after discontinuation of ICIs. Herein, we report a case of delayed immune-related SC caused by pembrolizumab.

CASE PRESENTATION

Chief complaints

A 68-year-old male patient was admitted to our hospital with abdominal pain and fever.

Imaging examinations

Contrast-enhanced computed tomography and endoscopic ultrasonography revealed swelling and wall thickening of the gallbladder and dilatation of the common bile duct without obstruction (Figure 1A-D). Endoscopic retrograde cholangiopancreatography revealed a dilated common bile duct and irregularly narrowed right intrahepatic bile duct (Figure 1E). Intraductal ultrasonography demonstrated diffuse wall thickening from the right bile duct to the common bile duct (Figure 1F).

Laboratory examinations

Laboratory tests revealed increased levels of white blood cells [10100/μL (3300-8600/μL)], C-reactive protein [4.88 mg/dL (0.00-0.18 mg/dL)], aspartate transaminase [31 U/L (13–30 U/L)], alanine transaminase (ALT) [50 U/L (7–23 U/L)], gamma-glutamyl transpeptidase [205 U/L (13-64 U/L)], and alkaline phosphatase (ALP) [996 U/L (38-113 U/L)]. Total bilirubin was within the normal range [0.7 mg/dL (0.4-1.5 mg/dL)], and blood cultures showed no bacterial infection. The serum immunological markers, including antinuclear antibody, antimitochondrial antibody, and anti-smooth muscle antibody were negative, and the immunoglobulin G4 Level (59 mg/dL) was within the normal range.

Physical examination

The patient's body temperature was 38.9 °C, but the other vital signs were stable. Regarding the pulmonary and cardiac examination, no obvious abnormality was observed. Physical examination revealed epigastric tenderness without rebound tenderness or Murphy's sign. No jaundice or palpable masses were observed.

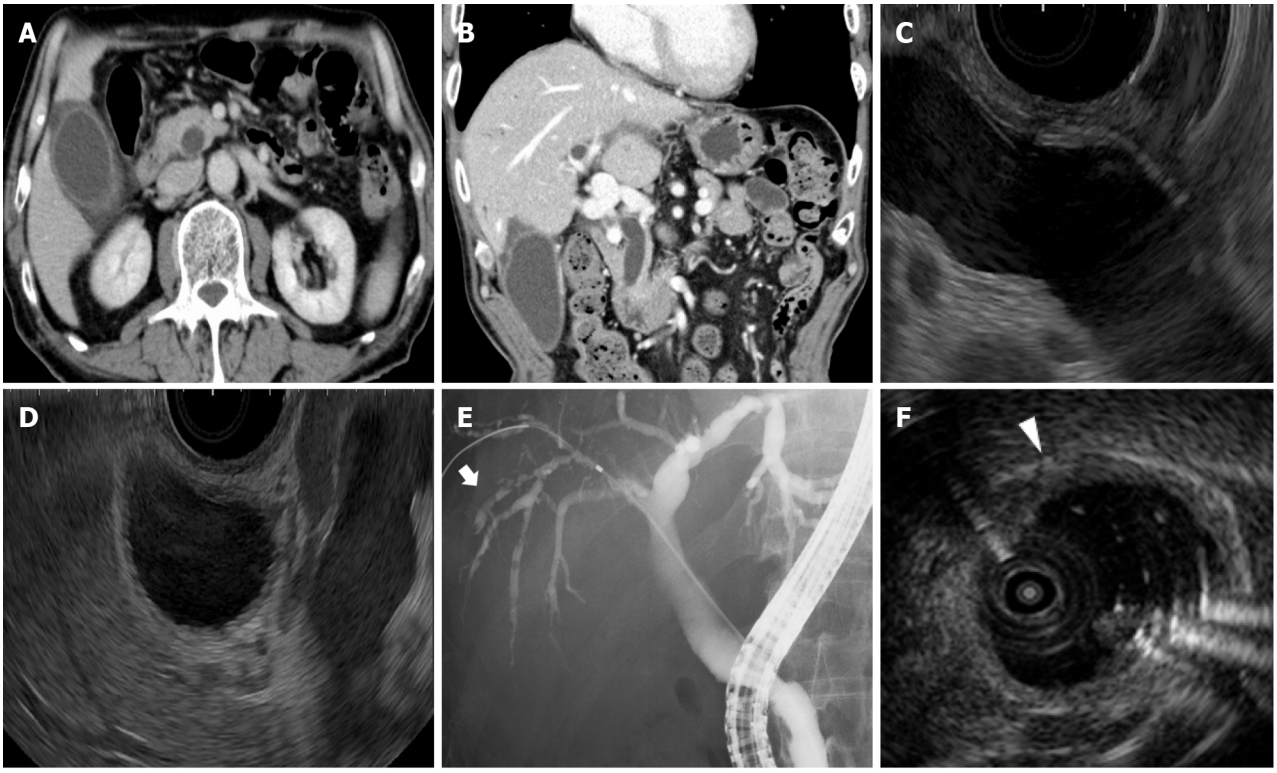

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Figure 1 Imaging examinations of the gallbladder and common bile duct. A, B: Contrast-enhanced computed tomography shows swelling and wall thickness of the gallbladder and common bile duct; C, D: Endoscopic ultrasonography shows dilatation of the common bile duct without obstruction and wall thickness of the gallbladder; E: Endoscopic retrograde cholangiopancreatography shows a dilated common bile duct and irregularly narrowed right intrahepatic bile duct (white arrow); F: Intraductal ultrasonography shows the wall thickness from the right bile duct to the common bile duct (white arrowhead).

Personal and family history

No information was available regarding his family history.

History of past illness

The patient has laparoscopic inguinal hernia repair twenty years ago.

History of present illness

The patient was diagnosed with stage IV lung adenocarcinoma (wild-type epidermal growth factor receptor and anaplastic lymphoma kinase, programmed death ligand 1 positive expression) and received first-line treatment with cisplatin and pemetrexed sodium hydrate. Four months after the initiation of first-line chemotherapy, the patient received second-line treatment with pembrolizumab because of disease progression. Three months later (three cycles of pembrolizumab), carboplatin and nab-paclitaxel were administered as third-line treatments. Four months after discontinuation of pembrolizumab (3 mo after initiation of third-line chemotherapy), the patient was admitted to our hospital with abdominal pain and fever.

FINAL DIAGNOSIS

The common bile duct biopsy showed intraepithelial infiltration of lymphocytes, and $CD8^+$ T cells were more predominant in the biliary epithelium than $CD4^+$ T cells (Figure 2A-C). Percutaneous ultrasonography-guided liver biopsy was also performed for differential diagnosis of other liver diseases. The liver biopsy showed infiltration of predominantly $CD8^+$ T cells in the portal area, but the periductal "onion-skin" fibrosis characteristic of primary sclerosing cholangiopathies, and panlobular hepatitis or isolated central zonal necrosis characteristic of acute hepatitis were not detected (Figure 2D-G). According to these imaging and pathological findings, the patient was diagnosed with immune-related SC induced by pembrolizumab.

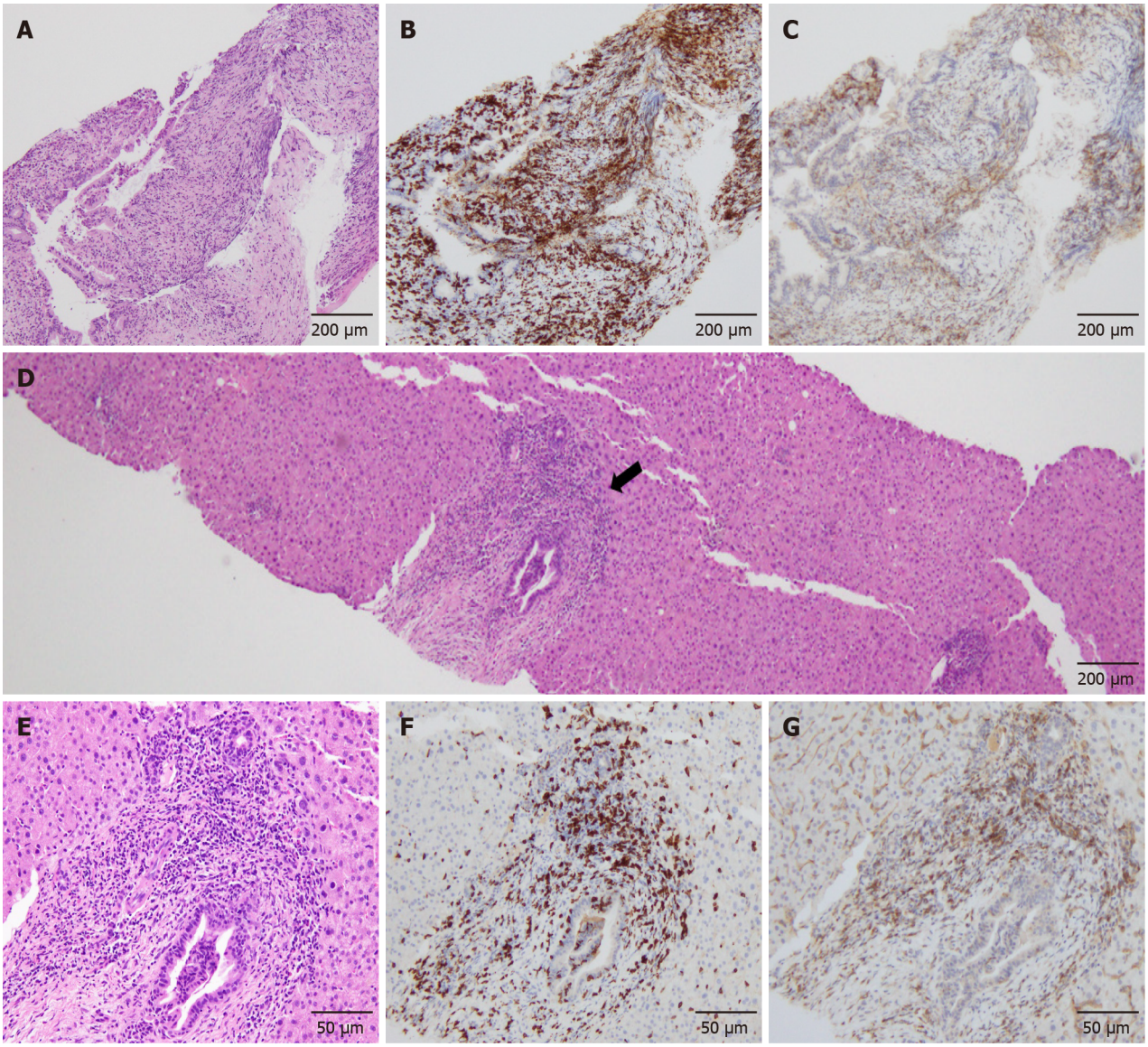

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Figure 2 Pathological findings of the common bile duct and liver. A: Hematoxylin–eosin staining (× 40): Intraepithelial infiltrations of lymphocytes are observed; B and C: CD8 (B) and CD4 (C) staining (× 40): Infiltration of $CD8^+$ T cells are predominant in the biliary epithelium compared to $CD4^+$ T cells; D and E: Hematoxylin–eosin staining: On liver biopsy, inflammation of the hepatic parenchyma is not observed. The periductal "onion-skin" fibrosis is not observed in the portal area (black arrow); F and G: CD8 (F) and CD4 (G) staining (× 200): In a zoomed-in view of the portal area, predominant infiltration of $CD8^+$ T cells, compared with $CD4^+$ T cells, is observed.

TREATMENT

Endoscopic nasobiliary drainage was performed and administration of 25 mg (0.5 mg/kg) of methylprednisolone was initiated. However, grade 3 elevation of ALP and grade 1 elevation of ALT were still present, and the cholangiography findings did not improve.

OUTCOME AND FOLLOW-UP

Because of disease progression, no further treatment was administered, and the patient was referred for palliative care.

DISCUSSION

ICIs enhance antitumor immunity by blocking negative regulators of T cell function that exist on both

immune and tumor cells. Although these agents can lead to remarkable responses, their use may also be associated with irAEs. A previous study showed that most irAEs occur in the first few months of treatment, but late-onset toxicity even after ICI discontinuation is also possible[4]. To the best of our knowledge, no case of immune-related SC after pembrolizumab discontinuation has been reported.

Kawakami *et al*[5] revealed that immune-related cholangitis was characterized by: (1) Localized extrahepatic bile duct dilation without obstruction; (2) Diffuse hypertrophy of the extrahepatic bile duct wall; (3) A dominant increase in the biliary tract enzymes ALP and gamma-glutamyl transpeptidase relative to the hepatic enzymes aspartate and alanine aminotransferase; (4) Normal or reduced levels of the serum immunological markers antinuclear antibody, antimitochondrial antibody, smooth muscle antibody, and immunoglobulin G4; (5) Pathological findings of biliary tract cluster of differentiation $CD8^+$ T cell infiltration from liver biopsy; and (6) A moderate to poor response to steroid therapy. The present case met all these characteristics of immune-related cholangitis.

In contrast, SC developed while carboplatin and nab-paclitaxel were administered. Previous studies reported that chemotherapy-induced SC and drug-induced SC appeared to be one or more strictures of the large bile ducts, mainly the common hepatic duct and the right and left hepatic ducts, usually sparing the common bile duct and smaller intrahepatic ducts[6,7]. Histologically, Weber *et al*[8] showed that a shift in the $CD4^+/CD8^+$ cell ratio in favor of $CD8^+$ cytotoxic T lymphocytes is useful in discriminating irAEs from other conditions (*e.g.*, autoimmune diseases and drug-induced injury). In our case, an irregularly narrowed right intrahepatic bile duct and infiltration of predominantly $CD8^+$ T cells were observed. Therefore, we diagnosed the patient with SC induced by pembrolizumab on the basis of the imaging and pathological findings of previous reports.

Recently, new reports of irAEs emerging after discontinuation of immunotherapy, a clinical diagnostic complex termed delayed immune-related events (DIRE), have surfaced[9]. The first case of DIRE after discontinuation of pembrolizumab was reported in 2013[10], and various late-onset irAEs have also been reported in the literature[11]. Anti-PD-1 antibodies continue to bind to lymphocytes several weeks after the last infusion of PD-1 inhibitors, which explains the development of irAEs after discontinuation of ICI therapy[12]. Although there have been some reports of DIRE after discontinuation of pembrolizumab, no cases of delayed immune-related SC induced by pembrolizumab have been reported.

Previous studies showed that steroid therapy was recommended for the treatment of irAEs, but was not useful for the treatment of immune-related SC[4,13]. Ursodeoxycholic acid (UDCA) and other anti-inflammatory agents, including immunomodulators and infliximab, have been considered for the treatment of irAEs, but their efficacy and response have been reported to be insufficient for the treatment of immune-related SC[14,15], and more cases may be needed to evaluate the usefulness of these drugs for immune-related SC. Biliary drainage has been reported to be ineffective for immune-related SCs[5,16,17]. It remains uncertain why there is a poor response to steroid therapy and biliary drainage for immune-related SC, and further studies are necessary to establish the treatment for immune-related SC.

This indicates that immune-related SC can occur later, similar to other hepatic irAEs. The imaging features and pathological findings of immune-related SC have become almost clear, but the mechanism and onset time of immune-related SC have not been elucidated. Further studies and accumulation of cases are necessary to establish appropriate diagnostic criteria and management strategies, and caution should be exercised for late-onset irAEs after discontinuation of ICIs.

CONCLUSION

Immune-related sclerosing cholangitis may have a late onset, and such cases occurring after discontinuation of immune checkpoint inhibitors should be carefully managed.

FOOTNOTES

Author contributions: All authors contributed to the study conception and design; Tanaka T, Tsujimae M, Yamada Y, and Kobayashi T performed the material preparation, data collection and analysis; Tanaka T wrote the first draft of the manuscript; Sakai A designed the study concept; Masuda A and Kodama Y were involved in study supervision and revised the manuscript; All authors commented on previous versions of the manuscript, read and approved the final manuscript.

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REFERENCES

1 **Brooling J**, Leal R. Secondary Sclerosing Cholangitis: a Review of Recent Literature. *Curr Gastroenterol Rep* 2017; **19**: 44 [PMID: 28752474 DOI: 10.1007/s11894-017-0583-8]
2 **Hofmann L**, Forschner A, Loquai C, Goldinger SM, Zimmer L, Ugurel S, Schmidgen MI, Gutzmer R, Utikal JS, Göppner D, Hassel JC, Meier F, Tietze JK, Thomas I, Weishaupt C, Leverkus M, Wahl R, Dietrich U, Garbe C, Kirchberger MC, Eigentler T, Berking C, Gesierich A, Krackhardt AM, Schadendorf D, Schuler G, Dummer R, Heinzerling LM. Cutaneous, gastrointestinal, hepatic, endocrine, and renal side-effects of anti-PD-1 therapy. *Eur J Cancer* 2016; **60**: 190-209 [PMID: 27085692 DOI: 10.1016/j.ejca.2016.02.025]
3 **Khoja L**, Day D, Wei-Wu Chen T, Siu LL, Hansen AR. Tumour- and class-specific patterns of immune-related adverse events of immune checkpoint inhibitors: a systematic review. *Ann Oncol* 2017; **28**: 2377-2385 [PMID: 28945858 DOI: 10.1093/annonc/mdx286]
4 **de La Rochefoucauld J**, Noël N, Lambotte O. Management of immune-related adverse events associated with immune checkpoint inhibitors in cancer patients: a patient-centred approach. *Intern Emerg Med* 2020; **15**: 587-598 [PMID: 32144552 DOI: 10.1007/s11739-020-02295-2]
5 **Kawakami H**, Tanizaki J, Tanaka K, Haratani K, Hayashi H, Takeda M, Kamata K, Takenaka M, Kimura M, Chikugo T, Sato T, Kudo M, Ito A, Nakagawa K. Imaging and clinicopathological features of nivolumab-related cholangitis in patients with non-small cell lung cancer. *Invest New Drugs* 2017; **35**: 529-536 [PMID: 28317087 DOI: 10.1007/s10637-017-0453-0]
6 **Sandrasegaran K**, Alazmi WM, Tann M, Fogel EL, McHenry L, Lehman GA. Chemotherapy-induced sclerosing cholangitis. *Clin Radiol* 2006; **61**: 670-678 [PMID: 16843750 DOI: 10.1016/j.crad.2006.02.013]
7 **Visentin M**, Lenggenhager D, Gai Z, Kullak-Ublick GA. Drug-induced bile duct injury. *Biochim Biophys Acta Mol Basis Dis* 2018; **1864**: 1498-1506 [PMID: 28882625 DOI: 10.1016/j.bbadis.2017.08.033]
8 **Weber JS**, Yang JC, Atkins MB, Disis ML. Toxicities of Immunotherapy for the Practitioner. *J Clin Oncol* 2015; **33**: 2092-2099 [PMID: 25918278 DOI: 10.1200/JCO.2014.60.0379]
9 **Couey MA**, Bell RB, Patel AA, Romba MC, Crittenden MR, Curti BD, Urba WJ, Leidner RS. Delayed immune-related events (DIRE) after discontinuation of immunotherapy: diagnostic hazard of autoimmunity at a distance. *J Immunother Cancer* 2019; **7**: 165 [PMID: 31269983 DOI: 10.1186/s40425-019-0645-6]
10 **Hanrahan P**, Van Der Westhuizen A, Collins S, Owens D, Hersey P. Delayed autoimmune effects in patients treated with antibodies against the checkpoint inhibitor PD1. *J Dtsch Dermatol Ges* 2013; **11**: 16 [DOI: 10.1111/ddg.12015_4]
11 **Kanaoka K**, Moriizumi K, Okada H, Iwahashi K, Tsuji H, Yasuoka H, Minami S. Pembrolizumab-Induced Delayed-Onset Hepatitis. *Case Rep Gastroenterol* 2020; **14**: 586-592 [PMID: 33250701 DOI: 10.1159/000509953]
12 **Osa A**, Uenami T, Koyama S, Fujimoto K, Okuzaki D, Takimoto T, Hirata H, Yano Y, Yokota S, Kinehara Y, Naito Y, Otsuka T, Kanazu M, Kuroyama M, Hamaguchi M, Koba T, Futami Y, Ishijima M, Suga Y, Akazawa Y, Machiyama H, Iwahori K, Takamatsu H, Nagatomo I, Takeda Y, Kida H, Akbay EA, Hammerman PS, Wong KK, Dranoff G, Mori M, Kijima T, Kumanogoh A. Clinical implications of monitoring nivolumab immunokinetics in non-small cell lung cancer patients. *JCI Insight* 2018; **3** [PMID: 30282824 DOI: 10.1172/jci.insight.59125]
13 **Onoyama T**, Takeda Y, Yamashita T, Hamamoto W, Sakamoto Y, Koda H, Kawata S, Matsumoto K, Isomoto H. Programmed cell death-1 inhibitor-related sclerosing cholangitis: A systematic review. *World J Gastroenterol* 2020; **26**: 353-365 [PMID: 31988594 DOI: 10.3748/wjg.v26.i3.353]
14 **Zhu GQ**, Shi KQ, Huang GQ, Wang LR, Lin YQ, Braddock M, Chen YP, Zhou MT, Zheng MH. A network meta-analysis of the efficacy and side effects of UDCA-based therapies for primary sclerosing cholangitis. *Oncotarget* 2015; **6**: 26757-26769 [PMID: 26378046 DOI: 10.18632/oncotarget.5610]
15 **Puzanov I**, Diab A, Abdallah K, Bingham CO 3rd, Brogdon C, Dadu R, Hamad L, Kim S, Lacouture ME, LeBoeuf NR, Lenihan D, Onofrei C, Shannon V, Sharma R, Silk AW, Skondra D, Suarez-Almazor ME, Wang Y, Wiley K, Kaufman HL, Ernstoff MS; Society for Immunotherapy of Cancer Toxicity Management Working Group. Managing toxicities associated with immune checkpoint inhibitors: consensus recommendations from the Society for Immunotherapy of Cancer (SITC) Toxicity Management Working Group. *J Immunother Cancer* 2017; **5**: 95 [PMID: 29162153 DOI: 10.1186/s40425-017-0300-z]

16 **Kashima J**, Okuma Y, Shimizuguchi R, Chiba K. Bile duct obstruction in a patient treated with nivolumab as second-line chemotherapy for advanced non-small-cell lung cancer: a case report. *Cancer Immunol Immunother* 2018; **67**: 61-65 [PMID: 28913619 DOI: 10.1007/s00262-017-2062-3]

17 **Koya Y**, Shibata M, Shinohara N, Nebuya S, Oe S, Honma Y, Senju M, Sato N, Harada M. Secondary sclerosing cholangitis with hemobilia induced by pembrolizumab: Case report and review of published work. *Hepatol Res* 2019; **49**: 950-956 [PMID: 30861263 DOI: 10.1111/hepr.13329]

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LETTER TO THE EDITOR

Prednisolone induced pneumatosis coli and pneumoperitoneum

Serene S N Goh, Vishal Shelat

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Abstract

Pneumatosis intestinalis (PI) is defined as the presence of gas within the submucosal or subserosal layer of the gastrointestinal tract. It is a radiologic sign suspicious for bowel ischemia, hence non-viable bowel must be ruled out in patients with PI. However, up to 15% of cases with PI are not associated with bowel ischemia or acute abdomen. We described an asymptomatic patient with prednisolone-induced PI and modified the Naranjo score to aid in a surgeon's decision-making for emergency laparotomy *vs* non-operative management with serial assessment in patients who are immunocompromised due to long-term steroid use.

Key Words: Benign pneumatosis; Pneumatosis coli; Pneumatosis intestinalis; Prednisolone

Core Tip: We described an asymptomatic patient with prednisolone-induced pneumatosis intestinalis and modified the Naranjo score to aid in a surgeon's decision-making for emergency laparotomy *vs* non-operative management with serial assessment in patients who are immunocompromised due to long-term steroid use.

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TO THE EDITOR

We read with interest the report by Azzaroli *et al*[1], who conservatively managed two patients with benign pneumatosis intestinalis (PI). We would like to share a similar

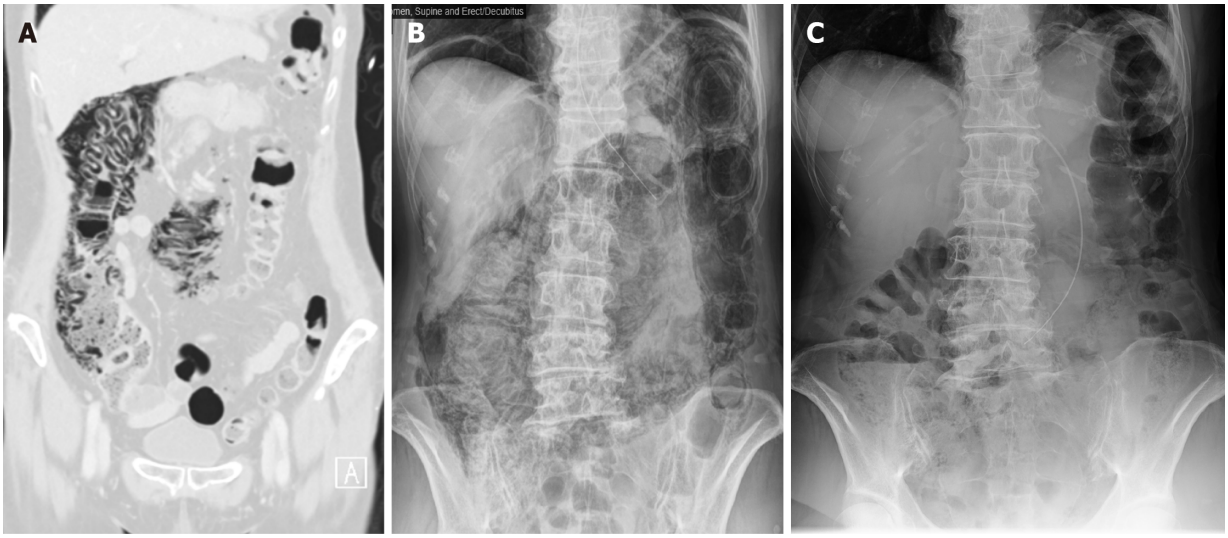

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Figure 1 Computed tomography of abdomen and pelvis and serial erect abdominal radiographs showing interval improvement in pneumatosis coli and resolution of pneumoperitoneum. A: First admission day. Pneumatosis coli from cecum to transverse colon; B: 2 wk after admission. Progression of pneumatosis coli and pneumoperitoneum; C: 2 wk Post-hyperbaric oxygen therapy. Resolution of pneumoperitoneum and pneumatosis coli.

clinical case with prednisolone-induced pneumatosis coli and propose a modified Naranjo score for prednisolone-induced pneumatosis.

A 71-year-old lady with dysphagia and diplopia symptoms was diagnosed with Neuromyelitis Optica (NMO). Treatment with prednisolone 20 mg once daily improved her diplopia. Nasogastric tube (NGT) feeding was commenced due to malnourishment from dysphagia. The chest radiograph for NGT placement showed pneumoperitoneum, and she was referred urgently to the surgical unit. She was asymptomatic, afebrile with normal hemodynamics. Abdomen was soft and non-tender. Leukocyte count, procalcitonin, lactate, and arterial blood gas were normal. A computed tomography of abdomen and pelvis (CTAP) with intravenous and NGT contrast confirmed pneumoperitoneum and pneumatosis coli from cecum to splenic flexure (Figure 1). There was no contrast extravasation, portal venous gas, inflammatory pathology, or mesenteric ischemia. Non-operative management with nil enteral feeding, serial abdominal examination, serum tests, and abdominal radiographs (AXR) was done. The patient remained asymptomatic with normal serum tests. A repeat CTAP showed minimal improvement of pneumoperitoneum. A follow-up AXR two weeks later showed worsening of pneumatosis coli. Hyperbaric oxygen therapy (HBOT) was arranged. Five HBOT sessions were performed at 2.2 atmospheric pressure for 90 min. Her abdominal girth reduced from 79 to 73 cm with minimal AXR improvement. Prednisolone was weaned over next five days and she was discharged well on oral diet. At two-weeks outpatient follow-up, AXR showed improvement (Figure 1).

Corticosteroid therapy remains the cornerstone for the treatment of autoimmune diseases. The true incidence of benign PI as an ADR secondary to corticosteroids is unknown[2,3]. The hypothesis is due to atrophy of lymphoid follicles in the bowel wall. Although PI occurred after prednisolone's commencement in our patient, we did not initially stop prednisolone in balancing risk *vs* benefits for NMO therapy. When PI worsened, HBOT was offered due to concerns for secondary bowel ischemia from PI. The HBOT regimen was similar to that described by Feuerstein *et al*[4], who suggested at least three sessions. As our patient's PI improved but did not resolve fully after 5 HBOT sessions, we reduced prednisolone dose. After two weeks of cessation, PI resolved, similar to a report described by Choi *et al* [5].

According to the Naranjo score (adverse drug reaction probability scale) of 6, PI was probably caused by prednisolone in our patient. Naranjo score recommends isolation of drug in toxic concentrations in body fluid, response to placebo administration, and drug rechallenge to evaluate for the occurrence of symptoms. These three criteria are not routinely done due to practical and safety reasons[6]. We propose a modified Naranjo score (Tables 1 and 2) for prednisolone-induced pneumatosis which replaces these three criteria with the following: (1) No symptoms or signs of abdominal pathology; (2) Serum investigations for inflammatory markers (*e.g.*, C-reactive protein and procalcitonin) must be normal; and (3) Imaging studies should rule out hollow viscus perforation or inflammatory abdominal pathology as a cause for PI. With the modified Naranjo score, the causal link of PI due to prednisolone becomes definite. We propose validation of modified Naranjo score.

Table 1 Modified Naranjo score-pneumatosis intestinalis specific score

Question	Yes/No/Do not know	Score
Are there previous conclusive reports on this reaction?	Yes	1
Did the adverse event appear after the suspected drug was administered?	Yes	2
Did the adverse reaction improve when the drug was discontinued, or a specific antagonist was administered?	Yes	1
Are there alternative causes (other than the drug) that could on their own have caused the reaction?	No	0
Was the reaction more severe when the dose was increased or less severe when the dose was decreased?	Yes	1
Did the patient have a similar reaction to the same or similar drugs in any previous exposure?	No	0
Did any objective evidence confirm the adverse event?	Yes	1
Were there any symptoms or signs of abdominal pathology? (instead of isolation of drug in toxic concentrations in body fluid)	No	1
Were the serum inflammatory markers normal? (instead of drug rechallenge to evaluate for reoccurrence of symptoms)	Yes	1
Did imaging studies rule out hollow viscus perforation or inflammatory abdominal organ pathology? (instead of response to placebo administration)	Yes	1
Total score		**9 (definite)**

Table 2 Interpretation of scores

Total score	Interpretation of scores
≥ 9	Definite
5 to 8	Probable
1 to 4	Possible
≤ 0	Doubtful

FOOTNOTES

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REFERENCES

1 **Azzaroli F**, Turco L, Ceroni L, Galloni SS, Buonfiglioli F, Calvanese C, Mazzella G. Pneumatosis cystoides intestinalis. *World J Gastroenterol* 2011; **17**: 4932-4936 [PMID: 22171137 DOI: 10.3748/wjg.v17.i44.4932]
2 **Berritto D**, Crincoli R, Iacobellis F, Iasiello F, Pizza NL, Lassandro F, Musto L, Grassi R. Primary pneumatosis intestinalis of small bowel: a case of a rare disease. *Case Rep Surg* 2014; **2014**: 350312 [PMID: 25478280 DOI: 10.1155/2014/350312]
3 **Paradkar S**. Reported Adverse Drug Reactions During the Use of Corticosteroids in a Tertiary Care Hospital. *Ther Innov Regul Sci* 2019; **53**: 128-131 [PMID: 29759019 DOI: 10.1177/2168479018776262]

4 **Feuerstein JD**, White N, Berzin TM. Pneumatosis intestinalis with a focus on hyperbaric oxygen therapy. *Mayo Clin Proc* 2014; **89**: 697-703 [PMID: 24797647 DOI: 10.1016/j.mayocp.2014.01.026]
5 **Choi JY**, Cho SB, Kim HH, Lee IH, Lee HY, Kang HS, Lee SY. Pneumatosis intestinalis complicated by pneumoperitoneum in a patient with asthma. *Tuberc Respir Dis (Seoul)* 2014; **77**: 219-222 [PMID: 25473410 DOI: 10.4046/trd.2014.77.5.219]
6 **Shelat VG**. Sulfasalazine induced acute pancreatitis in a patient with prior cholecystectomy. *Postgrad Med J* 2021; **97**: 738-739 [PMID: 32848081 DOI: 10.1136/postgradmedj-2020-138648]

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LETTER TO THE EDITOR

Is patient satisfaction sufficient to validate endoscopic anti-reflux treatments?

Mauro Bortolotti

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Abstract

Endoscopic anti-reflux treatment is emerging as a new option for gastro-esophageal reflux disease (GERD) treatment in patients with the same indications as for laparoscopic fundoplication. There are many techniques, the first of which are transoral incisionless fundoplication (TIF) and nonablative radio-frequency (STRETTA) that have been tested with comparative studies and randomized controlled trials, whereas the other more recent ones still require a deeper evaluation. The purpose of the latter is to verify whether reflux is abolished or significantly reduced after intervention, whether there is a valid high pressure zone at the gastroesophageal junction, and whether esophagitis, when present, has disappeared. Unfortunately in a certain number of cases, and especially in the more recently introduced ones, the evaluation has been based almost exclusively on subjective criteria, such as improvement in the quality of life, remission of heartburn and regurgitation, and reduction or suspension of antacid and antisecretory drug consumption. However, with the most studied techniques such as TIF and STRETTA, an improvement in symptoms better than that of laparoscopic fundoplication can often be observed, whereas the number of acid episodes and acid exposure time are similar or higher, as if the acid refluxes are better tolerated by these patients. The suspicion of a local hyposensitivity taking place after anti-reflux endoscopic intervention seems confirmed by a Bernstein test at least for STRETTA. This examination should be done for all the other techniques, both old and new, to identify the ones that reassure rather than cure. In conclusion, the evaluation of the effectiveness of the endoscopic anti-reflux techniques should not be based exclusively on subjective criteria, but should also be confirmed by objective examinations, because there might be a gap between the improvement in symptoms declared by the patient and the underlying pathophysiologic alterations of GERD.

Key Words: Endoscopic anti-reflux treatment; Transoral incisionless fundoplication; Nonablative radio-frequency; Anti-reflux mucosectomy; Gastro-esophageal reflux disease; Laparoscopic Nissen fundoplication

Core Tip: Endoscopic anti-reflux treatments are being increasingly used instead of anti-reflux surgery. However, most of them have been evaluated only on the ground of subjective symptoms, without performing any objective examination. Furthermore, some also appear to be more effective than surgery in improving acid reflux symptoms, even if their acid exposure is worse, suggesting a reduced sensitivity. The Bernstein test performed after nonablative radio-frequency seems to confirm this hypothesis. Hence, to verify the effectiveness of these esophageal anti-reflux interventions, in addition to evaluating the symptoms before and after the intervention, it is necessary to perform objective examinations.

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TO THE EDITOR

In the past few years, endoscopic anti-reflux treatments have increasingly caught the attention of many gastroenterological centers, as they offer a minimally invasive option for patients with gastroesophageal reflux disease (GERD) who show refractoriness or intolerance to proton pump inhibitors (PPI), or who refuse to take lifelong medication, but want to avoid a surgical intervention, such as the classical laparoscopic fundoplication (LF). A recent article[1] has provided an up-to-date review of the technical aspects, clinical effectiveness, and safety of the main endoscopic anti-reflux procedures including transoral incisionless fundoplication (TIF), nonablative radio-frequency (STRETTA), and Medigus ultrasonic surgical endostapler (MUSE), together with the following still experimental techniques, such as full-thickness endoscopic plication device, anti-reflux mucosectomy (ARMS), anti-reflux mucosal ablation, and band-assisted ligation techniques. However, two observations can be made after consulting the literature studies on the effectiveness of these systems.

First, in most studies the efficacy of an endoscopic anti-reflux technique has been evaluated almost exclusively considering subjective symptoms, such as the improvement in quality of life, remission of heartburn and regurgitation, and reduction or suspension of antacid and antisecretory drugs consumption, to the extent that it has been deemed a success when the patient halved the dose of PPI used prior to intervention.

Second, in most studies where these endoscopic interventions are compared with LF or PPI therapy, symptom improvement after endoscopic treatments is frequently greater than that obtained with LF, even when the 24 h pH recording shows a worse result.

This statement can be easily verified in the systematic review and network meta-analysis of Richter *et al*[2] from seven randomized controlled trials (RCTs) with a total of 1128 patients, concerning a comparison of laparoscopic Nissen fundoplication (LNF) *vs* TIF or PPI treatment. The results indicate that TIF has the highest likelihood of increasing the patient's health-related quality of life (HRQL) followed by LNF, whereas the LNF has the highest likelihood of increasing the percentage of time with distal esophageal pH > 4, followed by PPI and then by TIF, which, in addition, has a higher likelihood than LNF for persistent esophagitis.

Also in the randomized controlled trial of Witteman *et al*[3] performed in 60 patients to evaluate the effectiveness of TIF in comparison with the PPI treatment, GERD symptoms after 6 mo are significantly more improved in the TIF group than in the PPI group, despite the similar improvement in distal esophageal acid exposure, whereas the pH normalization for TIF group is 50% with respect to 63% for the PPI group.

A systematic review and network meta-analysis[4] comprising 516 patients from 10 RCTs compared the anti-reflux efficacy of Stretta, TIF, and PPI. Both STRETTA and TIF are significantly superior to PPI in improving GERD-HRQL and heartburn scores, whereas PPI is better in decreasing the percentage of time with pH < 4.0 when compared with TIF.

Also in the study of He *et al*[5], the symptom score improvement was significantly higher in the STRETTA group of 26 patients compared with the PPI group of 21 patients after 6 mo, whereas both interventions improved, without significant differences, the 24 h pH parameters, including the number of acid episodes, acid exposure time, and DeMeester score.

Furthermore, the absence of correlation between the improvement in GER symptoms and the decrease in acid reflux in patients treated with STRETTA is highlighted by the following two studies.

Coron *et al*[6] in an article comparing the results in 20 patients undergoing STRETTA and 16 patients treated with PPI report that GERD-HRQL scores do not differ between groups, whereas no significant

change in esophageal acid exposure is noted between baseline and 6 mo after STRETTA treatment. So they came to the conclusion that the efficacy of the STRETTA treatment does not seem to be related to a decrease in esophageal acid exposure. In addition, the study of Arts *et al*[7] shows that 3 mo after STRETTA procedure on 11 patients, the symptom score was significantly improved, whereas no changes were observed in esophageal acid exposure.

In view of all these results, we may be led to believe that the greater improvements in GER symptoms observed after TIF and STRETTA applications as compared with LF and PPI treatments, despite the scarce improvement in GER objective parameters, likely depend on another factor, that could be identified with a decreased visceral sensitivity. The hypothesis that symptom improvement in these patients depends on a decreased esophageal acid sensitivity is confirmed, at least for STRETTA, by the study of Arts *et al*[8]. They found that 6 mo after the STRETTA procedure in 13 patients, the mean time needed to induce heartburn during esophageal acid perfusion of the Bernstein test increased from 9.5 ± 2.3 (mean ± SD) to 18.1 ± 4.4 min ($P < 0.01$) , whereas five had become insensitive to 30 min acid perfusion, *vs* none at baseline ($P = 0.04$).

Also in the case of TIF, the dissociation between symptoms and objective parameters, if compared with those of LF and PPI, suggests the post-intervention appearance of a hyposensitive condition, similar to that of STRETTA, although a Bernstein test is necessary to confirm this supposition.

The cause of this local hyposensitivity could lie in an effect of radiofrequency (RF) energy of STRETTA on the myenteric and submucosal nervous plexuses carrying sensory receptors, acetylcholine stimulatory and non-adrenergic, non-cholinergic (NANC) inhibitory neurons, as well as on the two branches of the vagus nerve, which pass in the subadventitial space at the esophagogastric junction level. RF energy inducing a thermal injury promoting submucosal fibrosis and muscularis propria hypertrophy, should also act on nervous tissues impairing the inhibitory NANC neurons with a decrease in transient LES relaxations, while increasing its yield pressures[9]. It is likely that RF energy could also act on sensitive neurons of the esophageal wall. In addition, a slight excess of RF energy or a tissue weakness may also cause damage to the vagus nerve passing below, causing delayed gastric emptying and gastroparesis reported by some authors[10-13]. With regard to the other endoscopic procedures, there are no appropriate comparative studies with LNF and PPI treatments, except for the study of Wong *et al*[14], who compared Nissen fundoplication with ARMS. But they did not perform objective measurements for reflux and only evaluated GERD-HRQL and reflux symptoms up to 2 years, without observing any difference between the two groups.

In conclusion, the evaluation of the effectiveness of any endoscopic anti-reflux technique should not be based exclusively on subjective criteria, such as a good response to PPI suspension, remission of heartburn, and GERD-HRQL improvement, but should also be established with objective examinations, such as 24 h pH monitoring, and, if possible, manometric measurement of the high pressure zone in the distal esophagus, besides endoscopy for the assessment of esophagitis. The good capacity of TIF and STRETTA to improve the reflux symptoms and the quality of life more than LF and PPI treatments, is a double-edged weapon, because a scarce prevention of acid reflux in the long term may expose the patient to the risk that more or less serious alterations will pass unnoticed. This risk could also be taken with the other more recent endoscopic techniques, which, as previously mentioned, should be compared with LF or PPI treatments and possibly examined by the Bernstein test.

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FOOTNOTES

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REFERENCES

1 **Rodríguez de Santiago E**, Albéniz E, Estremera-Arevalo F, Teruel Sanchez-Vegazo C, Lorenzo-Zúñiga V. Endoscopic anti-reflux therapy for gastroesophageal reflux disease. *World J Gastroenterol* 2021; **27**: 6601-6614 [PMID: 34754155 DOI: 10.3748/wjg.v27.i39.6601]
2 **Richter JE**, Kumar A, Lipka S, Miladinovic B, Velanovich V. Efficacy of Laparoscopic Nissen Fundoplication vs Transoral Incisionless Fundoplication or Proton Pump Inhibitors in Patients With Gastroesophageal Reflux Disease: A Systematic Review and Network Meta-analysis. *Gastroenterology* 2018; **154**: 1298-1308.e7 [PMID: 29305934 DOI: 10.1053/j.gastro.2017.12.021]
3 **Witteman BP**, Conchillo JM, Rinsma NF, Betzel B, Peeters A, Koek GH, Stassen LP, Bouvy ND. Randomized controlled trial of transoral incisionless fundoplication vs. proton pump inhibitors for treatment of gastroesophageal reflux disease. *Am J Gastroenterol* 2015; **110**: 531-542 [PMID: 25823768 DOI: 10.1038/ajg.2015.28]
4 **Xie P**, Yan J, Ye L, Wang C, Li Y, Chen Y, Li G. Efficacy of different endoscopic treatments in patients with gastroesophageal reflux disease: a systematic review and network meta-analysis. *Surg Endosc* 2021; **35**: 1500-1510 [PMID: 33650003 DOI: 10.1007/s00464-021-08386-1]
5 **He S**, Xu F, Xiong X, Wang H, Cao L, Liang N, Jing X, Liu T. Stretta procedure versus proton pump inhibitors for the treatment of nonerosive reflux disease: A 6-month follow-up. *Medicine (Baltimore)* 2020; **99**: e18610 [PMID: 32011441 DOI: 10.1097/MD.0000000000018610]
6 **Coron E**, Sebille V, Cadiot G, Zerbib F, Ducrotte P, Ducrot F, Pouderoux P, Arts J, Le Rhun M, Piche T, Bruley des Varannes S, Galmiche JP; Consortium de Recherche Indépendant sur le Traitement et L'exploration du Reflux Gastro-oesophagien et de L'endobrachyoesophage (CRITERE). Clinical trial: Radiofrequency energy delivery in proton pump inhibitor-dependent gastro-oesophageal reflux disease patients. *Aliment Pharmacol Ther* 2008; **28**: 1147-1158 [PMID: 18616516 DOI: 10.1111/j.1365-2036.2008.03790.x]
7 **Arts J**, Bisschops R, Blondeau K, Farré R, Vos R, Holvoet L, Caenepeel P, Lerut A, Tack J. A double-blind sham-controlled study of the effect of radiofrequency energy on symptoms and distensibility of the gastro-esophageal junction in GERD. *Am J Gastroenterol* 2012; **107**: 222-230 [PMID: 22108449 DOI: 10.1038/ajg.2011.395]
8 **Arts J**, Sifrim D, Rutgeerts P, Lerut A, Janssens J, Tack J. Influence of radiofrequency energy delivery at the gastroesophageal junction (the Stretta procedure) on symptoms, acid exposure, and esophageal sensitivity to acid perfusion in gastroesophagal reflux disease. *Dig Dis Sci* 2007; **52**: 2170-2177 [PMID: 17436101 DOI: 10.1007/s10620-006-9695-y]
9 **Sowa P**, Samarasena JB. Nonablative Radiofrequency Treatment for Gastroesophageal Reflux Disease (STRETTA). *Gastrointest Endosc Clin N Am* 2020; **30**: 253-265 [PMID: 32146945 DOI: 10.1016/j.giec.2019.12.006]
10 **Richards WO**, Scholz S, Khaitan L, Sharp KW, Holzman MD. Initial experience with the stretta procedure for the treatment of gastroesophageal reflux disease. *J Laparoendosc Adv Surg Tech A* 2001; **11**: 267-273 [PMID: 11642661 DOI: 10.1089/109264201317054546]
11 **Dughera L**, Navino M, Cassolino P, De Cento M, Cacciotella L, Cisarò F, Chiaverina M. Long-Term Results of Radiofrequency Energy Delivery for the Treatment of GERD: Results of a Prospective 48-Month Study. *Diagn Ther Endosc* 2011; **2011**: 507157 [PMID: 22110288 DOI: 10.1155/2011/507157]
12 **Houston H**, Khaitan L, Holzman M, Richards WO. First year experience of patients undergoing the Stretta procedure. *Surg Endosc* 2003; **17**: 401-404 [PMID: 12436238 DOI: 10.1007/s00464-002-8923-6]
13 **Aziz AM**, El-Khayat HR, Sadek A, Mattar SG, McNulty G, Kongkam P, Guda MF, Lehman GA. A prospective randomized trial of sham, single-dose Stretta, and double-dose Stretta for the treatment of gastroesophageal reflux disease. *Surg Endosc* 2010; **24**: 818-825 [PMID: 19730952 DOI: 10.1007/s00464-009-0671-4]
14 **Wong HJ**, Su B, Attaar M, Kuchta K, Stearns S, Linn JG, Haggerty SP, Denham W, Ujiki MB. Anti-reflux mucosectomy (ARMS) results in improved recovery and similar reflux quality of life outcomes compared to laparoscopic Nissen fundoplication. *Surg Endosc* 2021; **35**: 7174-7182 [PMID: 33237465 DOI: 10.1007/s00464-020-08144-9]

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LETTER TO THE EDITOR

Endoluminal vacuum-assisted therapy as a treatment for anastomotic leakage in colorectal surgery

Maria Michela Chiarello, Valentina Bianchi, Pietro Fransvea, Giuseppe Brisinda

Specialty type: Gastroenterology and hepatology

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Abstract

Anastomotic leakage (AL) has a wide range of clinical features ranging from radiological only findings to peritonitis and sepsis with multiorgan failure. An early diagnosis of AL is essential in order to establish the most appropriate treatment for this complication. Despite AL continues to be a dreadful complication after colorectal surgery, there has been no consensus on its management. However, based on patient's presentation and timing of the AL, there has been a gradual shift to a more conservative management, keeping surgery as the last option Reoperation for sepsis control is rarely necessary especially in those patients who already have a diverting stoma at the time of the leak. A nonoperative management is usually preferred in these patients. There are several treatment options, also for patients without a stoma who do not require a reoperation for a contained pelvic leak, including recently developed endoscopic procedures, such as clip placement or endoluminal vacuum-assisted therapy. More conservative treatments could be an option in patients who are clinically stable or in presence of a small defect.

Key Words: Anastomotic leakage; Colorectal cancer; Colorectal surgery; Mortality; Morbidity

Core Tip: The authors of the review have a remarkable clinical experience and scientific authority in colorectal surgery and related complications. The authors focus their attention on endoluminal vacuum therapy to treat anastomotic leakage in colorectal surgery. The authors highlight that most studies are heterogeneous in term of success rate definition, salvage and long-term results. Furthermore, there is paucity of comparative studies and thus definitive conclusions are not warranted at present time, as pointed out by the authors in their narrative review.

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TO THE EDITOR

We were pleased to read the high-level article on treatment of colorectal anastomotic leakage (AL) with endoluminal vacuum therapy (EVT), published by Vignali and De Nardi[1]. The authors have a remarkable clinical experience and scientific authority in colorectal surgery and related complications[2-4].

We agree with the authors that AL is still the worst complication of colorectal surgery today. The consequences of AL can severely alter functional outcomes and oncological results[5]. In the case of AL, the severity of the clinical picture is extremely variable. Some AL have no impact on the patient, while others present with sepsis and can be fatal[6-8].

The incidence of AL is variable[1,5,9]. We believe that this variability in incidence is related to several factors. In accordance with the literature data, we believe that variables such as neoplastic or inflammatory disease, timing of the operation, the distance of the tumor from the anal merge, the clinical presentation with signs of visceral perforation or intestinal obstruction, the local characteristics of the tumor and the surgery performed are important for the postoperative onset of AL. In this context, the need for a common definition appears to be fundamental. AL occurs when a surgical anastomosis fails. In this circumstance, the intestinal contents leak out of the surgical connection. A pelvic abscess close to the anastomosis must be considered as AL. The classification of severity degrees proposed by the International Study Group of Rectal Cancer (ISREC)[10] is the most widely adopted. ISREC[10] proposes a classification of AL into three types (A, B and C) of increasing severity according to the therapeutic management it requires (conservative management in type A; non-surgical management in type B; surgical treatment in type C).

We believe that the onset of AL is important. The time to onset of AL affects both the severity of the complication and also the treatment of the complication itself. Colonic or rectal stump ischemia due to excessive preparation of the stump itself with consequent interruption of the perianastomotic supply of microvascular blood or tension in the anastomotic site is usually responsible for the early onset of AL [11]. On the other hand, the late presentation of AL is not linked to surgical technique problems but rather to clinical conditions of impaired tissue healing, such as local sepsis, malnutrition, the intake of immunosuppressive drugs, severe obesity and exposure to radiation[9,12]. In most patients, AL that occurs early is associated with more severe clinical symptoms. Faecal contamination of the peritoneum is frequently observed in these patients and the incidence of emergency laparotomies is higher. In conditions of worsening sepsis, mortality rates increase. By contrast, in late forms of AL, clinical manifestations will be characterized by the appearance of a pelvic abscess[13,14].

Furthermore, we believe that early diagnosis of AL is essential in order to establish the most appropriate treatment for this complication[15]. Despite AL continues to be a dreadful complication after colorectal surgery, there has been no consensus on its management[5]. Today, however, there is an increasing use of more conservative management. It is preferred to keep surgery as a last resort. Obviously this attitude is related to the presentation of the patient and the timing of the AL[16-18]. In agreement with Vignali and De Nardi[1], we believe that saving the anastomosis through the use of conservative treatments represents a valid therapeutic option to be used especially in clinically stable patients or in the presence of a small defect.

The authors focus their attention on EVT. Its principle is based on the application of topic negative pressure to drain the cavity and to prevent the development of chronic sinus. The paragraph on the description of the device is interesting and exhaustive, too. We agree with Vignali and De Nardi[1] that several reviews, and meta-analyses have been published so far with promising results. Nevertheless, the analysis of most documents yields mixed results, especially when the success rate, anastomosis salvage rates, and long-term outcomes are taken into account. Furthermore, the paucity of comparative studies does not allow definitive conclusions to be drawn, as pointed out by the authors in their narrative review.

There are several treatment options, including recently developed endoscopic procedures, such as stent or clip placement or EVT. These procedures can be used primarily in patients who already have a diverting stoma at the time of the leak. These treatments also find application in patients with extraperitoneal anastomosis and in those without ostomy who do not require further surgery due to a contained pelvic loss (type B of the ISREC classification). EVT is performed endoscopically. An open-pored polyurethane sponge is positioned in the leakage cavity through the anastomotic defect. The main advantages of the treatment are represented by a less invasive approach and continuous drainage. Treatment favors granulation and vascularity; furthermore, it determines a mechanical reduction in the size of the abscess cavity[19-21]. The main indication for the use of the EVT are extraperitoneal, low leaks that are difficult to drain. A disadvantage to the systematic adoption of EVT is the fact that the sponges must be periodically changed, usually more than 8 times in 4-6 wk. Anastomotic necrosis and stricture are the most common complications of the procedure.

The AL treatment should be tailored taking into consideration many factors, giving that there is no universally accepted management flowchart for the optimal treatment of this complication. Non-operative management is usually preferred in patients with extraperitoneal anastomosis without sepsis and peritonitis and in those who underwent proximal faecal diversion at the initial operation[5]. Excluding complications directly related to the endoluminal device, the factors associated with the failure of a conservative approach using EVT are mainly represented by neoadjuvant therapy, lack of a protective stoma before treatment and male sex. However, it must be emphasized that most of these are well known risk factors for AL in general. The timing of EVT can influence success, significantly. A high success rate occurs when endoluminal therapy is started early (within 6 wk) after the onset of AL[22, 23]. Riss *et al*[24] found that 25% of patients assessed after primary successful EVT in a multicentre study developed recurrent abscesses after a median follow-up of 17 mo. No study in the literature has focused attention on oncological and functional outcomes.

It seems obvious to us that in case of AL it is better to prevent its complications rather than treat them once they have arisen. Unfortunately, we believe that complications after colorectal surgery are still inevitable. We are sure that also Vignali and De Nardi[1] agree with our orientation.

Moreover, surgeons have employed several intraoperative techniques to assess integrity of the colorectal anastomoses in order to minimize the risks of postoperative complications. Basic mechanic patency tests (traditional air leak testing, saline leak, and methylene blue leak tests), endoscopic visualization techniques, and more recently, micro perfusion assessment technology are the most commonly used methods. Other methods commonly adopted to evaluate the blood perfusion of the intestinal segments to be used for the creation of the anastomosis are the visual evaluation of the color of the intestinal wall, the presence of visible peristalsis and bleeding from the marginal arteries. These tests are limited. None of these tests allow viewing of the lumen. The anastomosis is assessed by occluding the proximal lumen and then filling the intraluminal cavity with air or fluid and checking for leakage[2,25].

Intraoperative endoscopy was used to visualize the anastomosis. However, the use of the method is not so widespread. Furthermore, the results of intraoperative endoscopy are not of univocal interpretation. A recent systematic review evaluating the results of five non-randomized controlled trials, in which a total of over 900 patients were enrolled, documented an incidence rate of AL of 7.7% in patients with documented AL at intraoperative endoscopy, with no significant difference in postoperative AL rate compared to patients with negative intraoperative endoscopy for AL[26].

Nowadays, a promising and increasingly used new technology is intra-operative fluorescence angiography with indocyanine green[2,27-29]. Evidence for the impact of intraoperative fluorescence angiography in reducing AL after colorectal anastomosis is growing. The procedure allows direct visualization of tissue perfusion. It may help to prevent AL. The use of indocyanine green fluorescence angiography leads to a significant reduction in AL compared to standard intraoperative methods to assess anastomotic blood perfusion in colorectal surgery. Moreover, especially in patients with low or ultra-low rectal resection, the use of indocyanine green fluorescence decreases the need for surgical reintervention for AL.

A diverting stoma ideally protects a low colorectal anastomosis. However, there is a lot of controversy surrounding the use of a protective stoma. The first controversy is certainly about which is the most optimal type of ostomy, an ileostomy or a colostomy[30]. We believe that ostomy is useful to reduce clinical symptoms of AL by increasing the percentage of sub-clinical dehiscence, but not modifying the overall percentage. Moreover, no significant benefit of a diverting stoma for reducing the risk of AL has been showed in some large-volume studies. Usually, we tend to protect with an ostomy the patients who underwent neoadjuvant radiotherapy and those with an ultralow colorectal anastomosis. In recent years, this dogma has been challenged. Several authors questioning the need of an ostomy for all patients with an ultralow anastomosis.

We believe that the use of a transanastomotic drainage tube (TDT) may be useful in preventing the onset of AL. Regarding the TDT, several studies showed no difference in AL rate between patients with and without one[31]. Other literature observations, instead, have documented a reduction in frequency of AL in patients with TDT[32-35]. Prophylactic TDT presents fewer risks of complication than derivative stoma. It was thought to lower the risk of AL, *per* as documented in a systematic review and meta-analysis pooling 1772 patients undergoing rectal anterior resection[36]. The same conclusions were obtained in another systematic review and meta-analysis, including patients with diverting stoma[37].

Therefore, prophylactic TDT could constitute an efficient method to prevent AL in high-risk patients without exposing them to the complications of a diverting stoma. However, it is necessary to conduct an RCT on a large number of patients in order to obtain definitive results from the comparison of the two techniques.

AL after rectal surgery is a fearsome complication with considerable mortality and morbidity. Many factors are related to the onset of AL in the postoperative period. Some of these factors cannot be changed in the least by medical intervention. It is paramount to identify leaks early to minimize the potential morbidities of this complication. Despite advances in combating surgical infections, new devices for bowel reapproximation, better understanding of risk factors for anastomotic complications, and improved perioperative care, we continue to struggle with the occurrence and management of this complication. Technology will likely never be developed that would allow surgeons to prevent the onset of AL in the postoperative period. The use of the TDT prevents the formation of AL. This is a simple method that could avoid performing diverting ileostomies. AL can be further reduced by fluorescence angiography. This method, while bringing significant intraoperative changes in surgical strategies, allows the direct evaluation of the vascularization of the intestinal segments. In the meantime, it's necessary promptly diagnose AL. Likewise, the knowledge of all the therapeutic possibilities, from the least invasive ones to surgery, is necessary in order to treat AL and improve patient outcomes by reducing the incidence of functional disorders or the impact on survival.

FOOTNOTES

Author contributions: Chiarello MM and Brisinda G conceived the original idea; Fransvea P and Bianchi V performed a comprehensive review of all available literature and summarized the data; Brisinda G and Chiarello MM meet the criteria for authorship established by the International Committee of Medical Journal Editors and verify the validity of the results reported; all authors read and approved the final manuscript.

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REFERENCES

1 **Vignali A**, De Nardi P. Endoluminal vacuum-assisted therapy to treat rectal anastomotic leakage: A critical analysis. *World J Gastroenterol* 2022; **28**: 1394-1404 [PMID: 35582677 DOI: 10.3748/wjg.v28.i14.1394]
2 **De Nardi P**, Elmore U, Maggi G, Maggiore R, Boni L, Cassinotti E, Fumagalli U, Gardani M, De Pascale S, Parise P, Vignali A, Rosati R. Intraoperative angiography with indocyanine green to assess anastomosis perfusion in patients undergoing laparoscopic colorectal resection: results of a multicenter randomized controlled trial. *Surg Endosc* 2020; **34**: 53-60 [PMID: 30903276 DOI: 10.1007/s00464-019-06730-0]
3 **Vignali A**, De Nardi P. Multidisciplinary treatment of rectal cancer in 2014: where are we going? *World J Gastroenterol* 2014; **20**: 11249-11261 [PMID: 25170209 DOI: 10.3748/wjg.v20.i32.11249]
4 **Vignali A**, Ghirardelli L, Di Palo S, Orsenigo E, Staudacher C. Laparoscopic treatment of advanced colonic cancer: a case-matched control with open surgery. *Colorectal Dis* 2013; **15**: 944-948 [PMID: 23398664 DOI: 10.1111/codi.12170]
5 **Chiarello MM**, Fransvea P, Cariati M, Adams NJ, Bianchi V, Brisinda G. Anastomotic leakage in colorectal cancer surgery. *Surg Oncol* 2022; **40**: 101708 [PMID: 35092916 DOI: 10.1016/j.suronc.2022.101708]
6 **Paun BC**, Cassie S, MacLean AR, Dixon E, Buie WD. Postoperative complications following surgery for rectal cancer. *Ann Surg* 2010; **251**: 807-818 [PMID: 20395841 DOI: 10.1097/SLA.0b013e3181dae4ed]
7 **Alves A**, Panis Y, Lelong B, Dousset B, Benoist S, Vicaut E. Randomized clinical trial of early *vs* delayed temporary stoma closure after proctectomy. *Br J Surg* 2008; **95**: 693-698 [PMID: 18446781 DOI: 10.1002/bjs.6212]
8 **Fazio VW**, Zutshi M, Remzi FH, Parc Y, Ruppert R, Fürst A, Celebrezze J Jr, Galanduik S, Orangio G, Hyman N, Bokey L, Tiret E, Kirchdorfer B, Medich D, Tietze M, Hull T, Hammel J. A randomized multicenter trial to compare long-term functional outcome, quality of life, and complications of surgical procedures for low rectal cancers. *Ann Surg* 2007; **246**:

481-8; discussion 488 [PMID: 17717452 DOI: 10.1097/SLA.0b013e3181485617]
9 **Brisinda G**, Vanella S, Cadeddu F, Civello IM, Brandara F, Nigro C, Mazzeo P, Marniga G, Maria G. End-to-end *vs* end-to-side stapled anastomoses after anterior resection for rectal cancer. *J Surg Oncol* 2009; **99**: 75-79 [PMID: 18985633 DOI: 10.1002/jso.21182]
10 **Rahbari NN**, Weitz J, Hohenberger W, Heald RJ, Moran B, Ulrich A, Holm T, Wong WD, Tiret E, Moriya Y, Laurberg S, den Dulk M, van de Velde C, Büchler MW. Definition and grading of anastomotic leakage following anterior resection of the rectum: a proposal by the International Study Group of Rectal Cancer. *Surgery* 2010; **147**: 339-351 [PMID: 20004450 DOI: 10.1016/j.surg.2009.10.012]
11 **Park JS**, Choi GS, Kim SH, Kim HR, Kim NK, Lee KY, Kang SB, Kim JY, Kim BC, Bae BN, Son GM, Lee SI, Kang H. Multicenter analysis of risk factors for anastomotic leakage after laparoscopic rectal cancer excision: the Korean laparoscopic colorectal surgery study group. *Ann Surg* 2013; **257**: 665-671 [PMID: 23333881 DOI: 10.1097/SLA.0b013e31827b8ed9]
12 **Sparreboom CL**, van Groningen JT, Lingsma HF, Wouters MWJM, Menon AG, Kleinrensink GJ, Jeekel J, Lange JF; Dutch ColoRectal Audit group. Different Risk Factors for Early and Late Colorectal Anastomotic Leakage in a Nationwide Audit. *Dis Colon Rectum* 2018; **61**: 1258-1266 [PMID: 30239395 DOI: 10.1097/DCR.0000000000001202]
13 **Lim SB**, Yu CS, Kim CW, Yoon YS, Park IJ, Kim JC. Late anastomotic leakage after low anterior resection in rectal cancer patients: clinical characteristics and predisposing factors. *Colorectal Dis* 2016; **18**: O135-O140 [PMID: 26888300 DOI: 10.1111/codi.13300]
14 **Lim SB**, Yu CS, Kim CW, Yoon YS, Park IJ, Kim JC. The types of anastomotic leakage that develop following anterior resection for rectal cancer demonstrate distinct characteristics and oncologic outcomes. *Int J Colorectal Dis* 2015; **30**: 1533-1540 [PMID: 26260482 DOI: 10.1007/s00384-015-2359-7]
15 **Chiarello MM**, Brisinda G. A Commentary on "Diagnostic accuracy of procalcitonin on POD3 for the early diagnosis of anastomotic leakage after colorectal surgery: A meta-analysis and systematic review" (Int. J. Surg. 2022; 100: 106592). *Int J Surg* 2022; **101**: 106624 [PMID: 35472516 DOI: 10.1016/j.ijsu.2022.106624]
16 **Ahmad NZ**, Abbas MH, Khan SU, Parvaiz A. A meta-analysis of the role of diverting ileostomy after rectal cancer surgery. *Int J Colorectal Dis* 2021; **36**: 445-455 [PMID: 33064212 DOI: 10.1007/s00384-020-03771-z]
17 **Hüser N**, Michalski CW, Erkan M, Schuster T, Rosenberg R, Kleeff J, Friess H. Systematic review and meta-analysis of the role of defunctioning stoma in low rectal cancer surgery. *Ann Surg* 2008; **248**: 52-60 [PMID: 18580207 DOI: 10.1097/SLA.0b013e318176bf65]
18 **Wang Z**, Yang YS, Zhao XF. A novel multi-modal approach for prevention and treatment of anastomotic leakage after low anterior resection in rectal cancer patients. *Asian J Surg* 2022; **45**: 539-541 [PMID: 34642051 DOI: 10.1016/j.asjsur.2021.09.008]
19 **Bobkiewicz A**, Krokowicz L, Banasiewicz T, Borejsza-Wysocki M. Endoscopic vacuum therapy with instillation (iEVT) - a novel endoscopic concept for colorectal anastomotic leak and perianal complications. *Wideochir Inne Tech Maloinwazyjne* 2020; **15**: 560-566 [PMID: 33294070 DOI: 10.5114/wiitm.2020.93204]
20 **Kühn F**, Wirth U, Zimmermann J, Beger N, Hasenhütl SM, Drefs M, Heiliger C, Burian M, Werner J, Schiergens TS. Endoscopic vacuum therapy for in- and outpatient treatment of colorectal defects. *Surg Endosc* 2021; **35**: 6687-6695 [PMID: 33259019 DOI: 10.1007/s00464-020-08172-5]
21 **Shalaby M**, Emile S, Elfeki H, Sakr A, Wexner SD, Sileri P. Systematic review of endoluminal vacuum-assisted therapy as salvage treatment for rectal anastomotic leakage. *BJS Open* 2019; **3**: 153-160 [PMID: 30957061 DOI: 10.1002/bjs5.50124]
22 **Weidenhagen R**, Gruetzner KU, Wiecken T, Spelsberg F, Jauch KW. Endoscopic vacuum-assisted closure of anastomotic leakage following anterior resection of the rectum: a new method. *Surg Endosc* 2008; **22**: 1818-1825 [PMID: 18095024 DOI: 10.1007/s00464-007-9706-x]
23 **van Koperen PJ**, van Berge Henegouwen MI, Rosman C, Bakker CM, Heres P, Slors JF, Bemelman WA. The Dutch multicenter experience of the endo-sponge treatment for anastomotic leakage after colorectal surgery. *Surg Endosc* 2009; **23**: 1379-1383 [PMID: 19037698 DOI: 10.1007/s00464-008-0186-4]
24 **Riss S**, Stift A, Meier M, Haiden E, Grünberger T, Bergmann M. Endo-sponge assisted treatment of anastomotic leakage following colorectal surgery. *Colorectal Dis* 2010; **12**: e104-e108 [PMID: 19508536 DOI: 10.1111/j.1463-1318.2009.01885.x]
25 **Rausa E**, Zappa MA, Kelly ME, Turati L, Russo A, Aiolfi A, Bonitta G, Sgroi LG. A standardized use of intraoperative anastomotic testing in colorectal surgery in the new millennium: is technology taking over? *Tech Coloproctol* 2019; **23**: 625-631 [PMID: 31302816 DOI: 10.1007/s10151-019-02034-6]
26 **Hirst NA**, Tiernan JP, Millner PA, Jayne DG. Systematic review of methods to predict and detect anastomotic leakage in colorectal surgery. *Colorectal Dis* 2014; **16**: 95-109 [PMID: 23992097 DOI: 10.1111/codi.12411]
27 **Amagai H**, Miyauchi H, Muto Y, Uesato M, Ohira G, Imanishi S, Maruyama T, Tochigi T, Okada K, Maruyama M, Matsubara H. Clinical utility of transanal indocyanine green near-infrared fluorescence imaging for evaluation of colorectal anastomotic perfusion. *Surg Endosc* 2020; **34**: 5283-5293 [PMID: 31820154 DOI: 10.1007/s00464-019-07315-7]
28 **Benčurik V**, Škrovina M, Martínek L, Bartoš J, Macháčková M, Dosoudil M, Štěpánová E, Přibylová L, Briš R, Vomáčková K. Intraoperative fluorescence angiography and risk factors of anastomotic leakage in mini-invasive low rectal resections. *Surg Endosc* 2021; **35**: 5015-5023 [PMID: 32970211 DOI: 10.1007/s00464-020-07982-x]
29 **Mangano A**, Gheza F, Chen LL, Minerva EM, Giulianotti PC. Indocyanine Green (Icg)-Enhanced Fluorescence for Intraoperative Assessment of Bowel Microperfusion During Laparoscopic and Robotic Colorectal Surgery: The Quest for Evidence-Based Results. *Surg Technol Int* 2018; **32**: 101-104 [PMID: 29611153]
30 **Güenaga KF**, Lustosa SA, Saad SS, Saconato H, Matos D. Ileostomy or colostomy for temporary decompression of colorectal anastomosis. *Cochrane Database Syst Rev* 2007; CD004647 [PMID: 17253517 DOI: 10.1002/14651858.CD004647.pub2]
31 **Zhao S**, Zhang L, Gao F, Wu M, Zheng J, Bai L, Li F, Liu B, Pan Z, Liu J, Du K, Zhou X, Li C, Zhang A, Pu Z, Li Y, Feng B, Tong W. Transanal Drainage Tube Use for Preventing Anastomotic Leakage After Laparoscopic Low Anterior Resection in Patients With Rectal Cancer: A Randomized Clinical Trial. *JAMA Surg* 2021; **156**: 1151-1158 [PMID:

34613330 DOI: 10.1001/jamasurg.2021.4568]
32 **Choy KT**, Yang TWW, Heriot A, Warrier SK, Kong JC. Does rectal tube/transanal stent placement after an anterior resection for rectal cancer reduce anastomotic leak? *Int J Colorectal Dis* 2021; **36**: 1123-1132 [PMID: 33515307 DOI: 10.1007/s00384-021-03851-8]
33 **Wang Z**, Liang J, Chen J, Mei S, Liu Q. Effectiveness of a Transanal Drainage Tube for the Prevention of Anastomotic Leakage after Laparoscopic Low Anterior Resection for Rectal Cancer. *Asian Pac J Cancer Prev* 2020; **21**: 1441-1444 [PMID: 32458653 DOI: 10.31557/APJCP.2020.21.5.1441]
34 **Kawada K**, Takahashi R, Hida K, Sakai Y. Impact of transanal drainage tube on anastomotic leakage after laparoscopic low anterior resection. *Int J Colorectal Dis* 2018; **33**: 337-340 [PMID: 29270785 DOI: 10.1007/s00384-017-2952-z]
35 **Matsuda M**, Tsuruta M, Hasegawa H, Okabayashi K, Kondo T, Shimada T, Yahagi M, Yoshikawa Y, Kitagawa Y. Transanal drainage tube placement to prevent anastomotic leakage following colorectal cancer surgery with double stapling reconstruction. *Surg Today* 2016; **46**: 613-620 [PMID: 26231480 DOI: 10.1007/s00595-015-1230-3]
36 **Yang Y**, Shu Y, Su F, Xia L, Duan B, Wu X. Prophylactic transanal decompression tube *vs* non-prophylactic transanal decompression tube for anastomotic leakage prevention in low anterior resection for rectal cancer: a meta-analysis. *Surg Endosc* 2017; **31**: 1513-1523 [PMID: 27620910 DOI: 10.1007/s00464-016-5193-2]
37 **Chen H**, Cai HK, Tang YH. An updated meta-analysis of transanal drainage tube for prevention of anastomotic leak in anterior resection for rectal cancer. *Surg Oncol* 2018; **27**: 333-340 [PMID: 30217286 DOI: 10.1016/j.suronc.2018.05.018]

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