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REVIEW

Ductular reaction in non-alcoholic fatty liver disease: When Macbeth is perverted

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Abstract

Non-alcoholic fatty liver disease (NAFLD) or metabolic (dysfunction)-associated fatty liver disease is the leading cause of chronic liver diseases defined as a disease spectrum comprising hepatic steatosis, non-alcoholic steatohepatitis (NASH), liver fibrosis, cirrhosis, and hepatic carcinoma. NASH, characterized by hepatocyte injury, steatosis, inflammation, and fibrosis, is associated with NAFLD prognosis. Ductular reaction (DR) is a common compensatory reaction associated with liver injury, which involves the hepatic progenitor cells (HPCs), hepatic stellate cells, myofibroblasts, inflammatory cells (such as macrophages), and their secreted substances. Recently, several studies have shown that the extent of DR parallels the stage of NASH and fibrosis. This review summarizes previous research on the correlation between DR and NASH, the potential interplay mechanism driving HPC differentiation, and NASH progression.

Key Words: Ductular reaction; Non-alcoholic steatohepatitis; Hepatic progenitor cells; Cell differentiation; Inflammatory cells; Liver fibrosis

Core Tip: This is the first review focusing on recent advances in the relationship of hepatic cells with ductular reaction (DR), in fatty liver-related steatohepatitis and fibrosis. Recent advances in DR, a common compensatory reaction in liver injury, shed light on the effects of hepatic progenitor cells, hepatic stellate cells, myofibroblasts, inflammatory cells, and their secreted substance. In particular, hepatic progenitor cell differentiation was thoroughly discussed in developing steatohepatitis and fibrosis. This review summarizes the correlation between DR and steatohepatitis and fibrosis, the advanced stages of non-alcoholic fatty liver disease, or metabolic (dysfunction) related fatty liver disease.

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INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD), which affects approximately 25% of adults worldwide, is the leading cause of chronic liver diseases[1]. NAFLD refers to a disease spectrum including hepatic steatosis, non-alcoholic steatohepatitis (NASH), liver fibrosis, cirrhosis, and hepatic carcinoma[2]. In early 2020, an international expert group led a consensus-driven process to develop a more appropriate term for NAFLD, and the term "metabolic (dysfunction) related fatty liver disease (MAFLD)" was recommended[3]. NASH/MASH is characterized by ≥ 5% hepatic steatosis, hepatocyte injury or necrosis, and inflammation[2,4]. NASH is a critical stage in NAFLD development and is associated with NAFLD prognosis; thus, it has become the focus of NAFLD research. NASH is the second most common indication for liver transplantation in the United States[1]. The occurrence and progress of NASH are related to several factors such as glucose and lipid metabolism, immune response, and gut microbiota[5-7]. The diagnosis and severity classification of NASH depends on histopathological examination. The main pathological features of NASH are hepatocyte balloon degeneration, inflammatory infiltration, Mallory-Den K corpuscle, and zone 3 fibrosis[2,8]. Some studies have shown that neutrophil infiltration and portal inflammatory infiltration are also characteristics of NASH[9,10].

Ductular reaction (DR) is a compensatory reaction commonly detected in various liver injuries[11], involving the participation of hepatic progenitor cells (HPCs), hepatic stellate cells (HSCs), myofibroblasts, inflammatory cells (such as macrophages), and their secreted substances. Among them, the proliferation and differentiation of HPCs are the core of DR[12]. DR is commonly found in the livers of NASH patients. Moreover, there is a parallel relationship between DR and the severity of inflammation and fibrosis in NASH patients[13-15], suggesting that DR has an important role in the progression of NASH.

Based on clinical investigations, the present review summarizes the correlation between DR and NASH. It discusses the shaped HPC differentiation fate in the context of NASH and its influence on NASH progression.

OVERVIEW OF DUCTULAR REACTION AND CORRELATION BETWEEN HPC AND DR

DR is a compensatory reaction in the portal area caused by biliary diseases, viral hepatitis, NAFLD, acute fulminant liver failure, *etc*[16]. DR is heterogeneous in both pathology and pathophysiology. Desmet divided DR into four types based on pathology: Type 1, Type 2A, Type 2B, and Type 3[17].

Type 1 is predominant in acute complete bile duct (BD) obstruction, alpha-naphtyl isothiocyanate intoxication, and cytokine (*e.g.*, interleukin 6)-induced ductular increase. It results from the proliferation of preexisting cholangiocytes. Type 1 causes the biliary tubes to elongate, branch out, and widen their lumens, allowing them to adjust to the swelling and inflammation of the portal mesenchyme. Type 2A has been interpreted as "ductular metaplasia of hepatocytes." It is often detected in periportal areas, most characteristically, in chronic cholestatic conditions. In lasting cholestasis, bile acids increase the number of cholangiocytes, which promote the development of pericellular fibrosis, and in this way, it enhances bile ductular metaplasia of hepatocytes. Of note, Type 1 and Type 2A can be reversed when the causative trigger is eliminated; the ductular structures are cleared by apoptosis; and the associated fibrosis is ameliorated to a considerable extent. Prolonged hypoxia induces Type 2B, which manifests in areas of parenchymal hypoxia, specifically in the centrolobular region of liver lobules and the centronodular region of cirrhotic nodules. Although often slower in development, its microscopic pattern is comparable to that of Type 2A in terms of ductular metaplasia or dedifferentiation of mature hepatocytes, which is associated with myofibroblast-induced fibrosis. Type 3 occurs in cases of massive loss of parenchymal cells and is characterized by the activation and proliferation of HPCs located in the

ductules and canals of Hering. As bipotential cells, HPCs can differentiate into hepatocytes and BD cells [17].

There is consensus that the fate of HPC differentiation is the core of DR, determining the pathological type of DR and affecting disease development[18]. Epithelial cell adhesion molecule and the neural cell adhesion molecule/sex-determining region Y-Box 9 (SOX9) have been previously considered markers of HPCs, cytokeratin-7 (CK7) and CK19 have been used to identify cholangiocytes, and albumin and hepatic nuclear factor 4-alpha have been considered markers of hepatocytes[19-21]. HPCs located in the Hering canal typically differentiate into biliary cells in a normal liver[18] but do not lead to DR. HPCs are activated and differentiate into hepatocytes or biliary cells during liver injury. For example, HPCs differentiate into hepatocytes in acute fulminant hepatic failure and contribute to liver regeneration[22, 23]. CK7 immunohistochemistry is also positive in HPCs, which can predict liver injury severity; for instance, HPCs differentiate into CK7+ cells in the portal area in chronic hepatitis C and exacerbate liver injury[13,14,24-26]. Furthermore, a similar phenomenon has been found in hepatitis B virus-injected murine models[27]. In addition, DR is significantly associated with hepatocellular carcinoma peritumoral hepatic inflammation, liver fibrosis, tumor node metastasis classification stage, and poor prognosis[28]. Hepatocyte-derived ductular HPCs can give rise to hepatocellular carcinoma *via* concomitant activation of yes-associated protein (YAP) and transcriptional coactivator with PDZ-binding motif transcription factors. Autophagy suppresses the formation of hepatocyte-derived cancer-initiating HPCs in the liver[29].

HPCs are activated in the majority of liver diseases[30]. During liver injury, a ubiquitous DR affects the differentiation *vs* dedifferentiation type of HPCs, depending on the severity of the liver injury[31]. Proliferating BDs in DR are misshapen, lack an apparent lumen, and are associated with increased portal inflammation and fibrosis[19,32]. It has been previously demonstrated that HPC activation is sufficient to regenerate a large proportion of the liver parenchyma using targeted deletion of mouse double minute 2 (MDM2) in mouse hepatocytes. This kind of HPC activation may be induced by the tumor necrosis factor-like weak inducer of apoptosis (TWEAK)/fibroblast growth factor-inducible 14 pathway[33]. Interestingly, in the hepatocyte-specific β-catenin knockout model, hepatocytes lose their regenerative capacity, and cholangiocytes still express β-catenin. β-catenin-positive cholangiocytes (differentiated HPCs) differentiate into β-catenin-positive small hepatocytes, which then proliferate and repopulate the liver[34,35]. A previous study reported that YAP levels are increased in NAFLD patients and NAFLD mouse models[36]. A recent study showed that the DR reaction is more intense and hepatocytes trans-differentiate into cholangiocytes protected from cholestatic damage by activating Hippo-YAP in the Tjp2 cKO mouse model (more susceptible to cholic acid-induced liver injury) fed 3,5-diethoxycarbonyl-1,4-dihydrocollidine (DDC)[37]. A murine BD ligation model of liver fibrosis showed that heme oxygenase-1-mediated pro-resolution M2 polarization of macrophages protects the liver from excessive DR and fibrosis with the ligand of numb protein X1 as the key downstream factor[38]. Interestingly, recent studies have shown that HPCs can promote angiogenesis by secreting vascular endothelial growth factor (VEGF) *via* the secretin/secretin receptor/microRNA 125b (miR-125b) axis [39]. However, recent studies have shown that DR cells can promote angiogenesis through slit guidance ligand 2-roundabout 1 signaling channels in various chronic liver diseases (CLDs), contrary to VEGF [40]. Another study showed that the signaling of apelin/APJ (G protein-coupled apelin receptor) can promote intrahepatic angiogenesis[41].

The impact of DR on liver diseases is a double-edged sword. HPCs can be activated and differentiated into hepatocytes to participate in liver regeneration in the case of massive loss of parenchymal cells. Conversely, the activation of HPCs may play a role in the activation of HSCs and the infiltration of inflammatory cells in DR in most CLDs, which can lead to further liver injury, including cirrhosis and tumorigenesis[14,25,42,43].

Correlation between NASH and DR

A state of NAFLD begins with healthy liver parenchyma (steatosis in < 5% of hepatocytes) and then progresses to steatosis in > 5% of hepatocytes with the initiation of DR. The condition progresses to a severe stage with scar tissue accumulation, elevated steatosis, and hepatic ballooning[43]. In recent years, DR has attracted considerable attention in NASH research. It is worth noting that although DR can assist in repairing liver injury by aiding in HPC activation and differentiation, its impact on the progression of chronic liver disease associated with NASH may not always be favorable, especially when liver regeneration capacity is impaired. In fact, in some cases, DR-induced differentiation may even contribute to the occurrence and progression of inflammation and liver fibrosis in NASH. In 2007, Richardson *et al*[14] analyzed data from 118 liver specimens (107 from NAFLD patients and 11 from normal liver) and found that DR commonly existed in NASH, especially in patients with fibrosis. Multivariate analysis demonstrated that the extent of DR was independently associated with hepatocyte replicative arrest [odds ratio (OR) = 6.5] and fibrosis stage (OR = 17.9). Moreover, they further found that the expansion of HPCs was significantly correlated with NASH activity score[14]. In 2013, based on biopsy specimens from 56 adults with NAFLD (10 with steatosis and 46 with NASH) from Austria and the United States, Skoien *et al*[44] found that both centrilobular fibrosis and portal fibrosis stages were positively associated with the extent of DR. In 2018, multicenter observational studies of 90 NAFLD patients showed that DR was identified in 90% of biopsy samples, and its extent was correlated with

fibrosis stage[15]. Similarly, Gadd *et al*[13] also found that DR appeared in almost all NASH patients, and its grade was significantly associated with pathological liver progression. Similar to the results in adult NAFLD, DR can also be found in pediatric NAFLD, and its extent and/or HPC expansion were significantly correlated with fibrosis degree[44-46].

DR also exists in animal NAFLD models. In an 8-wk methionine/choline-deficient (MCD) diet mouse model and a 16-wk western diet mouse model, the number of YAP+, CK19+ reactive-appearing ductular cells, and HPCs were significantly increased with the severity of hepatocyte injury and inflammation[47]. A recent study based on mouse models indicated that during NASH development, YAP activation occurred earlier than DR but they were spatiotemporally correlated. Murine YAP activation may promote hepatocyte dedifferentiation during NASH development[48]. Morell *et al*[49] also established an 8-wk MCD diet mouse model and found that DR extent and HPC number increased steadily over time in the portal and lobular areas. Furthermore, the extent of DR rose significantly in a 12-wk western diet and carbon tetrachloride-treated mouse model, which led to severe NASH-related fibrosis. DR can also occur in other NAFLD animal models, such as rats and monkeys[50,51]. Although some animal models are particularly useful, especially for studying liver regeneration, many features of DR in humans are significantly different from those of animals[18]. The contrasting anatomical features of the two species likely account for this distinction. In humans, cholangiocytes are classified based on the diameter of the biliary tract, which can vary from small to medium to large, resulting in different sizes of the cells. Unlike humans, rodents have small BDs and large BDs, lined by small BDs and large BD cells, respectively, with distinct functional properties[52].

Interestingly, the location of DR varies in different NAFLD patient populations. In pediatric NAFLD patients, DR often appears in the portal/periportal area. In a retrospective study involving 30 children and adolescents with biopsy-proven NAFLD, CK7-positive HPCs localized at the portal-parenchymal interface, *i.e.* the periportal site[45]. Similarly, a cohort study of 32 children and adolescents with biopsy-proven NAFLD showed that DR commonly occurred in the portal area[46]. In another pediatric NAFLD study, the authors gathered 38 biopsy specimens from NASH children in three United Kingdom medical centers. They found DR at the interface between the parenchyma and portal areas in 36 NASH patients[44]. Similarly, portal DR can also occur in adult NAFLD patients[13-15]. However, in adult NAFLD patients, CK7+ cells and/or CK7+ structures can be found in the centrilobular area. Interestingly, CK7+ cells and/or CK7+ structures in centrilobular zones universally occurred in several other CLDs (including chronic viral hepatitis, autoimmune hepatitis, drug-induced liver injury, *etc*), which was termed centrilobular DR[53-55]. Both centrilobular DR and periportal DR were also found in adult NAFLD studies and showed a significant correlation with NASH progression[15,55,56]. Importantly, centrilobular DR was also located, and the correlation of fibrosis stage with centrilobular DR was much stronger than with periportal DR (regression coefficient: 1.856 *vs* 0.646)[15].

The difference in DR localization between pediatric NAFLD and adult NAFLD is plausible. In children, pediatric NASH is characterized by portal inflammation and/or fibrosis[57-59]. Since it is acknowledged that periportal DR is closely related to NASH progression in pediatric NAFLD, the localization of DR in the portal area is reasonable. The concept of centrilobular DR seemingly contradicts the localization characteristic (portal area) in the classic DR definition in adults. However, this phenomenon might be explained from the following two perspectives. From the pathology standpoint, centrilobular fibrosis, *i.e.* zone 3 fibrosis, is one of the typical pathological features of adult NASH[8]. Therefore, DR – a process related to fibrosis – would emerge in the centrilobular area by fibrosis location. Regarding the underlying pathophysiological mechanism, it has been postulated that CK7+ cells/structures in centrilobular DR might stem from hepatocytes through metaplastic response and/or dedifferentiation[55,60]. Hence, the concept of DR in NAFLD should be expanded to cover centrilobular DR[17]. In a cross-sectional analysis, it was found that centrilobular DR was highly correlated with the stage of fibrosis in adult non-alcoholic steatohepatitis[15]. In addition, centrilobular was the dominant injury pattern, presumably due to pressure induced by mechanical injury[53]. Besides, in NASH, the different underlying impact between centrilobular DR and periportal DR on disease development remains to be clarified.

DR microenvironment and HPC differentiation fate in NASH

The DR microenvironment, composed of parenchymal cells, mesenchymal cells, inflammatory cells, and their secreted substances, participates in the activation, proliferation, and differentiation of HPCs[12,61,62]. Different components drive HPC differentiation fate in different directions (Figure 1). Previous studies have indicated that HPCs reside in a specialized microenvironment (niche), which is crucial in determining their cell fate. Laminins, as part of the extracellular matrix (ECM), control the expansion of HPCs in an undifferentiated state, and hence DR, during liver injury. Other studies have demonstrated that HSCs and myofibroblasts might play an essential role in the differentiation of HPCs towards the cholangiocyte cell phenotype, while macrophages may participate in HPC differentiation into hepatocyte phenotypes[12,63]. A previous study showed that estimated glomerular filtration rate (EGFR) ligands were present in the liver microenvironment. In animal models lacking EGFR catalytic activity, the expansion of HPCs can be observed after DDC-induced liver damage, indicating that the lack of EGFR may promote HPC differentiation into hepatocytes, and thus liver regeneration[64]. However, it is noteworthy that the differentiation of HPCs is not modulated by a single factor but by a

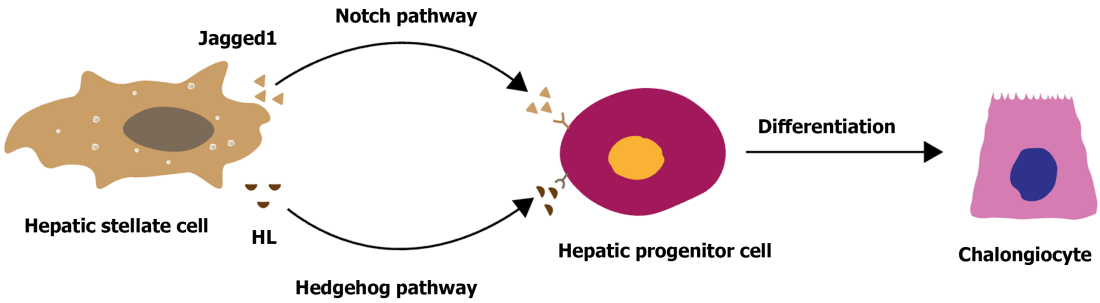

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Figure 1 Factors contributing to the differentiation of hepatic progenitor cells in non-alcoholic fatty liver disease and potential pathways associated with hepatic progenitor cells-mediated non-alcoholic fatty liver disease progression.

complicated cellular and molecular network in liver diseases. HPCs tend to differentiate into biliary cell phenotypes in NASH, which may involve the participation of HSCs, myofibroblasts, macrophages, and natural killer T (NKT) cells[13-15,18,44]. At the molecular level, Notch and Hedgehog pathways may be the critical pathways in HPC differentiation into the biliary cell phenotype in NASH patients and mice [16,19,65] (Figure 1).

HSC and HPC differentiation fate in NASH

HSCs, located in the space of Disse, are the critical cells for liver fibrosis development and progression [66,67]. HSCs maintain a quiescent phenotype in normal liver but they can be activated by multiple factors in NAFLD, such as inflammatory cells, damaged hepatocytes, oxidative stress, *etc*[66]. Activated HSCs can acquire a myofibroblast phenotype and increase ECM production, contributing to NASH progression[67].

HSC fibrogenic activation promotes HPC differentiation into hepatocytes to restore mass and function[68]. A subfamily of the inhibitor of apoptosis protein family, survivin (also called baculoviral inhibitor of apoptosis repeat containing-5), has minimal expression in differentiated cells and is associated with cell division. Activated HSCs and HPCs can express survivin. Survivin protein is upregulated with increasing fibrogenic activation of HSCs from their quiescent state. Survivin protein can suppress the fibrotic response of HSCs. At this point, the regenerative capacity of hepatocytes is diminished, followed by replenishment with survivin-expressing HPCs, which differentiate into hepatocytes to promote liver regeneration[68].

HSCs also play an essential role in NAFLD-related DR, possibly by inducing HPCs to differentiate into CK7+ and/or CK19+ cells[12,17,69,70]. In NAFLD, the emergence of DR is accompanied by a significant increase in HSCs and ECM in the DR microenvironment, and the number of HSCs is associated with the DR stage and CK7+ HPC expansion[13]. A similar association between HSC and DR can also be found in other liver diseases, such as hepatitis C infection and primary biliary cirrhosis[13,16]. Further studies have partially explained the underlying mechanism of HSC-mediated HPC differentiation[25,69].

Primary studies have shown that HSC-mediated HPC differentiation may involve the Notch and Hedgehog pathways. In the DR microenvironment, activated HSCs can upregulate the Notch pathway in HPCs by expressing Jagged1 (a Notch pathway ligand)[60,63], leading to the expression of Notch pathway target genes such as hes-related family bHLH transcription factor with YRPW motif 1 and hairy and enhancer of split homolog-1[63,71,72]. Increased Notch target gene expression can further increase the expression of hepatic nuclear factor 1β (HNF1β) and HNF6, consequently contributing to HPC differentiation into biliary cells and BD formation[73-75]. Similarly, activated HSCs can upregulate the Hedgehog pathway in HPCs by expressing HL (a ligand of the Hedgehog pathway), leading to an increase in the Gli transcription factor family (Gli1, Gli2, and Gli3)[76]. Furthermore, Gli2 can translocate to the nucleus and promote target gene transcription[77,78], whose activation can promote the proliferation and differentiation of HPCs into CK7+ cells[79-83]. Elevated activity of Notch and Hedgehog pathways was analogous to disease severity in studies of both mouse models of NASH and patients with NASH[48,79,84], indicating the potential role of Notch and Hedgehog pathways in HSC-mediated HPC differentiation (Figure 2).

Macrophages and HPC differentiation fate in NASH

Emerging evidence suggests that macrophages are a heterogeneous population of cells. There are two types of macrophages: Resident macrophages, *i.e.* Kupffer cells, originating from yolk sac-derived erythroid, myeloid progenitors in the fetal liver; and infiltrating macrophages originating from bone marrow-derived circulating monocytes[7]. In NAFLD, macrophages can be activated and differentiated into two types of macrophages: M1 and M2 macrophages[7]. M1 macrophages secrete pro-inflammatory cytokines and have high phagocytic activity, whereas M2 macrophages secrete immune-suppressive but

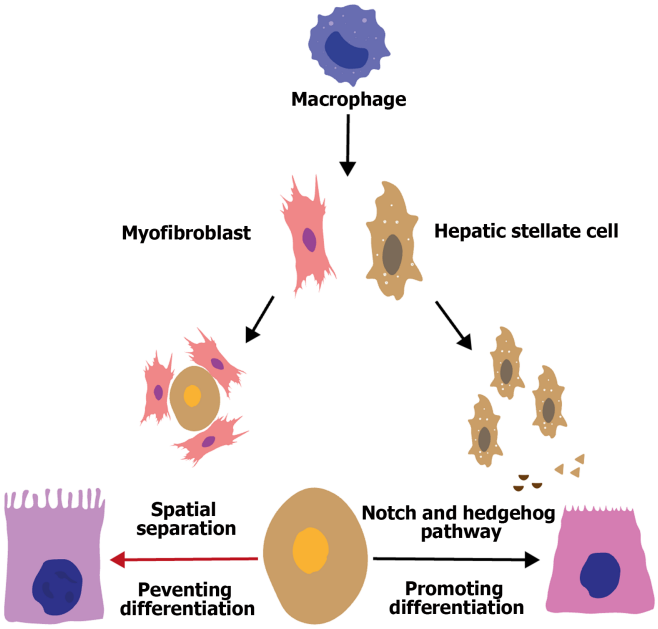

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Figure 2 Hepatic progenitor cell-mediated hepatic progenitor cell differentiation may involve the Notch and Hedgehog pathways.

pro-fibrogenic cytokines[85,86].

Although it is universally acknowledged that macrophages play a critical role in NAFLD progression, the relationship between macrophages and HPC differentiation in NAFLD-related DR remains elusive. Macrophages were found to promote HPC differentiation into hepatocytes in the DDC diet mouse model, and the Wnt/β-catenin pathway was the key mechanism in this process[69,83,87]. After phagocytosis of the hepatocyte debris, macrophages increase the expression and secretion of Wnt3a (a ligand of the Wnt/β-catenin pathway), activating the Wnt/β-catenin pathway in HPCs[12,63]. Therefore, β-catenin can translocate to the nucleus and bind its co-activators (*e.g.*, CREB-binding protein), promoting the expression of target genes such as SOX9, MYC, and Twist-related protein 1, all of which are associated with HPC differentiation into hepatocytes[63,88]. Studies have shown that HPCs activate during chronic liver injury when hepatocyte proliferation is insufficient to reach homeostasis. During transforming growth factor (TGF)-induced apoptosis in a fibrogenic environment, HPC expands due to a balance between proliferation and apoptosis, which is favorable in a fibrogenic climate. Mitogens that trigger HPC expansion overlap significantly with pro-inflammatory cytokines released by hepatic macrophages including tumor necrosis factor, interferon gamma (IFN-γ), interleukin 6 (IL-6), and TWEAK. Human amnion epithelial cell-treated NASH mice showed a reduction in both HPC and macrophage numbers and expression levels of HPC mitogens and macrophage-released cytokines[89]. In NAFLD patients, macrophages increased significantly in the DR area, and macrophage infiltration was mainly related to the expansion of CK7+ HPCs and fibrosis stage, indicating the potential role of the macrophage in the HPC differentiation fate[13,46]. However, in the context of liver diseases, the role of macrophages in determining HPC differentiation fate is still unclear. Deduced from the aforementioned basic studies, the increased macrophage infiltration in the DR area of NAFLD patients may promote the differentiation of HPCs into hepatocytes. Nonetheless, according to pathological findings, the actual characteristic of NAFLD-related DR is HPC differentiation into cholangiocytes. Therefore, this seemingly contradictory phenomenon might be explained from the following two perspectives.

The regulation of macrophage-mediated HPC differentiation fate may vary across different disease contexts, which is one potential explanation. Disease pathogenesis in the DDC diet mouse model is highly distinct from NAFLD pathogenesis. Therefore, the functional state of macrophages in NAFLD might be correspondingly specific to that in the DDC diet mouse model. Second, the crosstalk between macrophages and HSCs in NAFLD may predominantly contribute to the differentiation of HPCs into cholangiocytes. It has been well established in NAFLD that macrophages can express multiple pro-fibrotic factors (such as platelet-derived growth factors subunit B and TGF-β), contributing to the proliferation and activation of HSCs and myofibroblasts[7,66,90-92]. Notably, macrophages were near HSCs in the DR area in NAFLD patients, indicating a potential promotive effect of macrophages in driving HPC differentiation into cholangiocytes by activating HSCs[13,46].

Conversely, HSCs might hinder macrophage-mediated HPC differentiation into hepatocytes by interrupting the interaction between macrophages and HPCs in spatial separation. In a biliary regeneration model, HPCs were surrounded by a thick sheath-like layer of myofibroblasts and collagen I, which excluded macrophages from forming a close association with HPCs[63]. Similar sheath-like

structures might also exist in NAFLD; however, further studies in NAFLD patients are needed to validate the potential existence of this structure in the DR area. In summary, macrophages may participate in NAFLD-related DR onset and development through crosstalk with cells such as HPCs and HSCs. However, its specific role and related mechanisms warrant further investigation (Figure 3).

Mast cells and HPC differentiation fate in NASH

According to recent studies, NAFLD/NASH development is primarily influenced by the interaction between DR and mast cells (MCs)[93,94]. MCs may promote NAFLD/NASH progression by activating Kupffer cells and HSCs with histamine[94]. Recruitment of MCs is a characteristic of BD injury. It has been proven that knocking down or inhibiting the expression of MCs can effectively reduce DR[95,96]. MC-derived TGF-β1 is a critical regulator of hepatobiliary damage, and blockage of TGF-β1 can ameliorate DR and other features of cholestatic liver injury[97]. MCs were found to promote microvesicular steatosis development *via* the miR-144-3p/aldehyde dehydrogenase 1 family, member A3 (ALDH1A3) signaling pathway in a Western diet mouse model with NASH[98]. Reduced ALDH1A3 expression promotes lipid peroxidation associated with liver fibrosis and steatosis and a reduction in β-oxidation of free fatty acids[99].

Moreover, miR-144-3p showed increased expression in insulin resistance in NASH. Meanwhile, DR expansion in mouse models of Western diet with NASH is more sensitive. The phenotypic changes are associated with the secretion of insulin-like growth factor 1 by cholangiocytes, driving peribiliary infiltration and MC activation. Consistent with this finding, MCs from NASH patients accumulate in the portal area, directly correlating with fibrosis stage[93]. A more relevant study discovered that inhibiting MCs reduced DR, inflammation, fibrosis, and recovery from liver injury after MC injection[94].

Previous studies have demonstrated that elevated farnesoid X receptor (FXR) expressed by MCs can be detected in primary sclerosing cholangitis, primary biliary cholangitis, and NAFLD[100-102]. MC-FXR plays a critical role in liver injury and DR in a cholestasis model, where MCs express FXR and infiltrate the liver promoting liver fibrosis during cholestasis and triggering biliary injury. After migration and activation, MCs induce DR and senescence through paracrine interactions with cholangiocytes. Moreover, the MC-FXR signaling pathway modulates the biliary senescence/senescence-associated secretory phenotype and histamine H1- and H2-receptor signaling pathways to regulate total bile acid and then affects DR and liver injury[103]. According to these studies, MCs are corrected with DR in various liver diseases and may affect the differentiation of HPCs through macrophages, HSCs, and fibroblasts. However, the mechanism by which MCs influence HPC differentiation remains obscure.

ECM and HPC differentiation fate in NASH

ECM – a supporting structure for organs, tissues, and cells-represents a complex protein network including fibrillar and non-fibrillar collagen, laminin, fibronectin, *etc*[104]. ECM proteins can play a vital role in HPC differentiation fate. For example, loss of the basement membrane, a cell-supporting structure, is correlated with the increased level of HNF4 in HPCs, indicating the differentiation of HPCs into hepatocytes[105]. In addition, laminin can upregulate the expression of the biliary marker gene and downregulate hepatocyte transcription factor C/EBPa in HPCs, driving HPC differentiation into cholangiocytes[106]. A recent study based on mouse models of chronic parenchymal damage showed that iloprost reduces laminin deposition and enhances the differentiation of HPCs into hepatocytes [107]. The disruption of integrin β6, an adhesion receptor that interacts with fibronectin and TGF-β1, inhibits the response of HPCs to tissue damage. Significant ECM deposition, such as collagen deposition, is commonly found in NAFLD-related fibrosis[67,108]. Therefore, the accumulation of ECM during the development of NAFLD may contribute to HPC differentiation and the formation of DR.

Hepatocyte senescence and HPC differentiation fate in NASH

Cellular senescence, a cell cycle arrest response, is mediated by the induction of cyclin-dependent kinase inhibitors p21 and p16[109,110]. In NAFLD, hepatocyte senescence involves multiple factors, such as oxidative stress and inflammation, and is characterized by increased p21 levels[111,112]. Interestingly, hepatocyte senescence, *i.e*. replicative arrest, may activate HPC proliferation and differentiation. Oxidative stress induces hepatocyte senescence with consequent cell cycle arrest and impaired regeneration[113]. A recent study demonstrated that oxidative stress can affect HPC differentiation, and the redox is regulated by various transcription factors, of which nuclear factor (erythroid-derived 2)-like 2 (NRF2) plays a crucial role in HPC differentiation, and its activation can inhibit oxidative stress. As stemness is maintained in HPCs through constitutive NRF2 activation, it is inhibited when HPCs are activated during liver injury, *e.g.*, NASH.

Interestingly, NRF2 inhibition increases the transplantation efficiency of human HPCs[114]. In an MDM2-deleted mouse model, server hepatocyte senescence was characterized by a high p21 level and resulted in significant HPC proliferation and differentiation into hepatocytes[33]. However, in NAFLD patients and the choline-deficient and ethionine-supplemented (CDE) diet mouse model, mild hepatocyte senescence was also identified by a lower p21 level and was positively correlated with DR stage and CK7+ HPC expansion, conversely indicating a potential role of hepatocyte senescence in HPC differentiation into cholangiocytes[14,33]. To reconcile these apparently conflicting findings, some

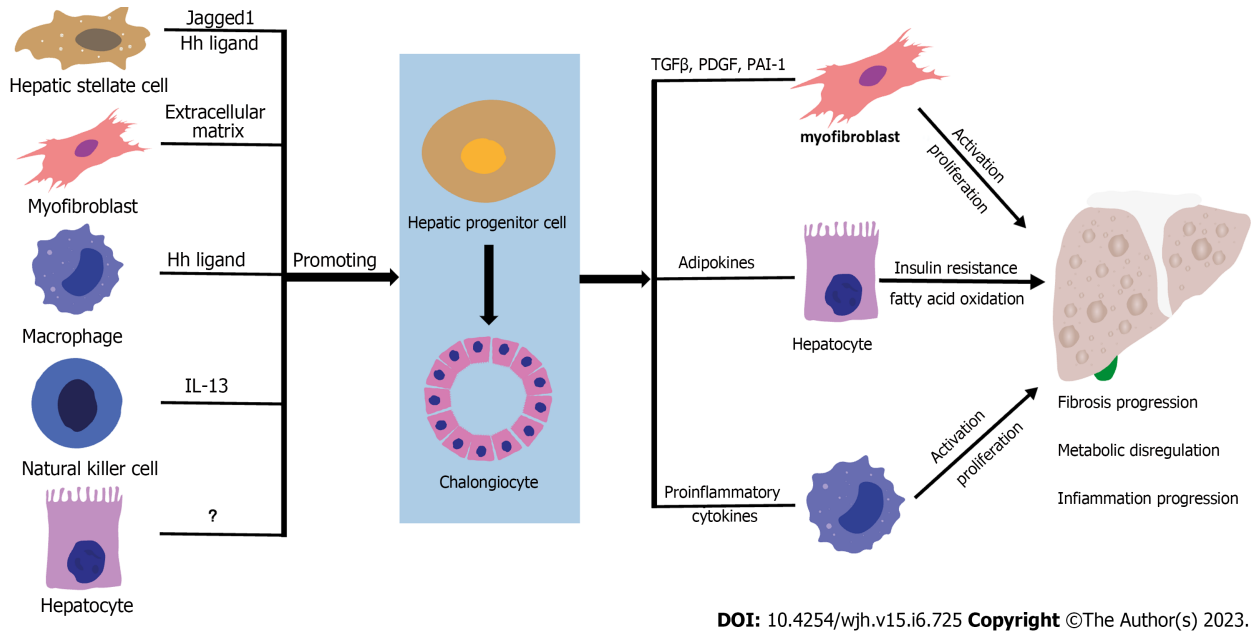

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Figure 3 Potential role of macrophages in hepatic progenitor cell differentiation fate in non-alcoholic fatty liver disease.

experts have suggested that the absence of hepatocyte senescence may enable hepatocytes to undergo self-regeneration without relying on HPC-mediated regeneration[33]. In addition, hepatocytes are the primary source of liver regeneration in a healthy liver, while HPCs do not participate in normal liver regeneration. Therefore, it might be further speculated that aging and healthy hepatocytes may regulate HPC differentiation. Nevertheless, the mechanism by which aging hepatocytes and/or healthy hepatocytes regulate HPC differentiation fate is yet to be elucidated.

NKT cells and HPC differentiation fate in NASH

NKT cells – a type of innate immune cell in the liver – can participate in the development of liver inflammation and fibrosis[115]. In NAFLD, NKT cells significantly increase in the DR area, and their infiltration extent correlates with both NASH severity and DR stage[80,116]. Conversely, liver biopsies of HBV patients often reveal a pronounced DR and diminished expression of IFN-γ, which is caused by NKT cells. Nevertheless, treatment with IFN-γ has been shown to ameliorate DR in these patients[117]. However, the role of NKT cells in HPC differentiation fate is unclear in NAFLD-related DR. There is evidence suggesting a promotive role of NKT cells in HPC differentiation into cholangiocytes in liver injury models. In these studies, NKT cells increased the expression of IL-13 and the production of Hedgehog ligands, which may drive HPC differentiation into cholangiocytes[80,118-121]. Nevertheless, it is unclear whether NKT cells are required for HPC differentiation into biliary cells in NASH.

Potential role of HPC differentiation in aggravating NASH

In addition to the impact of the NASH-related DR microenvironment on HPC differentiation fate, differentiated HPCs can aggravate inflammation and fibrosis progression in NASH. As aforementioned, there is a close correlation between HPC expansion and NASH progression, indicating the potential role of differentiated HPCs in aggravating NASH. Moreover, the promotive role of differentiated HPCs in NASH inflammation and fibrosis progression has been proven in NASH-related animal models. Although the underlying mechanism has yet to be fully understood, it may involve the participation of HSCs, macrophages, adipokines, and the epithelial-mesenchymal transition (EMT) (Figure 1).

Differentiated HPCs may participate in HSC-mediated NASH-related fibrosis by promoting HSC activation and proliferation. Increased hepatic levels of several factors, such as PDGF, connective tissue growth factor (CTGF), and Hedgehog ligands, have been found in NAFLD animal models[60,122,123]. In basic studies, HPCs are one of the sources of PDGF, CTGF, and Hedgehog ligands[81,122]. The promotive role of these molecules in enhancing HSC proliferation, accumulation, and ECM production has been well established[81,124-126]. Therefore, these pathways may be involved in HPC-mediated HSCs activation in NASH aggravation.

In addition to directly promoting HSC and myofibroblast activation, HPCs may undergo the EMT towards myofibroblasts, consequently leading to hepatic fibrosis progression. EMT is a cell reprogramming process from the epithelial to mesenchymal phenotype[76,77,127]. EMT in hepatocytes, cholangiocytes, and HSCs can be found in various liver diseases and is related to hepatic fibrosis[76,128,129]. A proportion of HPCs can go through the EMT, which is characterized by the upregulation of mesenchymal cell markers [such as alpha-smooth muscle actin (α-SMA) and S100 calcium-binding

protein A4) and downregulation of epithelial cell markers (such as CK7 and CK19)[130-133]. Differentiated HPCs (CK7+] that highly express α-SMA can be found in NAFLD, indicating the presence of HPC-originated EMT and its potential contribution to fibrosis pathogenesis[79]. The onset of EMT in HPCs may involve the Hedgehog pathway activity and TGF-β[79]. Notably, whether high expression of a-SMA or collagen in HPCs can be regarded as the EMT remains controversial. This is because a recent lineage tracing study, using an α-fetoprotein Cre mouse model, provided strong evidence against the existence of HPC-myofibroblast transition[134]. Therefore, further basic studies regarding the origination of α-SMA and CK7 double-positive cells are warranted.

Differentiated HPCs can promote macrophage-mediated inflammation in NASH. Studies have shown that macrophages play an essential role in NASH aggravation[7]. As previously mentioned, significant macrophage infiltration was detected in the NAFLD-related DR area. The number of macrophages is significantly associated with the extent of DR and HPC expansion, indicating that HPCs have a potential role in macrophage recruitment[13]. Primary studies have proven that multiple factors, such as chemokines and pro-inflammatory cytokines, are involved in HPC-mediated macrophage recruitment [7,135-137]. For example, HPCs can contribute to macrophage recruitment by increasing C-C motif chemokine ligand 2 and C-X3-C motif chemokine ligand 1 expression and promote macrophage polarization into M1-type by secreting IL-1, IL-6, and IFN-γ, consequently exacerbating hepatic inflammation[7,135-137]. Therefore, these cytokines may participate in HPC-mediated macrophage infiltration and activation in NASH.

Metabolic dysregulation is a major hallmark in the pathophysiological process of NAFLD, and differentiated HPCs exacerbate by causing dysregulation of the secretion of adipokines, leading to an increase in NASH progression. Adipokines, including adiponectin, leptin, and resistin, contribute to NAFLD development by modulating glycolipid metabolism, inflammatory response, and HSC activation[138]. Although adipokines are mainly produced by adipose tissues, they have also been found to secrete adiponectin and resistin[45,139]. Notably, in NASH, differentiated HPCs increase resistin expression and downregulate adiponectin expression. Moreover, resistin expression in HPCs is positively correlated with the severity of NAFLD.

By contrast, adiponectin expression in HPCs was found to be negatively correlated with the severity of NAFLD, indicating that adipokines play a role in HPC-mediated NASH progression[45]. Adiponectin can suppress hepatic lipogenesis and the production of proinflammatory cytokines but can stimulate insulin secretion and fatty acid oxidation in the liver[140,141]. By contrast, resistin reduces peripheral insulin sensitivity and promotes the expression of proinflammatory cytokines[138,142]. In NASH, adipokine dysregulation aggravates insulin resistance, worsening liver inflammation and injury, which also increases HSC activation, thereby aggravating NASH[45,143-145]. Therefore, the NAFLD-related microenvironment can cause the dysregulation of adipokine expression in HPCs, leading to NAFLD-related metabolic dysregulation.

CONCLUSION

Studies conducted in the past 100 years have shown that DR may be a compensatory reaction to liver injury, but the correlation between DR and NAFLD needs to be sufficiently studied. The expected prevalence of DR in NAFLD patients, and more importantly, the close relationship between DR and the progression of inflammation and fibrosis in NASH, remain to be clarified. Although DR promotes liver regeneration[54,146], it remodels the NASH microenvironment, which aggravates rather than alleviates NASH severity, similar to the initially upright "Macbeth" getting perverted under a corruptive lure. In NAFLD, HPC proliferation and differentiation, the core processes in DR pathogenesis, might be triggered by NAFLD-related liver injury. The cells (such as HSCs and macrophages) and their secreted substances may drive the differentiation of HPCs into cholangiocytes. Conversely, differentiated HPCs may, in turn, aggravate NASH through multiple pathways, which may involve the participation of HSCs, macrophages, adipokines, and the EMT. The involvement of these cells in the interaction between DR and NASH pathogenesis may form a 'vicious circle,' presumably leading to further progression of hepatic inflammation and fibrosis.

However, the bilateral interaction between DR and NAFLD remains to be further verified. For the DR caused by NAFLD, the majority of previous findings about NAFLD-related DR were primarily obtained through observational studies. Several signaling pathways are involved in DR (*e.g.*, Notch, Hedgehog, TWEAK), and it was recently discovered that long non-coding RNA/p300 could influence DR progression[147]. However, how these pathways promote the pathogenesis of DR in the context of NAFLD remains unclear. We are still determining whether the pathways mentioned above are involved in DR-related NAFLD. The key factors driving HPC differentiation in NAFLD need to be further investigated. In addition, in terms of the impact of DR on the pathogenesis of NAFLD, considering our limited understanding of the core molecular mechanism driving DR, it is difficult to provide a direct and exact intervention towards the DR onset, which hinders establishment of a causal effect of DR on NAFLD progression. Therefore, we need further investigations to deepen our understanding of the core and characteristic pathways of DR, to achieve the development of DR-targeted intervention in NAFLD-

related studies. More importantly, the underlying mechanisms of both NAFLD-caused DR and HPC-mediated NAFLD progression may be important targets for treating NAFLD.

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REVIEW

Recent advances in pathophysiology, diagnosis and management of hepatorenal syndrome: A review

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Abstract

Hepatorenal syndrome with acute kidney injury (HRS-AKI) is a form of rapidly progressive kidney dysfunction in patients with decompensated cirrhosis and/or acute severe liver injury such as acute liver failure. Current data suggest that HRS-AKI occurs secondary to circulatory dysfunction characterized by marked splanchnic vasodilation, leading to reduction of effective arterial blood volume and glomerular filtration rate. Thus, volume expansion and splanchnic vasoconstriction constitute the mainstay of medical therapy. However, a significant proportion of patients do not respond to medical management. These patients often require renal replacement therapy and may be eligible for liver or combined liver-kidney transplantation. Although there have been advances in the management of patients with HRS-AKI including novel biomarkers and medications, better-calibrated studies, more widely available biomarkers, and improved prognostic models are sorely needed to further improve diagnosis and treatment of HRS-AKI.

Key Words: Hepatorenal syndrome; Pathophysiology; Diagnosis; Management; Review

Core Tip: Hepatorenal syndrome (HRS) is a specific form of acute kidney injury that occurs in the presence of severe acute liver injury (*e.g.*, acute liver failure or severe alcoholic hepatitis), decompensated cirrhosis, or acute on chronic liver failure and is particularly associated with poor prognosis. Here, we reviewed some of the recent advancements in the diagnosis and treatment of HRS.

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INTRODUCTION

Acute kidney injury (AKI) is a common complication in patients with cirrhosis and has been reported in 20%-50% of the hospitalized patients with cirrhosis[1,2]. Within the spectrum of AKI in cirrhosis, hepatorenal syndrome (HRS) with AKI (HRS-AKI) has by far the worst prognosis[3]. HRS-AKI is a rapidly progressive type of AKI with a median survival of few weeks[3,4]. Although most commonly seen in the setting of cirrhosis, HRS-AKI can occur in patients with acute liver injury such as acute liver failure or severe alcoholic hepatitis[3,5]. Observations of acute progressive kidney injury without significant preexisting kidney dysfunction, minimal histological abnormalities, and reversible angiographic changes in renal vasculature led to the hypothesis that HRS-AKI is a functional and potentially reversible phenomenon caused by hemodynamic instability, splanchnic vasodilation and renal vasoconstriction[6-10]. However, more recent data have shown the pathophysiology is more complex[10-14].

In this review, we examine the current understanding of the mechanisms of kidney injury in patients with cirrhosis, discuss the contemporary classification of AKI in patients with cirrhosis, and review the recent advances in diagnosis and management of HRS-AKI.

PATHOPHYSIOLOGY

HRS-AKI is primarily due to an unbalanced but potentially reversible cirrhosis-induced circulatory dysfunction without structural kidney damage[11-14]. This understanding of HRS pathophysiology is supported by clinical findings including the return to normal kidney function occurring commonly after liver transplantation, successful kidney transplant using donors with HRS, and normal postmortem histology and angiography[9,10,15,16]. Direct experimental evaluation of the pathophysiology of HRS remains lacking due to lack of a fitting animal model. The primary mechanism of renal injury, splanchnic hypoperfusion, triggers a physiologic response including sodium retention and renal vasoconstriction. These mechanisms generally cannot compensate to maintain perfusion and as the disease progresses, eventually contribute to circulatory dysfunction and thus worsen renal function. The compensatory mechanisms, maladaptive responses, and their role in disease progression will be detailed in the following sections.

Cirrhosis-induced circulatory dysfunction

Cirrhosis causes elevated intrahepatic vascular resistance and a paradoxical splanchnic vasodilatation due to increased production of mediators such as nitric oxide and prostacyclins[17-20]. Compensatory hyperdynamic circulation initially preserves the effective intravascular volume in early stages of cirrhosis. As the cirrhosis progresses or during times of acute stress, the hyperdynamic cardiac circulation cannot compensate for the splanchnic vasodilation resulting in activation of other compensatory mechanisms. The renin-angiotensin-aldosterone system (RAAS), the sympathetic nervous system (SNS), and at later stages, the non-osmotic secretion of arginine vasopressin are subsequently activated to maintain effective intravascular volume[21-24]. These responses cause vasoconstriction as well as water and sodium retention in an attempt to counteract vasodilation and maintain adequate intravascular volume. However, with further disease progression and in the absence of effective treatment, these compensatory mechanisms not only will fail to adequately counterbalance the vasodilation but begin to contribute to renal dysfunction. Renal vasoconstriction begins to impair renal blood flow and therefore worsens renal function. Volume retention contributes to worsening portal hypertension, which in turn worsens the underlying physiology which initiates and perpetuates HRS.

An interesting and specific finding in HRS-AKI is the sequence and distribution of microvascular changes in the kidney itself. In the early stages of cirrhosis and in the presence of mild portal hypertension, as renal blood flow decreases, resistive indices (RIs) measured by Doppler ultrasound show a gradual increase starting from the main renal artery (hilum) toward the cortical arteries, sparing the outer cortex parenchyma[25-27]. By contrast, in later stages and in the presence of severe portal hypertension, the RI gap between hilum and cortex disappears and cortical ischemia occurs[26,27]. Therefore, cortical ischemia is considered to be a hallmark feature of HRS-AKI. Another potential contributing factor to HRS-AKI in cirrhosis is abnormal renal vascular response to stimuli and altered autoregulation of kidney blood flow. Despite decreased renal blood flow, the vasoconstrictor effect of angiotensin II on the efferent arterioles and vasodilator effect of nitric oxide on the afferent arterioles

preserve adequate pressure in the glomeruli to keep glomerular filtration rate (GFR) within normal limits[26]. However, as cirrhosis advances, GFR starts to decline presumably due to the disruption of nitric oxide production and progressive cortical ischemia caused by the very same compensatory mechanisms explained above. Animal models have also shown a blunted vasodilatory response to bradykinin and an augmented vasoconstrictive response to noradrenaline[28,29]. If the decreased renal blood flow is not reversed quickly, then the persistent vasoconstriction and ischemia could lead to acute tubular necrosis (ATN), which may not improve even after the adequate renal blood flow has been restored. The potential for permeant dysfunction as a result of an acute insult from HRS-AKI illustrates the importance of early diagnosis and treatment.

Cirrhotic cardiomyopathy

Cirrhosis-induced cardiac dysfunction or cirrhotic cardiomyopathy is cardiac dysfunction and abnormal response to stimuli in patients with advanced cirrhosis in the absence of structural cardiac disease. This phenomenon is observed in up to 50% of cirrhotic patients with varying degree of severity[30-32]. As mentioned earlier, the compensatory response to the splanchnic vasodilation includes both increase in cardiac output and activation of RAAS and SNS. However, chronic activation of RAAS and SNS may result in impaired cardiac response to stress, diastolic dysfunction, electrophysiological abnormalities (*e.g.,* prolonged QT interval), and eventually decreased cardiac output[30-33]. Although cardiac compensatory mechanisms may be able to maintain adequate profusion under normal circumstances, they can collapse under physiologic and pathologic stressors such as infections (particularly spontaneous bacterial peritonitis), bleeding, or inappropriate use of medications such as β-blockers, diuretics and angiotensin-converting enzyme inhibitors[31,33,34]. Because increased cardiac output is an essential compensatory mechanism to maintain renal profusion in the setting of vasodilation, reduced cardiac output can have significant negative effects on renal profusion and has been associated with development of HRS and poor outcomes in patients with HRS[33].

Inflammation

In recent years, the notion that decompensated cirrhosis is a constant inflammatory state has emerged and a growing body of evidence shows pro-inflammatory cytokines and chemokines such as interleukin 6 (IL-6), IL-8 and tumor necrosis factor alpha (TNF-α) may play a central role in the organ dysfunction in patients with cirrhosis[35-38]. The levels of pro-inflammatory cytokines increase with disease progression as a response to sterile (non-infectious) inflammation or infectious inflammation[37,39]. Sterile inflammation typically manifests with systemic inflammatory response syndrome and is mainly driven by danger-associated molecular patterns such as high-mobility group protein B1. In patients with cirrhosis infectious, inflammation is mainly driven by pathogen-associated molecular patterns from gut-derived bacterial translocation but can also be associated with other sources of infection[38-40]. The inflammatory response may have prognostic value in predicting progression of AKI and mortality[40, 41]. Furthermore, levels of certain inflammatory mediators in the serum (IL-6, TNF-α, vascular adhesion protein-1) and urine (monocyte chemoattractant protein-1, neutrophil gelatinase–associated lipocalin) may help to differentiate AKI-HRS from other causes of AKI such as prerenal azotemia. In addition to changes in the systemic inflammatory environment, patients with decompensated cirrhosis have increased expression of inflammatory receptors such as toll-like receptor 4 (TLR-4) in the kidneys[38, 42]. These subtle structural changes can lead to exaggerated tubulointerstitial, glomerular, and vascular injuries in response to relatively minor hemodynamic changes or occult infections[43,44].

Other factors

In addition to circulatory dysfunction and inflammation, there is evidence that other factors contribute to the development of HRS. Bile cast nephropathy or cholemic nephropathy in decompensated cirrhosis may contribute to renal dysfunction as patients with higher bilirubin have lower response to therapy in HRS[45,46]. However, the causative relationship between bile cast nephropathy and renal dysfunction in HRS has not been clearly established. Relative adrenal insufficiency in cirrhosis, formerly called hepatoadrenal syndrome, is a relatively common phenomenon occurring in 24%-47% of patients with decompensated cirrhosis. The lack of normal adrenal function impairs the compensatory response to hypoperfusion and increases the risk of AKI-HRS[47]. Although glucocorticoid supplementation may improve outcomes in patients with relative adrenal insufficiency and septic shock, the effect of supplementation on AKI-HRS outcomes has not been evaluated rigorously. Figure 1 summarizes the pathophysiology of HRS-AKI

DIAGNOSIS

In recent years, the definition and diagnostic criteria of AKI-HRS have been adjusted and novel diagnostic biomarkers have been proposed[48-50]. Two forms of HRS are currently recognized: HRS-AKI (formerly known as HRS type 1) and HRS-non-AKI (HRS-NAKI) (formerly known as HRS type 2). HRS-NAKI is further divided into two subtypes: HRS - acute kidney disease (HRS-AKD) defined as

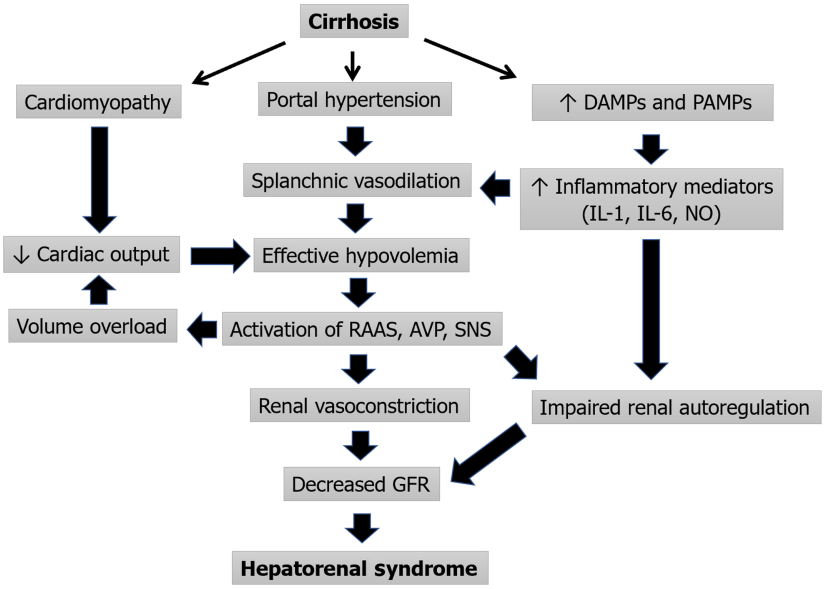

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Figure 1 Pathophysiology of hepatorenal syndrome. AVP: Arginine vasopressin; DAMPs: Danger-associated molecular patterns; GFR: Glomerular filtration rate; PAMPs: Pathogen-associated molecular patterns; RAAS: Renin angiotensin aldosterone system; SNS: Sympathetic nervous system.

estimated GFR (eGFR) < 60 mL/min per 1.73 m^2 for less than 3 months and HRS-chronic kidney disease (HRS-CKD) defined as eGFR < 60 mL/min per 1.73 m^2 for more than 3 months. Currently, the American Association for the Study of Liver Diseases (AASLD) defines AKI-HRS according to the International Club of Ascites (ICA) criteria as an increase in serum creatinine (SCr) ≥ 0.3 mg/dL within 48 h or increase ≥ 1.5 times from baseline SCr that is known or presumed to have occurred within the preceding 7 d. The other diagnostic criteria for HRS-AKI have remained largely unchanged from HRS type 1 including cirrhosis with ascites, no response after 2 consecutive days of diuretic withdrawal and plasma volume expansion with albumin infusion (1 g/kg body weight per day), absence of shock, no current or recent use of nephrotoxic drugs (non-steroidal anti-inflammatory drugs, aminoglycosides, or iodinated contrast media), and no signs of structural kidney injury. Evidence for structural kidney disease includes proteinuria (> 500 mg per day), microhematuria (> 50 red blood cells per high-power field), and/or abnormal renal ultrasonography. The updated criteria have adopted lower thresholds of creatinine increase and no absolute minimum creatinine necessary for diagnosis primarily to facilitate earlier identification of patients at risk for poor outcomes. Despite improvement in the HRS-AKI criteria, they do not differ for patients with underlying CKD, which can make diagnosis more challenging for these patients.

Novel diagnostic biomarkers

Despite improved understanding of the pathogenesis and diagnostic criteria of HRS-AKI, it remains a diagnosis of exclusion and requires a period of observation after diuretic/nephrotoxic medication withdrawal. Establishing the diagnosis can be difficult due to the similar presentations of other causes of AKI such as prerenal AKI and ATN. In addition, AKI-HRS can result in ATN as the disease progresses, which further complicates distinguishing the two entities[51]. Although the ICA criteria and its proposed treatment algorithm try to address this issue, accurate differentiation may not be feasible in a timely fashion especially when patients' condition is rapidly changing. Thus, there is an unmet need for biomarkers to quickly and accurately differentiate HRS-AKI from other causes of AKI and stratify risk. Accurately predicting renal function in patients with cirrhosis is also essential as this would allow for earlier identification of patients with renal dysfunction. However, accurately estimating GFR is challenging as standard SCr and Cr-based equations are unreliable in patients with cirrhosis because sarcopenia, impaired production of Cr (the precursor of SCr), and increased Cr filtration are common and result in the underestimation of renal dysfunction[52,53]. A new model, the royal free hospital cirrhosis GFR, which includes sodium, presence of ascites, blood urea nitrogen, and international normalized ratio in the equation, has been suggested to be more accurate for estimating renal function in this population. However external validation in large cohorts has not been completed[54]. There are several novel biomarkers under investigation to improve diagnosis and prognostication in AKI-HRS including plasma cystatin C, urinary neutrophil gelatinase-associated lipocalin (uNGAL), interleukin-18 (IL-8), kidney injury molecule-1, and liver-type fatty acid-binding protein and albumin[55-60]. Among these biomarkers, uNGAL, IL-18 and cystatin C appear to be the most promising biomarkers. IL-18 and uNGAL can differentiate ATN from other types of AKI and predict mortality in cirrhosis[59,60]. Specifically, uNGAL is identified in the most recent 2021 AASLD guidelines as the most promising

biomarker in distinguishing HRS-AKI from ATN and suggests measuring it on day 3 from onset of renal dysfunction for greatest accuracy[49]. Plasma cystatin C (the most commonly used marker besides SCr) predicts HRS and mortality in patients with cirrhosis[59,60]. An additional advantage to cystatin C is that it has become more widely performed, can yield results relatively quickly, and can predict GFR more accurately in patients with sarcopenia[59]. Using biomarkers including uNGAL, IL-18, liver fatty acid-binding protein (L-FABP), and albumin in a combination panel may improve their ability to differentiate between HRS and ATN as well as predict AKI progression and death[55]. Specifically, the biomarker combination of cystatin C and uNGAL and predictive models MELD-cystatin C and MELD-NGAL have shown the potential for improving diagnosis and risk stratification, making them attractive topics for future research[58,60]. MicroRNAs (*e.g.*, microRNA-122) and metabolomics signature associated with hepatorenal dysfunction (4-acetamidobutanoate, trans-aconitate, 1-methylhistidine, glucuronate, N4-acetylcytidine, 3-ureidopropionate, 3-methoxytyramine sulfate, cytidine, S-adenosylhomocysteine, and myo-inositol) have shown promising results predicting mortality and kidney dysfunction in small studies but need validation in large prospective cohorts[61,62]. Tables 1 and 2 summarize commonly used methods of estimating GFR and the novel biomarkers and equations for diagnosis of AKI in cirrhosis. Although these novel markers are promising, they are not all readily available, often do not have standard cut-off values, have values that do not correlate with specific stages of AKI, and have cut-off values that vary by type of AKI. Therefore, standardization and validation are needed in prospective studies. In addition, given the importance of early diagnosis and intervention, biomarkers need to have a rapid turn-around to be clinically useful, which is often not the case in many, especially smaller, institutions. However, they have the potential to allow for earlier more specific diagnosis, which facilitates more aggressive intervention, and when appropriate, evaluation for liver transplant.

TREATMENT

Despite the growth of knowledge in pathogenesis and shifts in definition and prognostication, HRS-AKI is still associated with high morbidity and mortality. The mainstay of therapy includes volume expansion and vasoconstrictors. However, there have been changes in the availability and data supporting the use of terlipressin recently. If medical management fails, renal replacement therapy and eventually organ transplant should be considered.

Medical management

In patients with cirrhosis and AKI, when precipitating factors are excluded first patients are generally treated with diuretic withdrawal and a 48-hour volume expansion with albumin (1 g/Kg, 100 g maximum). Historically subsequent treatment has been variable depending on the availability of terlipressin. In places where terlipressin is not available, a combination of midodrine (α_1-receptor agonist), octreotide (splanchnic vasoconstrictor), and albumin are typically used outside the intensive care unit (ICU) and low-dose noradrenaline with albumin are used in the ICU. Terlipressin (vasopressin agonist), which can be administered peripherally, has been used for many years outside the United States and has demonstrated a higher response rate than albumin alone or midodrine, octreotide, and albumin combination regimen and comparable to noradrenaline plus albumin[63-73]. When co-administered with albumin, terlipressin has better outcomes than terlipressin alone, which may be due to some of the anti-inflammatory and immunomodulatory properties of albumin besides oncotic volume expansion[74,75]. Terlipressin has been studied extensively in prospective studies, randomized trials, and meta-analyses and its efficacy in reversal of HRS-AKI (Tables 1 and 2) and as a result has been approved for the treatment of HRS outside the United States for several years[76-80]. However, despite mounting evidence for the benefit of terlipressin, including initial data from the United States-based CONFRIM trial, the United States Food and Drug Administration (FDA) rejected terlipressin due to safety concerns as recently as 2020. This was controversial at the time not only because of terlipressin's wide approval outside the United States but also because the FDA subcommittee on Cardiovascular and Renal Drugs Advisory Committee voted 8-7 in favor of approval. After post-hoc analyses of the CONFIRM trial with proposed changes to mitigate the risk of safety events, terlipressin was ultimately approved in the United States for the treatment of HRS-AKI in 2022, although with several warnings. The CONFIRM trial showed terlipressin was more effective than placebo in reversal of HRS (32% *vs* 17%), although there was no statistical difference in death at 90 d. However, the trial also highlighted safety considerations when using terlipressin. Respiratory failure (14% *vs* 5%) and death within 90 d due to respiratory disorders (11% *vs* 2%) was more common in the terlipressin group compared to in the placebo group. As a result, terlipressin is contraindicated in patients with ongoing ischemia (coronary, peripheral, or mesenteric) and hypoxia or worsening respiratory symptoms. For all patients, continuous pulse oximetry is recommended to monitor the development of respiratory failure. The FDA also recommends paying close attention to volume status as this may predispose patients to respiratory failure and consider discontinuing terlipressin in patients who develop fluid overload. Liver transplantation was performed in 29% of the placebo group compared to 23% in the terlipressin group.

Table 1 Methods of estimating glomerular filtration rate and the novel equations for diagnosis of acute kidney injury in cirrhosis

Ref.	Equation		Variables	Advantage
	Cr-based			
Cockcroft *et al*[93]	Cockcroft-Gault	1976	Age, SCr, sex, weight	
Levey *et al*[94]	MDRD-4	2006	Age, SCr, sex, ethnicity	
Levey *et al*[95]	MDRD-6	2007	Age, SCr, sex, ethnicity, BUN, albumin	
Levey *et al*[96]	CKD-Epi	2009	Age, SCr, sex, ethnicity	
Kalafateli *et al*[54]	The royal free hospital	2017	Age, SCr, sex, ascites, BUN, Na, INR	
	Cystatin C-based		Age, sex, cystatin C	Equations including cystatin C are more accurate in patients with sarcopenia and advanced liver disease
Hoek *et al*[97]		2003		
Larsson *et al*[98]	CKD Epi-Cystatin C	2004		
Inker *et al*[99]		2012		
	Cr-Cystatin C-based		Age, sex, cystatin C, SCr, ethnicity	
Stevens *et al*[100]	CKD EPI -Cr Cystatin C	2008		
Inker *et al*[99]		2012		
Mindikoglu *et al*[101]		2016		

ACLF: Acute on chronic liver failure; AKI: Acute kidney injury; BUN: Blood urea nitrogen; CKD: Chronic kidney disease; INR: International normalized ratio; MELD: Model for end-stage liver disease; Na: Sodium; SCr: Serum creatinine; UTI: Urinary tract infection.

Because of the possibility that terlipressin treatment resulted in clinical change, which precluded patients from transplant, the FDA added a warning label indicating that terlipressin-induced adverse events may make a patient ineligible for liver transplant and risks of using terlipressin may outweigh benefits in patients with MELD ≥ 35. An additional warning was issued for patients with severe acute on chronic liver failure (ACLF grade 3), because the likelihood of adverse events was higher and the response to treatment diminished[72,81]. These findings suggest that renal replacement therapy and liver transplant evaluation should be considered early in patients with high baseline SCr and ACLF grade. Tables 3 and 4 summarize clinical trials and meta-analyses on terlipressin effects on HRS-AKI, although it should be noted that no meta-analyses include the CONFIRM trial.

Norepinephrine, although not FDA-approved for the treatment of HRS-AKI, has shown efficacy and is frequently used off label for the treatment of HRS, especially in the United States where terlipressin was not available until recently. The need for central venous administration and close hemodynamic monitoring generally limits its use to the ICU. Comparison of terlipressin and norepinephrine has been limited to single-center open-label studies, but has not shown a clear difference in the reversal of HRS or mortality[81,82]. Given the lack of clear benefit of terlipressin over norepinephrine, higher cost of terlipressin, warnings issued by the FDA, and established practice patterns using norepinephrine in the United States, it is unclear how quickly and widely terlipressin will be adopted.

Transjugular intrahepatic portosystemic shunt

There is interest in using transjugular intrahepatic portosystemic shunt (TIPS) treatment of HRS-AKI because it can improve portal hypertension and cardiac output, two of the central causes of HRS-AKI. Although data regarding the role of TIPS in HRS generally involves small numbers of patients, a meta-analysis of 128 patients treated with TIPS for HRS showed improvement in renal function in 93% of patients with HRS-AKI. The significance of this finding is difficult to assess as there was no comparison group, significant heterogeneity, and high mortality[83]. There is also significant risk associated with TIPS insertion in patients with HRS-AKI including 90-d mortality of 25%-80%[84]. However, it is difficult to determine to what degree the high mortality was the result of TIPS. Given the lack of prospective or larger well-conducted retrospective analysis of TIPS for HRS as well as high procedural risks and complications associated with TIPS insertion in patients with HRS-AKI, it remains difficult to accurately identify patients who will benefit. Perhaps the clearest benefit of TIPS in the management of HRS-AKI lies in prevention by ameliorating portal hypertension. This is supported by a lower incidence of HRS in patients with diuretic resistant ascites treated with TIPS compared to those treated with serial paracentesis (9% *vs* 31%)[85].

Table 2 Methods of estimating glomerular filtration rate and the novel biomarkers for diagnosis of acute kidney injury in cirrhosis

Ref.	Biomarker	Year Published-Patient population	Advantage(s)	Limitation(s)
Fagundes *et al*[102]	NGAL and/or IL-18	2012-Cirrhosis	Best supporting data; can differentiate HRS-AKI and ATN; predicts AKI progression; predicts mortality; NGAL has good performance in patients with ACLF	Increased in inflammation and infections (UTI); lack of standard cut-offs
Verna *et al*[103]		2012-Cirrhosis		
Tsai *et al*[104]		2013-Cirrhosis		
Gungor *et al*[105]		2014-Cirrhosis		
Belcher *et al*[55]		2014-Cirrhosis		
Barreto *et al*[106]		2014-Cirrhosis		
Qasem *et al*[56]		2014-Cirrhosis		
Treeprasertsuk *et al*[107]		2015-Cirrhosis		
Ariza *et al*[57]		2015-Cirrhosis		
Markwardt *et al*[59]	Cystatin C	2017-Cirrhosis	Predicts AKI progression; predicts short-term mortality; Used in combination with MELD score (MELD-cystatin score)	Increases in CKD
Maiwall *et al*[60]		2017-Cirrhosis		
Jaques *et al*[108]		2019-Cirrhosis		
Belcher *et al*[55]	KIM-1	2014-Cirrhosis	Predicts AKI progression; Predicts short-term mortality	Low sensitivity and specificity for differentiating causes of AKI
Ariza *et al*[57]		2015-Cirrhosis		
Belcher *et al*[55]	L-FABP	2014-Cirhrosis	Predicts AKI progression; predicts short-term mortality	Increased in CKD; poor performance in differentiating causes of AKI
Jiang *et al*[109]		2018-Cirrhosis		
Belcher *et al*[55]	Albumin	2014-Cirrhosis	Can differentiate HRS-AKI and ATN; good performance in ACLF; predicts short-term mortality; readily available	Decreased level in advanced cirrhosis

ACLF: Acute on chronic liver failure; AKI: Acute kidney injury; ATN: Acute tubular necrosis; CKD: Chronic kidney disease; HRS-AKI: Hepatorenal syndrome with acute kidney injury; IL-18: Interleukin 18; KIM-1: Kidney injury molecule 1; L-FABP: Liver-type fatty acid-binding protein; MELD: Model for end-stage liver disease; NGAL: Neutrophil gelatinase-associated lipocalin; SCr: Serum creatinine; UTI: Urinary tract infection.

Renal replacement therapy

Renal replacement therapy (RRT), usually in the form of continuous hemodialysis, is the second-line treatment in patients with HRS-AKI who fail medical management and often regarded as a bridge to organ transplant since it does not address the underlying physiology of HRS. Additionally, RRT does not improve survival in patients with HRS-AKI after failure of medical management[86]. Based on current evidence, RRT is best reserved for potential liver transplant candidates or if HRS-AKI is due to a potentially reversible condition such as infection or bleeding. In patients who are not transplant candidates or if the inciting cause is unclear or unlikely to be reversed, palliative care should be considered prior to initiation of RRT.

Artificial liver support systems

Liver support systems, including molecular adsorbent recirculating system and extracorporeal liver assist device, are forms of albumin dialysis where albumin recirculate as a scavenger of bacterial products and inflammatory cytokines have been considered for HRS-AKI. Thus far, there are no clear benefits in AKI-HRS and studies have shown mixed results regarding improving renal blood flow and survival[48]. Thus, further studies are needed before its use can be officially recommended in HRS-AKI but it may be considered as a bridge to transplant in selected patients.

Organ transplant

Liver transplant is considered definitive treatment for HRS-AKI because it reverses the underlying pathophysiology causing renal impairment. This is evidenced by renal recovery in up to 75% of patients with HRS after liver transplant alone (LTA)[87,88]. The strongest predictor of non-recovery of HRS-AKI is the duration of pretransplant dialysis, with each additional day of pretransplant dialysis increasing the risk of non-recovery by 6%[89]. Other pre-transplant factors associated with lack of renal recovery after LTA are older age, higher baseline SCr, prolonged ischemia during transplant, exposure to nephrotoxic agents, diabetes, and development of ATN[87-91]. Unfortunately, 6%-10% of patients with HRS who have LTA will develop end-stage renal disease by 1-year post-transplant[87-89]. Therefore, it

Table 3 Results of studies using vasoconstrictor therapy in patients with hepatorenal syndrome with acute kidney injury

Ref.	Study design	Treatment	Alb	HRS reversal (%)	Mortality (%)
Uriz *et al*[110], 2000	Prospective	Terlipressin	Yes	77	Not defined
Halimi *et al*[111], 2002	Retrospective	Terlipressin	No	72	Not defined
Moreau *et al*[112], 2002	Retrospective	Terlipressin	Yes	58	Not defined
Ortega *et al*[75], 2002	Prospective	Terlipressin	Yes	77	Not defined
Duvoux *et al*[113], 2002	Prospective	NE	Yes	83	Not defined
Solanki *et al*[60], 2003	Randomized	Terlipressin *vs* placebo	Yes	42 *vs* 0	58 *vs* 100
Alessandria *et al*[64], 2007	Randomized	Terlipressin *vs* NE	Yes	83 *vs* 70	25 *vs* 20
Neri *et al*[65], 2008	Randomized	Terlipressin *vs* placebo	Yes	81 *vs* 19	27 *vs* 58
Sharma *et al*[66], 2008	Randomized	Terlipressin *vs* NE	Yes	50 *vs* 50	45 *vs* 45
Sanyal *et al*[67], 2008	Randomized	Terlipressin *vs* placebo	Yes	34 *vs* 13	57 *vs* 62
Martin-Llahi *et al*[68], 2008	Randomized	Terlipressin *vs* placebo	Yes	44 *vs* 9	74 *vs* 83
Singh *et al*[69], 2012	Randomized	Terlipressin *vs* NE	Yes	39 *vs* 43	70 *vs* 65
Cavallin *et al*[70], 2015	Randomized	Terlipressin *vs* MID plus OCT	Yes	70 *vs* 29	30 *vs* 32
Cavallin *et al*[71], 2016	Randomized	Terlipressin infusion *vs* terlipressin bolus	Yes	56 *vs* 46	59 *vs* 43
Boyer *et al*[72], 2016	Randomized	Terlipressin *vs* placebo	Yes	24 *vs* 15	33 *vs* 35
Wong *et al*[73], 2019	Randomized	Terlipressin *vs* placebo	Yes	29 *vs* 16	73 *vs* 71
Wong *et al*[81], 2021	Randomized	Terlipressin *vs* placebo	Yes	32 *vs* 16	51 *vs* 45

Alb: Albumin; HRS: Hepatorenal syndrome; HRS-AKI: Hepatorenal syndrome with acute kidney injury; MID: Midodrine; NE: Norepinephrine; OCT: Octreotide.

Table 4 Results of recent meta-analyses comparing terlipressin to other vasoconstrictor therapies in hepatorenal syndrome with acute kidney injury

Ref.	Study design	Number of studies	HRS reversal	Mortality benefit	Data quality
Facciorusso *et al*[76], 2017	Meta-analysis	13	Same as NE; better than Alb+OCT; better than Alb+MID+OCT	Possible short-term benefits	Very low to low
Israelsen *et al*[77], 2017	Meta-analysis	10	Same as NE; better than Alb+OCT; better than Alb+MID+OCT	No difference	Very low to low
Nanda *et al*[78], 2018	Meta-analysis	13	Same as NE; better than Alb+OCT; better than Alb+MID+OCT	No difference	Poor to good
Wang *et al*[79], 2018	Meta-analysis	18	Same as NE; better than Alb+OCT; better than Alb+MID+OCT	Confers short-term benefits	Low to high
Best *et al*[80], 2019	Meta-analysis	25	Same as NE; better than Alb+OCT; better than Alb+MID+OCT	No difference	Very low to low

Alb: Albumin; HRS: Hepatorenal syndrome; HRS-AKI: Hepatorenal syndrome with acute kidney injury; MID: Midodrine; NE: Norepinephrine; OCT: Octreotide.

is essential to consider the likelihood of renal recovery after LTA and if the patient would benefit more from simultaneous liver-kidney transplant (SLKT). Currently, the main indications for SLKT are AKI requiring RRT or GFR < 25 mg/dL for more than 4-6 wk (guidelines vary) and CKD, commonly defined as GFR < 30 mg/dL at the time of listing with a GFR persistently < 60 mg/dL for at least 90 d[48,89]. The decision regarding LTA or SLKT must also weigh the potential benefit for the individual patient with consideration of the principles of just and equitable organ allocation. In an attempt to balance these factors agencies responsible for organ allocation have set specific guidelines in their respective countries [92].

CONCLUSION

Although the primary cause of HRS remains circulatory dysfunction resulting in impaired renal profusion, there is now an improved understanding of the role of other factors including the inflammatory environment and cardiac dysfunction. This has contributed to the development of better biomarkers for earlier and more accurate diagnosis of HRS. Despite not being widely available the offer promise that effective treatment can be applied during the critical early stages of the disease where there is the greatest potential for benefit. Medical treatment remains primarily vasoactive medications and albumin, and have not yet been able to exploit the improved understanding of pathophysiology. However, the approval of terlipressin in the United States and clearer delineation of patients most likely to benefit from this therapy offers hope for improved medical management in the future. Despite advances in medical treatment, liver transplantation remains the most definitive treatment and should be considered early in the disease course as delay can increase the risk of incomplete renal recovery after transplant.

FOOTNOTES

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REVIEW

Treatment of liver fibrosis: Past, current, and future

Chun-Ye Zhang, Shuai Liu, Ming Yang

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Abstract

Liver fibrosis accompanies the progression of chronic liver diseases independent of etiologies, such as hepatitis viral infection, alcohol consumption, and metabolic-associated fatty liver disease. It is commonly associated with liver injury, inflammation, and cell death. Liver fibrosis is characterized by abnormal accumulation of extracellular matrix components that are expressed by liver myofibroblasts such as collagens and alpha-smooth actin proteins. Activated hepatic stellate cells contribute to the major population of myofibroblasts. Many treatments for liver fibrosis have been investigated in clinical trials, including dietary supplementation (*e.g.*, vitamin C), biological treatment (*e.g.*, simtuzumab), drug (*e.g.*, pegbelfermin and natural herbs), genetic regulation (*e.g.*, non-coding RNAs), and transplantation of stem cells (*e.g.*, hematopoietic stem cells). However, none of these treatments has been approved by Food and Drug Administration. The treatment efficacy can be evaluated by histological staining methods, imaging methods, and serum biomarkers, as well as fibrosis scoring systems, such as fibrosis-4 index, aspartate aminotransferase to platelet ratio, and non-alcoholic fatty liver disease fibrosis score. Furthermore, the reverse of liver fibrosis is slowly and frequently impossible for advanced fibrosis or cirrhosis. To avoid the life-threatening stage of liver fibrosis, anti-fibrotic treatments, especially for combined behavior prevention, biological treatment, drugs or herb medicines, and dietary regulation are needed. This review summarizes the past studies and current and future treatments for liver fibrosis.

Key Words: Liver fibrosis; Molecular mechanism; Therapeutic targets; Treatments; Clinical trials

Core Tip: Liver fibrosis accompanies the progression of chronic liver diseases independent of their etiologies. The initiation and progression of liver fibrosis are mainly driven by liver inflammation, cell death, and metabolic dysregulation, which cause the activation of hepatic stellate cells and excessive accumulation of extracellular matrix proteins. Without effective treatments, liver fibrosis can lead to cirrhosis and primary liver cancer. To date, current therapeutic options for liver fibrosis are limited to prevent the initial causing factors for liver inflammation, hepatocyte cell death, and oxidative stress. However, the reverse of liver fibrosis is slowly and frequently impossible for advanced fibrosis or cirrhosis. To avoid the life-threatening stage of liver fibrosis, anti-fibrotic treatments including biological, medicines, dietary change, and behavior prevention are needed, especially for combined therapy.

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INTRODUCTION

Liver fibrosis accompanies the progression of chronic liver diseases independent of etiologies[1], such as hepatitis viral infection, alcohol abuse, and metabolic-associated fatty liver disease (MAFLD). It is commonly associated with liver injury, inflammation, and cell death. Abnormal accumulation of extracellular matrix (ECM) components expressed by liver myofibroblasts, such as collagens and alpha-smooth actin proteins, are the markers of hepatic fibrogenesis[2]. Activated hepatic stellate cells (HSCs) contribute to the major population of myofibroblasts in liver fibrosis[3]. Although many drugs have been investigated in clinical trials, there are no Food and Drug Administration (FDA)-approved treatments for liver fibrosis.

The activation of HSC is a complex pathogenesis in liver fibrosis[4]. Many factors including intrahepatic and extrahepatic factors can drive HSC activation to induce liver fibrosis. A variety of molecular signaling pathways are involved in the regulation of HSC activation[1,5], such as transforming growth factor-β (TGF-β), Toll-like receptors (TLRs), and epigenetic signals (*e.g.*, microRNAs, or miRNAs). The activation of HSCs can be divided into two phases, the initiation and perpetuation phases. RNA sequencing results have shown that fibrogenic transcriptional programs in the initiation phase are also active in the perpetuation phase; therefore, targeting the initial activation of HSC is also critically important for live fibrosis treatment[6].

In this review, the cellular and molecular mechanisms of liver fibrosis are reviewed. Importantly, the currently available treatments for liver fibrosis are summarized and discussed. Some pros and cons of available treatments are discussed. In addition, the future direction for liver fibrosis therapy is predicted.

INITIATION OF LIVER FIBROSIS: CELLULAR AND MOLECULAR MECHANISMS

Hepatic cell death

Liver cell death and inflammation are the initial events in chronic liver disease independent of etiologies. Many factors can cause liver injury and hepatic cell death and inflammation[7,8], including hepatitis viral infection, alcohol consumption, metabolic liver disease, abnormal bile acid products, and genetic factors. These pathogenic factors cause immune cell inflammation, hepatocyte death, mitochondrial dysfunction, and endoplasmic reticulum stress (Figure 1), resulting in HSC activation and differentiation to myofibroblasts to lead to liver fibrosis[5,9].

In non-alcoholic steatohepatitis (NASH), hepatocyte death results in the infiltration of monocyte-derived macrophages and upregulation of the expression of inflammatory cytokines[9], such as tumor necrosis factor α (TNF-α), TGF-β1, and interleukin-1β (IL-1β), Hepatocyte death can be classified into programed cell death including pyroptosis, apoptosis, necroptosis, ferroptosis, and autophagy-mediated cell death (Figure 2), as well as non-programed cell death (necrosis). In chronic liver disease, different types of cell death may be associated with the progression of liver fibrosis and end-stage of liver disease, such as hepatocellular carcinoma (HCC). Single-cell RNA sequencing coupled with spatial mapping approaches has been started to dissect the key cellular and molecular functions in liver disease [10].

Pyroptosis is an inflammatory cell death, associated with cell membrane rupture by cleaved gasdermin D[11]. The cleave of gasdermin D is induced by the activation of caspase-1 or caspase-11/4/5 [12]. For example, in mice with non-alcoholic fatty liver disease (NAFLD), feeding a high-fat diet can

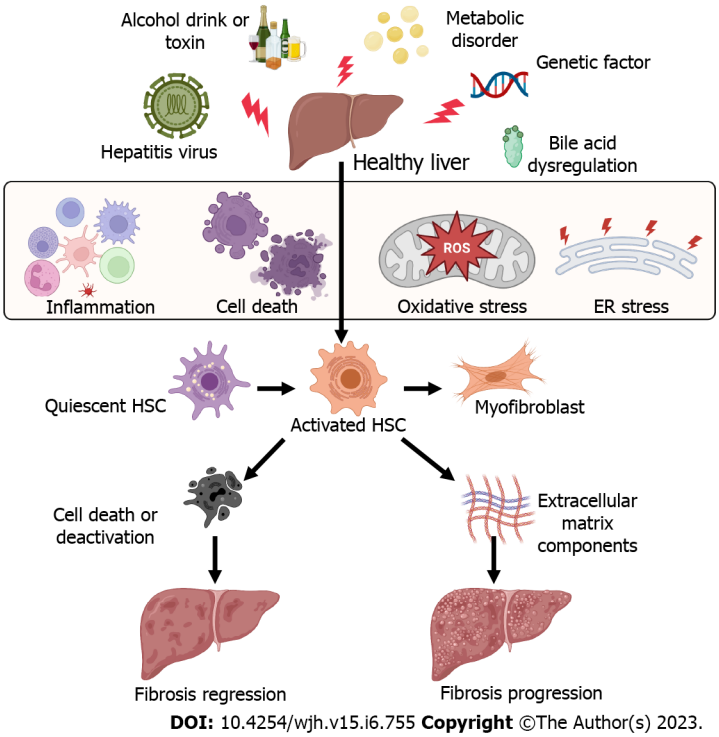

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Figure 1 Factors causing the activation of hepatic stellate cells and liver fibrosis. Many factors can cause liver injury and hepatic cell death and inflammation, including hepatitis viral infection, alcohol consumption, metabolic liver disease, abnormal bile acid products, and genetic factors. These pathogenic factors cause immune cell inflammation, hepatocyte death, oxidative stress, and endoplasmic reticulum stress, resulting in hepatic stellate cell activation and differentiation to myofibroblasts to lead to liver fibrosis. All cartoons in this figure were prepared using Biorender (https://biorender.com). ER: Endoplasmic reticulum; HSC: Hepatic stellate cell; ROS: Reactive oxygen species.

increase the expression of caspase-11 to cause pyroptosis of bone marrow monocyte-derived macrophages by cleave gasdermin D[13]. The expression of pyroptosis-related indicators including gasdermin D, IL-1β, and IL-18 has been shown to be increased in human patients with liver fibrosis and mice with CCl_4-induced fibrosis[14]. In addition, S100 calcium-binding protein A8 plays an essential role in macrophage pyroptosis in liver fibrosis, by inducing the expression of nucleotide-binding domain leucine-rich repeat-receptor, pyrin domain-containing-3 (NLRP3) inflammasome, pro-IL-1β, and pro-IL-18 *via* the activation of TLR4/nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) signaling pathway[14].

Apoptosis is a form of programmed cell death that occurs in all liver cell types. In hepatocytes, kindlin-2 deficiency can increase the apoptosis of hepatocytes, resulting in liver fibrosis and accumulation of ECM components by activating the TNF signaling pathway[15]. In NAFLD, microRNAs such as miR-22 from adipocyte-derived exosomes can cause hepatocyte apoptosis to increase hepatic inflammation, lipid accumulation, and fibrosis by regulating sirtuin 1 expression[16]. In contrast, promoting the apoptosis of HSCs can inhibit and reverse liver fibrosis. For example, treatment with gomisin D can inhibit CCl_4-induced HSC proliferation and activation in mice and increase HSC apoptosis to reduce liver fibrosis by regulating the platelet-derived growth factor receptor β signaling pathway[17].

Necroptosis is a regulated cell death that has the features of apoptosis and necrosis. Necroptosis can be induced by TLRs, interferons, and death receptors[18], which is mediated by receptor-interacting protein kinase-3 and its substrate mixed lineage kinase-like[19]. In addition, receptor-interacting serine/threonine-protein kinase 1 has an important role in this process (Figure 2). It contributes to hepatocyte death in NASH. Necroptotic hepatocytes cannot be removed by liver macrophages due to the activation of "don't-eat-me" signaling pathway, the CD47/signal regulatory protein alpha axis[20].

Ferroptosis is an intracellular iron-dependent lytic cell death which is different from apoptosis and necrosis[21,22]. Excessive iron accumulation and the inhibition of glutathione peroxidase 4 trigger ferroptosis, which causes cell plasma membrane rupture[23]. Accumulating research studies have demonstrated that ferroptosis is involved in different liver diseases, including alcoholic liver disease, NASH, cirrhosis, and cancer[24,25]. Several molecular signaling pathways are involved in ferroptosis in liver diseases, such as the nuclear factor erythroid 2-related factor 2[26], heat shock protein family A member 8[27], and nuclear receptor coactivator 4[28]. Pharmacological regulation of HSC ferroptosis is a therapeutic strategy for liver fibrosis[29]. For example, curcumol can induce ferroptosis of activated HSCs to inhibit liver fibrosis by inducing autophagy[28].

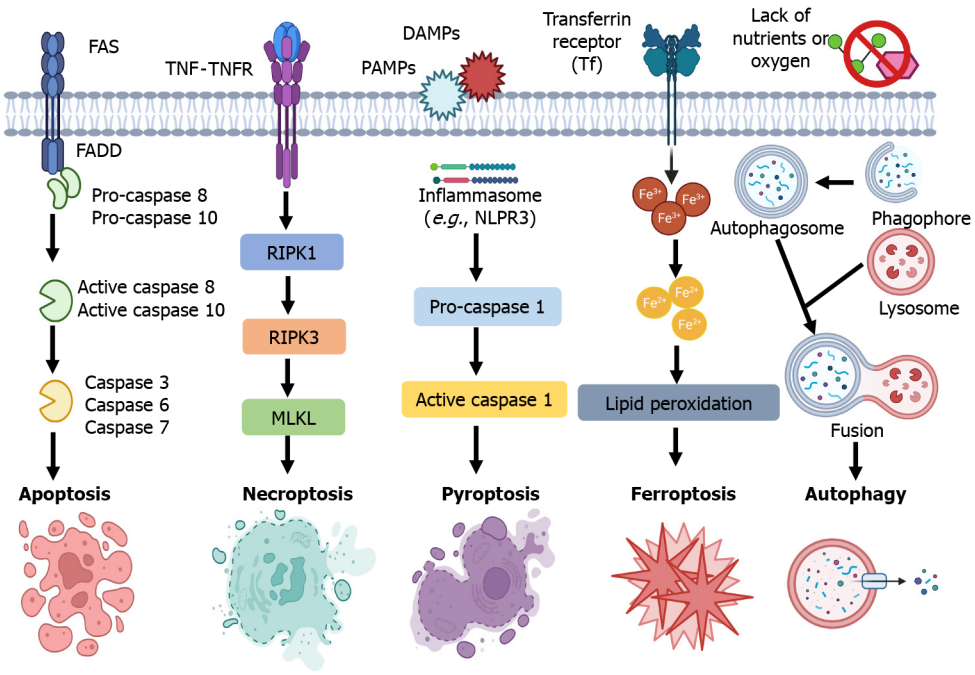

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Figure 2 Programmed cell death subtypes of hepatic cells, including apoptosis, necroptosis, pyroptosis, ferroptosis, and autophagy-mediated cell death. Apoptosis, necroptosis, pyroptosis, and ferroptosis are programmed forms of cell death, while necrosis is unprogrammed cell death. Autophagy-mediated cell death should be defined when autophagic flux is raised without the involvement of other types of programmed cell death, and pharmacological or genetic inhibition of autophagy blocks cell death. DAMPs: Danger-associated molecular patterns; FADD: Fas-associated protein with a death domain; FAS: Fas cell surface death receptor; MLKL: Mixed lineage kinase domain-like; NLRP3: Nod-like receptor family, pyrin domain containing 3; PAMPs: Pathogen-associated molecular patterns; RIPK1/3: receptor-interacting protein kinase 1/3; TNF: Tumor necrosis factor; TNFR: Tumor necrosis factor receptor. All cartoons in this figure were prepared using Biorender (https://biorender.com).

Furthermore, some dying cells develop autophagosomes that trigger apoptosis and necroptosis, namely autophagy-mediated cell death (ACD). However, ACD should be defined when some criteria are met[30], including: (1) Cell death happens without the involvement of other types of programmed cell death; (2) autophagic flux is raised; and (3) pharmacological or genetic inhibition of autophagy blocks cell death. Overall, the molecules implicated in the process and signaling pathways of hepatic cell death are potential targets for liver fibrosis.

Hepatic innate and adaptive immunity

Liver resident immune cells and infiltration of myeloid cells or circulating immune cells in the liver during chronic liver injury play key roles in the activation of HSCs. For example, in NASH liver, gut microbiota-diet interplay-resulted metabolite can activate liver macrophages to produce profibrotic factors[31], such as TGF-β1. Treatments such as astaxanthin can suppress the infiltration of monocyte-derived macrophages to suppress HSC activation, liver oxidative stress response, and hepatocyte death by decreasing the expression of proinflammatory cytokines[32], such as TNF-α, TGF-β1, and IL-1β. Another study also during NAFLD progression, mast cells can increase Western diet-induced biliary and liver damage to the development of microvesicular steatosis through microRNA (miR-144-3p)-targeted signaling pathway[33].

Adaptive immune cells including T and B cells have various roles in liver fibrosis. The imbalance of liver regulatory T cells and T helper (Th) cells, such as Th1 cells and Th17 cells plays an essential role in liver fibrosis, cirrhosis, and cancer[34]. The populations of multicytokine-producing CD4 T cells were significantly increased in the livers of patients with NASH compared with patients with NAFLD[35]. The cytokines produced by these CD4 T cells include TNF-α, IFN-γ, IL-17A, and IL-10. The phenotype of T cells is also important for their functions. One study showed that the reduction of tissue-resident memory $CD69^{+}CD103^{-}CD8$ T cells significantly decreased the resolution of fibrosis in NASH liver. These CD8 T cells can induce FasL/Fas-mediated apoptosis of HSCs[36]. Therefore, targeting immune cells or their secreted inflammatory cytokines is an optional treatment for liver fibrosis[37].

Furthermore, HSC activation is also associated with vascular aging[38], ischemia/reperfusion injury [39], and angiogenesis[40].

SIGNALING PATHWAYS AND MOLECULAR TARGETS FOR LIVER FIBROSIS TREATMENTS

Bile acid receptors

Farnesoid-X-receptor (FXR) and the G protein-coupled bile acid receptors are two widely studied bile acids regulating receptors, which play important roles in lipid and glucose metabolism, inflammation, fibrosis, and immune responses. Many FXR ligands have been investigated in clinical trials for NASH and liver fibrosis treatments, such as EDP-305[41,42], cilofexor[43,44], and MET409[45,46]. Hepatic concentrations of conjugated 12α-hydroxylated bile acids, such as taurodeoxycholate and glycodeoxycholate, were significantly increased in patients with NASH and mouse liver fibrosis models[47]. These bile acids contribute to HSC activation and liver fibrosis by regulating the signaling of G protein-coupled bile acid receptor 1, also known as TGR5.

Caspases

Caspases are involved in liver cell inflammation and hepatocyte cell death. Meanwhile, they play an essential role in liver fibrogenesis. For example, caspase 3-deficient mice on a methionine- and choline-deficient diet had reduced liver collagen production compared to wild-type mice[48]. Lipotoxicity can induce caspase-mediated apoptosis of hepatic cells and liver inflammation and injury in NAFLD. Treatment of caspase inhibitors such as emricasan (IDN-6556, a pan-caspase inhibitor) can reduce liver injury in patients with NAFLD[49].

Chemokine receptors

Chemokine receptors are commonly expressed by immune cells or inflammatory cells, which can be recruited during liver inflammation and fibrosis. For example, C-C chemokine receptor 2 (CCR2) and CCR5 are highly expressed by monocytes and subtypes of liver macrophages, which can be targeted to ameliorate liver fibrosis[50]. A recent study showed that the roles of CCR2 and CCR5 in liver macrophages are different during liver disease progression in mice with a hepatocyte-specific knock-out of NF-κB essential modulator. CCR2 oversees the recruitment of monocytes during liver injury, whereas CCR5 is needed to promote HSC activation[51].

In addition, many other chemokines and their receptors are involved in the pathogenesis of liver fibrosis, including C-X-C motif ligand 12 (CXCL12) /C-X-C receptor 4[52], chemokine (C-X3-C motif) ligand 1/C-X3-C receptor 1 (CX3CR1)[53], CCL19/CCR7[54], and CXCL12/atypical chemokine receptor 3[55].

Fibroblast growth factors

Fibroblast growth factor 15 (FGF15) is an important endocrine regulator for hepatic bile acid and lipid metabolism, which regulates gut-liver crosstalk in mice[56]. A combined treatment using an inhibitor of apical sodium-bile acid transporter (GSK233072) and adeno-associated virus 8-mediated hepatic FGF15 overexpression significantly can improve the therapeutic efficacy against NASH and fibrosis compared to either single treatment[57]. Bile acid nuclear receptor FXR plays an important role in the regulation of the expression of FGF15/19, bile acid homeostasis, and lipid metabolism, which is the target for NASH and liver fibrosis[58]. The expression of hepatic FXR and plasma FGF19 (the ortholog of mouse FGF15) was decreased in children with NASH compared to their expression in healthy subjects[59].

Galectins

Galectins are carbohydrate-binding proteins and play important roles in liver inflammation, immune response, and fibrosis. Galectin-1 (Gal-1) was shown to be highly expressed in the stroma of HCC by cancer-associated fibroblasts. Silencing Gal-1 in these fibroblasts can suppress inflammation and tumor progression[60]. The serum level of Gal-3 was increased in patients with advanced cirrhosis, and liver expression of Gal-3 was also correlated with liver disease severity and inflammation[61].

Lysyl oxidase family members

Lysyl oxidase (LOX) family members are extracellular copper-dependent enzymes, including LOX, lysyl oxidase-like 1 to 4 (LOXL1 to 4) members, which play important roles in the cross-linking of ECM proteins in fibrosis and carcinogenesis. Inhibition of pan-LOX family, LOX, LOXL1, or LOXL2 has been shown to prevent fibrogenesis and accelerate the reversal of liver fibrosis, as well as fibrosis in other organs. However, the roles of LOX family members as therapeutic targets for liver fibrosis need further to be evaluated[62].

NLRP3 inflammasome

Nucleotide-binding oligomerization (NLR) family pyrin domain-containing 3 (NLRP3) plays a pivotal role in liver fibrosis. Activation of NLRP3 can lead to the inflammatory response through the secretion of IL-1β and IL-18 and activation of caspase-1[63], which is involved in liver cell pyroptosis[64]. Activation of NLRP3 inflammasome can induce hepatocyte pyroptosis and liver fibrosis, while

inhibiting the activation of NLRP3 inflammasome can inhibit the development of NAFLD and NASH in animal models[65,66]. Activation of NLRP3 inflammasome in pyroptosis is mediated by canonical caspase-1-mediated signaling pathway and noncanonical caspase-11-mediated signaling pathway[67].

Peroxisome proliferator-activated receptors (PPARs)

PPARs, comprised of three subtypes PPARα, β/δ, and γ, play important roles in liver lipid metabolism, inflammation, and fibrosis[68-70]. The expression of liver PPARα was shown to be negatively correlated with NASH severity, visceral fat accumulation, and insulin resistance in human patients[71]. Treatment with PPARα/γ dual agonists decreased the concentrations of total cholesterol, triglyceride (TG), and inflammatory cytokine levels in serum, reduced hepatic steatosis, infiltration of inflammatory cells, and decreased the expression of lipogenic gene and NF-κB protein[72]. Another study showed that the levels of very low-density lipoprotein receptors (VLDLR) were increased in PPARβ/δ-deficient mice. In patients with hepatic steatosis, the mRNA levels of PPARβ/δ were suppressed and associated with an increase in VLDLR levels[73]. A pre-clinical study showed that treatment with pan-PPAR agonist lanifibranor can significantly decrease portal pressure and liver inflammation and induce fibrosis regression[74].

TGF-β/Smad

TGF-β/Smad is the most well-studied signaling pathway in fibrosis. SMAD proteins are essential intracellular effectors of TGF-β and show different roles in liver fibrosis[75], including pro-fibrotic functions (*e.g.*, SMAD3 and SMAD4) and protective functions (*e.g.*, SMAD2 and SMAD7). In addition, many studies have demonstrated that regulating the signaling pathway of TGF-β/Smad can prevent liver fibrosis[76], as well as the protein kinase B (PKB, or AKT)/Forkhead box O3 (FOXO3) signaling pathway.

Wnt/β-catenin

Proteins-derived from human amniotic mesenchymal stem cells, including insulin-like growth factor binding protein-3, Dickkopf-1, and DKK-3, can inhibit HSC activation by suppressing Wnt/β-catenin signaling pathway *in vitro*[77]. In vivo study also showed that treatment of niclosamide in rats can prevent CCl_4-induced liver fibrosis by inhibiting the Wnt/β-catenin pathway and glutaminolysis[78]. Another study also showed that Wnt3a can upregulate the expression of protein regulator of cytokinesis 1 to active β-catenin signaling to promote liver fibrosis[79]. The interaction of β-catenin/transcription factor 4 (TCF4) has been shown to increase during liver fibrosis in mice with bile duct ligation (BDL) [80]. Treatment with ICG-001, an inhibitor of the interaction between cyclic adenosine monophosphate response element binding protein binding protein and β-catenin, together with LF3, a small molecule antagonist that inhibits β-catenin/TCF4 transcriptional activity, can reduce liver fibrosis[80].

Yes-associated protein (YAP)

YAP plays a pivotal role in the sensitivity of HSCs to ferroptosis, apoptosis, and senescence in fibrotic livers. Selective depletion of YAP in myofibroblastic HSCs or activated HSCs can promote their senescence or apoptosis to reduce liver injury and fibrosis[81]. Taurocholic acid can induce the activation of HSCs through the sphingosine-1-phosphate receptor 2/YAP/p38 mitogen-activated protein kinase (p38 MAPK)[82].

CURRENT DIAGNOSIS FOR LIVER FIBROSIS

The golden standard method for liver fibrosis diagnosis is liver biopsy. Histological or histochemical staining can be used to stain the cells or the extracellular matrix proteins to identify liver fibrosis. Common histological staining methods for liver fibrosis evaluation are hematoxylin-eosin staining with Masson's trichrome or Sirius Red staining[83]. Due to the pain and the risk of potential complications of liver biopsy, non-invasive techniques (*e.g.*, elastography scanning) and biomarkers (*e.g.*, aminotransferase to platelet ratio (APRI): The aminotransferase/platelet ratio index) can be applied for diagnosing liver fibrosis[84]. Many available scoring systems can be applied for liver fibrosis diagnosis and evaluation, including fibrosis-4 index (FIB-4), APRI, and NAFLD fibrosis score (NFS)[85].

Imaging methods are commonly applied in the clinic to evaluate the progression of liver fibrosis. For example, ultrasound elastography techniques can be applied to characterize liver fibrosis and its stage in adult patients, such as vibration-controlled transient elastography, the most utilized and validated elastography method[86]. A meta-analysis study also showed that magnetic resonance elastography (MRE) and point-shear wave elastography (pSWE) can be applied for liver fibrosis diagnostic, and MRE is a more accurate imaging technique than pSWE[87]. The pooled sensitivities and specificities for MRE and pSWE were 0.94 (95% confidence level/CI: 0.89-0.97) and 0.95 (95%CI: 0.89-0.98), and 0.86 (95%CI: 0.80-0.90) and 0.88 (95%CI: 0.85-0.91), respectively. Their pooled summary receiver operating characteristic curves showed that the area under the curve (AUC) for MRE was 0.98 (95%CI: 0.96-0.99), whereas the AUC for pSWE was 0.93 (95%CI: 0.90-0.95). Another review paper has updated the conven-

tional and molecular imaging diagnostic methods for liver fibrosis[88]. In addition, artificial intelligence models have been applied for the diagnosis of liver fibrosis[89-91]. For example, the clinical features and imaging data collected from a patient can be analyzed for liver fibrosis diagnosis using a machine learning model.

Recently, studies also have shown that miRNAs, the single-stranded, non-coding RNAs containing 21 to 23 nucleotides, are involved in the pathogenesis of liver fibrosis, which are potential biomarkers for diagnosing liver fibrosis and therapeutic targets for liver fibrosis treatment[92]. The methods for liver fibrosis diagnosis have been reviewed in some recent publications[93-96]. Here, we will not discuss more details and will focus on the treatment options for liver fibrosis.

CURRENT TREATMENT OPTIONS FOR LIVER FIBROSIS

In this section, we review some different treatment options for liver fibrosis, such as biological intervention, anti-fibrotic drugs, and other treatment strategies. These treatments either target causing factors of liver fibrosis to accelerate the recovery of liver injury, or induce the balance of liver metabolism, such as anti-hepatitis viral infection, anti-cell death treatment, and regulators of lipid metabolism.

Biological intervention

Inhibition of LOXL2 in the fibrotic tumor microenvironment can synergistically increase the efficacy of sorafenib and 5-fluorouracil for liver cancer cells[97]. However, some treatments in clinical trials did not show promising results. For example, simtuzumab is a monoclonal antibody against LOXL2. In two phase 2b clinical trials, intravenous infusions of simtuzumab (200 or 700 mg) every other day for 48 wk and 96 wk did not show promising effects to decrease liver fibrosis and the progression of cirrhosis in patients with bridging fibrosis[98]. In a pilot clinical trial, intravenous treatment of simtuzumab (700 mg) every 2 wk for 22 wk did not improve liver biopsy fibrosis score for patients with advanced liver fibrosis[99].

Drug treatment

Aramchol, a partial inhibitor of hepatic stearoyl-CoA desaturase, has been shown to improve NASH and liver fibrosis in rodents and decrease liver triglycerides and fibrosis clinical trials[100].

Anti-hepatitis viral infection drugs: Inhibition of hepatitis viral infection can suppress liver inflammation and hepatocyte death to decrease liver injury, resulting in suppression of liver fibrosis. Drugs such as faldaprevir (also known as BI 201335)[101], ribavirin (HCV treatment)[102], and peginterferon alfa-2a (HBV treatment)[103,104], have been tested in clinical trials for the treatment of liver fibrosis. In addition, many other drugs have been evaluated or are under clinical trial evaluation against hepatitis viral infection[105-107], such as simeprevir, daclatasvir, and sofosbuvir.

Cenicriviroc: C-C chemokine receptors 2 and 5 dual antagonist, has been shown to improve liver fibrosis without worsening NASH compared to the placebo in phase 2 clinical trial (Clinicaltrials.gov, NCT02217475)[108]. A phase 3 clinical trial has been designed to confirm the efficacy and safety of cenicriviroc for liver fibrosis treatment in adults with NASH[109].

Cholangitis treatment: Obeticholic acid and ursodeoxycholic acid are the only two FDA-approved medicines for the treatment of primary biliary cholangitis[110], which have the potential to cholangitis-induced liver fibrosis.

Cyclophilin inhibitors: CRV431, a pan-cyclophilin inhibitor, can decrease liver fibrosis in mice treated with CCl_4 for 6 wk and mice with diet-induced NASH[111]. Another cyclophilin inhibitor NV556 also displays an antifibrotic effect in two mouse NASH models, the STAM model (streptozotocin plus a high-fat diet) and methionine- and choline-deficient diet-induced NASH model[112]. In addition, NV556 can also inhibit TGF-β1-induced activation of HSCs *in vitro*.

FGF regulators or analogues: Treatment of pegbelfermin (BMS-986036, 10 mg or 20 mg daily), a PEGylated human FGF21 analogue, can significantly decrease liver fat accumulation in patients with NASH without treatment-related severe adverse effects, and it can also improve liver fibrosis in patients with obesity and type 2 diabetes[113].

FXR agonists: Treatment of obeticholic acid (INT-747), a potent and orally active FXR agonist, can significantly ameliorate liver fibrosis and the histological and biological markers of NASH in patients with NASH[114].

Gal-3 inhibitors: GB1211, an inhibitor of Gal-3, can inhibit the differentiation of epithelial cells into myofibroblasts and macrophage or myofibroblast-induced fibrosis in the liver[115]. GR-MD-02 (belapectin), a galectin-3 inhibitor, has been shown to inhibit liver fibrosis and portal hypertension in rat

fibrosis mode, which is safe and well-tolerated in a phase 1 clinical trial. However, a phase 2b clinical trial showed that treatment of GR-MD-02 did not significantly improve liver fibrosis and reduce portal hypertension (hepatic venous pressure gradient) in patients with NASH[116]. Further studies are required to evaluate these treatments for liver fibrosis.

Glucagon-like peptide-1 (GLP-1) receptor agonist: GLP-1 analogues have been shown to have the effects to reduce liver fat accumulation, liver injury, and insulin resistance in mice with fatty liver disease. Clinical trial (ClinicalTrials.gov, NCT01237119) showed that treatment of GLP-1 analogue liraglutide was well tolerated and suppressed liver fibrosis progression in patients with NASH[117]. Another trial also showed that treatment of liraglutide markedly reduced liver fat content and body weight in patients with uncontrolled type 2 diabetes[118].

Pan-caspase inhibitor: Emricasan (IDN-6556), a pan-caspase inhibitor, can decrease liver cell apoptosis and inflammation and improve portal pressure in rats with CCl_4-induced cirrhosis[119]. However, a clinical trial (Clinicaltrials.gov, NCT03205345) did not show the efficacy of emaricasan against liver fibrosis, but it was safe and well-tolerated.

PPAR agonists: In rats with BDL-induced liver fibrosis, treatment of PPAR-γ agonist thiazolidinedione inhibited HSC activation and liver fibrosis by regulating fibrogenic factors, such as TGF-β1, platelet-derived growth factor, and connective tissue growth factor[120]. Farglitazar (GI262570), an agonist of peroxisome proliferator-activated receptor-gamma (PPARγ), can inhibit HSC activation.

Tropifexor: A non-bile acid FXR agonist, can potently inhibit cholestatic liver injury and fibrosis by enhancing the expression of FGF19 in the ileum and the expression of small heterodimer partner in the livers of piglets but inhibit cholesterol 7α-hydroxylase. In addition, tropifexor can increase the abundance of bile acid-biotransforming bacteria and later the amino acid composition in the intestine and decrease intestinal barrier injury in piglets with BLD[121]. Clinical trial (Clinicaltrials.gov, NCT02855164) also showed that treatment of tropifexor (10-90 μg) once daily for 12 weeks was safe and decreased the levels of alanine aminotransferase (ALT) and hepatic fat fraction (HFF) compared to baseline in a dose-dependent manner. The decrease of ALT and HFF can be sustained for up to 48 wk at high doses of tropifexor (140 μg and 200 μg once daily)[122].

Natural products or herbal medicines

Natural products or herbal medicines display diverse roles in the treatment of liver fibrosis. For example, a classical Traditional Chinese Medicine formula Yinchenhao decoction has been shown to ameliorate dimethylnitrosamine-induced liver fibrosis in rats and suppress liver cell apoptosis[123]. Another study showed that Xiaoyaosan decoction significantly reduced CCl_4-induced liver fibrosis in rats by regulating both TGF-β1/Smad and AKT/FOXO3 signaling pathways[76]. The major components of these herbal medicines such as Tanshinone IIA extracted from the traditional herbal medicine Salvia miltiorrhiza display broad biological activities, such as anti-inflammatory, antioxidant, antiangiogenic, and anticancer functions[124]. Furthermore, clinical trials also illustrate that these traditional medicine formula such as Fuzheng Huayu display therapeutic effects against hepatitis-B-caused cirrhosis in patients[125].

Dietary regulation or supplementation

Consumption of polyunsaturated fatty acids: The endogenous metabolites of n-3 polyunsaturated fatty acids such as 19,20-epoxy docosapentaenoic acid show a protective effect against liver fibrosis in mouse NASH models[126]. G protein-coupled receptors can be regulated by polyunsaturated fatty acids to reduce liver inflammation and fibrosis[127]. For example, supplementation of docosahexaenoic acid, an omega-3 fatty acid, can reduce liver inflammation and prevent liver fibrosis in diet-induced liver fibrosis model *via* G protein-coupled receptor 120 (GPR120) signaling, also known as free fatty acid receptor 4[128].

Probiotics: Treatment with probiotic *Lactobacillus rhamnosus* GG can significantly decrease liver inflammation and fibrosis by reducing the production of hepatic bile acids in mice with BLD[129].

Vitamins: The serum levels of vitamin C have been shown to be negatively associated with the odds of liver fibrosis in patients with NAFLD in United States adults[130]. Another study showed that a decreased serum level of vitamin B12 is associated with an increased risk of liver fibrosis in patients with NAFLD[131]. Treatment of Vitamin D_3 can alleviate liver injury and the expression of ECM proteins such as TGF-β and α-SMA in thioacetamide-induced hepatic fibrosis rat model[132]. The data from the National Health and Nutrition Examination Survey (2017-2018) also showed that levels of 25-Hydroxyvitamin D were inversely associated with liver fibrosis during NAFLD development and progression[133].

Antioxidant and anti-inflammatory agents: Supplementation of natural products with antioxidant and anti-inflammatory components can also ameliorate chronic liver disease to improve liver fibrosis and

inhibit cancer development[9], such as β-sitosterol and silymarin.

Bariatric surgery

Studies have shown that bariatric surgery (BS) can provide long-term benefits for the resolution of liver fibrosis. The two most common procedures of BS are laparoscopic Roux-en-Y-gastric and laparoscopic sleeve gastrectomy. For example, one study showed that NASH was resolved in 84% of patients (95%CI: 73.1%-92.2%) at year 5 post-BS treatment, while fibrosis was decreased in samples from 70.2% of patients (95%CI: 56.6%-81.6%) compared with baseline and fibrosis was disappeared in samples from 56% of all patients (95%CI: 42.4%-69.3%)[134]. BS has been shown to induce NASH disappearance in nearly 85% (95%CI: 75.8%-92.2%) of patients and to decrease fibrosis in 33.8% of patients (95%CI: 23.6%-45.2%) with NASH at 1 year after surgery[135]. Another clinical study showed that excessive weight loss shown in patients with cirrhosis with 73% (33%–167%), 85% (33%–190%), and 73% (29%–107%) after 1, 2, and 3 years of BS[136], respectively. Among 27 patients with cirrhosis, 3 patients had significant improvement in liver function and did not need liver transplantation, whereas 2 out of 27 patients had deleterious liver function post-BS treatment[136].

Genetic intervention

Gene therapy is a critical tool for disease treatment, including liver fibrosis and cancer. Noncoding RNAs, such as miRNAs and long noncoding RNAs, small interference RNAs, and circular RNAs are important. For example, the treatment of siRNA silencing CCR2 can regulate liver immune to inhibit the infiltration of profibrotic macrophages and neutrophils in murine fibrotic livers[137]. Another study showed that circRNA ASPH regulated liver fibrosis by binding miR-139-5p by regulating neurogenic locus notch homolog protein 1 (Notch 1) expression[138].

Transplantation of stem cells

Transplantation of umbilical cord Wharton's Jelly-derived mesenchymal stem cells to rats with CCl_4-induced hepatic fibrosis improved liver function, inflammation, and fibrosis *via* a paracrine mechanism possibly by targeting TGF-β1 signaling pathway[139]. Another study showed that transplantation of human umbilical cord blood mesenchymal stem cells substantially improved liver fibrosis in histopathological evaluation compared to that in the untreated group[140]. Infusions of hematopoietic stem cells into mice with methionine-choline-deficient diet- or CCl_4-induced liver fibrosis can reduce hepatic collagen production and the expression of α-smooth muscle actin[141,142].

Overall, there are several potent preventive and therapeutic treatments for liver fibrosis, including physical activity (*e.g.*, running), dietary change (*e.g.*, avoid of high-fat and high-sugar diet), dietary supplementation (*e.g.*, vitamin C), biological treatment (*e.g.*, simtuzumab), bariatric surgery (*e.g.*, Roux-en-Y-gastric procedure), drug (*e.g.*, pegbelfermin), change of gut microbiota (*e.g.*, probiotics), nanoparticles (*e.g.*, BMS-986263), genetic regulation (*e.g.*, non-coding RNAs), and transplantation of stem cells (*e.g.*, hematopoietic stem cells) (Figure 3).

CLINICAL TRIALS

In this section, we first review some completed clinical trials (Clinicaltrials.gov, Table 1). These treatments including biological treatment, drugs, dietary supplementation, and infusion of stem cells.

Future treatments

There is an unmet need for treatments for liver fibrosis due to the efficacy of available treatments. Some drugs with potent anti-fibrotic effects in pre-clinical models are now waiting to be further evaluated in clinical trials (Table 2). The promising preventive and therapeutic treatments for liver fibrosis, including treatment of hepatitis viral infection (*e.g.*, Peginterferon Alfa 2a), transplantation of mesenchymal stem cells, bariatric surgery for patients with obesity and NAFLD, dietary modification (*e.g.*, Mediterranean diet or Calorie-restricted diet).

Furthermore, deliver system can be applied to increase the efficiency of anti-fibrotic treatments. For example, BMS-986263, a lipid nanoparticle, has been applied to deliver small interfering RNA to degrade mRNA of heat shock protein 47, a key collagen chaperone involved in the pathogenesis of fibrosis. Treatment of MS-986263 in patients with HCV infection and sustained virologic response improved the Ishak score, the histology activity index score for levels of liver fibrosis[143]. Many other types of nanoparticles have been applied to treat liver fibrosis or its causing chronic liver disease, such as Fibroblast growth factor 2 conjugated superparamagnetic iron oxide nanoparticles[144], cerium oxide nanoparticles[145], and silymarin-conjugated gold nanoparticles[146].

Table 1 Completed clinical trials (Clinicaltrials.gov, accession date: March 1, 2023)

Method	Intervention	Trial number	Phase	Title	Condition
Biological	Peginterferon alfa-2b (SCH 54031)	NCT00049842	3	Prevention of Disease Progress in Chronic Hepatitis C Patients with Liver Fibrosis (Study P02570AM2)	Chronic HCV; Liver fibrosis
	PegIntron (peginterferon alfa-2b; SCH 54031) REBETOL (ribavirin; SCH 18908)	NCT00039871	3	PEG-Intron Plus Rebetol Treatment of Chronic Hepatitis C Subjects Who Failed Response to Alpha-Interferon Plus Ribavirin (Study P02370)	Hepatitis; HCV; Fibrosis; Liver cirrhosis
	Simtuzumab	NCT01707472	2	Study of Simtuzumab in HIV and/or Hepatitis C-Infected Adults with Liver Fibrosis	Liver fibrosis; HCV infection; HIV
	Simtuzumab	NCT01672853	2	Simtuzumab (GS-6624) in the Prevention of Progression of Liver Fibrosis in Adults with PSC	PSC
Dietary regulation	GK#10; Placebo	NCT01598064	N/A	Probiotics for Liver Cirrhosis with Portal Hypertension	Liver cirrhosis; Portal hypertension
Drug	Aramchol	NCT02279524	2	A Clinical Trial to Evaluate the Efficacy and Safety of Two Aramchol Doses Versus Placebo in Patients With NASH	Fatty liver; NASH; Liver fibrosis
	BI 201335	NCT01909778	1	Open Label Single Dose Phase I Trial of BI 201335 to Study Pharmacokinetics and Safety in Patients with Compensated Liver Cirrhosis	HCV; Liver cirrhosis
	BMS-986036; Placebo	NCT03486912	2	A Study of Experimental Medication BMS-986036 in Adults with NASH and Liver Cirrhosis	Hepatic cirrhosis; Liver fibrosis; NAFLD; NASH
	BMS-98603; Other: Placebo	NCT03486899	2	A Study of Experimental Medication BMS-986036 in Adults with NASH and Stage 3 Liver Fibrosis	Liver fibrosis; NAFLD; NASH
	BMS-986263 placebo	NCT03420768	2	A Study of Experimental Medication BMS-986263 in Adults with Advanced Hepatic Fibrosis After Cure of Hepatitis C	Hepatic cirrhosis; Liver fibrosis
	Cenicriviroc placebo	NCT02217475	2	Efficacy and Safety Study of Cenicriviroc for the Treatment of NASH in Adult Participants with Liver Fibrosis	NASH
	CRV431 placebo	NCT04480710	2	A Study of CRV431 Dosed Once Daily in NASH-induced F2 and F3 Subjects	NASH; Fibrosis; NAFLD
	Fuzheng Huayu placebo	NCT00854087	2	Assess the Antifibrotic Activity of Fuzheng Huayu in Chronic Hepatitis C Patients with Hepatic Fibrosis	Chronic HCV
	GI262570 placebo	NCT00244751	2	Antifibrotic Activity of GI262570 In Chronic Hepatitis C Subjects	Cirrhosis, liver
	GR-MD-02 placebo	NCT02462967	2	Clinical Trial to Evaluation the Safety and Efficacy of GR-MD-02 for the Treatment of Liver Fibrosis and Resultant Portal Hypertension in Patients with Nash Cirrhosis	Hypertension, portal
	GR-MD-02; Placebo	NCT02421094	2	Clinical Trial to Evaluate Efficacy of GR-MD-02 for Treatment of Liver Fibrosis in Patients with NASH With Advanced Fibrosis	NASH
	IDN-6556; Placebo	NCT02230670	2	A Study of IDN-6556 in Subjects with Liver Cirrhosis	Liver cirrhosis; Hepatic cirrhosis
	IDN-6556; Placebo	NCT02138253	2	A Trial of IDN-6556 in Post Orthotopic Liver Transplant for Chronic HCV	Liver fibrosis; Liver cirrhosis
	INT-747; Ursodeoxycholic acid; Placebo	NCT00550862	2	Study of INT 747 in Combination with URSO in Patients with PBC	Liver cirrhosis; Biliary injury
	Nitazoxanide; BID	NCT03656068	2	An Evaluation of the Safety and Efficacy of Nitazoxanide on Collagen Turnover in NASH Patients with Fibrosis	NASH; Fatty liver; Fibrosis; Compensated cirrhosis
	Placebo obeticholic acid	NCT00570765	2	Study of INT-747 as Monotherapy in Participants with PBC	Liver cirrhosis, biliary injury
	SEL; Simtuzumab	NCT02466516	2	Safety, Tolerability, and Efficacy of GS-4997 Alone or in Combination with Simtuzumab in Adults with NASH and Fibrosis Stages F2-F3	NASH
	Simeprevir; Daclatasvir;	NCT02349048	2	Study to Assess Efficacy, Safety, Tolerability and	HCV

	Sofosbuvir			Pharmacokinetics of Simeprevir, Daclatasvir and Sofosbuvir in Treatment-naive Participants with Chronic HCV Genotype 1 Infection	
	Tropifexor (LJN452) CVC	NCT03517540	2	Study of Safety, Tolerability, and Efficacy of a Combination Treatment of LJN452 and CVC in Adult Patients with NASH and Liver Fibrosis	NASH
	Peginterferon alfa-2a + Ribavirin; Peginterferon alfa-2a	NCT00006164	3	Long Term Interferon for Patients Who Did Not Clear HCV with Standard Treatment	Chronic HCV; Cirrhosis; Fibrosis; Hepatic cirrhosis
	OMACOR placebo oral capsule	NCT00760513	4	Treatment of non-Alcoholic Fatty Liver Disease With n-3 Fatty Acids	NAFLD
	Ceftriaxone normal saline	NCT04218695	4	Prophylactic Antibiotics in Admitted Cirrhotics	Cirrhosis, LIVER
	Proton pump inhibitors placebo	NCT03175731	4	PPIs and Gastroesophageal Varices in Liver Cirrhosis (PPIs: Proton pump inhibitors)	Liver cirrhosis; Hypertension, portal
Other	Human fetal liver cell transplantation	NCT01013194	1 or 2	Human Fetal Liver Cell Transplantation in Chronic Liver Failure	Liver cirrhosis
	G-colony stimulating factor and infusion of the mobilized monocyte cells	NCT01503749	1	Safety and Efficacy Study of Peripheral Blood Mononuc-leated Cells for Treatment of Liver Cirrhosis	Liver cirrhosis
	Leukapheresis; Infusion of stem cells *via* image-guided scan	NCT00147043	N/A	Adult Stem Cell Therapy in Liver Insufficiency	Liver cirrhosis

N/A: Not applicable; CVC: Cenicriviroc; HCV: Hepatitis C virus; HIV: Human immunodeficiency virus; NAFLD: non-alcoholic fatty liver disease; NASH: Nonalcoholic steatohepatitis; PBC: Primary biliary cirrhosis; PSC: Primary sclerosing cholangitis.

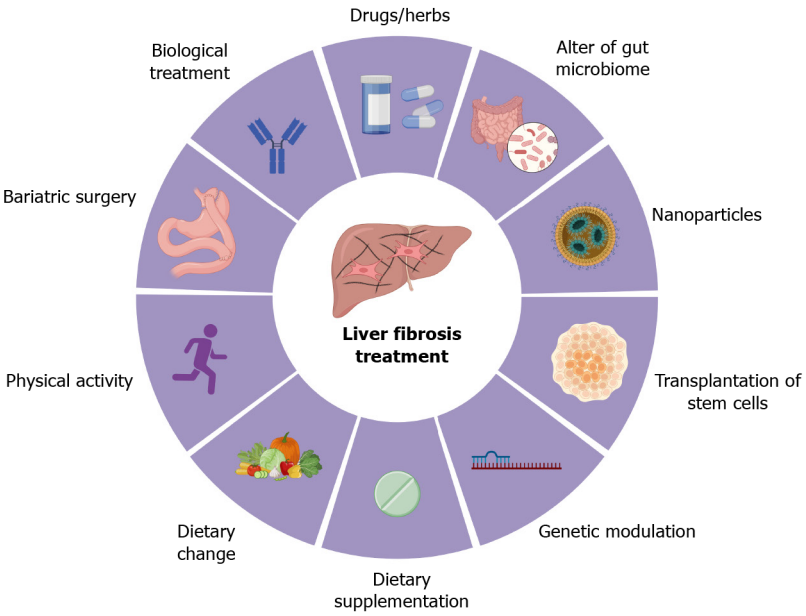

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Figure 3 Treatment options for liver fibrosis. Currently, the preventive and therapeutic treatments for liver fibrosis include physical activity (*e.g.*, running), dietary change (*e.g.*, avoid of high-fat and high-sugar diet), dietary supplementation (*e.g.*, vitamin C), biological treatment (*e.g.*, simtuzumab), bariatric surgery (*e.g.*, Roux-en-Y-gastric procedure), drugs and herb medicines (*e.g.*, pegbelfermin), change of gut microbiota (*e.g.*, probiotics), nanoparticles (*e.g.*, BMS-986263), genetic regulation (*e.g.*, non-coding RNAs), and transplantation of stem cells (*e.g.*, hematopoietic stem cells). All cartoons in this figure were prepared using Biorender (https://biorender.com).

CONCLUSION

Liver fibrosis accompanies the progression of chronic liver diseases independent of their etiologies. The initiation and progression of liver fibrosis are mainly driven by liver inflammation and hepatocyte or cholangiocyte injury and damage, resulting in the activation of HSCs and their differentiation into ECM protein-producing myofibroblasts. Thus, current therapeutic options for liver fibrosis are to prevent the initial causing factors for liver inflammation, hepatocyte cell death and oxidative stress. Unfortunately,

Table 2 Recruiting and active clinical trials (Clinicaltrials.gov, accession date: March 1, 2023)

Method	Interventions	NCT number	Phases	Title	Conditions
Drug	ZED1227; Placebo	NCT05305599	2	Different Doses of ZED1227 *vs* Placebo in NAFLD	NAFLD; Fibrosis
	Tropifexor; Licogliflozin; Placebo	NCT04065841	2	Efficacy, Safety, and Tolerability of the Combination of Tropifexor & Licogliflozin and Each Monotherapy, Compared with Placebo in Adult Patients with NASH and Liver Fibrosis	NASH; Liver fibrosis
	Tenofovir disoproxil Fumarate; PEG-Interferon alfa 2a	NCT03957629	N/A	Optimized Treatment of Peginterferon Alfa 2a in Treatment Experienced Patients with HBV-Related Liver Fibrosis	Hepatitis B; Fibrosis
	Sildenafil	NCT04908657	4	Sildenafil for Liver Fibrosis in Adolescents and Adults After Fontan Operation	Fibrosis
	Saroglitazar magnesium	NCT05011305	2	Saroglitazar Magnesium for the Treatment of NASH with Fibrosis	NASH; Fibrosis
	Saroglitazar magnesium	NCT05045482	1	Hepatic Impairment with Cirrhosis Due to Cholestatic Liver Disease	Hepatic impairment; Cirrhosis
	Rivaroxaban apixaban	NCT04874428	1	Direct Oral Anticoagulants (Rivaroxaban and Apixaban) in Patients with Liver Cirrhosis	Cirrhosis
	Resmetirom; Placebo	NCT05500222	3	A Phase 3 Study to Evaluate the Effect of Resmetirom on Clinical Outcomes in Patients with Well-compensated NASH Cirrhosis (MAESTRO-NASH-OUTCOMES)	NASH; Cirrhosis
	Rencofilstat; Placebo	NCT05402371	2	A Study to Evaluate the Efficacy and Safety of Rencofilstat in Subjects with NASH and Advanced Liver Fibrosis	NASH; Fibrosis, Liver NAFLD
	Placebo; Esomeprazole	NCT04448028	4	Stop of Proton-pump Inhibitor Treatment in Patients with Liver Cirrhosis - a Double-blind, Placebo-controlled Trial	Liver cirrhosis
	PHIN-214	NCT05490888	1	Single Dose Escalation of PHIN-214 in Child-Pugh A and B Liver Cirrhotics	Cirrhosis; Fibrosis; Hepatic ascites
	Placebo zibotentan + dapagliflozin	NCT05516498	2	Zibotentan and Dapagliflozin Combination, EvAluated in Liver Cirrhosis (ZEAL Study)	Cirrhosis
	L-ornithine; L-aspartate	NCT05737030	4	Effect of L-ornithine-L-aspertate (LOLA) on the Gut Microbiome	Cirrhosis
	Hydronidone capsules; The placebo capsules	NCT05115942	3	Hydronidone for the Treatment of Liver Fibrosis Associated with Chronic Viral Hepatitis B Phase 3 Trial	Liver fibrosis
	Growth hormone	NCT05253287	2/3	Growth Hormone in Decompensated Liver Cirrhosis	Cirrhosis; Fibrosis
	Empagliflozin 10 MG; Placebo pills	NCT05147090	4	Effects of Empagliflozin on Fibrosis and Cirrhosis in Chronic Hepatitis B	NAFLD; Cirrhosis; Fibrosis
	Cotadutide; Placebo	NCT05364931	2/3	A Study to Evaluate the Safety and Efficacy of Cotadutide Given by Subcutaneous Injection in Adult Participants with Non-cirrhotic Non-alcoholic Steato-hepatitis with Fibrosis	Non-cirrhotic NASH with Fibrosis
	Candesartan; Ramipril	NCT03770936	3	Effect of Some Drugs on Liver Fibrosis	Liver fibrosis
	Branched-chain amino acid; Placebo	NCT03633279	4	Treatment of Sarcopenia Improves the Muscle Mass and Muscle Strength of Patients with Liver Cirrhosis-Child C	Liver cirrhosis
	BMS-986263; Placebo	NCT04267393	2	Safety and Effectiveness of BMS-986263 in Adults with Compensated Cirrhosis (Liver Disease) From Nonalcoholic Steatohepatitis (NASH)	NASH
	AZD4831; Placebo	NCT05638737	2	A Study in Participants with Non-cirrhotic NASH With Fibrosis	Non-cirrhotic NASH with fibrosis
	Atorvastatin; Placebo	NCT05028829	2	Safety and Efficacy of Atorvastatin v. Placebo on HCC Risk	Liver fibrosis; Cirrhosis
Dietary supplement	Leucine enriched essential amino acid; Balanced amino acid	NCT03208868	N/A	Leucine-Enriched Essential Amino Acid Mixture to Reverse Muscle Loss in Cirrhosis	Cirrhosis

	supplement (BAA)				
	Hydroxy methyl butyrate; Balanced Amino Acids	NCT05166499	N/A	HMB Enriched Amino Acids to Reverse Muscle Loss in Cirrhosis	Cirrhosis
Biological	Umbilical cord-derived mesenchymal stem cell comprehensive treatment	NCT03945487	2	Mesenchymal Stem Cells Treatment for Decompensated Liver Cirrhosis	Decompensated liver cirrhosis
	Mesenchymal stem cell	NCT03254758	1/2	A Study of ADR-001 in Patients with Liver Cirrhosis	Decompensated liver cirrhosis
	Human umbilical cord-derived mesenchymal stem cells	NCT05227846	1	Human Umbilical Cord-derived Mesenchymal Stem Cells for Decompensated Cirrhosis (MSC-DLC-1)	Decompensated cirrhosis
	Human umbilical cord-derived mesenchymal stem cell infusion	NCT05331872	1	Umbilical Cord-derived Mesenchymal Stem Cell Infusion in the Management of Adult Liver Cirrhosis	Liver cirrhosis
	Fecal microbiota transplantation; Placebo	NCT04932577	2/3	Fecal Microbiota Transplantation for Liver Cirrhosis	Cirrhosis
	Cellgram-LC	NCT04689152	3	Clinical Trial to Evaluate the Efficacy and Safety of Cellgram-LC Administration in Patients with Alcoholic Cirrhosis	Alcoholic cirrhosis
	Autologous BM MSC	NCT03626090	1/2	Mesenchymal Stem Cell Therapy for Liver Cirrhosis	Cirrhosis
	Allogeneic umbilical cord mesenchymal stem cell	NCT04357600	1/2	Umbilical Cord Mesenchymal Stem Cell for Liver Cirrhosis Patient Caused by Hepatitis B	Cirrhosis
Other	Weight loss	NCT05104541	N/A	Impact of Weight Loss in Cirrhosis with Obesity and MAFLD	Liver cirrhosis
	Lifestyle therapy bariatric surgery	NCT03472157	N/A	A Randomized Controlled Study Evaluating Bariatric Surgery as a Treatment for Severe NASH With Advanced Liver Fibrosis in Non-severe Obese Patients	Surgery; Obesity; NASH; Cirrhosis
	Indo mediterranean diet calorie restricted diet	NCT05073588	N/A	Effect of Indo-Mediterranean Diet on Hepatic Steatosis and Fibrosis in NAFLD Children	NAFLD

N/A: Not applicable; HCC: Hepatocellular carcinoma; NAFLD: Non-alcoholic fatty liver disease; NASH: Nonalcoholic steatohepatitis.

the reverse of liver fibrosis is slowly and frequently impossible for advanced fibrosis or cirrhosis. Liver transplantation is the only therapeutic option for the late stage of liver cirrhosis and cancer. To avoid the life-threatening stage of advanced liver fibrosis and cirrhosis, anti-fibrotic treatments including biological, medicines, dietary change, and behavior prevention are needed. Currently, promising treatments for liver fibrosis are still the preventive strategies, such as treatment of hepatitis viral infection (*e.g.*, Peginterferon Alfa 2a), inhibition of the progression of MAFLD and obesity (*e.g.*, bariatric surgery), dietary modification (*e.g.*, Mediterranean diet or Calorie-restricted diet). In addition, nano-delivery systems have been applied to improve the treatment efficacy and specifically deliver the treatments. Pre-clinical and clinical evaluations for new treatments of liver fibrosis are required while we still lack currently effective strategies for liver fibrosis treatment. The treatment efficacy can be evaluated by histological staining methods, imaging methods, and serum biomarkers, as well as fibrosis scoring systems, such as FIB-4, APRI, and NFS. Although many anti-fibrotic candidate agents have shown robust effects in experimental animal models, their anti-fibrotic effects in clinical trials are less clear. The development of patient-derived organoid models for liver fibrosis may advance the development of compounds with anti-fibrotic properties in the future. In addition, new delivery systems can improve the efficacy of potent treatments and reduce the side effects of therapy. Meanwhile, additional clinical studies are required to confirm the efficacy and safety of treatments.

FOOTNOTES

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MINIREVIEWS

Tumor budding as a potential prognostic marker in determining the behavior of primary liver cancers

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Abstract

Hepatocellular (HCC) and intrahepatic cholangiocarcinoma (ICC), the most common primary tumors of the liver, are among the most important causes of cancer deaths worldwide. Because patients with primary liver tumors are frequently diagnosed at an advanced stage and have high mortality, many efforts have been made to identify new markers to determine their behavior and treatment, similar to those in other solid organ tumors. Recently, morphological assessment of tumor budding (TB) has been revealed as a promising prognostic finding to predict tumor behavior and survival across several different tumor types. Currently, the TB score in colorectal cancer has been revealed as an important parameter in pathology report protocols to determine the course of the disease. Regarding the liver, despite enormous data showing that many mechanisms involved in TB are associated with tumor behavior in both HCC and ICC, studies focusing on the role of TB in predicting the behavior and prognosis of these tumors have started to be investigated very recently. The purpose of this review is to present data about TB in primary tumors of the liver, pointing out the potential role of this parameter in determining the course of the disease, and emphasize the need to increase the number of further studies focusing on the evaluation of this parameter with an overview of the mechanisms involved in TB.

Key Words: Tumor budding; Hepatocellular carcinoma; Cholangiocarcinoma; Prognosis; Liver cancer; Epithelial-mesenchymal transition

Core Tip: This review aims to present recent data on the potential of tumor budding (TB) in determining tumor behavior in hepatocellular carcinoma and cholangiocarcinoma. Although the evidence from the published literature indicates that TB may be a promising prognostic factor for primary liver tumors, more multidisciplinary studies are needed to draw a conclusion. Besides, different assessment techniques in previous investigations indicate that a standard method should be established to provide a solid basis for further studies that may clarify whether this parameter will be included in pathology report protocols as in colorectal carcinoma in the near future.

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INTRODUCTION

Primary liver cancer is the seventh most common cancer worldwide and the fourth leading cause of cancer death[1]. Two types of liver cancer constitute a significant majority of cases: Hepatocellular carcinoma (HCC), originating from hepatocytes and usually accompanied by another underlying disease (75%-85%), and intrahepatic cholangiocarcinoma (ICC), arising from the bile duct epithelium (12%-15%)[2]. Their incidence rates are increasing in many countries and are expected to continue to rise in the next decade[3,4]. Considering that many patients are diagnosed at an advanced stage, there is a lack of current systemic therapy, especially for HCC, and the mortality rates are high, similar to that of other solid organ tumors. Thus, many efforts have been made to identify new markers to determine the course of the disease and the choice of treatment.

Recently, TB has emerged as a promising prognostic parameter to predict tumor behavior and survival across several tumor types[5,6]. After the international TB consensus conference, the first guideline for reporting TB was published in 2017[7]. Subsequently, the TB score in colorectal carcinoma (CRC) has been included as an important parameter in pathology report protocols[8]. These guidelines have also been confirmed to be helpful in cancers of the lung, stomach cancers, and ductal adenocarcinoma of the pancreas[9]. However, regarding primary liver tumors, studies focusing on the relationship between TB and clinicopathological parameters and prognosis are relatively new. Nevertheless, numerous studies have shown that many mechanisms involved in TB are associated with tumor behavior in HCC and ICC[10,11].

Therefore, this review aims to provide an overview of the events involved in TB, which is also observed in primary liver tumors. Additionally, this review presents the latest data in these tumors to draw attention to the potential role of this parameter in determining behavior and prognosis and underlines the need to increase the number of further studies focusing on the evaluation of this parameter.

GENERAL OVERVIEW OF THE MECHANISMS OF TB

During the invasion-metastasis process in cancers, tumor cells must undergo various changes to invade the surrounding tissue, transition to the vascular system, and finally engage in a parenchymal invasion of metastatic organs[12]. The mechanisms involved in TB are presented in Figure 1.

The epithelial-mesenchymal transition (EMT) program, which contributes to developmental events throughout embryogenesis, has been hypothesized to play a fundamental role in TB formation, particularly in the steps of cell dissociation and cell migration[10-14]. Indeed, accumulating evidence indicates that budding tumor cells might display the properties of cells undergoing EMT to acquire more invasive and migratory capacity.

E-cadherin, an essential cell-cell adhesion protein, plays a pivotal role in cellular dissociation. Therefore, the reports indicating a decrease or loss of expression of E-cadherin in the invasive margin and bud areas in many solid organ tumors, including esophageal, colon, pancreas, endometrial, and oral cancers, are not surprising[15-18]. In addition, the increase in the expression of EMT-related transcription factors in tumor buds that suppress the expression of this protein, including ZEB1, ZEB2, TWIST1, TWIST2, SNAI1 (SNAIL), and SNAI2 (SLUG), is also noted in many malignancies[17-19]. Recently, an increase in the expression of these transcription factors and a decrease in E-cadherin and b-catenin levels in tumor buds compared to tumor bulk have been observed in pancreatic and oral cancers [17,18]. In addition, it has been suggested that the decrease in β-catenin expression parallel to that of E-cadherin may be a finding of WNT-b-catenin signaling pathway activation in tumor buds[20-22].

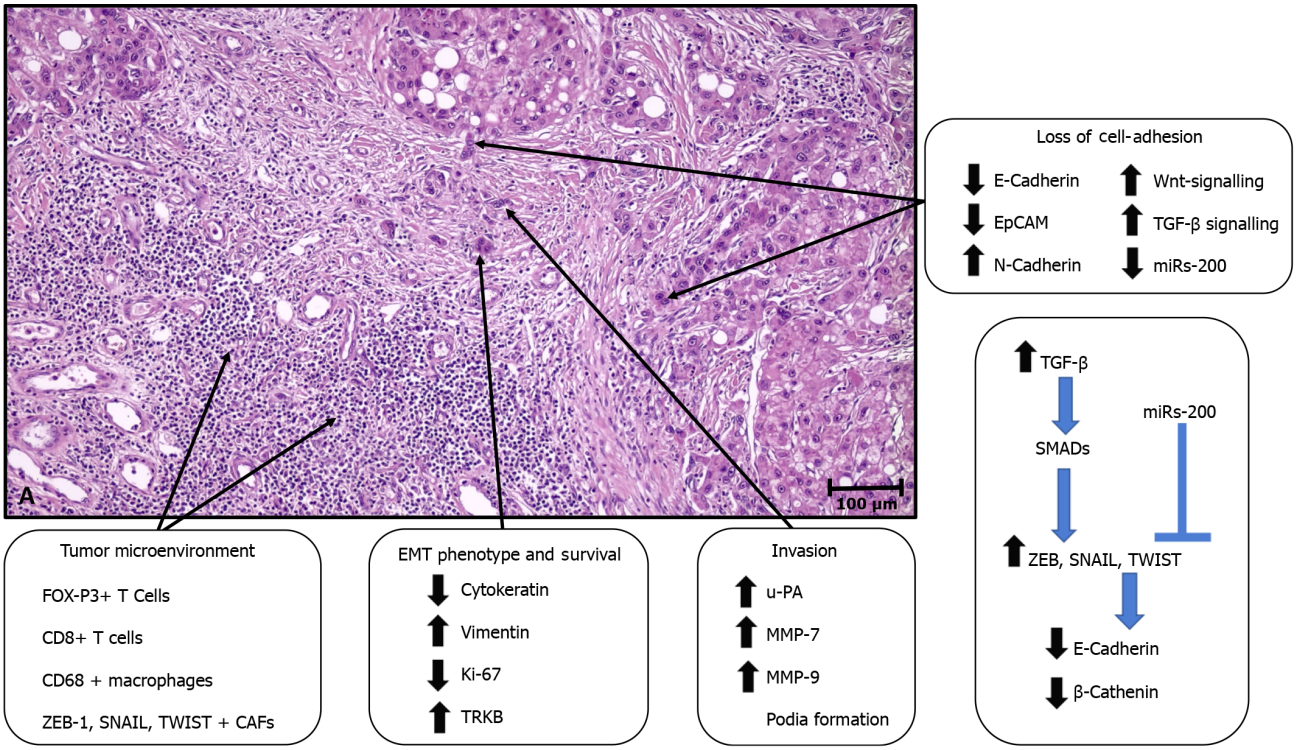

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Figure 1 Tumor budding in primary liver cancers. A: Tumor budding consisting of small clusters of 4 or fewer tumor cells present at the invasive edge in a case of hepatocellular carcinoma; B: The main processes and mechanisms involved in tumor budding. EMT: Epithelial to mesenchymal transition; EpCAM: Epithelial cell adhesion molecule; CAFs: Cancer-associated fibroblasts; MMP: Matrix metalloproteinase; TRKB: Tyrosine kinase receptor B; u-PA: Urokinase plasminogen activator.

Moreover, data have also shown that TGFβ signaling activation in buds can induce transcriptional repression of E-cadherin by inducing E-cadherin repressors, such as ZEB, TWIST, and SNAIL, *via* deregulation of SMADs[18,23]. However, the observation that different subtypes of EMT transcription factors are increased in some tumors highlights that not all of them should be expected to be increased together in tumor buds[24]. It has been shown that both E-cadherin and molecules such as CD44 and EpCam are lost in TB areas[25-27]. Signature changes in some miRNAs have also been shown to contribute to TB. In particular, changes in the miR-200 family have been noted[28-31]. The levels of miR-200, which has a suppressive effect on the ZEB family that induces E-cadherin expression, were significantly decreased in tumor buds of colorectal and pancreatic ductal adenocarcinoma[32,33].

In TB, the effect of EMT is not limited to cell dislocation; moreover, it significantly affects cell migration through cytoskeletal reorganization, increased cell-associated proteolytic activity, and reprogramming of gene expression[34]. Recently, many studies have shown that these changes are found in budding tumor cells, and marked differences in the expression of genes involved in integrin-mediated cell adhesion, cell migration, cytoskeletal changes, and extracellular matrix degradation have been noted[35].

A monomeric form of laminin 5 gamma 2, which plays a role in the anchorage of epithelial cells to the underlying basement membrane, has been found to increase during tumor invasion and in tumor buds [35,36]. This finding was associated with aggressive tumor behavior, especially in pulmonary[37,38] and colorectal cancers[39-42]. Moreover, in the latter, the dendritic extensions of budding tumor cells are positive for laminin 5 gamma 2, which is associated with vascular invasion[43,44]. In addition, in line with the findings that β-catenin induces gene expression of this protein by binding to TCF and LEF family transcription factors, decreased membranous β-catenin levels, increased nuclear β-catenin levels, positivity for laminin 5 gamma 2, and decreased E-cadherin expression were associated with TB[40,45]. These data indicate that altered expression of β-catenin may participate in multiple events in TB. In addition, other cell migration markers, including motility class III β-tubulin and high-mobility Group A family proteins, are more abundant in invasive and TB sites[46,47]. Furthermore, the expression of proteins such as matrix metalloproteinase 7, matrix metalloproteinase 9 urokinase plasminogen activator and cathepsin B, which degrade the matrix of cells, was found to be significantly increased in tumor buds[41,48-50]. In this region, various metastasis suppressors (such as rapidly accelerated fibrosarcoma kinase inhibitor protein and maspin) are frequently disrupted and/or downregulated in tumor buds compared to the primary tumor mass[51-54].

The survival of malignant cells in the tumor bud largely depends on their adaptation to a hypoxic environment. Studies have shown that these cells overexpress TRKB, a marker of resistance to cell death, and hypoxia-inducible factor 1α[55,56]. In addition, cells in tumor buds have either shallow levels or the absence of proliferation markers (such as Ki-67)[57,58]. These findings support the view that cell proliferation and migration are mutually exclusive processes and that the transition from cell proliferation to invasion may be triggered by hypoxia. Moreover, the fact that budding tumor cells frequently overexpress stem cell markers, such as LGR5, ALDH1, and CD44, suggests the self-renewal capacity of these cells, including those at metastatic sites[26,59-62].

There are also data showing that T cells in the peritumoral stroma (CD8+ T cells and FOXP3+ T cells)[63-65], EMT marker-positive cancer-associated fibroblasts[66-68], the engulfment of budding tumor cells by CD68+ macrophages, and the loss of MHC class I expression may play roles in TB[69-71].

From a morphological point of view, TB is defined as small clusters of 4 or fewer tumor cells at the interface of invasive carcinoma. Although different methods are performed, TB is usually evaluated by determining the most invasive area of the tumor (hot spot) at 20x magnification on hematoxylin and eosin-stained slides. Regardless of tumor type, buds in these areas are counted, and according to the recommendation of TBCC, TB is classified into three grades: Low, intermediate, and high[7].

In the context of HCC and ICC, there is evidence from numerous studies focusing on the mechanisms involved in TB outlined above. Among these, it is noteworthy that the number of studies focusing on the EMT in primary liver tumors is over 200 per year[10,11].

This is not surprising, given the considerable roles of the EMT in tumor behavior and progression[72-74]. Accordingly, the number of studies aiming to detect tumor aggression using comprehensive immunohistochemical and molecular methods far exceeds the number of studies focusing on TB, which can be easily detected as a simple, cost-effective morphological finding from resection materials.

TB IN HCC

Unfortunately, according to the literature, there are very few studies on the relationship between TB and tumor behavior and prognosis in HCC (Table 1). Kairaluoma *et al*[75] studied the prognostic value of TB, including 259 patients with HCC, in a retrospective cohort study from a single institution. TB is evaluated according to the hot spot method, which is recommended when investigating TB in CRC. The overall 5-year survival in bud-negative patients was higher (72.1%) than that in bud-positive patients (29.2%) (P = 0.009). In addition, the difference between the disease-specific 5-year survival rates of these two groups was also significant, 86.5% (in bud-positive patients) *vs* 35.1% (in bud-negative patients) (P = 0.002). Multivariate analysis demonstrated that TB is an independent prognostic factor in surgically treated cases.

However, this parameter was not correlated with clinicopathological factors. This is the only study investigating TB in HCC in a Western population, although it had some limitations, as noted by the authors. There were relatively few patients, yielding wide confidence intervals in the surgical cohort. Additionally, instead of looking for the optimal threshold value, the analysis was performed by making a negative/positive distinction in TB. Again, the absence of significant results in biopsy samples warrants further studies.

Another study was performed in China by Wei *et al*[76] to classify HCC based on TB and immune scores in 423 patients. The authors developed a prognosis-relevant immune score based on five types of immune cells. A classification based on TB grade and immune type was established (IS-TB type). To explore the association between IS-TB type and molecular alterations of HCC, tumor samples and adjacent nontumor tissues from 100 patients were investigated by whole-exome sequencing. TB was classified into three grades. In addition, cases were also divided into high-grade TB (with ≥ 10 buds) and low-grade TB (with 0 to 9 buds) groups. TB was an independent prognostic indicator for overall survival (OS) and disease-free survival (DFS) in the training and validation cohorts. They also observed that high-grade TB was significantly associated with EMT markers and had higher incidences in patients with nonsteatotic, nonfibrolamellar HCC, stromal active (high α-SMA expression), and immature tumors. A link between TB and EMT markers (E-cadherin and vimentin) confirmed the hypothesis that TB might represent the EMT process.

Because the role of the immune milieu of HCC as a prognostic feature is only starting to emerge, they also divided cases by an immune score established based on Z scores that included five parameters (CD8 stromal, PD-L1 stromal, mast-cell stromal, CD68 stromal, and FOXP3 stromal) for each patient. According to the cutoff value (0.04), patients were divided into immune type A and B groups. DFS and OS were better in the type A group than in the type B group in both the training and validation cohorts. The combination of TB grade and immune type cases was also divided into four groups: ISA-TB_{high} (type I), ISB-TB_{high} (type II), ISA-TB_{low} (type III), and ISB- TB_{low} (type IV). While cases within IS-TB type II showed the worst long-term survival, cases within IS-TB type III had the best OS and DFS. These findings are in line with previous observations that indicated that a high lymphocyte-to-TB ratio was a good prognostic factor and that the integration of both TILs and TB was advantageous in the prediction of long-term prognosis in colorectal cancers. These findings provide a rationale for the pathological

Table 1 Relationship of tumor budding with clinicopathologic parameters and survival in hepatocellular and cholangiocarcinomas

Ref.	Tumor	No.	Correlations	Prognosis
Kairaluoma *et al*[75]	HCC	47-R; 212-NR	Not observed; Not observed	OS: TB negative *vs* TB positive; DSS: TB negative *vs* TB positive
Wei *et al*[76]	HCC	423	Tumor subtypes, EMT related marker expression, FOXP3, PD-L1 and CD68 expressions; Frequent mast cell infiltration, p53 mutation (IS-TB type I); CTNNB1 mutation (IS-TB type IV)	DFS: Type II *vs* Type I + Type IV; Type III *vs* Type I + Type IV; OS: Type II *vs* Type I + Type IV; Type III *vs* Type I + Type IV
Okubo *et al*[77]	CCC	299	Dif $^{G1/G2}$ *vs* Dif ^{G3}	OS: TB negative *vs* TB positive
Ogino *et al*[78]	EHCC-PH; EHCC-DC	195; 115	Grade, T, LI, VI, PN, LNM, RSM; Grade, Higher T, LI, VI, PN, LNM	OS: TB low *vs* TB ıntermediate *vs* TB high; OS: TB low *vs* TB high
Tanaka *et al*[80]	ICC	107	Stage, Hilar invasion, Grade, VI, LNM, SM	RFS: TB negative *vs* TB positive; OS: TB negative *vs* TB positive
	Type 1	49	NP	RFS: Not prognostic; OS: Not prognostic
	Type 2	58	NP	RFS: Not prognostic; OS: TB negative *vs* TB positive
	EHCC-PH	54	LI	RFS TB negative *vs* TB positive; OS TB negative *vs* TB positive
	EHCC-DC	40	VI	RFS: Not prognostic; OS: Not prognostic
Ito *et al*[81]	EHCC-PH	78	Grade, T, LNM, M	
		36 NT	Combined HA/PV Resection, Grade, T, LNM, M	DSS: TB low *vs* TB high; RFS: TB low *vs* TB high
		42 WT	Not observed	DSS: TB low *vs* TB high; RFS: Not prognostic
Agostini-Vulaj *et al*[83]	EHCC; ICC	58; 54	Gender, Location, Grade, LNI, PNI, RSM; Gender, Location, Grade, LNI, PNI	DSS: TB ıntermediate *vs* TB high; RFS: TB ıntermediate *vs* TB high
Budau *et al*[84]	ICC	89	NP	OS: TB Low *vs* TB Intermediate *vs* TB High; RFS TB Low *vs* TB Intermediate *vs* TB High ITTB, PTTB, TB
Kosaka *et al*[85]	ICC	235	Size, Tumor type, Grade, VI, MBI, LNM	DSS: TB Low/Intermediate *vs* TB High; RFS: TB Low/Intermediate *vs* TB High
Nakayama *et al*[82]	EHCC-DC	65	T, LNM, LI, VI, ZEB-1 expression, stage	OS: TB Low *vs* TB High

CCC: Cholangiocarcinoma; DFS: Disease free survival; DSS: Disease specific survival; Dif: Differentiation; DM: Distant metastasis; EHCC-DC: Extrahepatic cholangiocarcinoma-distal; EHCC-PH: Extrahepatic cholangiocarcinoma-perihilar; EMT: Epithelial to mesenchymal transition; G: Grade; HA/PV: Hepatic artery and portal vein; HCC: Hepatocellular carcinoma; ICC: Intrahepatic cholangiocarcinoma; IS: Immunescore; ITTB: Intratumoral tumor budding; LI: Lymphatic invasion; LNM: Lymph node metastasis; M: Metastasis; MVI: Microvascular invasion; NCR: Noncurative resection; No: Number of cases; NR: Non-resectable; NT: Neoadjuvant therapy OS: Overall Survival; PN: Perineural invasion; PTTB: Peritumoral tumor budding; R: Resectable; RFS: Recurrence free survival; RSM: Residual tumor in surgical margin; T: Tumor invasion; TB: Tumor budding; VI: Type I: ISA-TB_{high}; Type II: ISB-TB_{high}; Type III: ISA-TB_{low}; Type IV: ISB-TB_{low}; VI: Vascular invasion; MBI: Major biliary invasion; WT: Without neoadjuvant therapy.

evaluation of the TME in addition to the current pathological classifications of HCC.

Another interesting finding of this study was the association between IS-TB type and molecular alterations. TP53 (mainly within IS-TB type I) and CTNNB1 (mainly within IS-TB type IV) mutations in two distinct HCC phenotypes exhibit different immune and pathological characteristics. While TP53 mutations were related to poor differentiation and a thick trabecular pattern, CTNNB1 mutations were associated with impaired antitumor immunity (immune type B), well-differentiated morphology, a pseudoglandular pattern, mature stroma, and low α-SMA (fibroblast activation protein) expression.

As noted above, despite the scarcity of studies examining TB in HCC, there is a wealth of data on the processes involved in this phenomenon.

TB IN CHOLANGIOCARCINOMA

Several studies focusing on TB in cholangiocarcinomas have recently been performed. The number of studies, including extrahepatic perihilar (EHCC-PH) and distal cholangiocarcinoma (EHCC-D) cases, exceeded the number of studies that included ICC cases in the study group. The characteristics and

results of these studies are summarized in Table 1.

In an earlier investigation of cholangiocarcinomas from all anatomical locations (CCC), TB was associated with the grade but not with the course of the disease[77]. However, in a more recent study, in addition to high grade, high TB was more frequently observed in males and patients with extrahepatic localization, perineural and lymphatic invasion, and presentation in settings with positive resection margins[78]. Moreover, TB is an independent prognostic factor for CCC. However, since TB scoring differed in these two studies, it is not possible to compare the results of one with the other (Tables 1 and 2), as noted by Regmi *et al*[79], who performed a meta-analysis of CCC samples from different locations, including tumors of the ampulla and gallbladder.

In EHCC-PH, TB is associated with tumor invasion, lymph node metastasis, perineural invasion, lymphovascular invasion, and positive resection margin status. It has also been shown to be an independent prognostic factor in determining the course of the disease in all of the studies[78,80,81]. In EHCC-D, higher TB was more frequent in tumors with deeper invasion, lymph node metastasis, and lymphovascular and perineural invasion[78,80,82]. The correlation between TB and stage and ZEB-1 expression was also noted[83]. Similar to EHCC-P, all but one study[81] showed that TB effectively determines the course of the disease, as shown by both univariate and multivariate analyses[77,78,81-84].

Regarding ICC, TB was shown to be correlated with stage, hilar invasion, grade, venous invasion, lymph node metastasis, and positive surgical margins, which are important parameters for determining the behavior of these tumors. Moreover, when ICCs were analyzed according to growth patterns, it was noted that 80% of mass-forming tumors had high TB. In contrast, this ratio was 16% and 2.3% in periductal infiltrating and intraductal growing subtypes, respectively[85]. In addition, the prognostic role of TB has been described[77,80,81,85]. Budau *et al*[84] analyzed TB using a three-tier grading system: high, intermediate, and low. While patients with low TB had the most favorable recurrence survival, high TB was associated with the most unfavorable outcomes.

Similarly, TB correlated significantly with the overall survival of patients in univariate and multivariate analyses ($P < 0.001$). In addition, their data demonstrated that in ICC, TB is significantly independent of the area of investigation (intratumoral or peritumoral). These findings indicate the possibility that TB assessment in preoperative tissue biopsies and in cases that would not be suitable for resection could be used to predict tumor behavior. Nevertheless, the evidence for intratumoral TB is still weak.

In another study, TB was observed to be a powerful prognostic factor for RFS and OS in ICC[80]. In patients stratified into negative and positive TB status, the median time to recurrence in cases with positive TB was 10.26 mo. This was significantly shorter than that of subjects with negative TB (35.57 mo), and the difference among median survival times was significant ($P < 0.001$). Furthermore, the results of the same study indicated that TB was a decisive and powerful prognostic factor for OS (HR: 4.547). Although these findings need to be supported by further large-scale studies, they suggest that TB may be an important prognostic parameter in these tumors.

Tanaka *et al*[80] presented an interesting finding about TB in ICC in an elegant study. When they evaluated TB by dividing ICC into two subgroups, Type 1 (hilar) and Type 2 (peripheral), according to the combined scores of mucin productivity and immunoreactivity of S100P, N-cadherin, and neural cell adhesion molecule, this parameter was determined to be a decisive prognostic factor in Type 2 but not in Type 1. They suggested that some differences exist in the biological behavior of these subtypes and pointed out that despite the prognostic importance of TB in ICC, its pathogenetic role in biliary tract carcinomas might differ by anatomic location. However, this finding needs to be supported in further studies. Nevertheless, the results of TB studies in ICC are similar and support the suggestion that TB is a relevant prognostic factor in the histopathological evaluation of these tumors.

Generally, different scoring methods have been used to investigate TB in cholangiocarcinomas. In a few studies, unlike the recommendation of TTBC, five cells were taken as the cutoff for the definition of TB[77,78,81]. The analyses were performed by categorizing the cases as negative vs. positive or low vs. high TB. In most other studies, including ICC cases, patients were assessed following the three-tiered system recommended by the TTBC for colorectal cancer[80,82-84]. However, different stratifications were used for further evaluations (Table 2). More recently, in an elegant study, Zlobec *et al*[86] observed that CRC without TB (TB0) is relatively frequent and provided additional information on tumor behavior, suggesting a new "zero budding" category for TB. There is currently no evidence about the prognostic value of TB0 in cholangiocarcinomas, and it would be interesting to conduct further studies in which this category is addressed separately.

Accumulated data indicate that the preferred staining method for scoring TB is HE. Recently, some studies on TB have reported that IHC is superior to HE regarding reproducibility and interobserver agreement in assessing this parameter in CRC. Regarding CCC, Ogino *et al*[78] obtained TB scores in HE-stained whole-tissue sections and PanCK immunostained tissue microarray (TMA) sections from 266 patients. They observed that the number of tumor buds in HE-stained slides was almost equal to that in PanCK-stained slides from TMA, with a strong correlation between them ($R = 0.763$, $P < 0.001$). This finding also supports that evaluating TB in HE-stained sections is a simple and reproducible method. Nevertheless, more studies are needed to standardize the assessment of TB in ICC because grading systems for this parameter vary between different types of cancer.

Table 2 Criteria applied for tumor budding in previous studies

Ref.	Tumor	Tumor budding criteria
Kairaluoma *et al* [75]	HCC	Evaluation was performed according to median values; Negative: No buds were found; Positive: At least one bud was present
Wei *et al*[76]	HCC	Association between TB and clinicopathological parameters; Grade 1 (0-4), Grade 2 (5-9), Grade 3 (≥ 10); For survival analysis; Low grade (0-9), High grade (≥ 10)
Okubo *et al*[77]	CCC	Negative: < 5 budding focus; Positive: ≥ 5 budding focus
Ogino *et al*[78]	EHCC-PH, EHCC-DC	Cut-off values of TB obtained by recursive partioning technique; For EHCC-PH; Low grade (0-4), Intermediate grade (5-11), High grade (≥ 12); For EHCC-DC; Low grade (0-4), High grade (≥ 5)
Tanaka *et al*[80]	ICC, EHCC-PH, EHCC-DC	Low grade (0-4), Intermediate grade (5-9), High grade (≥10)
Ito *et al*[81]	EHCC-PH	Low TB: < 5 budding focus; High TB: ≥ 5 budding focus
Agostini-Vulaj *et al*[83]	ICC, EHCC	Grade 1 (0-4), Grade 2 (5-9), Grade 3 (≥ 10)
Budau *et al*[84]	ICC	Grade 1 (0-4), Grade 2 (5-9), Grade 3 (≥ 10)
Kosaka *et al*[85]	ICC	Low grade (0-4), Intermediate grade (5-9), High grade (≥ 10)
Nakayama *et al* [82]	EHCC-DC	Low TB (0-4), High [TB Grade 2 (5-9) and 3 (≥ 10)]

CCC: Cholangiocarcinoma; EHCC-DC: Extrahepatic cholangiocarcinoma-distal; EHCC-PH: Extrahepatic cholangiocarcinoma-perihilar; HCC: Hepatocellular carcinoma; ICC: Intrahepatic cholangiocarcinoma.

In CRC, TB, combined with other established biomarkers, may allow us to discriminate between patients who would benefit from oncological resection and patients who will receive adjuvant therapy and to classify different therapeutic options, especially in advanced-stage patients[87]. Thus, TB can predict prognosis and regulate treatment options in primary liver cancers. However, the role of TB in the treatment of these tumors remains to be investigated.

CONCLUSION

This review highlights that TB may be a promising prognostic factor for primary liver tumors. However, its clinical value in managing patients should be established in multidisciplinary studies. Evidence also suggests that TB in HCC can identify and reclassify tumors of molecular subtypes with different behavioral characteristics. The differences in the classification of TB in primary liver tumors indicate that a standard and validated method should be established to provide a solid basis for large-scale clinicopathological studies for further evaluation. In addition, the precise determination of the value of budding tumor assessment with multiple further studies may allow us to clarify whether this parameter will be included in pathology report protocols as in CRC in the near future.

FOOTNOTES

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MINIREVIEWS

Role of vascular endothelial growth factor B in nonalcoholic fatty liver disease and its potential value

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Abstract

Nonalcoholic fatty liver disease (NAFLD) refers to fatty liver disease caused by liver injury factors other than alcohol. The disease is characterized by diffuse fat infiltration, including simple steatosis (no inflammatory fat deposition), nonalcoholic fatty hepatitis, liver fibrosis, and so on, which may cause liver cirrhosis, liver failure, and even liver cancer in the later stage of disease progression. At present, the pathogenesis of NAFLD is still being studied. The "two-hit" theory, represented by lipid metabolism disorder and inflammatory reactions, is gradually enriched by the "multiple-hit" theory, which includes multiple factors, such as insulin resistance and adipocyte dysfunction. In recent years, vascular endothelial growth factor B (VEGFB) has been reported to have the potential to regulate lipid metabolism and is expected to become a novel target for ameliorating metabolic diseases, such as obesity and type 2 diabetes. This review summarizes the regulatory role of VEGFB in the onset and development of NAFLD and illustrates its underlying molecular mechanism. In conclusion, the signaling pathway mediated by VEGFB in the liver may provide an innovative approach to the diagnosis and treatment of NAFLD.

Key Words: Nonalcoholic fatty liver disease; Vascular endothelial growth factor B; "Two-hit" theory; "Multiple-hit" theory; Obesity

Core Tip: Nonalcoholic fatty liver disease (NAFLD) is the most common liver disease with lipid accumulation caused by liver injury factors except alcohol. At present, vascular endothelial growth factor B (VEGFB) has been reported to play a special role in regulating lipid metabolism and improving the onset and development of NAFLD. Therefore, the use of VEGFB as a target for treatment has become the focus of current research. This review summarizes the role and potential mechanism of VEGFB in the pathogenesis of NAFLD to provide a theoretical basis for the clinical treatment of NAFLD.

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INTRODUCTION

With the growth of the economy and the increasing change in people's lifestyles, the prevalence and morbidity of nonalcoholic fatty liver disease (NAFLD) are rising rapidly worldwide. NAFLD occurs in one-fourth of the global population, and the highest incidence rate is in South America (31%) and the Middle East (32%), followed by Asia (27%), the United States (24%), and Europe (23%), while is not common in Africa (14%)[1]. In the United States, the number of NAFLD cases is expected to increase from 83.1 million in 2015 (approximately 24% of the population) to 100.9 million by 2030[2]. Because of its long course and high treatment cost, it has become a global medical and health problem.

NAFLD is an important cause of advanced liver disease, primary liver cancer, and liver transplantation and is also the world's fastest-growing cause of liver-related deaths[3]. In the United States, the burden of NAFLD-related cirrhosis is estimated to be twice that of hepatitis C virus (HCV) related cirrhosis, and it is expected to surpass HCV as the main indication for liver transplantation within 5 years[4]. In Asia, the incidence rate of hepatocellular carcinoma in patients with NAFLD is 1.8/1000 each year, and the total case fatality rate is 5.3/1000 each year[5]. In addition, insulin resistance, upregulation of insulin-like growth factor axis, downregulation of adiponectin expression, and elevated expression of tumor necrosis factor α (TNFα) caused by NAFLD may be potential factors to induce the development of tumors[6]. Meanwhile, NAFLD can also promote coronary atherosclerosis, significantly increase the risk of cardiomyopathy (mainly left ventricular hypertrophy), leading to valvular heart disease (mainly aortic valve and mitral valve), cardiac insufficiency, arrhythmia (mainly atrial fibrillation, prolonged QT interval) and some cardiac conduction system defects (such as an atrioventricular block)[7]. Therefore, more and more research is focusing on exploring the pathogenesis of NAFLD.

The physiological mechanism of NAFLD is very complex. The pathogenesis of early NAFLD is generally believed to be related to lipid metabolism and inflammatory reactions, which could not systematically and comprehensively explain the molecular mechanism and metabolic changes in NAFLD[8,9]. In recent years, studies have confirmed that insulin resistance is closely related to the pathogenesis of NAFLD[10]. In 2019, Lee *et al*[11] reported that NAFLD was related to liver and peripheral insulin resistance, leading to insufficient inhibition of liver insulin resistance, gluconeogenesis, reduced glycogen synthesis, and increased free fatty acid (FFA). Shi *et al*[12] confirmed that insulin resistance can promote the progression of liver fibrosis and NAFLD, and NAFLD can also accelerate insulin resistance in the liver.

With the deepening of research on NAFLD and the increasing understanding of its pathogenesis, it has been found that the onset of NAFLD is also related to "multiple-hit" such as liver insulin resistance, adipocyte dysfunction, gut microbiota imbalance, immune regulation imbalance, and dietary habits besides the "second-hit" caused by lipid metabolism disorder and inflammation reaction. Adolph *et al*[13] found that abnormal adiponectin secretion produced by adipocytes can aggravate high-fat diet (HFD)-induced obesity and related metabolic disorders, and the overexpression of adiponectin can hinder the progression of hepatic microsomal steatosis. Baker *et al*[14] found that the content of enzymes that can metabolize ethanol in the body of patients with NAFLD and intestinal flora imbalance increased significantly, which increased the permeability of the intestinal wall and was conducive to the entry of reactive oxygen species (ROS), bacterial endotoxins, ethanol and other toxic metabolites into the liver, resulting in increased liver damage andaccelerating the progression of NAFLD (Figure 1).

Early in 2008, Karpanen *et al*[15] unexpectedly found that vascular endothelial growth factor B (VEGFB) has a weak role in the vascular system but has a significant advantage in regulating lipid metabolism. In 2012, Hagberg *et al*[16] proved that targeting VEGFB as a novel treatment for insulin resistance and type 2 diabetes. In 2016, Robciuc *et al*[17] also found that transferring the VEGFB gene into HFD-induced obese mice can improve lipid metabolism and increase insulin supply and signal transduction. Hu *et al*[18] confirmed that VEGFB recombinant protein can reduce lipid accumulation and improve hyperlipidemia in NAFLD.

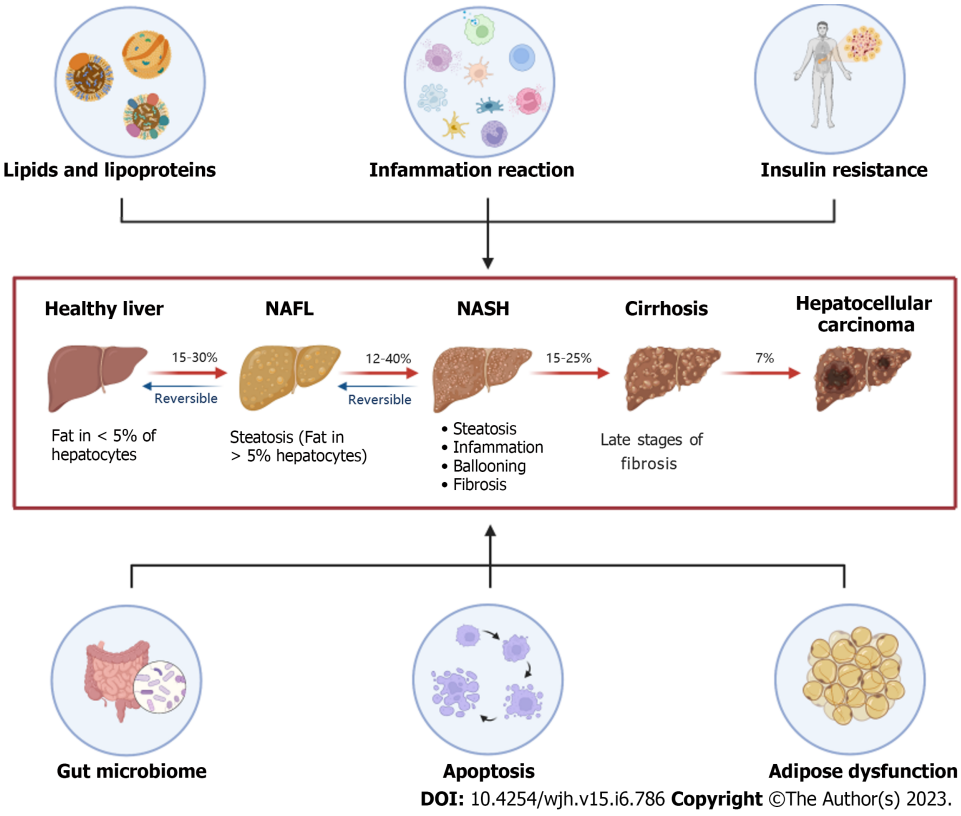

Figure 1 The "multiple-hit" theories are involved in the progress of nonalcoholic fatty liver disease. Lipids and lipoproteins represent the "first-hit", while the inflammation reaction illustrates the "second-hit" in the development of nonalcoholic fatty liver disease (NAFLD). Six aspects including lipids and lipoproteins, inflammation reaction, insulin resistance, gut microbiome, apoptosis, and adipose dysfunction have a common influence on the pathophysiological mechanism of NAFLD. NAFL: Non-alcoholic fatty liver; NASH: Non-alcoholic steatohepatitis.

The regulatory role of VEGFB in the occurrence and development of metabolic diseases has attracted many scholars' attention. In this review, we mainly focus on the underlying mechanism of VEGFB in the onset of NAFLD and analyze how VEGFB participates in the "multiple-hit" of NAFLD by regulating lipid metabolism, inflammatory reactions, adipocyte dysfunction, and cell apoptosis. First, we introduce the positive regulatory effect of VEGFB on lipid metabolism and discuss how it affects fatty acid oxidation and lipid synthesis under the mediation of Adenosine 5'-monophosphate (AMP)-activated protein kinase (AMPK) signaling. Then, we summarize the role of VEGFB in anti-inflammation in NAFLD and further discuss the current mechanism of VEGFB in insulin resistance and the impact of targeted therapy. Finally, we also explain the controversial role of VEGFB in metabolic diseases and estimate whether VEGFB-mediated signal transduction could provide a theoretical and experimental basis for the pathogenesis of NAFLD and help identify potential treatment targets.

THE NOVEL ROLE OF VEGFB IN NAFLD

VEGFB is a special type of vascular endothelial growth factor. The total length of the VEGFB gene is 1197 bp, with 7 exons, and the total length of the CDS region is 566 bp, with two subtypes, VEGFB^{167} and VEGFB^{186}[19]. The VEGFB^{167} homotype has a similar effect to that of VEGFB^{186}[17]. VEGFB is a glycoprotein that forms a homodimer through the covalent binding of disulfide bonds. It needs to combine with a high-affinity tyrosine kinase receptor to exert biological effects[20]. The VEGF family includes seven members, VEGF-A, VEGF-B, VEGF-C, VEGF-D, VEGF-E, VEGF-F, and placental growth factor[21]. The VEGF receptor family includes VEGFR1, VEGFR2, VEGFR3, and neuropilin 1/2 (NRP1/2). VEGFA can combine with VEGFR1 or VEGFR2 to play a role in promoting angiogenesis[22]. Unlike other members of the VEGF family, the effect of VEGFB on vascular endothelial growth is not obvious. The biological function of VEGFB is exerted by forming a complex with VEGFR1. Moreover, the combination of VEGFB and NRP1 can also induce a series of reactions through a paracrine mechanism [23]. In recent years, studies have shown that the VEGFB/VEGFR1 pathway has therapeutic potential for obesity, type 2 diabetes, and other lipid metabolism disorder-related diseases[17].

VEGFB mainly exists in the heart, skeletal muscle, brown adipose tissue, and other tissues with high metabolic activity and plays a role in regulating blood vessel distribution and lowering blood lipids[24]. The level of VEGFB in the liver is also significantly higher than that in tissues with general metabolic

activity. Shang *et al*[25] found that cardiac-specific overexpression of VEGFB can reduce the activity of lipoprotein lipase and improve the metabolic level of myocardial cells. Wagenmakers *et al*[26] showed that VEGFB can control the expression of fatty acid transport protein (FATP) in the capillary endothelium and connect the uptake of endothelial FFA with the oxidation ability of the skeletal muscle to potentially prevent the accumulation of skeletal muscle lipotoxic FFA. Robciuc *et al*[17] confirmed that the complex of VEGFB and VEGFR1 can reshape the vascular distribution in adipose tissue and improve the insulin function of obese mice.

VEGFB also plays a biological role in the liver by forming a complex with VEGFR1. Cordeiro *et al*[27] showed that targeting VEGFB can effectively prevent lipid deposition in peripheral tissues in animal models. Hu *et al*[18] observed that the complex of VEGFB and VEGFR1 can increase the oxidation level of fatty acids in liver tissue and hepatocytes and reduce obesity-related hyperlipidemia and fatty liver disease in HFD-induced liver. Li *et al*[28] found that inhibiting VEGFB gene expression in liver tissue not only increased the weight and body fat rate of obese mice but also led to pathological changes, such as hepatocyte steatosis and liver fibrosis. These studies suggest that VEGFB is involved in the onset and development of simple steatosis and liver fibrosis in NAFLD (Figure 2).

VEGFB PARTICIPATES IN REGULATING THE "FIRST HIT" IN NAFLD

In 1998, Day *et al*[29] first proposed the "two-hit" theory of the pathogenesis of NAFLD. The "first hit" of NAFLD mainly involves lipid metabolism disorder caused by various factors. Hepatotoxicity is caused by FFA, which leads to an increase in the permeability of the cell membrane, destruction of mitochondrial function, and inhibition of related enzymes to produce genotoxicity. As the disease progresses, excess FFA undergoes β oxidation in mitochondria. When the capacity of the mitochondria to β oxidize FFA is overloaded, excess FFA accumulates in the liver and aggravates the steatosis of hepatocytes. Meanwhile, the triglyceride (TG) synthesized by excess FFA in the liver cannot be converted into very low-density lipoprotein for transport to the peripheral adipose tissue for storage. Therefore, TG can only be stored in the liver and eventually aggravate the onset of liver steatosis. Reducing lipid accumulation and restoring the balance of lipid metabolism are the key methods to improve the "first hit" of NAFLD.

The research findings on the role of VEGFB in improving the lipid disorder of the heart, skeletal muscle, and brown adipose tissue provide the theoretical and experimental basis for VEGFB to participate in the regulation of hepatic lipid metabolism in NAFLD. Shang *et al*[25] observed that rat heart lipoprotein lipase activity and lipid accumulation were decreased and insulin function was improved after cardiac-specific overexpression of VEGFB. Li *et al*[30] found that VEGFB can enhance the expression of FATP1 and FATP4 in C2C12 cells, promote the oxidation of FFA and the decomposition of TG in C2C12 myotubes, and inhibit the re-esterification of FFA to reduce lipid accumulation in myotubes. Chen *et al*[31] found that after inhibition of adipose-specific VEGFB, mice increased in size with more white adipose tissue, and the form and function of fat changed from those of brown adipose tissue to those of white adipose tissue, which indicated that VEGFB was the main regulator of the growth and function of fat.

Some scholars have proposed that the signaling pathway triggered by the combination of VEGFB with its receptor can promote lipid flow in the body, which may become a promising target to prevent the accumulation of ectopic lipids. In 2020, Tong *et al*[32] showed that VEGFB can reduce the levels of TG and FFA in the liver to prevent HFD-induced fatty liver disease by producing E. coli-expressed recombinant tPep-VEGFB. In 2021, Hu *et al*[18] found that recombinant VEGFB protein reduced the increase in high-density lipoprotein and low-density lipoprotein in the liver caused by HFD and reduced liver hyperlipidemia.

The mechanism of NAFLD involves multiple signaling pathways, of which the AMPK signaling pathway plays a key role in de novo synthesis and fatty acid oxidation[33]. Harjes *et al*[34] confirmed that the combination of VEGFB with its receptor VEGFR1 can activate AMPK, FATP3, and FATP4 to potentially promote the usage of FFA. AMPK activation is regulated by its upstream molecule Ca^{2+}/Calmodulin-dependent protein kinase β (CaMKKβ), which responds to increased intracellular calcium content[35]. Extracellular calcium ions enter the cell through a calcium channel carrier. The elevated intracellular calcium level causes the conformational change of CaMKKβ and the phosphorylation of AMPK[36]. Jia *et al*[37] showed that a high concentration of VEGFB recombinant protein can increase the level of calcium ions in MIN6 cells to increase insulin secretion. Li *et al*[28] suggested that inhibiting the expression of VEGFB in the liver can reduce the expression level of CaMKKβ and then affect the phosphorylation level of AMPK induced by CaMKKβ.

AMPK can control lipid metabolism by regulating its downstream molecules after its phosphorylation[38]. AMPK can directly phosphorylate and inhibit the activation of acetyl coenzyme A carboxylase (ACC), the rate-limiting enzyme that inhibits the synthesis of fatty acids, thereby activating carnitine palmitate transferase (CPT1) and transferring fatty acids into mitochondria for β oxidation[39, 40]. AMPK can also negatively regulate the expression of sterol-regulatory element-binding protein-1c (SREBP1c), downregulate the level of desaturase [stearoyl-CoA desaturase-1 (SCD1)], and inhibit the

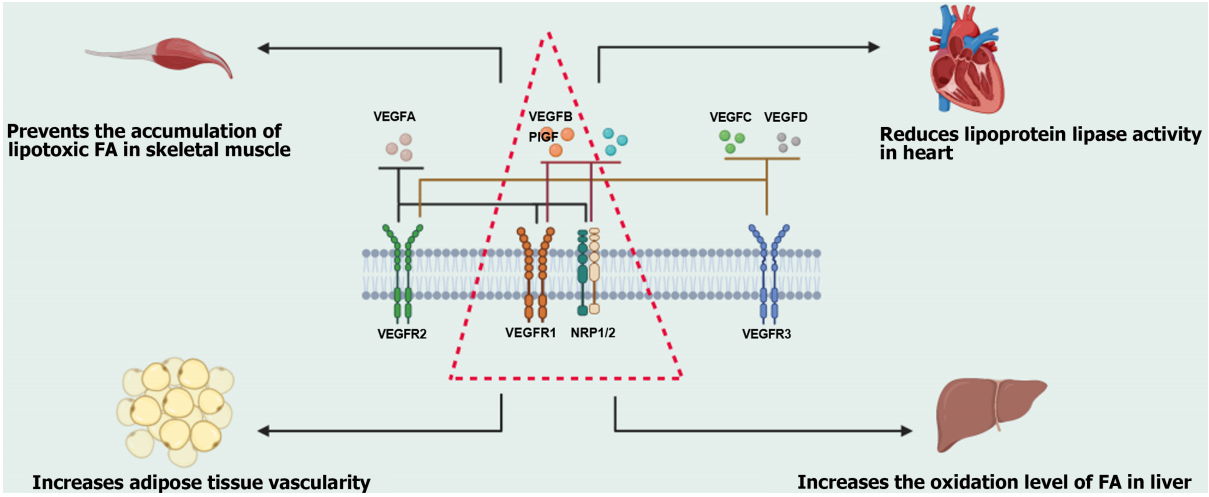

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Figure 2 The vascular endothelial growth factor family and its receptors, and the biological function of vascular endothelial growth factor B. The vascular endothelial growth factor (VEGF) family includes VEGFA, vascular endothelial growth factor B (VEGFB), VEGFC, VEGFD, and so on. The VEGF receptor family includes VEGFR1, VEGFR2, VEGFR3, and neuropilin 1/2 (NRP1/2). VEGFA combines with VEGFR1, VEGFR2, or NRP1/2. VEGFB and placental growth factor combine with VEGFR1 or NRP1/2. VEGFC and VEGFD combine with VEGFR2 or VEGFR3 to exert their biological functions. VEGFB can prevent the accumulation of lipotoxic free fatty acid (FFA) in skeletal muscle, reduce lipoprotein activity in the heart, increase adipose tissue vascularity, and increase the oxidation level of FFA in the liver. PIGF: Placental growth factor; FA: Fatty acid; NRP1/2: Neuropilin 1/2; VEGF: Vascular endothelial growth factor; VEGFB: Vascular endothelial growth factor B.

synthesis of fatty acids and TG[41]. Hu *et al*[18] showed that VEGFB recombinant protein can upregulate the AMPK/ACC/CPT1 signaling pathway in the liver by binding to VEGFR1, promoting FFA oxidation and reducing lipid deposition. That study also found that VEGFB can simultaneously upregulate the expression levels of the lipid oxidation-related genes PPARα, PGC-1α, HSL, ACO, and CPT1 and that the downregulation of lipid synthesis can inhibit weight gain under HFD conditions and improve obesity-related hyperlipidemia and fatty liver disease[18]. Li *et al*[28] also found that VEGFB knockout can downregulate the CaMKKβ-mediated AMPK/ACC/CPT1 signaling pathway to inhibit fatty acid oxidation and activate the AMPK/SREBP1/SCD1 signaling pathway to promote lipid synthesis, thus affecting the level of lipid metabolism (Figure 3).

VEGFB PARTICIPATES IN REGULATING THE "SECOND HIT" IN NAFLD

Liver lipid accumulation induces overloaded lipid catabolism, causing lipid peroxidation. Excessive lipid peroxidation leads to oxidative stress, making it the "second hit" to the progression of NAFLD, which can accelerate inflammation and hepatocyte damage. Nuclear factor-kappa B (NF-kB) signaling plays an important role in the macrophage-mediated liver inflammatory response[42]. Research has confirmed that NF-kB can be activated by FFA in patients with NAFLD, and as the severity of NAFLD increases, the activity of NF-kB increases[43]. Moreover, liver mitochondrial dysfunction also accelerates the occurrence and development of NAFLD. The compensated acceleration of β oxidation in mitochondria can produce a large number of ROS[44]. When the antioxidant system mainly composed of reduced glutathione fails to eliminate ROS in time, oxidative stress develops[45], and a large number of peroxides are generated, which aggravate hepatocyte damage[46]. The apoptotic bodies produced by hepatocyte apoptosis are engulfed by Kupffer cells to decrease the activity of endothelial nitric oxide synthase (eNOS), which affects Mitochondrial function.

VEGFB can induce cell proliferation and differentiation, tumor immunity, and other biological effects through the signaling pathway mediated by the tyrosine-protein kinase receptor[47]. Kusuhara *et al*[48] observed that the VEGFR1 signal in monocytes and macrophages was significantly affected by the upregulation of VEGFB under inflammatory conditions. Akiyoshi U confirmed that VEGFR1 can regulate AKT signaling and affect the activity of NF-KB and eNOS respectively to regulate macrophage migration and mitochondrial function[49]. Mehlem *et al*[50] found that VEGFB signaling is involved in regulating pathological lipid accumulation in diabetes, obesity, and cardiovascular disease and mainly affects mitochondrial genes related to the regulation of fatty acid intake. Cao *et al*[51] showed that VEGFB/IL-17 inhibits the expression of fatty acid transporters to reduce the accumulation of renal lipids and inhibit renal oxidative stress and mitochondrial dysfunction, thus improving the inflammatory response. Shen *et al*[52] also found that VEGFB/IL-22 can not only regulate glucose and lipid metabolism but also reduce inflammation and ROS accumulation. Robciuc *et al*[17] transduced the

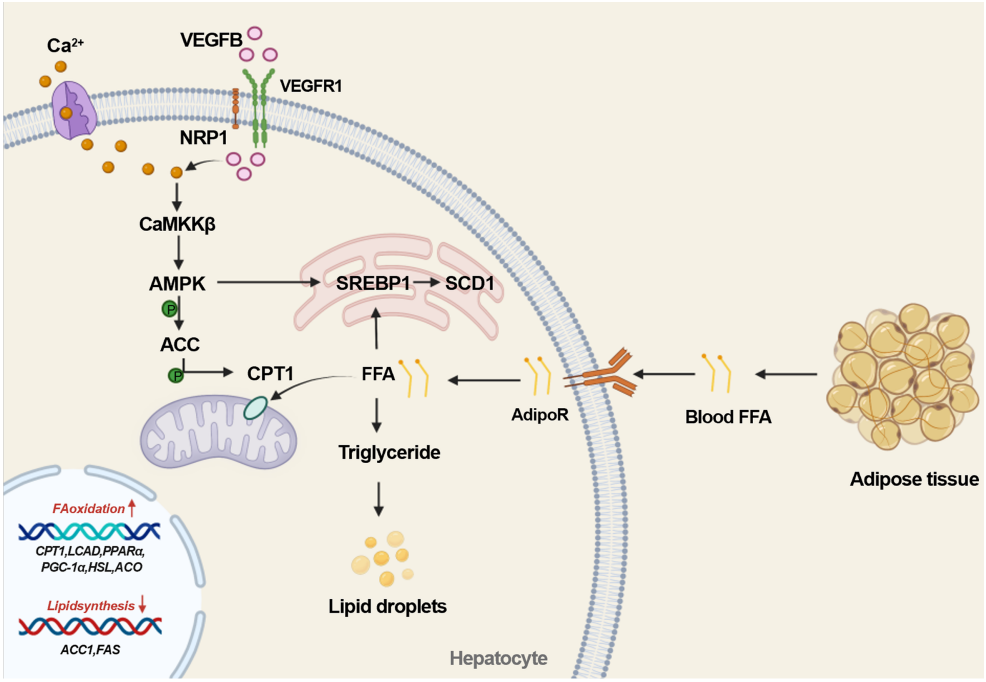

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Figure 3 Vascular endothelial growth factor B regulates lipid metabolism in the "first hit" of nonalcoholic fatty liver disease *via* the activated protein kinase signaling pathway. Vascular endothelial growth factor B (VEGFB) performs its biological function by combining with VEGFR1. Once it enters the cell, it activates Calmodulin-dependent protein kinase β (CaMKKβ), which is induced by an increase in intracellular Ca^{2+} content. VEGFB activates the CaMKKβ-mediated activated protein kinase (AMPK)/A carboxylase (ACC)/carnitine palmitate transferase (CPT1) signaling pathway and related genes, such as CPT1 and long-chain acyl coenzyme A dehydrogenase, which regulate FFA oxidation in mitochondria. VEGFB activates AMPK/SREBP1/SCD1 and related genes, such as ACC1 and FAS, to inhibit lipid synthesis in the endoplasmic reticulum. CaMKKβ: Calmodulin-dependent protein kinase β; AMPK: Adenosine 5'-monophosphate (AMP)-activated protein kinase; ACC: A carboxylase; CPT1: Carnitine palmitate transferase-1; FFA: Free fatty acid; NRP1/2: Neuropilin 1/2; SCD1: Stearoyl-CoA desaturase-1; SREBP1: Sterol-regulatory element-binding protein-1; VEGFB: Vascular endothelial growth factor B; VEGF: Vascular endothelial growth factor; VEGFB: Vascular endothelial growth factor B; FAS: fatty acid synthase; ACO: Acyl Coenzyme A Oxidase; HSL: hormone-sensitive lipase; LCAD: long-chain acyl-CoA dehydrogenase; PPARα: proliferator-activated receptor-α; PGC-1α: peroxidase proliferator activator receptor γ coactivator 1α.

VEGFB gene into mice to inhibit obesity-related inflammation and improve metabolic health.

The lipid deposition caused by the "first hit" can lead to an inflammatory cascade, causing the "second hit" to hepatocyte damage and accelerating pathological changes in NAFLD. VEGFB can affect inflammatory response by regulating lipid metabolism in NAFLD, thereby affecting the "first hit" and improving the "second hit" in NAFLD.

VEGFB PARTICIPATES IN REGULATING THE "MULTIPLE-HIT" IN NAFLD

"Multiple-hit" theory believes that the pathological mechanism of NAFLD involves insulin resistance, adipocyte dysfunction, gut microbiota disorder, aggregation of inflammatory factors, mitochondrial dysfunction, lipotoxicity, endoplasmic reticulum stress, and so on[53]. These factors collaborate and overlap with each other, accelerating hepatocyte damage and ultimately developing into cirrhosis, liver cancer, and end-stage liver failure. Insulin resistance is a common metabolic abnormality in patients with NAFLD and is considered the first step in the development of NAFLD[54]. Studies have shown that the activation of insulin receptor substrate 1 (IRS1) protein is downregulated and SREBP-1c is upregulated when insulin resistance occurs, which ultimately increases the expression of de novo synthesis of lipids, thus increasing the transport of FFA to the liver[55]. Meanwhile, hyperinsulinemia can inhibit the β-oxidative of FFA to further promote lipid accumulation in the liver. Excessive lipid accumulation in the liver can disrupt the homeostasis of glucose metabolism[56]. Hepatic insulin resistance participates in the inhibition of forkhead box protein 1 (FOXO1) and serine/threonine kinase (GSK-3) through phosphoinositide 3-kinase (PI3K)/protein kinase B (AKT), reduces Phosphoenolpyruvate carboxykinase (PEPCK) and glucose 6 phosphatase (G6Pase) levels in the liver, which promotes gluconeogenesis and inhibits glycogen synthesis[57] (Figure 4). Recent studies have shown that VEGFB plays an active role in regulating metabolic diseases related to insulin resistance. Robciuc *et al*[17] found that VEGFB can increase the sensitivity of peripheral insulin and improve insulin resistance. Hu *et al*[18] found that VEGFB can reduce insulin resistance by reducing the content of FFA and total cholesterol, thus improving the disorder of lipid metabolism in NAFLD.

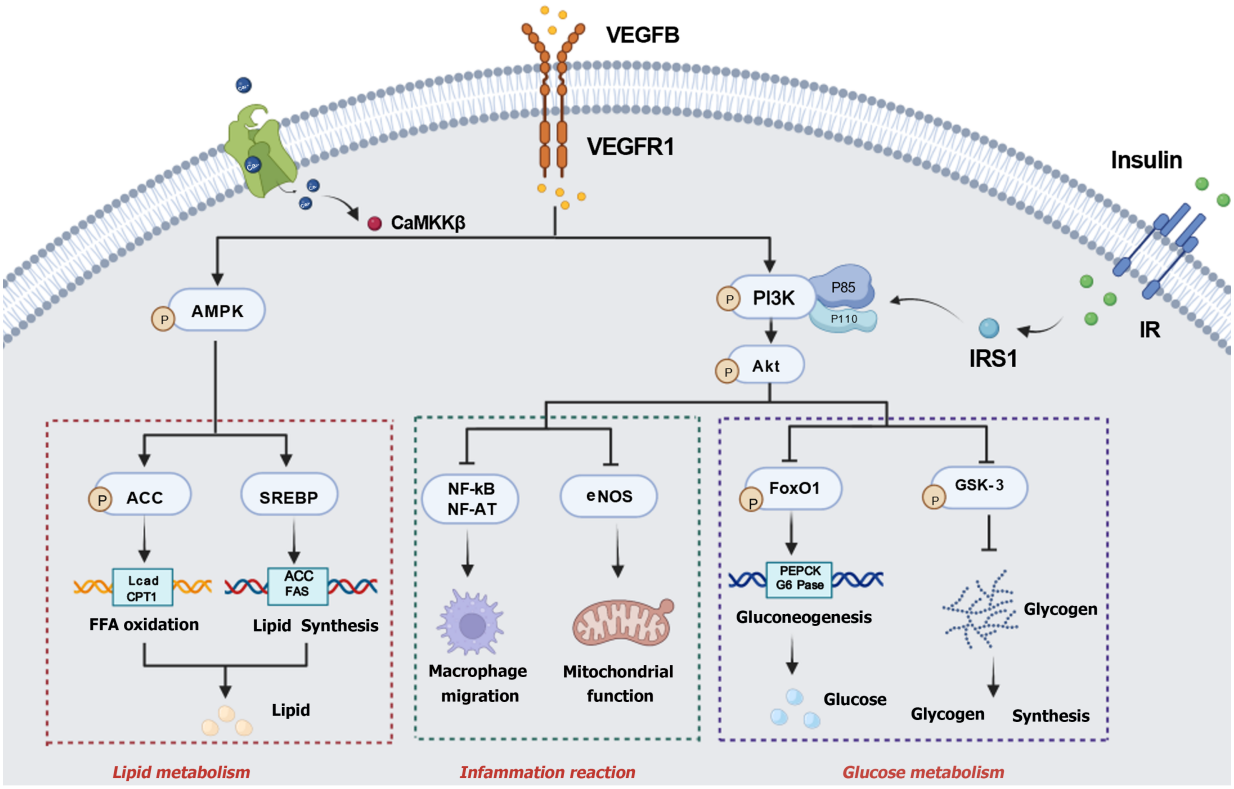

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Figure 4 Vascular endothelial growth factor B participates in the "multiple-hit" of nonalcoholic fatty liver disease. Vascular endothelial growth factor B (VEGFB) regulates lipid metabolism, inflammation reaction, and glucose metabolism, which co-exist in the nonalcoholic fatty liver disease progression. VEGFB activates the AMPK phosphorylation to regulate free fatty acid oxidation and lipid synthesis. Long-term lipid metabolism disorders will cause inflammatory reactions and glucose metabolism disorders. VEGFB promotes the phosphorylation of protein kinase B (AKT) *via* combining with the VEGFR1 to affect macrophage migration and mitochondrial inflammation reaction. Meanwhile, VEGFB/VEGFR1 also plays an important role in inhibiting gluconeogenesis and promoting glycogen synthesis by activating the phosphorylation of AKT to regulate glucose metabolism. CaMKKβ: Calmodulin-dependent protein kinase β; VEGFR1: Vascular endothelial growth factor receptor-1; VEGFB: Vascular endothelial growth factor B; IR: Insulin receptor; IRS1: Insulin receptor substrate-1; PI3K: Phosphoinositide 3-kinase; AKT: Protein kinase B; AMPK: Adenosine 5'-monophosphate (AMP)-activated protein kinase; ACC: A carboxylase; CPT1: Carnitine palmitate transferase-1; Lcad: long-chain acyl-CoA dehydrogenase; SREBP: Sterol-regulatory element-binding protein; FAS: fatty acid synthase; NF-kB: Nuclear factor-kappa B; NF-AT: Nuclear factors of activated T; eNOS: endothelial nitric oxide synthase; FoxO1: Forkhead box protein-1; GSK-3: Serine/threonine kinase-3; PEPCK: Phosphoenolpyruvate carboxykinase; G6Pase: Glucose 6 phosphatase;

Insulin resistance has been proven to be an activator of cell apoptosis[58]. Cell apoptosis, an injury factor in the "multiple hits", is a common and important mechanism of NAFLD lesions and liver injury. Li *et al*[59] believe that the decrease in the number of hepatocytes may be due to apoptosis, and excessive apoptosis of hepatocytes is an important sign of NAFLD/NASH patients. As a member of the vascular growth factor family, VEGFB participates in many angiogenesis-dependent diseases, and its pathogenesis is related to cell apoptosis. Williams *et al*[60] showed that inhibition of VEGFB leads to increased apoptosis in cardiomyocytes of patients with diabetes. Dai *et al*[61] also demonstrated that VEGFB plays an antiapoptotic role in the context of tumors.

Adipocyte dysfunction has also been confirmed to be closely related to the pathogenesis of NAFLD [62]. The degree of adipocyte dysfunction is consistent with abnormal metabolism in NAFLD. Martina Rudnicki's study confirms that male mice fed with HFD exhibit adipocyte dysfunction[63]. Robciuc *et al* [17] has shown that the VEGFB/VEGFR1 pathway can be used to enhance vascular distribution in adipose tissue, which improves metabolic health and obesity. The VEGFB gene may affect the occurrence and development of NAFLD by affecting the expansion and loss of adipose tissue.

CONCLUSION

The role of VEGFB in regulating metabolic diseases, such as NAFLD, has attracted increasing attention from scholars. Research has shown that VEGFB can reduce lipid accumulation and restore insulin sensitivity in NAFLD. VEGFB activates the AKT signaling pathway by combining with VEGFR1, inhibits FOXO1 and GSK3 genes, blocks gluconeogenesis, accelerates glycogen synthesis, and improves insulin resistance. VEGFB not only improves liver insulin resistance, but also activates the AMPK signaling pathway, thereby activating the ACC signal to inhibit the expression of SREBP protein,

improving fatty acid oxidation, inhibiting lipid synthesis, and restoring lipid metabolism balance. The activated AKT protein inhibits nuclear factors and proteins such as NF-kB or eNOS after phosphorylation, regulates inflammatory factors such as macrophages and liver mitochondrial function, reduces the occurrence of inflammatory reactions in hepatocytes, and prevents the progression of NAFLD[43].

Although more and more studies support that VEGFB can be a new target for the treatment of NAFLD and type 2 diabetes, some studies have shown that VEGFB has not played a positive role in regulating lipid metabolism and insulin resistance. Ning *et al*[64] confirmed that the changes in VEGFB did not affect glucose metabolism or lipid uptake. Hagberg *et al*[65] suggested that VEGFB gene deletion can prevent ectopic lipid deposition and ameliorate dyslipidemia. Falkevall *et al*[66] showed that inhibition of VEGFB signaling can target liver steatosis by inhibiting lipolysis and preventing the development of NAFLD.

At present, the understanding of the role of VEGFB in regulating NAFLD and its mechanism remains controversial and is not completely clear. So more research focuses on the mechanism of VEGFB in the occurrence and development of NAFLD, which will provide a new idea for the study of pathophysiological mechanisms and therapeutic targets of NAFLD.

FOOTNOTES

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ORIGINAL ARTICLE

Retrospective Study

Acute pancreatitis in liver transplant hospitalizations: Identifying national trends, clinical outcomes and healthcare burden in the United States

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Abstract

BACKGROUND

Acute pancreatitis (AP) in liver transplant (LT) recipients may lead to poor clinical outcomes and development of severe complications.

AIM

We aimed to assess national trends, clinical outcomes, and the healthcare burden of LT hospitalizations with AP in the United States (US).

METHODS

The National Inpatient Sample was utilized to identify all adult (≥ 18 years old) LT hospitalizations with AP in the US from 2007–2019. Non-LT AP hospitalizations served as controls for comparative analysis. National trends of hospitalization characteristics, clinical outcomes, complications, and healthcare burden for LT hospitalizations with AP were highlighted. Hospitalization characteristics, clinical outcomes, complications, and healthcare burden were also compared between the LT and non-LT cohorts. Furthermore, predictors of inpatient mortality for LT hospitalizations with AP were identified. All *P* values ≤ 0.05 were considered statistically significant.

RESULTS

The total number of LT hospitalizations with AP increased from 305 in 2007 to 610 in 2019. There was a rising trend of Hispanic (16.5% in 2007 to 21.1% in 2018, P-trend = 0.0009) and Asian (4.3% in 2007 to 7.4% in 2019, p-trend = 0.0002) LT hospitalizations with AP, while a decline was noted for Blacks (11% in 2007 to 8.3% in 2019, P-trend = 0.0004). Furthermore, LT hospitalizations with AP had an increasing comorbidity burden as the Charlson Comorbidity Index (CCI) score ≥ 3 increased from 41.64% in 2007 to 62.30% in 2019 (P-trend < 0.0001). We did not find statistically significant trends in inpatient mortality, mean length of stay (LOS), and mean total healthcare charge (THC) for LT hospitalizations with AP despite rising trends of complications such as sepsis, acute kidney failure (AKF), acute respiratory failure (ARF), abdominal abscesses, portal vein thrombosis (PVT), and venous thromboembolism (VTE). Between 2007–2019, 6863 LT hospitalizations with AP were compared to 5649980 non-LT AP hospitalizations. LT hospitalizations with AP were slightly older (53.5 *vs* 52.6 years, *P* = 0.017) and had a higher proportion of patients with CCI ≥ 3 (51.5% *vs* 19.8%, *P* < 0.0001) compared to the non-LT cohort. Additionally, LT hospitalizations with AP had a higher proportion of Whites (67.9% *vs* 64.6%, *P* < 0.0001) and Asians (4% *vs* 2.3%, *P* < 0.0001), while the non-LT cohort had a higher proportion of Blacks and Hispanics. Interestingly, LT hospitalizations with AP had lower inpatient mortality (1.37% *vs* 2.16%, *P* = 0.0479) compared to the non-LT cohort despite having a higher mean age, CCI scores, and complications such as AKF, PVT, VTE, and the need for blood transfusion. However, LT hospitalizations with AP had a higher mean THC ($59596 *vs* $50466, *P* = 0.0429) than the non-LT cohort.

CONCLUSION

In the US, LT hospitalizations with AP were on the rise, particularly for Hispanics and Asians. However, LT hospitalizations with AP had lower inpatient mortality compared to non-LT AP hospitalizations.

Key Words: Liver transplantation; Pancreatitis; Mortality; Cost; Length of stay

Core Tip: Liver transplant (LT) is a lifesaving intervention for patients with end-stage liver disease. Acute pancreatitis (AP) in LT recipients may lead to poor clinical outcomes and development of severe complications. In this study, we noted an increase in LT hospitalizations with AP at a national level from 305 in 2007 to 610 in 2019 with a rising trend for Hispanics and Asians. However, there was no trend for inpatient mortality, mean length of stay and mean total healthcare charge. After a comparative analysis, LT hospitalizations with AP had lower inpatient mortality compared to the non-LT cohort despite a higher mean age, comorbidity burden, and presence of complications.

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INTRODUCTION

Acute pancreatitis (AP), an inflammatory response to injury of the pancreas, is one of the leading causes of hospitalization amongst gastrointestinal disorders in the United States (US). In the general population, the incidence of AP is estimated to be 40-50 per 100000 persons and there are approximately 275000 AP hospitalizations annually in the US[1,2]. Risk factors implicated in the development of AP include cholelithiasis, heavy alcohol use (4-5 drinks daily for > 5 years), hypertriglyceridemia (> 1000 mg/dL), smoking, medications, autoimmune diseases, genetic predispositions, blunt/penetrating abdominal trauma, viral infections, and therapeutic endoscopic procedures such as endoscopic retrograde cholangiopancreatography (ERCP), among others[3-10]. The pathogenesis of AP is multifactorial, but ultimately involves the unregulated activation of proteolytic enzymes within the pancreas eventually leading to pancreatic ductal obstruction, subsequent inflammation, and in severe cases a systemic-inflammatory response syndrome[11]. The characteristic clinical features of AP include nausea, vomiting, loss of appetite, and epigastric abdominal pain radiating to the back[12]. A diagnosis of AP can be established by the presence of any two of the following three criteria: (1) Characteristic epigastric abdominal pain; (2) serum lipase and/or amylase greater than three times the upper limit of normal; and (3) evidence of AP on abdominal imaging[13]. Over the years, AP hospitalizations are on a rise in the US, with mortality rates ranging from 1%-2% and over 2.5 billion dollars being spent annually on healthcare costs[1,14].

Liver transplant (LT) has revolutionized management for chronic end-stage liver disease with excellent results. Since the first LT in 1967, the procedure has saved close to 500000 Life-years among patients with acute fulminant hepatic failure, hepatocellular carcinoma (HCC), and end-stage liver disease[15,16]. The recipients of the procedure have excellent survival rates. Per the Scientific Registry of Transplant Recipients data, the overall patient survival rate after deceased donor LT was 90% and 77% at 1 year and 5 years, respectively[17]. Moreover, the graft survival rate at 1 year and 5 years after LT was noted to be 89.6% and 72.8%, respectively[18].

AP is an important risk factor for poor surgical outcomes in patients with LT. Studies have reported an incidence rate ranging from 3%-8% for post-LT pancreatitis[19,20]. Common risk factors implicated in the development of post-LT pancreatitis include hepatitis B infection as an indication of transplant, re-transplantation, duration of venous bypass, hypotension with longer procedural time, utilization of ERCP, type of biliary reconstruction, intraoperative calcium chloride administration, and use of an aorto-hepatic graft[19,21,22]. Additionally, surgical manipulation, immunosuppression, infections, and biliary complications before LT may also increase the risk of developing post-LT pancreatitis[23]. In LT recipients, peri-transplant pancreatitis is associated with a two-fold increased risk of mortality[24]. Furthermore, early AP in LT recipients (within 1-2 mo of LT) may have mortality rates as high as 67% [25]. Given the acute-organ shortage worldwide, we must identify LT hospitalizations at high risk of developing AP to maximize patient survival.

Although studies investigating post-LT pancreatitis currently exist, they are primarily limited to small single-center experiences[19,20,22,25-27]. Hence, this study was designed to investigate trends in hospitalization characteristics and clinical outcomes for LT hospitalizations with AP. Furthermore, we performed a comparative analysis between LT and non-LT hospitalizations with AP to determine the influence of LT on clinical outcomes and healthcare burden. Predictors of inpatient mortality for LT hospitalizations with AP were also identified.

MATERIALS AND METHODS

Design and data source

This retrospective study derived the study population from the National Inpatient Sample (NIS) for 2007–2019 which was coded using the International Classification of Diseases, 9th and 10th Revision, Clinical Modification (ICD-9/10- CM) diagnosis codes, and procedure codes. The NIS, maintained by the Healthcare Cost and Utilization Project (HCUP), is one of the largest, publicly available, multi-ethnic databases in the US. HCUP is a family of healthcare databases, related software tools, and products developed through a Federal-State-Industry partnership and sponsored by the Agency for Healthcare Research and Quality. The NIS enables medical researchers to analyze data on more than seven million hospital stays each year in the US. It approximates a 20-percent stratified sample of all discharges from US community hospitals, excluding rehabilitation and long-term acute care hospitals. The NIS database is publicly available at: https://www.hcup-us.ahrq.gov/.

Study population and outcome measures

We utilized the NIS to identify all adult (≥ 18 years old) LT hospitalizations with AP in the US from 2007–2019. National trends of hospitalization characteristics, clinical outcomes, complications, and the healthcare burden were highlighted. Furthermore, non-LT AP hospitalizations served as controls for a comparative analysis of hospitalization characteristics, clinical outcomes, complications, and the healthcare burden with the LT cohort. Predictors of inpatient mortality for LT hospitalizations with AP were also identified.

Statistical analysis

Statistical analysis was conducted using SAS 9.4 (SAS Institute Inc, Cary, NC, United States) to account for weights in the stratified survey design of the NIS. During the statistical estimating process, weights were considered by incorporating the variables for strata, weight to discharges and cluster. Descriptive statistics including mean (± standard error) for continuous variables, and count (%) for categorical variables were provided after statistical analysis. The Cochran-Armitage trend tests were implemented to test the trends for proportions of binary variables. The trends for the averages of age, mean length of stay (LOS) and mean total healthcare charge (THC) were examined by using linear regression. The Rao-Scott design-adjusted chi-square test examined the association between binary variables in LT and non-LT hospitalizations with AP. F-statistics from the weighted regression model was used to test the differences in age, mean LOS, and mean THC in LT and non-LT hospitalizations with AP. Adjusted hazard ratios with 95% confidence interval were obtained through Cox proportional hazards regression to identify factors that influenced mortality. All analytical results were considered statistically significant when *P* values were less than or equal to 0.05.

Ethical considerations

The NIS database lacks patient and hospital-specific identifiers to protect patient privacy and maintain anonymity. Hence, our study was exempt from Institutional Review Board (IRB) approval as per guidelines put forth by our IRB for analysis of database studies.

RESULTS

Trends of hospitalization characteristics for LT hospitalizations with AP

There was an increase in the total number of LT hospitalizations with AP from 305 in 2007 to 610 in 2019. We did not find a statistically significant trend for gender or mean age; however, there was an increasing trend of LT hospitalizations with AP for patients aged ≥ 65 years (Table 1). Furthermore, LT hospitalizations with AP had an increasing comorbidity burden as the Charlson Comorbidity Index (CCI) score ≥ 3 increased from 41.64% in 2007 to 62.30% in 2019 (P-trend < 0.0001). Interestingly, we also noted a rising trend of LT hospitalizations with AP from 58.89% in 2007 to 82.79% in 2019 at urban teaching hospitals.

Racial differences in the trends of LT hospitalizations with AP were apparent. Whites made up a majority of the study cohort (Table 1) without a statistically significant trend. We noted an overall increasing trend of Hispanic (16.49% in 2007 to 21.09% in 2018, P-trend = 0.0009) and Asian (4.27% in 2007 to 7.44% in 2019, P-trend = 0.0009) LT hospitalizations with AP (Table 1 and Figure 1). However, Black LT hospitalizations with AP had a declining trend from 11% to 8.26%, P-trend = 0.0004) (Table 1).

Trends of clinical outcomes, healthcare burden and complications for LT hospitalizations with AP

We did not find a statistically significant trend for inpatient mortality, mean LOS, and mean THC for LT hospitalizations with AP (Table 2). However, we observed a rising trend of complications such as sepsis (1.25% in 2007 to 18.03% in 2019, P-trend < 0.0001), acute kidney failure (AKF) (17.13% to 34.43%, P-trend < 0.0001), acute respiratory failure (ARF) (1.44% to 6.56%, P-trend = 0.0002), abdominal abscesses

Table 1 Trends of hospitalization characteristics for liver transplant hospitalizations with acute pancreatitis in the United States from 2007–2019, *n* (%)

Epidemiologicalvariable	Years													Trend (*P* value)
	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	
Total number of hospitalizations	305	600	550	520	453	590	455	500	460	650	505	665	610	--
Mean age in yr (standard error)	53.08 (1.52)	51.00 (1.35)	52.01 (1.59)	51.32 (1.45)	51.79 (1.49)	52.02 (1.10)	52.86 (1.20)	55.16 (1.22)	50.27 (1.31)	51.84 (1.11)	53.70 (1.26)	53.32 (1.12)	54.73 (1.20)	No trend (0.1256)
Age groups (yr)														
18–34	30 (9.94)	82 (13.62)	76 (13.77)	62 (11.90)	50 (11.07)	80 (13.56)	45 (9.89)	55 (11.00)	90 (19.57)	105 (16.15)	50 (9.90)	70 (10.53)	55 (9.02)	No trend (0.1345)
35–49	61 (19.82)	180 (29.97)	117 (21.38)	111 (21.37)	84 (18.46)	145 (24.58)	100 (21.98)	90 (18.00)	115 (25.00)	120 (18.46)	100 (19.80)	185 (27.82)	165 (27.05)	No trend (0.2379)
50–64	164 (53.56)	236 (39.30)	270 (49.09)	281 (54.15)	276 (60.97)	280 (47.46)	255 (56.04)	225 (45.00)	170 (36.96)	295 (45.38)	260 (51.49)	265 (39.85)	200 (32.79)	Decrease (< 0.0001)
65–79	46 (14.90)	103 (17.12)	87 (15.76)	62 (11.86)	38 (8.40)	85 (14.41)	55 (12.09)	120 (24.00)	75 (16.30)	125 (19.23)	85 (16.83)	135 (20.30)	190 (31.15)	Increase (< 0.0001)
≥ 80	< 11 (1.78)	0 (0.00)	0 (0.00)	< 11 (0.71)	< 11 (1.09)	0 (0.00)	0 (0.00)	< 11 (2.00)	< 11 (2.17)	< 11 (0.77)	< 11 (1.98)	< 11 (1.50)	0 (0.00)	Increase (0.0157)
Gender														
Male	163 (53.30)	278 (46.32)	311 (56.61)	328 (63.08)	270 (59.48)	325 (55.08)	260 (57.14)	235 (47.00)	275 (59.78)	380 (58.46)	270 (53.47)	370 (55.64)	340 (55.74)	No trend (0.1383)
Female	143 (46.70)	322 (53.68)	238 (43.39)	192 (36.92)	184 (40.52)	265 (44.92)	195 (42.86)	265 (53.00)	185 (40.22)	270 (41.54)	235 (46.53)	295 (44.36)	270 (44.26)	No trend (0.1383)
Race														
White	139 (59.81)	387 (72.63)	310 (66.45)	322 (66.37)	285 (71.89)	395 (71.17)	290 (65.91)	375 (78.95)	265 (61.63)	390 (64.46)	360 (75.00)	385 (60.16)	405 (66.94)	No trend (0.0517)
Black	26 (11.00)	48 (9.04)	69 (14.68)	99 (20.39)	58 (14.49)	35 (6.31)	45 (10.23)	40 (8.42)	20 (4.65)	90 (14.88)	50 (10.42)	60 (9.38)	50 (8.26)	Decrease (0.0004)
Hispanic	38 (16.49)	63 (11.85)	54 (11.58)	43 (8.80)	39 (9.75)	65 (11.71)	70 (15.91)	35 (7.37)	95 (22.09)	80 (13.22)	35 (7.29)	135 (21.09)	80 (13.22)	Increase (0.0009)
Asian	< 11 (4.27)	20 (3.77)	0 (0.00)	11 (2.19)	< 11 (2.57)	35 (6.31)	20 (4.55)	15 (3.16)	20 (4.65)	30 (4.96)	< 11 (2.08)	25 (3.91)	45 (7.44)	Increase (0.0002)
Other	20 (8.42)	14 (2.71)	34 (7.28)	11 (2.26)	< 11 (1.31)	25 (4.50)	15 (3.41)	< 11 (2.11)	30 (6.98)	15 (2.48)	25 (5.21)	35 (5.47)	25 (4.13)	No trend (0.406)
CCI														
CCI = 1	130 (42.71)	232 (38.75)	174 (31.68)	147 (28.25)	138 (30.35)	185 (31.36)	130 (28.57)	155 (31.00)	150 (32.61)	160 (24.62)	120 (23.76)	160 (24.06)	145 (23.77)	Decrease (< 0.0001)
CCI = 2	48 (15.65)	101 (16.81)	106 (19.32)	155 (29.77)	61 (13.38)	115 (19.49)	115 (25.27)	130 (26.00)	90 (19.57)	110 (16.92)	75 (14.85)	115 (17.29)	85 (13.93)	Decrease (0.0036)
CCI ≥ 3	127 (41.64)	267 (44.44)	269 (49.01)	218 (41.98)	255 (56.27)	290 (49.15)	210 (46.15)	215 (43.00)	220 (47.83)	380 (58.46)	310 (61.39)	390 (58.65)	380 (62.30)	Increase (< 0.0001)
Hospital region														

Northeast	36 (11.70)	209 (34.76)	82 (14.99)	61 (11.73)	89 (19.75)	100 (16.95)	60 (13.19)	35 (7.00)	65 (14.13)	95 (14.62)	115 (22.77)	115 (17.29)	85 (13.93)	Decrease (< 0.0001)
Midwest	64 (20.97)	98 (16.26)	213 (38.69)	134 (25.71)	144 (31.79)	130 (22.03)	125 (27.47)	140 (28.00)	130 (28.26)	125 (19.23)	130 (25.74)	140 (21.05)	130 (21.31)	Decrease (0.0063)
South	105 (34.47)	121 (20.22)	119 (21.68)	244 (47.04)	169 (37.29)	185 (31.36)	180 (39.56)	205 (41.00)	170 (36.96)	270 (41.54)	145 (28.71)	265 (39.85)	230 (37.70)	Increase (< 0.0001)
West	100 (32.86)	173 (28.77)	135 (24.64)	81 (15.52)	51 (11.18)	175 (29.66)	90 (19.78)	120 (24.00)	95 (20.65)	160 (24.62)	115 (22.77)	145 (21.80)	165 (27.05)	No trend (0.2338)
Hospital bed-size														
Small	43 (13.91)	48 (7.97)	23 (4.59)	34 (6.64)	23 (5.15)	30 (5.08)	60 (13.19)	45 (9.00)	65 (14.13)	60 (9.23)	40 (7.92)	90 (13.53)	85 (13.93)	Increase (< 0.0001)
Medium	68 (22.16)	93 (15.44)	103 (20.26)	91 (17.70)	58 (13.10)	120 (20.34)	75 (16.48)	135 (27.00)	100 (21.74)	120 (18.46)	120 (23.76)	195 (29.32)	155 (25.41)	Increase (< 0.0001)
Large	195 (63.93)	459 (76.59)	381 (75.16)	389 (75.67)	363 (81.75)	440 (74.58)	320 (70.33)	320 (64.00)	295 (64.13)	470 (72.31)	345 (68.32)	380 (57.14)	370 (60.66)	Decrease (< 0.0001)
Hospital location and teaching status														
Rural	18 (5.97)	57 (9.51)	20 (3.98)	41 (7.98)	35 (7.95)	65 (11.02)	30 (6.59)	45 (9.00)	35 (7.61)	35 (5.38)	15 (2.97)	65 (9.77)	25 (4.10)	Decrease (0.0238)
Urban nonteaching	107 (35.14)	145 (24.21)	171 (33.68)	156 (30.35)	84 (18.89)	190 (32.20)	140 (30.77)	70 (14.00)	85 (18.48)	125 (19.23)	110 (21.78)	110 (16.54)	80 (13.11)	Decrease (< 0.0001)
Urban teaching	180 (58.89)	398 (66.28)	316 (62.34)	317 (61.67)	325 (73.16)	335 (56.78)	285 (62.64)	385 (77.00)	340 (73.91)	490 (75.38)	380 (75.25)	490 (73.68)	505 (82.79)	Increase (< 0.0001)
Disposition														
Discharge Home	276 (90.47)	473 (78.88)	397 (72.25)	359 (69.05)	333 (73.51)	440 (74.58)	345 (75.82)	395 (79.00)	375 (81.52)	525 (80.77)	370 (73.27)	490 (73.68)	455 (74.59)	No trend (0.1111)
Transfer to short-term hospital	< 11 (3.06)	46 (7.74)	37 (6.72)	68 (13.01)	35 (7.81)	50 (8.47)	25 (5.49)	35 (7.00)	40 (8.70)	30 (4.62)	60 (11.88)	30 (4.51)	35 (5.74)	No trend (0.0657)
Transfer to another facility (Includes SNF and ICF)	< 11 (3.25)	20 (3.28)	34 (6.11)	26 (5.09)	13 (2.97)	25 (4.24)	< 11 (2.20)	< 11 (1.00)	10 (2.17)	45 (6.92)	< 11 (0.99)	70 (10.53)	15 (2.46)	No trend (0.0532)
Home health care	< 11 (3.22)	30 (5.07)	51 (9.32)	62 (11.87)	42 (9.25)	60 (10.17)	60 (13.19)	50 (10.00)	30 (6.52)	45 (6.92)	55 (10.89)	55 (8.27)	60 (9.84)	Increase (0.0426)
Discharge against medical advice	0 (0.00)	25 (4.16)	11 (2.00)	< 11 (0.98%)	< 11 (1.11)	< 11 (1.69)	15 (3.30)	<11 (2.00)	< 11 (1.09)	0 (0.00)	< 11 (0.99)	20 (3.01)	25 (4.10)	No trend (0.1735)

CCI: Charlson comorbidity index; ICF: Intermediate care facility; SNF: Skilled nursing facility.

(0% in 2007 to 0.82% in 2019, P-trend = 0.0006), portal vein thrombosis (PVT) (0% to 4.10%, P-trend < 0.0001) and venous thromboembolism (VTE) (1.82% to 7.38%, P-trend < 0.0001) for LT hospitalizations with AP. Moreover, there was a decline in the need for blood transfusion from 6.09% in 2007 to 0% in 2019 (P-trend < 0.0001) for LT hospitalizations with AP.

Table 2 Trends of outcomes for liver transplant hospitalizations with acute pancreatitis in the United States from 2007–2019, *n* (%)

Outcomes	Years													Trend (*P* value)
	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	
Inpatient mortality	0 (0.00)	< 11 (0.87)	20 (3.60)	0 (0.00)	24 (5.35)	< 11 (0.85)	0 (0.00)	< 11 (1.00)	0 (0.00)	< 11 (0.77)	< 11 (1.98)	0 (0.00)	20 (3.28)	No trend (0.3879)
Length of stay (d)	5.62	5.44	7.09	6.02	7.85	5.40	8.53	4.53	4.86	7.33	6.62	6.23	4.55	No trend (0.6905)
Total healthcare charge ($)	36413	53418	50432	53115	68247	42107	95774	41613	42319	79746	80479	65054	56011	No trend (0.1946)
Complications														
Pancreatic psuedocyst	< 11 (3.41)	35 (5.88)	34 (6.13)	26 (5.06)	14 (3.01)	< 11 (1.69)	20 (4.40)	< 11 (2.00)	< 11 (1.09)	20 (3.08)	0 (0.00)	50 (7.52)	30 (4.92)	No trend (0.1273)
Abdominal abscess	0 (0.00)	0 (0.00)	0 (0.00)	11 (2.07)	< 11 (1.00)	0 (0.00)	< 11 (1.10)	< 11 (1.00)	0 (0.00)	< 11 (0.77)	0 (0.00)	20 (3.01)	< 11 (0.82)	Increase (0.0006)
Sepsis	< 11 (1.25)	41 (6.91)	46 (8.37)	30 (5.69)	32 (7.15)	20 (3.39)	35 (7.69)	30 (6.00)	40 (8.70)	60 (9.23)	55 (10.89)	70 (10.53)	110 (18.03)	Increase (< 0.0001)
Acute renal failure	52 (17.13)	121 (20.10)	149 (27.09)	123 (23.74)	143 (31.62)	160 (27.12)	130 (28.57)	145 (29.00)	155 (33.70)	205 (31.54)	180 (35.64)	245 (36.84)	210 (34.43)	Increase (< 0.0001)
Acute respiratory failure	< 11 (1.44)	37 (6.10)	21 (3.79)	21 (4.06)	28 (6.28)	0 (0.00)	15 (3.30)	< 11 (2.00)	25 (5.43)	55 (8.46)	20 (3.96)	40 (6.02)	40 (6.56)	Increase (0.0002)
Need for blood transfusion	19 (6.09)	72 (12.03)	33 (6.10)	40 (7.61)	91 (20.09)	95 (16.10)	45 (9.89)	35 (7.00)	30 (6.52)	20 (3.08)	20 (3.96)	25 (3.76)	0 (0.00)	Decrease (< 0.0001)
Portal vein thrombosis	0 (0.00)	< 11 (1.69)	< 11 (0.86)	0 (0.00)	0 (0.00)	0 (0.00)	< 11 (1.10)	< 11 (2.00)	< 11 (1.09)	15 (2.31)	15 (2.97)	15 (2.26)	25 (4.10)	Increase (< 0.0001)
Venous thromboembolism	< 11 (1.82)	< 11 (0.85)	35 (6.29)	16 (3.02)	< 11 (1.00)	< 11 (1.69)	< 11 (1.10)	< 11 (1.00)	< 11 (2.17)	25 (3.85)	25 (4.95%)	50 (7.52)	45 (7.38)	Increase (< 0.0001)

Comparative analysis of hospitalization characteristics for LT and non-LT hospitalizations with AP

Between 2007–2019, there were 6863 LT hospitalizations with AP which were compared to 5649980 non-LT AP hospitalizations. LT hospitalizations with AP had a slightly higher mean age (53.5 *vs* 52.55 years, $P = 0.017$) compared to the non-LT cohort. Furthermore, LT hospitalizations with AP also had a higher proportion of males (55.43% *vs* 51.13%, $P = 0.0046$) and patients with a CCI score ≥ 3 (51.46% *vs* 19.76%, $P < 0.0001$) compared to non-LT hospitalizations (Table 3). A majority of LT hospitalizations with AP were at large (69.47%), urban teaching (69.73%) hospitals.

Racial differences were observed between the LT and non-LT cohorts. We noted a higher proportion of Whites (67.91% *vs* 64.57%, $P < 0.0001$) and Asians (3.95% *vs* 2.3%, $P < 0.0001$) in the LT cohort, while there was a higher proportion of Blacks and Hispanics in the non-LT cohort (Table 3).

Comparative analysis of clinical outcomes, healthcare burden and complications for LT and non-LT hospitalizations with AP

Overall, the inpatient mortality for LT hospitalizations with AP was lower (1.37% *vs* 2.16%, $P = 0.0479$) than the non-LT cohort (Table 4). We did not find a statistical difference in the inpatient mortality rates after stratifying for age, gender, or race. Although the mean LOS was comparable between both groups, the mean THC was higher for LT hospitalizations with AP ($59596 *vs* $50466, P-trend = 0.0429) compared to the non-LT cohort. Furthermore, LT hospitalizations with AP also had a higher proportion

Table 3 Comparative analysis of hospitalization characteristics for liver and non-liver transplant hospitalizations with acute pancreatitis in the United States from 2007–2019, *n* (%)

Outcomes	Liver transplant hospitalizations with acute pancreatitis	Non-liver transplant hospitalizations with acute pancreatitis	*P* value
Total number of hospitalizations	6863	5649980	
Mean age ± standard error (yr)	53.50 (0.04)	52.55 (0.39)	0.017
Age group (yr)			< 0.0001
18–34	12.38	16.20	
35–49	22.91	26.36	
50–64	46.29	29.97	
65–79	17.55	18.43	
≥ 80	0.86	9.03	
Gender			0.0046
Male	55.43	51.13	
Female	44.57	48.87	
Race			≤ 0.0001
White	67.91	64.57	
Black	10.85	16.11	
Hispanic	13.11	13.12	
Asian	3.95	2.30	
Other	4.16	3.90	
Charlson comorbidity index			< 0.0001
CCI = 1	29.53	28.27	
CCI = 2	19.02	13.65	
CCI ≥ 3	51.46	19.76	
Hospital region			0.0753
Northeast	16.71	16.45	
Midwest	24.80	22.02	
South	35.10	40.16	
West	23.38	21.37	
Hospital bed-size			< 0.0001
Small	9.49	17.96	
Medium	21.04	28.13	
Large	69.47	53.91	
Hospital location and teaching status			< 0.0001
Rural	7.15	12.46	
Urban nonteaching	23.12	36.23	
Urban teaching	69.73	51.32	
Disposition			< 0.0001
Routine (Home)	76.26	77.39	
Transfer to short-term hospital	7.30	3.10	
Transfer to another type of facility (Includes SNF and ICF)	4.20	7.64	
Home health care	8.89	6.69	

Discharge against medical advice	1.98	3.00

CCI: Charlson comorbidity index; ICF: Intermediate care facility; SNF: Skilled nursing facility.

Table 4 Comparative analysis of clinical outcomes for liver and non-liver transplant hospitalizations with acute pancreatitis in the United States from 2007–2019, *n* (%)

Outcomes	Liver transplant hospitalizations with acute pancreatitis	Non-liver transplant hospitalizations with acute pancreatitis	*P* value
Inpatient mortality	1.37	2.16	0.0479
Gender-specific inpatient mortality			
Male	1.43	2.34	0.1107
Female	1.31	1.97	0.2396
Race specific inpatient mortality			
White	1.48	2.23	0.1403
Black	1.45	2.00	0.6469
Hispanic	0.00	1.60	---
Asian	1.99	3.20	0.6289
Others	4.01	2.23	0.3868
Age group specific inpatient mortality			
18-34	0.60	0.64	0.9583
35-49	0.90	1.04	0.7855
50-64	1.12	2.08	0.0921
65-79	3.26	3.60	0.7784
≥ 80	0.00	5.46	---
Length of stay (d)	6.14	5.80	0.3189
Total healthcare charge ($)	59596	50466	0.0429
Complications (out of total hospitalizations)			
Pancreatic psuedocyst	3.85	5.46	0.0259
Abdominal abscess	0.81	0.53	0.1925
Sepsis	8.35	8.78	0.5834
Acute renal failure	29.41	14.91	< 0.0001
Acute respiratory failure	4.61	5.67	0.1018
Cholangiocarcinoma	0.21	0.11	0.2545
Need for blood transfusion	7.65	4.75	< 0.0001
Portal vein thrombosis	1.53	0.64	< 0.0001
Venous thromboembolism	3.50	2.19	0.0011

of patients with complications such as AKF (29.41% *vs* 14.91%, $P < 0.0001$), need for blood transfusion (7.65% *vs* 4.75%, $P < 0.0001$), PVT (1.53% *vs* 0.64%, $P < 0.0001$) and VTE (3.5% *vs* 2.19%, $P = 0.0011$) compared to non-LT hospitalizations; however, the non-LT cohort had a higher proportion of patients with pancreatic pseudocysts (5.46% *vs* 3.85%, $P = 0.0259$) (Table 4).

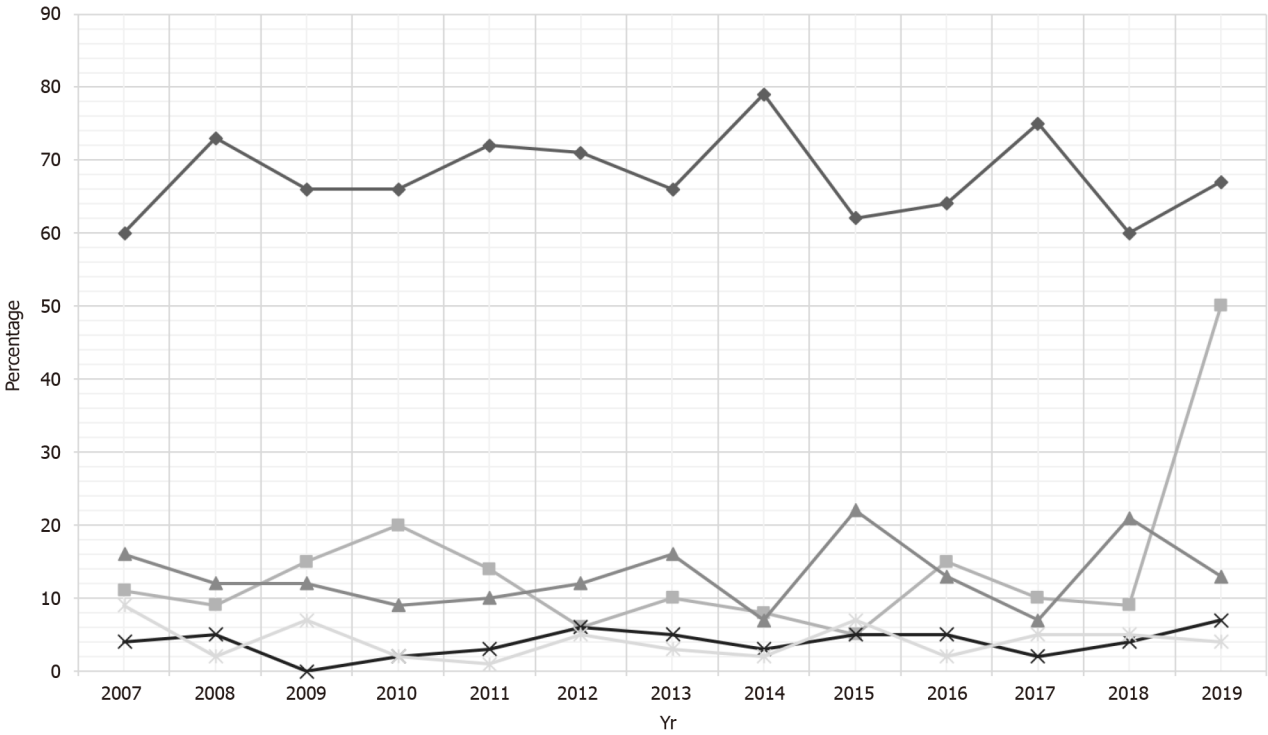

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Figure 1 Racial trends for liver transplant hospitalizations with acute pancreatitis in the United States from 2007–2019.

Predictors for inpatient mortality for LT hospitalizations with AP

After a regression analysis, Hispanics were noted to have lower odds of inpatient mortality compared to Whites (Table 5). Furthermore, after adjusting for all other variables, every one-point increase in the CCI score was associated with a 67.8% increase in inpatient mortality for LT hospitalizations with AP (Table 5). The presence of complications such as pancreatic pseudocysts (aHR: 14.158, 95%CI 1.642-122.094, $P = 0.016$), sepsis (aHR: 13.960, 95%CI 2.163-90.093, $P < 0.0001$), AKF (aHR: 2.684, 95%CI 1.109-6.494, $P = 0.029$), ARF (aHR: 24.758, 95%CI 1.063-576.522, $P = 0.046$), need for blood transfusion (aHR: 150.340, 95%CI 17.049-1325.754, $P < 0.0001$) and VTE (aHR: 75.422, 95%CI 1.637-3475.134, $P = 0.027$) were also associated with higher odds inpatient mortality for LT hospitalizations with AP after adjusting for all other variables.

DISCUSSION

AP is a well-known clinical entity. Although it has been thoroughly studied in the general population, there is a significant paucity of data on AP in solid-organ transplant recipients, particularly those undergoing LT. This is the only study in current literature that investigates trends, clinical outcomes, and the healthcare burden of LT hospitalizations with AP at a national level. In this study, we noted an increase in LT hospitalizations with AP with a rising trend for ethnic minorities *i.e.* Hispanics and Asians; however, we did not find a statistically significant trend of inpatient mortality, mean LOS and mean THC. Although the LT cohort was slightly older and had a higher comorbidity burden, the overall inpatient mortality was lower (1.37% *vs* 2.16%, $P = 0.0479$) compared to the non-LT cohort. Furthermore, LT hospitalizations with AP had a higher proportion of patients with AKF, PVT, VTE, and the need for blood transfusion compared to the non-LT cohort. Increasing CCI and the presence of pancreatic pseudocysts, sepsis, ARF, AKF, VTE, and the need for blood transfusion were associated with increased odds of inpatient mortality for LT hospitalizations with AP. With the increasing rates of liver transplants being performed and relative organ shortage in the US, it is vital to understand patient characteristics, outcomes, and complications of LT hospitalizations with AP to potentially reduce adverse clinical outcomes in these high-risk individuals[18].

As per data available from United Network for Organ Sharing (UNOS), the total number of LT increased from 6494 in 2007 to 8896 in 2019[18]. However, in our study, the total number of LT hospitalizations with AP increased disproportionally, essentially doubling in the same time frame. In the US, the rates of LT for patients ≥ 65 years of age have also been on the rise as there is a general consensus that LT in the elderly is feasible with acceptable short-term and long-term results[28,29]. Similarly, in this study, we noted an increase in the rates of LT hospitalizations with AP for patients > 65 years of age

Table 5 Predictors of inpatient mortality for liver transplant hospitalizations with acute pancreatitis in the United States from 2007–2019

Variable	Adjusted hazard ratio	95%CI	*P* value
Gender			
Male	Reference		
Female	0.596	(0.150, 2.365)	0.461
Race			
White	Reference		
Black	0.306	(0.017, 5.368)	0.418
Hispanic	< 0.001	(< 0.001, < 0.001)	< 0.0001
Asian	0.042	(< 0.001, 14.916)	0.289
Other	0.064	(< 0.001, 10.989)	0.295
Charlson comorbidity index	1.678	(1.055, 2.668)	0.029
Hospital region			
Northeast	Reference		
Midwest	1.574	(0.148, 16.692)	0.706
South	1.435	(0.230, 8.955)	0.699
West	1.723	(0.423, 7.014)	0.447
Hospital bed size			
Small	Reference		
Medium	1.427	(0.093, 21.893)	0.798
Large	1.974	(0.093, 42.139)	0.663
Hospital location and teaching status			
Rural	Reference		
Urban nonteaching	< 0.001	(< 0.001, 0.048)	0.003
Urban teaching	0.551	(0.053, 5.689)	0.617
Complications (reference = Without the complication)			
Pancreatic psuedocyst	14.158	(1.642, 122.094)	0.016
Abdominal abscess	< 0.001	(< 0.001, < 0.001)	< 0.0001
Sepsis	13.960	(2.163, 90.093)	0.006
Acute renal failure	2.684	(1.109, 6.494)	0.029
Acute respiratory failure	24.758	(1.063, 576.522)	0.046
Need for blood transfusion	150.340	(17.049, 1325.754)	< 0.0001
Portal vein thrombosis	< 0.001	(< 0.001, < 0.001)	< 0.0001
Venous thromboembolism	75.422	(1.637, 3475.134)	0.027

(Table 1). However, it should be noted that AP carries a higher morbidity and mortality burden in the elderly population at baseline, and this is compounded in organ transplant recipients[30].

In the US, there was an increase in LT for Hispanics and Asians from 912 in 2007 to 1498 in 2019 and 325 in 2007 to 363 in 2019, respectively as per the UNOS registry. Current literature lacks data on the racial distribution of AP in LT recipients, particularly for ethnic minorities *i.e.* Blacks, Hispanics, and Asians. However, studies have demonstrated that ethnic minorities, at baseline, are at a higher risk of developing AP and have greater severity of disease compared to the general population[2,31-35]. In our study, there was an increasing trend of Hispanic and Asian LT hospitalizations with AP (Figure 1) which was disproportionate to the increase in LT for this population. Interestingly, Black LT hospitalizations with AP were noted to have a declining trend between 2007–2019. After a comparative analysis, we observed a higher proportion of Asians in the LT cohort, while there was a higher proportion of Blacks and Hispanics in the non-LT cohort. The exact reason for this variable racial distribution is currently unknown but needs further investigation through large, multi-center prospective studies.

Furthermore, we emphasize the need for early recognition and prompt treatment of AP in Hispanic and Asian LT hospitalizations to prevent adverse clinical outcomes.

Statistics have demonstrated continuous improvements in survival rates for liver transplant recipients [36-38]. Over the last few decades, AP-related mortality has also declined due to prompt recognition and improvement in management strategies[1,39]. However, prior literature offers conflicting evidence on ethnic variations in AP-related mortality with some studies reporting increased mortality rates in Whites, while others noted higher mortality rates in Blacks among the general population[14,40]. There continues to be a significant paucity of data on mortality for AP in LT recipients in current literature. In our study, we did not find a statistically significant trend for inpatient mortality in LT hospitalizations with AP (Table 2). Interestingly, after a comparative analysis, LT hospitalizations with AP had lower inpatient mortality rates compared to the non-LT cohort despite a higher mean age, greater comorbidity burden, and higher proportion of patients with complications. Furthermore, we did not find a statistical difference in the inpatient mortality rates after stratifying for age, gender, or race. The exact reason for lower inpatient mortality rates in LT hospitalizations with AP is unknown. However, it may, in part, be due to increased vigilance for complications in these high-risk hospitalizations, overall improvements in management strategies, and a multi-disciplinary team approach for management of these highly complex patients. Additional multi-center prospective studies are needed to further investigate these findings. Nonetheless, lower mortality suggests improved survival rates for LT hospitalizations which is in line with current literature.

Healthcare utilization by LT recipients is on the rise. A study by Habka *et al*[41] in 2015 predicted that the cost of LT will increase by 33% in 10 years and 81% in the next 20 years. The inpatient cost of management of AP has also almost doubled from 1996 ($3.9 billion) to 2016 ($7.7 billion)[42]. On the contrary, the utilization of the inpatient service (bed days per prevent case) for AP has declined over the years[42]. No data currently exists on healthcare utilization for AP in LT recipients. In our study, we did not find a statistically significant trend in mean LOS and mean THC for LT hospitalizations with AP indicating that the healthcare burden has remained relatively stable over the years despite a higher proportion of patients with complications such as sepsis, AKF, ARF, PVT, VTE, and abdominal abscesses. After a comparative analysis, the mean LOS was comparable between the LT and non-LT cohorts; however, the mean THC for the LT cohort was $9130 higher than that of the non-LT cohort. This may, in part, be attributed to a higher proportion of patients with complications in the LT cohort compared to the non-LT cohort requiring a higher level of care and multi-disciplinary team management (Table 4). Furthermore, after adjusting for all other variables, increasing CCI, and the presence of complications such as pancreatic pseudocysts, sepsis, ARF, AKF, VTE, and need for blood transfusions were associated with higher odds of inpatient mortality for LT hospitalizations with AP. These findings somewhat mirror predictors of inpatient mortality for AP that have been reported in previous population-based studies[43].

Our study has several strengths and a few limitations. Our study population, which was drawn from one of the largest, publicly available, multi-ethnic databases in the US, is a key strength of this study. This is the only study in the current literature that offers a national perspective on hospitalization characteristics, clinical outcomes, complications, and the healthcare burden of LT hospitalizations with AP over 13 years, compared to other single-center experiences which offer limited information. Through a comprehensive and unique analysis technique, we were also able to compare LT and non-LT hospitalizations to understand the influence of AP on LT hospitalizations thereby giving gastroenterologists real world data. Furthermore, as the NIS covers approximately 97% of the US population, the results of our study are applicable to all LT hospitalizations with AP in the US.

However, we do acknowledge the limitations associated with our study. The retrospective study design makes our study susceptible to the biases that are associated with retrospective studies. Additionally, the NIS database does not contain information on the indication of liver transplant, time from LT to development of AP, disease severity, hospital course, treatment aspects of the disease, time from any procedure to development of complications, procedural complications (pre, intra, and post), intraprocedural operator preferences, or performance of any procedure. Lastly, the NIS is an administrative database that uses ICD codes to store data; hence, the possibility of human coding errors always exists. Despite these limitations, our large sample size, unique analysis technique, and multi-faceted outcomes add valuable data to limited literature.

CONCLUSION

LT is a lifesaving procedure for chronic end-stage liver disease patients. However, the development of post-LT pancreatitis may lead to poor surgical outcomes and development of complications. In our study, we noted an increase in LT hospitalizations with AP, particularly for ethnic minorities *i.e.* Hispanics and Asians; however, there was no trend for inpatient mortality. We also did not find a statistically significant trend mean LOS and mean THC indicating that healthcare utilization has remained relatively stable for LT hospitalizations with AP between 2007–2019. On comparison, LT hospitalizations with AP had lower inpatient mortality compared to non-LT AP hospitalizations despite

a higher proportion of patients that were older, had CCI ≥ 3, and had complications such as AKF, PVT, VTE, and need for blood transfusion. Increasing CCI, presence of pancreatic pseudocysts, sepsis, ARF, ARF, VTE, and need for blood transfusion were identified to be independent predictors of inpatient mortality for LT hospitalizations with AP.

ARTICLE HIGHLIGHTS

Research background

The development of Acute Pancreatitis (AP) in Liver Transplant (LT) recipients may be associated with poor clinical outcomes and severe complications.

Research motivation

Although studies investigating post-LT pancreatitis currently exist, they are primarily limited to small single-center experiences. Currently, a national perspective in the United States (US) does not exist. Therefore, this study was designed to investigate trends and outcomes of LT hospitalization with AP.

Research objectives

We aimed to assess national trends of hospitalization characteristics, clinical outcomes, and the healthcare burden of LT hospitalizations with AP in the US. Non-LT hospitalizations with AP were also identified as controls to compare hospitalization characteristics, clinical outcomes, and the healthcare burden with the LT cohort. Furthermore, predictors of inpatient mortality for LT hospitalizations with AP were identified.

Research methods

The National Inpatient Sample was utilized to identify LT and non-LT hospitalizations with AP. The Cochran-Armitage trend was used to test the trends for proportions of binary variables. Linear regression examined the trends for the averages of age, mean length of stay (LOS), and mean total healthcare charge (THC). Rao-Scott design-adjusted chi-square test examined the association between binary variables in LT and non-LT Hospitalizations with AP. F-statistics were used to test the differences in age, mean LOS, and mean THC in LT and non-LT Hospitalizations with AP. Cox proportional hazards regression was used to identify factors that influenced mortality.

Research results

The total number of LT hospitalizations with AP increased from 305 in 2007 to 610 in 2019. We did not find statistically significant trends in inpatient mortality, mean LOS, and mean THC for LT hospitalizations with AP. LT hospitalizations with AP had lower inpatient mortality compared to the non-LT cohort despite having a higher mean age, comorbidity burden, and complications. Increasing CCI, presence of pancreatic pseudocysts, sepsis, acute respiratory failure, acute renal failure, venous thromboembolism, and need for blood transfusion were independent predictors of inpatient mortality for LT hospitalizations with AP.

Research conclusions

LT is a lifesaving procedure for chronic end-stage liver disease patients. In the US, LT hospitalizations with AP increased between 2007 to 2019, particularly for Hispanics and Asians. However, LT hospitalizations with AP had lower inpatient mortality compared to non-LT AP hospitalizations.

Research perspectives

This is the only study in the current literature that offers a national perspective on hospitalization characteristics, clinical outcomes, complications, and the healthcare burden of LT hospitalizations with AP in the US.

FOOTNOTES

Author contributions: Dahiya DS, Jahagirdar V, Chandan S, Inamdar S, Sharma N, and Al-Haddad M contributed to conception and design; Dahiya DS, Cheng CI, and Al-Haddad M contributed to administrative support; Dahiya DS and Cheng CI contributed to provision, collection, and assembly of data; all Authors contributed to review of literature, drafting the manuscript, revision of key components of the manuscript, final approval of manuscript, agreement to be accountable for all aspects of the work.

Institutional review board statement: The NIS database lacks patient and hospital-specific identifiers to protect patient privacy and maintain anonymity. Hence, our study was exempt from Institutional Review Board (IRB) approval as

per guidelines put forth by our IRB for analysis of database studies.

Informed consent statement: The data for this study was collected from the National Inpatient Sample (NIS) database. As the NIS database lacks patient-specific and hospital-specific identifiers, this study did not require informed consent. The NIS database is available at: https://www.hcup-us.ahrq.gov.

Conflict-of-interest statement: All the authors have no conflict of interest to declare.

Data sharing statement: The NIS database is publicly available at: https://www.hcup-us.ahrq.gov/.

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ORIGINAL ARTICLE

Observational Study

Lower alanine aminotransferase levels are associated with increased all-cause and cardiovascular mortality in nonalcoholic fatty liver patients

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Abstract

BACKGROUND

Serum alanine aminotransferase (ALT) levels are often considered a marker to evaluate liver disease and its severity.

AIM

To investigate the association between ALT levels and all-cause and cause-specific mortality in patients with nonalcoholic fatty liver disease (NAFLD).

METHODS

The Third National Health and Nutrition Examination Survey (NHANES-III) from 1988 to 1994 and NHANES-III-related mortality data from 2019 onward were used to obtain the necessary data for the study. NAFLD was defined as hepatic steatosis, as diagnosed by ultrasound, with no other liver diseases. ALT levels were categorized into four groups according to the different recommended upper limits of normal (ULN) in men and women: < 0.5 ULN, 0.5-1 ULN, 1-2 ULN, and ≥ 2 ULN. The hazard ratios for all-cause mortality and cause-specific mortality were analyzed using the Cox proportional hazard model.

RESULTS

Multivariate logistic regression analysis demonstrated that the odds ratio of NAFLD correlated positively with increased serum ALT levels. In patients with NAFLD, all-cause mortality and cardiovascular mortality were the highest when ALT was < 0.5 ULN, yet cancer-related mortality was the highest when ALT was ≥ 2 ULN. The same results could be found in both men and women. Univariate analysis showed that severe NAFLD with normal ALT levels had the highest all-cause and cause-specific mortality, but the difference was not statistically significant after adjustment for age and multivariate factors.

CONCLUSION
The risk of NAFLD was positively correlated with ALT level, but all-cause and cardiovascular mortality were the highest when ALT was < 0.5 ULN. Regardless of the severity of NAFLD, normal or lower ALT levels were associated with higher mortality than elevated ALT levels. Clinicians should be aware that high ALT levels indicate liver injury, but low ALT levels are associated with a higher risk of death.

Key Words: Non-alcoholic fatty liver disease; Alanine aminotransferase; Mortality; NHANES-III

Core Tip: The risk of nonalcoholic fatty liver disease (NAFLD) was positively correlated with alanine aminotransferase (ALT) level, but all-cause and cardiovascular mortality were the highest when ALT < 0.5 upper limits of normal. Regardless of the severity of NAFLD, normal or lower ALT levels are associated with higher mortality than elevated ALT levels. Clinicians should be aware of not only high ALT, indicating liver injury, but also low ALT associated with higher risk of death.

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INTRODUCTION

The number of patients suffering from nonalcoholic fatty liver disease (NAFLD) has reached one billion worldwide, replacing viral hepatitis as the most common chronic liver disease[1-3], as well as increasing the risk of hepatocellular carcinoma[4]. Research has demonstrated that NAFLD is no longer restricted to the liver itself but is also the main manifestation of metabolic syndrome in the liver, which is closely related to obesity, dyslipidemia, hypertension, type 2 diabetes, insulin resistance, and cardiovascular disease[5,6]. The all-cause mortality of patients with NAFLD is significantly increased, and cardiovascular disease, malignant tumors, and end-stage liver disease are the main causes of death in patients with NAFLD[7-9]. Therefore, it is very important to find an effective treatment for NAFLD.

The elevation of alanine aminotransferase (ALT) is a measure of liver disease activity and liver injury severity. Several studies have shown that an increase in ALT levels has a close correlation with an increased risk of NAFLD. It has also been proven to be an independent predictor of NAFLD and is related to nonalcoholic steatohepatitis and advanced liver fibrosis[10-12]. Research has shown that elevated ALT levels are also positively correlated with metabolic syndrome-related diseases, such as cardiovascular disease and type 2 diabetes[13,14]. Based on these previous findings, it could be inferred that all-cause and cardiovascular mortality are correlated with elevated ALT levels. However, this conclusion appears to be inconsistent and controversial[15-17]. Elevated ALT levels are related to mortality from various causes in some studies[18,19], but not in others[20,21]. In contrast, some studies have indicated that normal or lower ALT levels appear to be negatively correlated with a higher risk of all-cause mortality[22,23]. Moreover, the relationship between ALT levels and all-cause and cause-specific mortality in patients with NAFLD has not been fully reported. Therefore, this study focuses on the relationship between serum ALT levels and all-cause mortality and cause-specific mortality in patients with NAFLD in the Third National Health and Nutrition Examination Survey (NHANES-III) database, so as to further explore the clinical significance of using ALT as a potential treatment target and to improve the survival and prognosis of patients with NAFLD.

MATERIALS AND METHODS

Dataset and study population

The NHANES-III produced a national dataset that evaluates the health status of people in the United States, and it was conducted in two phases (1988-1991 and 1991-1994). The NHANES-III conducted interviews, physical examinations, and laboratory tests, and, at the same time, it used ultrasonic examination to evaluate hepatic steatosis, which is the main reason the authors chose this dataset. Data from NHANES-III was also linked to death certificates from the National Death Index (NDI) as of

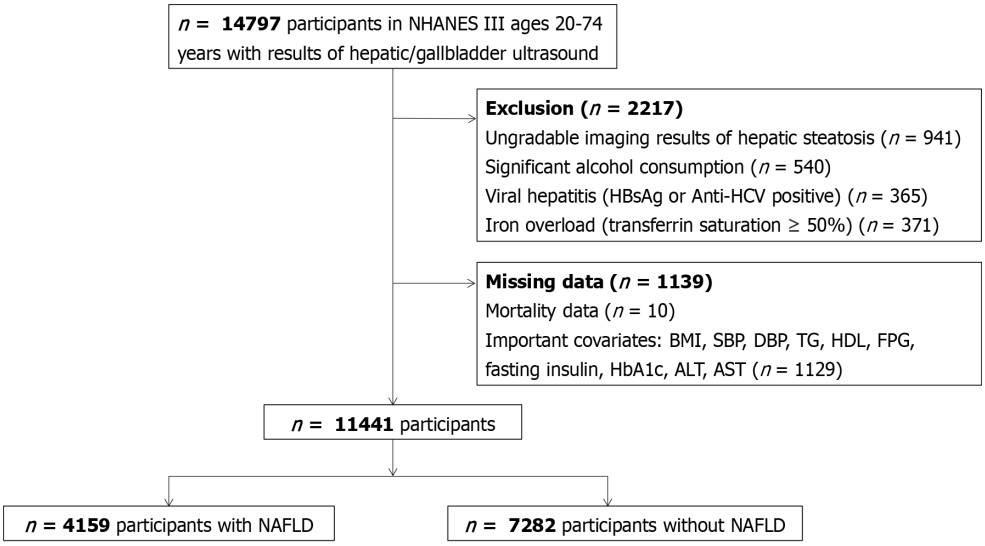

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Figure 1 Flow-chart of the Study. NHANE III: The National Health and Nutrition Examination Survey III (1988-1994); HBsAg: Hepatitis B surface antigen; HCV: Hepatitis C virus; BMI: Body mass index; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; TC: Total cholesterol; TG: Triglyceride; HDL: High-density lipoprotein cholesterol; FPG: Fasting plasma glucose; HbA1c: Glycosylated hemoglobin; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; NAFLD: Nonalcoholic fatty liver disease.

December 31, 2019, allowing for mortality analysis. The NHANES protocol was approved by the Ethics Review Committee of the National Center for Health Statistics, which obtained informed consent from all subjects.

Among the adult participants (aged 20-74 years old) in the NHANES-III survey with liver/gallbladder ultrasound and laboratory test results (n = 14797), individuals without a liver ultrasound steatosis grade and those with significant alcohol consumption (men > 21 drinks/week, women > 14 drinks/week), viral hepatitis (serum hepatitis B surface antigen and/or serum hepatitis C antibody positive), or iron overload (transferring saturation ≥ 50%) (n = 2217) were excluded from this study. Individuals with incomplete mortality information and certain important indicators were also excluded (n = 1139). Finally, this study included a total of 11,441 individuals aged 20-74 years (Figure 1).

Clinical variables

An elevated ALT level was defined as ALT > 30 U/L in males and > 19 U/L in females, and the ALT levels were classified into four groups [< 0.5 upper limits of normal (ULN), 0.5-1 ULN, 1-2 ULN, ≥ 2 ULN]. Race/ethnicity was categorized as non-Hispanic white, non-Hispanic black, Mexican–American, or other. Diabetes was defined as a high fasting blood sugar (> 126 ng/dL), a high glycosylated hemoglobin (HbA1c) (> 6.5%), or a history of diabetes and/or use of diabetic medication. A homeostasis model assessment of insulin resistance > 2.5 was considered as insulin resistant[24]. Hypertension was defined as systolic blood pressure ≥ 140 mmHg or diastolic blood pressure ≥ 90 mmHg and/or treatment with antihypertensive medication. A self-reported questionnaire on the frequency and amount of alcohol consumption was used to identify drinking status[25]. If people answered "no" to having done any of the following activities in the previous month, they were classified as being "sedentary" in terms of sports activities: jogging/running, cycling, swimming, aerobic exercise, other dancing, aerobics, yard work/gardening, weightlifting, or other sports[26].

Definition of NAFLD

The gallbladder ultrasound image files were reviewed by three board-certified clinicians to assess hepatic steatosis. In this study, NAFLD was defined as any degree (mild to severe) of steatosis, according to five criteria, without a competing etiology for secondary liver steatosis.

Mortality

Participants who were aged over 20 years in the NHANES-III were followed up for passive mortality as of December 31, 2019. Probability matching was performed using NDI records to assess the death status (including the date of death) and the cause of death; the potential cause of death 113 code was used to code deaths before 1998, and deaths between 1999 and 2015 were coded according to the Ninth Revision of the International Classification of Diseases. The Centers for Disease Control and Prevention restricted the liver-related mortality data in the NHANES III for public use.

Statistical analysis

All data were analyzed using SPSS 27.0. Weighted analyses were performed using NHANES survey weights[27]. The Student's *t*-test was used to compare continuous variables, and the Rao-Scott chi-square was used to test categorical variables. Multivariate logistic regression was used to confirm an independent relationship between ALT status and NAFLD after adjustment for potential clinical and demographic variables. Cox proportional hazards regression analysis was used to analyze all-cause and cause-specific mortality. A P value < 0.05 was considered to have a significant statistical difference.

RESULTS

Baseline characteristics of patients

Of the 11441 NHANES-III survey participants (mean age, 44.4 years; male, 44.6%) enrolled in this study, the prevalence of NAFLD was 36.4%, among which moderate to severe steatosis accounted for 22.8%. In this cohort, 84.8% of the participants had normal ALT levels, and 15.2% had elevated ALT levels. Table 1 summarizes the baseline characteristics of the overall population and patients with NAFLD. In the whole population, compared with the individuals with normal ALT levels, those with elevated ALT levels were more likely to be young people, women, or Mexican Americans, those who did little physical activity, and those who were diabetic and insulin resistant. In addition, body mass index (BMI), waist circumference, TC, TG, HbA1c, FPG, and AST were higher in these people, and HDL was lower. The prevalence of NAFLD was higher in individuals with elevated ALT levels compared with those with normal ALT levels (59% *vs* 32.3%, P < 0.001). In patients with NAFLD, compared with individuals with normal ALT levels, those with elevated ALT levels were also younger, more likely to be women, Mexican American, diabetic, insulin resistant, and they had higher BMI, waist circumference, TC, TG, and AST levels. The proportion of patients with moderate and severe steatosis was higher (47.7% and 30.1%, respectively). When ALT was classified into different levels (< 0.5 ULN, 0.5-1 ULN, 1-2 ULN, and ≥ 2 ULN), in the univariate model, the incidence of NAFLD increased with the increase in ALT level [odds ratio (OR): 1, 1.65, 3.56, and 6.72 respectively, P < 0.001]. When adjusted for age, this relationship still existed. Multivariate analysis showed that with the increase in ALT level, the risk of NAFLD increased by 37%, 128%, and 217%, respectively (Supplementary Table 1).

ALT levels and mortality in the overall population

The average follow-up time of the 11,441 individuals was 23.8 years. A total of 3,976 people died during the follow-up period, and cardiovascular disease (n = 1104) and cancer (n = 953) were the two leading causes of death. The results of the Cox proportional hazards regression analysis of the overall population are given in Table 2. The univariate analysis showed that all-cause mortality gradually decreased with the increase in ALT level [hazard ratio (HR): 0.76, 0.66, and 0.60, respectively, P < 0.001]. When considering known demographic variables and traditional risk factors, all-cause mortality was still the highest when ALT was at the lowest level (< 0.5 ULN). With regard to NAFLD, in univariate analysis, patients with NAFLD had a 41% higher risk of all-cause death than the patients who did not have NAFLD [HR: 1.41, 95% confidence interval (CI): 1.33-1.51, P < 0.001]. However, NAFLD was no longer correlated with all-cause mortality when other demographics and covariates were controlled.

When mortality was limited to cardiovascular disease (Table 3), the multivariable analysis indicated that elevated ALT levels were correlated with decreased cardiovascular mortality in individuals overall and in patients with NAFLD, but there was no significant statistical difference when ALT was ≥ 2 ULN. Death caused by other diseases, such as respiratory and cerebrovascular diseases, with different ALT levels also showed a similar result. The analysis of cancer-related mortality had a different result; when ALT was ≥ 2 ULN, the risk of cancer-related death in the whole population increased by 11% and, in patients with NAFLD, it increased by 39%, but there was no significant statistical difference.

ALT levels and mortality in patients with NAFLD

Further research was done concerning the patients with NAFLD (Supplementary Table 2 in the ESM). Because the ULN of the ALT level differs in men and women, an analysis was performed according to gender. The baseline characteristics of the enrolled patients with NAFLD are summarized in Supplementary Table 3 in the ESM. A total of 1932 men and 2227 women are included, with an average age of 47.9 and 45.8 years, respectively. The median ALT level was 25 U/L in men and 18 U/L in women. In most patients with NAFLD, ALT levels were within the normal range, of which 0.5-1 ULN was the most common (found in 49.9% of males and 50.9% of females, respectively). All-cause mortality and cause-specific mortality were investigated based on ALT levels in men and women. In male patients with NAFLD, the univariate model showed that all-cause and cancer-related mortality decreased with the increase in ALT level, but the age-adjusted and multivariate models showed that mortality increased when ALT was ≥ 2 ULN. However, the difference was not statistically significant. Regarding death caused by cardiovascular disease and other causes, the three models showed that the risk of death decreased with the increase of ALT level. Among female patients, cancer-related mortality was the

Table 1 Baseline characteristics of the overall population and nonalcoholic fatty liver disease patients with normal or elevated alanine aminotransferase level

	Overall population (*n* = 11441)			NAFLD population (*n* = 4159)		
	Normal ALT level (*n* = 9698)	Elevated ALT level (*n* = 1743)	*P* value	Normal ALT level (*n* = 3131)	Elevated ALT level (*n* = 1028)	*P* value
Age (yr)	44.4 ± 0.17	41.5 ± 0.35	< 0.001	48.0 ± 0.29	43.2 ± 0.45	< 0.001
Sex, male (%)	4397 (45.3)	710 (40.7)	< 0.001	1496 (47.8)	436 (42.4)	0.003
Race/ethnicity, *n* (%)			< 0.001			< 0.001
Non-Hispanic White	3725 (38.4)	527 (30.2)		1186 (37.9)	300 (29.2)	
Non-Hispanic Black	2950 (30.4)	325 (18.6)		861 (27.5)	143 (13.9)	
Mexican American	2620 (27.0)	815 (46.8)		967 (30.9)	540 (52.5)	
Others	403 (4.2)	76 (4.4)		117 (3.7)	45 (4.4)	
Waist circumference (cm)	92.3 ± 0.15	98.3 ± 0.35	< 0.001	97.9 ± 0.30	102.6 ± 0.45	< 0.001
BMI (kg/m^2)	27.0 ± 0.06	29.7 ± 0.15	< 0.001	28.9 ± 0.12	31.4 ± 0.20	< 0.001
Hypertension, *n* (%)	3892 (40.1)	698 (40.0)	0.946	1434 (45.8)	458 (44.6)	0.486
Diabetes, *n* (%)	836 (8.6)	253 (14.5)	< 0.001	476 (15.2)	214 (20.8)	< 0.001
HOMA-IR, *n* (%)	3652 (37.7)	1117 (64.1)	< 0.001	1712 (54.7)	785 (76.4)	< 0.001
Fasting plasma glucose (mmol/L)	5.6 ± 0.02	5.9 ± 0.06	< 0.001	6.0 ± 0.05	6.3 ± 0.09	0.002
HbA1c (%)	5.5 ± 0.01	5.7 ± 0.03	< 0.001	5.8 ± 0.02	5.9 ± 0.05	0.027
TG (mg/dL)	135.2 ± 1.0	182.4 ± 3.2	< 0.001	165.9 ± 2.09	207.0 ± 4.49	< 0.001
Total cholesterol (mg/dL)	204.2 ± 0.44	209.7 ± 1.07	< 0.001	208.6 ± 0.80	212.6 ± 1.37	< 0.001
HDL (mg/dL)	51.3 ± 0.15	47.0 ± 0.36	< 0.001	48.3 ± 0.27	45.0 ± 0.47	< 0.001
ALT (IU/L)	13.7 ± 0.06	39.5 ± 0.63	< 0.001	14.9 ± 0.10	41.7 ± 0.90	< 0.001
AST (IU/L)	19.0 ± 0.06	34.2 ± 0.64	< 0.001	19.5 ± 0.11	35.6 ± 0.92	< 0.001
Albumin (g/L)	41.4 ± 0.04	41.7 ± 0.09	0.007	41.1 ± 0.07	41.8 ± 0.11	< 0.001
Smoked at least 100 cigarettes, *n* (%)	4872 (50.2)	752 (43.3)	< 0.001	1637 (52.3)	448 (43.6)	< 0.001
Sedentary lifestyle, *n* (%)	2811 (29.0)	583 (33.4)	< 0.001	1048 (33.5)	373 (36.3)	0.099
NAFLD, *n* (%)	3131 (32.3)	1028 (59.0)	< 0.001			
Mild	1326 (13.7)	229 (13.1)		1326 (42.4)	229 (22.3)	
Moderate	1268 (13.1)	490 (28.1)		1268 (40.5)	490 (47.7)	
Severe	537 (5.5)	309 (1.7)	< 0.001	537 (17.2)	309 (30.1)	< 0.001

Categorical values are shown as *n* (%). Continuous variables are shown as mean ± SEs. Elevated alanine aminotransferase (ALT) level was defined as ALT > 30 U/L in men or > 19 U/L in women. BMI: Body mass index; HOMA-IR: Homeostasis model assessment of insulin resistance; HbA1c: Glycosylated hemoglobin; TG: Triglyceride; HDL: High-density lipoprotein cholesterol; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; NAFLD: Nonalcoholic fatty liver disease.

highest in the univariate, age-adjusted, and multivariate models when ALT was ≥ 2 ULN, which was similar to the results in the whole population with NAFLD (Tables 4 and 5).

The different models and mortality in patients with NAFLD

The different NAFLD statuses (mild to moderate or severe) were then combined with the different ALT levels (normal or elevated) for further analysis of all-cause and cause-specific mortality. In patients with NAFLD, the univariate and age-adjusted models showed that severe NAFLD with normal ALT levels had the highest all-cause mortality (HR: 1.45, $P < 0.001$ and 1.11, $P = 0.139$), but this relationship no longer existed after multivariable adjustment (HR: 0.98, $P = 0.727$) (Table 6). In terms of cause-specific mortality, Model 1 (mild to moderate NAFLD with normal ALT level) and Model 3 (severe NAFLD with normal ALT level) had a higher risk of death than Model 2 (mild to moderate NAFLD with

Table 2 Association between alanine aminotransferase level or nonalcoholic fatty liver disease status and all-cause mortality in overall population

ALT level	Unadjusted		Age-adjusted		Multivariate-adjusted	
	HR (95%CI)	*P* value	HR (95%CI)	*P* value	HR (95%CI)	*P* value
< 0.5 ULN	1		1		1	
0.5-1 ULN	0.76 (0.71-0.81)	< 0.001	0.74 (0.70-0.80)	< 0.001	0.76 (0.71-0.81)	< 0.001
1-2 ULN	0.66 (0.60-0.74)	< 0.001	0.73 (0.66-0.81)	< 0.001	0.70 (0.63-0.79)	< 0.001
≥ 2 ULN	0.60 (0.49-0.74)	< 0.001	0.92 (0.75-1.14)	0.442	0.74 (0.57-0.97)	0.017
NAFLD						
No NAFLD	1		1		1	
NAFLD	1.41 (1.33-1.50)	< 0.001	1.13 (1.06-1.20)	< 0.001	0.99 (0.92-1.06)	0.758

The multivariate model was adjusted for age, sex, race/ethnicity, body mass index, waist circumference, aspartate aminotransferase, albumin, triglyceride, total cholesterol, high-density lipoprotein cholesterol, smoking status, diabetes, hypertension, and sedentary lifestyle. CI: Confidence interval; HR: Hazard ratio; ULN: Upper limits of normal; ALT: Alanine aminotransferase; NAFLD: Nonalcoholic fatty liver disease.

Table 3 Association of alanine aminotransferase level, cardiovascular disease, cancer-related and others-related mortality stratified by the presence/absence of nonalcoholic fatty liver disease

	Total population		No NAFLD		NAFLD	
	HR (95%CI)	*P* value	HR (95%CI)	*P* value	HR (95%CI)	*P* value
ALT level						
Cardiovascular						
< 0.5 ULN	1		1		1	
0.5-1 ULN	0.74 (0.65-0.85)	< 0.001	0.82 (0.68-0.97)	0.022	0.66 (0.52-0.82)	< 0.001
1-2 ULN	0.63 (0.50-0.79)	< 0.001	0.78 (0.56-1.09)	0.149	0.55 (0.39-0.78)	< 0.001
≥ 2 ULN	0.63 (0.35-1.12)	0.113	0.70 (0.26-1.86)	0.469	0.68 (0.31-1.50)	0.342
Cancer						
< 0.5 ULN	1		1		1	
0.5-1 ULN	0.74 (0.64-0.86)	< 0.001	0.70 (0.58-0.84)	< 0.001	0.85 (0.66-1.11)	0.230
1-2 ULN	0.67 (0.52-0.86)	0.002	0.61 (0.42-0.88)	0.009	0.77 (0.54-1.12)	0.171
≥ 2 ULN	1.11 (0.67-1.86)	0.682	0.86 (0.35-2.11)	0.738	1.39 (0.70-2.75)	0.346
Others						
< 0.5 ULN	1		1		1	
0.5-1 ULN	0.89 (0.80-0.99)	0.029	0.92 (0.81-1.05)	0.227	0.80 (0.67-0.95)	0.011
1-2 ULN	0.98 (0.83-1.16)	0.809	1.25 (0.98-1.60)	0.074	0.73 (0.57-0.93)	0.012
≥ 2 ULN	0.75 (0.51-1.09)	0.131	0.69 (0.33-1.45)	0.331	0.53 (0.32-0.89)	0.015

The multivariate model was adjusted for age, sex, race/ethnicity, body mass index, waist circumference, aspartate aminotransferase, albumin, triglyceride, total cholesterol, high-density lipoprotein cholesterol, smoking status, diabetes, hypertension, and sedentary lifestyle. Others: Chronic lower respiratory diseases, accidents (unintentional injuries), cerebrovascular diseases, Alzheimer's diseases, diabetes mellitus, influenza and pneumonia, nephritis, nephrotic syndrome and nephrosis and all other causes (residual). CI: Confidence interval; HR: Hazard ratio; ULN: Upper limits of normal; ALT: Alanine aminotransferase; NAFLD: Nonalcoholic fatty liver disease.

elevated ALT level) and Model 4 (severe NAFLD with elevated ALT level). Further analysis was made according to gender (Supplementary Tables 4 and 5). Among male patients with NAFLD, those with severe NAFLD and elevated ALT levels had higher cancer-related mortality (HR: 1.18, $P = 0.602$). The risk factors of ALT at the lowest and highest levels were explored (Supplementary Table 6 in the ESM). In male patients, the univariate analysis indicated that the age of those with ALT ≥ 2 ULN was

Table 4 Association of alanine aminotransferase level, all-cause, cardiovascular disease, cancer-related and others-related mortality among nonalcoholic fatty liver disease patients in men

Mortality outcome, ALT level	No. of death, *n* (%)	Unadjusted		Age-adjusted		Multivariate-adjusted	
		HR (95%CI)	*P* value	HR (95%CI)	*P* value	HR (95%CI)	*P* value
All-cause							
< 0.5 ULN	306 (61.1)	1		1		1	
0.5-1 ULN	436 (45.2)	0.60 (0.52-0.70)	< 0.001	0.69 (0.59-0.80)	< 0.001	0.68 (0.58-0.80)	< 0001
1-2 ULN	115 (30.8))	0.37 (0.30-0.46)	< 0.001	0.62 (0.50-0.77)	< 0.001	0.60 (0.47-0.76)	< 0001
≥ 2 ULN	21 (22.6)	0.26 (0.16-0.40)	< 0.001	0.69 (0.44-1.09)	0.11	0.72 (0.42-1.21)	0.214
Cardiovascular disease							
< 0.5 ULN	102 (20.4)	1		1		1	
0.5-1 ULN	119 (22.3)	0.50 (0.39-0.65)	< 0.001	0.58 (0.44-0.76)	< 0.001	0.57 (0.43-0.77)	< 0.001
1-2 ULN	34 (9.1)	0.34 (0.23-0.50)	< 0.001	0.58 (0.39-0.87)	0.007	0.55 (0.33-0.92)	0.022
≥ 2 ULN	3 (3.2)	0.11 (0.04-0.36)	< 0.001	0.33 (0.10-1.05)	0.06	0.51 (0.13-1.97)	0.331
Cancer							
< 0.5 ULN	67 (13.4)	1		1		1	
0.5-1 ULN	103 (10.7)	0.65 (0.48-0.89)	0.006	0.73 (0.54-1.00)	0.051	0.79 (0.57-1.10)	0.169
1-2 ULN	25 (6.7)	0.37 (0.23-0.58)	< 0.001	0.62 (0.39-0.99)	0.047	0.76 (0.45-1.29)	0.308
≥ 2 ULN	5 (5.4)	0.28 (0.11-0.69)	0.006	0.81 (0.32-2.05)	0.654	1.05 (0.35-3.12)	0.935
Others							
< 0.5 ULN	137 (44.8)	1		1		1	
0.5-1 ULN	214 (49.1)	0.81 (0.65-1.00)	0.048	0.81 (0.66-1.01)	0.058	0.73 (0.57-0.92)	0.921
1-2 ULN	56 (48.7)	0.68 (0.50-0.92)	0.014	0.75 (0.55-1.03)	0.073	0.57 (0.38-0.85)	0.851
≥ 2 ULN	13 (61.9)	0.70 (0.39-1.23)	0.212	0.76 (0.42-1.37)	0.361	0.40 (0.19-0.84)	0.844

The multivariate model was adjusted for age, sex, race/ethnicity, body mass index, waist circumference, aspartate aminotransferase, albumin, triglyceride, total cholesterol, high-density lipoprotein cholesterol, smoking status, diabetes, hypertension, and sedentary lifestyle. Others: Chronic lower respiratory diseases, accidents (unintentional injuries), cerebrovascular diseases, Alzheimer's diseases, diabetes mellitus, influenza and pneumonia, nephritis, nephrotic syndrome and nephrosis and all other causes (residual). CI: Confidence interval; HR: Hazard ratio; ULN: Upper limits of normal; ALT: Alanine aminotransferase.

significantly lower than that of those with ALT < 0.5 ULN, with more patients aged 20-39 years and fewer patients aged over 60 years. The multivariable-adjusted analysis also indicated that age was a protective factor in male patients. However, in female patients, no significant statistical difference was seen between the two age groups.

DISCUSSION

The main findings of the present large cohort study were that elevated serum ALT levels were closely related to the increased risk of NAFLD but did not increase the risk of all-cause and cardiovascular mortality in the whole population and in patients with and without NAFLD. On the contrary, in the patients with NAFLD, all-cause and cardiovascular mortality was the highest when ALT was < 0.5 ULN, and this was the same for both sexes and all ages. Cancer-related mortality was the highest when ALT was ≥ 2 ULN, but there was no significant statistical difference. With different degrees of NAFLD (mild to moderate or severe), whether the ALT level was elevated or not seems to have had no significant effect on all-cause and cause-specific mortality.

Previous research has shown that NAFLD is related to a higher risk of all-cause mortality[28]. In addition, the correlation between NAFLD and increased risk of cardiovascular events was proved in a meta-analysis[29]. Cardiovascular disease, malignant tumors, and end-stage liver disease are the main causes of death in patients with NAFLD[7,8], but cardiovascular disease is the primary cause of death [7]. Therefore, it is of great importance to recognize the significant influence of metabolic complications

Table 5 Association of alanine aminotransferase level, all-cause, cardiovascular disease, cancer-related and others-related mortality among nonalcoholic fatty liver disease patients in women

Mortality outcome, ALT level	No. of death, *n* (%)	Unadjusted		Age-adjusted		Multivariate-adjusted	
		HR (95%CI)	*P* value	HR (95%CI)	*P* value	HR (95%CI)	*P* value
All-cause							
< 0.5 ULN	144 (34.0)	1		1		1	
0.5-1 ULN	440 (38.8)	1.16 (0.96-1.40)	0.127	0.88 (0.73-1.06)	0.176	0.91 (0.74-1.10)	0.348
1-2 ULN	187 (36.0)	1.05 (0.85-1.31)	0.632	0.83 (0.67-1.03)	0.095	0.76 (0.60-0.97)	0.027
≥ 2 ULN	46 (30.7)	0.89 (0.64-1.24)	0.484	1.10 (0.79-1.53)	0.585	0.82 (0.52-1.31)	0.416
Cardiovascular disease							
< 0.5 ULN	41 (9.7)	1		1		1	
0.5-1 ULN	119 (10.5)	1.10 (0.77-1.58)	0.594	0.82 (0.58-1.17)	0.282	0.86 (0.59-1.26)	0.44
1-2 ULN	442 (8.1)	0.83 (0.54-1.28)	0.41	0.65 (0.42-1.00)	0.051	0.62 (0.38-1.02)	0.061
≥ 2 ULN	99 (6.0)	0.61 (0.30-1.26)	0.182	0.78 (0.38-1.61)	0.504	0.85 (0.29-2.43)	0.756
Cancer							
< 0.5 ULN	31 (7.3)	1		1		1	
0.5-1 ULN	97 (8.6)	1.18 (0.79-1.77)	0.425	0.93 (0.62-1.40)	0.728	1.02 (0.66-1.57)	934
1-2 ULN	37 (7.1)	0.96 (0.60-1.55)	0.877	0.76 (0.47-1.23)	0.261	0.83 (0.49-1.44)	0.482
≥ 2 ULN	15 (10.0)	1.34 (0.72-2.48)	0.352	1.38 (0.74-2.57)	0.309	1.49 (0.61-3.68)	0.395
Others							
< 0.5 ULN	71 (49.7)	1		1		1	
0.5-1 ULN	224 (50.9)	0.91 (0.70-1.20)	0.526	0.92 (0.71-1.20)	0.549	0.89 (0.67-1.18)	0.411
1-2 ULN	108 (57.8)	1.01 (0.75-1.37)	0.935	1.08 (0.80-1.46)	0.627	0.85 (0.61-1.20)	0.366
≥ 2 ULN	22 (47.8)	0.99 (0.62-1.61)	0.978	1.16 (0.72-1.88)	0.548	0.62 (0.30-1.29)	0.199

The multivariate model was adjusted for age, sex, race/ethnicity, body mass index, waist circumference, aspartate aminotransferase, albumin, triglyceride, total cholesterol, high-density lipoprotein cholesterol, smoking status, diabetes, hypertension, and sedentary lifestyle. Others: Chronic lower respiratory diseases, accidents (unintentional injuries), cerebrovascular diseases, Alzheimer's diseases, diabetes mellitus, influenza and pneumonia, nephritis, nephrotic syndrome and nephrosis and all other causes (residual). CI: Confidence interval; HR: Hazard ratio; ULN: Upper limits of normal; ALT: Alanine aminotransferase.

on NAFLD, especially as seen in patients with NAFLD who died of cardiovascular disease. Since ALT is often considered a marker to evaluate the activity of liver disease and the severity of liver injury in clinical practice, many believe that monitoring ALT levels can be an effective means of following the progression of NAFLD[30,31]. Because of the invasive nature of liver biopsy, clinicians often take ALT levels as the basis of liver protection treatment. The association between elevated ALT levels and increased mortality has been reported in several epidemiological studies[18,32]. It seems to be a reasonable inference that elevated ALT levels may increase the risk of death. However, in different countries and cohorts, the opposite relationship between ALT levels and all-cause mortality has been observed. One study of NHANES-III data found that the relationship between ALT levels and all-cause mortality was similar to a U-shaped curve[22]. Another large cohort study also indicated that ALT at the lowest levels (≤ 10 U/L) and elevated ALT levels (> 40 U/L) were associated with increased all-cause mortality, especially among people aged over 60 years[33]. Two recent studies also showed that lower ALT levels within the normal range were related to a higher risk of all-cause and cardiovascular mortality in the elderly population[34,35]. These findings consistently indicate that there is a certain non-linear correlation between ALT levels and all-cause mortality although some unmeasured or residual confounding cannot be excluded.

The results of the current study showed that in patients with NAFLD, the ALT levels of the vast majority, both male and female, are within the normal range, and the number of patients with an ALT level in the range of 0.5-1 ULN is the largest, while the number in the range ≥ 2 ULN is the smallest. In addition, nearly half of the male patients with NAFLD with an ALT level < 0.5 ULN were elderly, while nearly 3/4 of those with an ALT level ≥ 2 ULN were young. Another study also showed that the ALT level first increased and then decreased with age[36], which may explain why in patients with NAFLD

Table 6 Association of different models, all-cause, cardiovascular disease, cancer-related and others-related mortality among nonalcoholic fatty liver disease patients

Mortality outcome	No. of death, *n* (%)	Unadjusted		Age-adjusted		Multivariate-adjusted	
		HR (95%CI)	*P* value	HR (95%CI)	*P* value	HR (95%CI)	*P* value
All-cause							
Model 1	1055 (41.9)	1		1		1	
Model 2	248 (31.2)	0.68 (0.59-0.78)	< 0.001	0.89 (0.77-1.02)	0.1	0.87 (0.74-1.01)	0.067
Model 3	271 (53.7)	1.45 (1.26-1.65)	< 0.001	1.11 (0.97-1.27)	0.139	0.98 (0.85-1.12)	0.727
Model 4	121 (35.5)	0.82 (0.68-0.99)	0.037	0.91 (0.75-1.10)	0.312	0.81 (0.66-0.99)	0.042
Cardiovascular disease							
Model 1	309 (12.3)	1		1		1	
Model 2	68 (8.6)	0.64 (0.49-0.83)	< 0.001	0.86 (0.66-1.12)	0.254	0.89 (0.65-1.20)	0.437
Model 3	72 (14.3)	1.30 (1.01-1.68)	0.045	0.99 (0.76-1.27)	0.907	0.82 (0.63-1.07)	0.146
Model 4	20 (5.9)	0.46 (0.30-0.73)	< 0.001	0.52 (0.33-0.82)	0.005	0.53 (0.33-0.87)	0.011
Cancer							
Model 1	241 (9.6)	1		1		1	
Model 2	50 (6.3)	0.60 (0.44-0.81)	0.001	0.74 (0.55-1.01)	0.055	0.86 (0.62-1.21)	0.387
Model 3	57 (11.3)	1.31 (0.98-1.75)	0.067	1.02 (0.76-1.36)	0.904	1.01 (0.75-1.37)	0.928
Model 4	32 (9.4)	0.94 (0.65-1.36)	0.743	1.02 (0.70-1.47)	0.938	1.07 (0.71-1.61)	0.755
Others							
Model 1	504 (47.8)	1		1		1	
Model 2	130 (53.4)	0.94 (0.77-1.13)	0.492	1.03 (0.84-1.25)	0.799	0.93 (0.74-1.16)	0.506
Model 3	142 (53.4)	1.28 (1.06-1.55)	0.009	1.25 (1.04-1.51)	0.019	1.15 (0.95-1.40)	0.159
Model 4	69 (57.0)	1.09 (0.85-1.41)	0.483	1.14 (0.89-1.47)	0.312	0.89 (0.67-1.20)	0.453

Model 1: Mild to moderate nonalcoholic fatty liver disease (NAFLD) with normal alanine aminotransferase (ALT) level; Model 2: Mild to moderate NAFLD with elevated ALT Level; Model 3: Severe NAFLD with normal ALT level; Model 4: Severe NAFLD with elevated ALT Level. The multivariate model was adjusted for age, sex, race/ethnicity, body mass index, waist circumference, aspartate aminotransferase, albumin, triglyceride, total cholesterol, high-density lipoprotein cholesterol, smoking status, diabetes, hypertension, and sedentary lifestyle. Others: Chronic lower respiratory diseases, accidents (unintentional injuries), cerebrovascular diseases, Alzheimer's diseases, diabetes mellitus, influenza and pneumonia, nephritis, nephrotic syndrome and nephrosis and all other causes (residual). CI: Confidence interval; HR: Hazard ratio.

the mortality rate is the highest when ALT is < 0.5 ULN. That is to say, during the follow-up period of over 31 years, individuals with an ALT level < 0.5 ULN at baseline were mostly elderly, so the rate of all-cause and cause-specific death outcomes was higher. However, most of the patients with ALT level ≥ 2 ULN at baseline were young people, so the rate of endpoint events was low. However, in the age-adjusted model and multivariable model, the results were still approximately the same, which shows that other factors besides age may play a more important role, and this possibility needs further study.

Three mechanisms might explain the underlying association between ALT levels and death. First, as an enzyme, ALT plays a vital role in converting L-alanine and α- ketoglutarate to pyruvate and glutamic acid in the heart, liver, kidney, skeletal muscle, and brain, and a low ALT level increases the risk of death by reducing the catalytic capacity for the vital metabolic steps of amino acid metabolism and gluconeogenesis[37-40]. Second, a low ALT level indicates a vitamin B6 deficiency, and epidemiological evidence indicates that a deficiency of vitamin B6 can increase the risk of cardiovascular disease, immune dysfunction, depression, and neurocognitive impairment[41,42]. Finally, other unmeasured confounding factors, rather than vitamin B6, may lead to death.

It was also found that the BMI of male and female patients with NAFLD with ALT levels < 0.5 ULN were 26.8 kg/m^2 and 27.8 kg/m^2, respectively. Recent epidemiological studies have shown that mild obesity (BMI: 23-29 kg/m^2) has a protective effect on overall mortality[43,44]. However, the underlying mechanism is unclear and needs further research.

Some limitations exist to the current study. First, it is a cross-sectional observation of the relationship between ALT levels and NAFLD, so the time correlation cannot be determined. Second, the diagnosis of NAFLD was carried out by hepatic ultrasound, which does not distinguish fat accumulation of less than

30%[45]. However, the advantages of ultrasound include safety, repeatability, low cost, high sensitivity, and specificity[46]. Therefore, ultrasound is considered a first-line imaging technology in epidemiological research and clinical practice[47]. Third, despite the adjustments of multiple covariates affecting all-cause and cause-specific mortality, some unmeasured or residual confounding factors may still exist. It was also not possible to extract specific information about liver-related mortality (due to NHANES), and obtaining such data could provide more clarity. Finally, since the participants in NHANES-III represent the population from 1988 to 1994, there may have been a much lower prevalence of diabetes and NAFLD than would be expected today.

However, this study also has its strengths. This is the first study focusing on ALT levels and all-cause and cause-specific mortality in patients with NAFLD. In a large cohort of the United States population, there are rich demographic and metabolic variables, with a median follow-up period of 23.8 years. Unlike other NHANES cycles that lack liver ultrasound results, NHANES-III includes liver steatosis diagnosed by ultrasound, which makes the diagnosis of NAFLD more accurate than that using non-invasive laboratory markers.

CONCLUSION

In conclusion, the present study's findings confirmed that all-cause and cardiovascular mortality in patients with NAFLD was the highest when ALT was < 0.5 ULN, and, regardless of the severity of the NAFLD, normal or lower ALT levels are associated with higher mortality than elevated ALT levels. This knowledge may be of importance to clinicians who, because of the invasive nature of liver perforation, often use ALT levels as the basis for liver protection treatment. According to the results of this study, in terms of treatment, it may not be beneficial to reduce ALT levels as low as possible. The findings suggest that, on the contrary, clinicians should be more vigilant of patients with NAFLD who have ALT levels < 0.5 ULN.

ARTICLE HIGHLIGHTS

Research background

Serum alanine aminotransferase (ALT) levels are greatly important in the liver disease but the role ALT levels play in the nonalcoholic fatty liver disease (NAFLD) is not clear.

Research motivation

This study aimed to investigate the association between ALT levels and all-cause and cause-specific mortality in patients with NAFLD.

Research objectives

To give the clinicians a hint about the patients with NAFLD who have lower ALT levels.

Research methods

The Third National Health and Nutrition Examination Survey (NHANES-III) from 1988 to 1994 and NHANES-III-related mortality data from 2019 onward were used to obtain the necessary data for the study. NAFLD was defined as hepatic steatosis, as diagnosed by ultrasound, with no other liver diseases. ALT levels were categorized into four groups according to the different recommended upper limits of normal (ULN) in men and women: < 0.5 ULN, 0.5-1 ULN, 1-2 ULN, and ≥ 2 ULN. The hazard ratios for all-cause mortality and cause-specific mortality were analyzed using the Cox proportional hazard model.

Research results

In patients with NAFLD, all-cause mortality and cardiovascular mortality were the highest when ALT was < 0.5 ULN, yet cancer-related mortality was the highest when ALT was ≥ 2 ULN. The same results could be found in both men and women. Univariate analysis showed that severe NAFLD with normal ALT levels had the highest all-cause and cause-specific mortality, but the difference was not statistically significant after adjustment for age and multivariate factors, both the underlying mechanism is unclear and needs further research.

Research conclusions

The risk of NAFLD was positively correlated with ALT level, but all-cause and cardiovascular mortality were the highest when ALT was < 0.5 ULN. Regardless of the severity of NAFLD, normal or lower ALT levels were associated with higher mortality than elevated ALT levels. Clinicians should be aware that high ALT levels indicate liver injury, but low ALT levels are associated with a higher risk of death.

Research perspectives

The underlying mechanism about the lower ALT levels and high mortality death is unclear and needs further research.

FOOTNOTES

Author contributions: Feng B designed the study; Zheng JR and Wang ZL contributed to the acquisition, analysis and interpretation of data and drafted the manuscript; Jiang SZ contributed to the critical revision of the manuscript for important intellectual content and Chen HS contributed to the study supervision; all authors have made a significant contribution to this study and have approved the final manuscript.

Institutional review board statement: The NHANES protocol was approved by the Ethics Review Committee of the National Center for Health Statistics, which obtained informed consent from all subjects.

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.

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ORIGINAL ARTICLE

Randomized Clinical Trial

Randomized intervention and outpatient follow-up lowers 30-d readmissions for patients with hepatic encephalopathy, decompensated cirrhosis

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Abstract

BACKGROUND

We previously reported national 30-d readmission rates of 27% in patients with decompensated cirrhosis (DC).

AIM

To study prospective interventions to reduce early readmissions in DC at our tertiary center.

METHODS

Adults with DC admitted July 2019 to December 2020 were enrolled and randomized into the intervention (INT) or standard of care (SOC) arms. Weekly phone calls for a month were completed. In the INT arm, case managers ensured outpatient follow-up, paracentesis, and medication compliance. Thirty-day

readmission rates and reasons were compared.

RESULTS

Calculated sample size was not achieved due to coronavirus disease 2019; 240 patients were randomized into INT and SOC arms. 30-d readmission rate was 33.75%, 35.83% in the INT *vs* 31.67% in the SOC arm (P = 0.59). The top reason for 30-d readmission was hepatic encephalopathy (HE, 32.10%). There was a lower rate of 30-d readmissions for HE in the INT (21%) *vs* SOC arm (45%, P = 0.03). There were fewer 30-d readmissions in patients who attended early outpatient follow-up (n = 17, 23.61% *vs* n = 55, 76.39%, P = 0.04).

CONCLUSION

Our 30-d readmission rate was higher than the national rate but reduced by interventions in patients with DC with HE and early outpatient follow-up. Development of interventions to reduce early readmission in patients with DC is needed.

Key Words: Decompensated cirrhosis; Hospital readmissions; Interventions

Core Tip: Our 30-d readmission rate was higher than the national rate but reduced by interventions in patients with decompensated cirrhosis (DC) with hepatic encephalopathy and early outpatient follow-up. Development of interventions to reduce early readmission in patients with DC is needed.

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INTRODUCTION

Cirrhosis affects approximately 5 million annually[1] and has been reported to be the 8th leading cause of death with more than 40000 deaths annually in the United States[2]. A study on the burden of gastrointestinal (GI), liver, and pancreatic diseases in the United States revealed that liver diseases had the highest mortality at 3.1%[3]. In addition to high mortality, cirrhosis is also associated with high morbidity. The sequelae of decompensated cirrhosis (DC) are often managed during hospital admissions and include volume overload in the form of ascites, edema or hepatic hydrothorax, portal hypertension leading to bleeding esophageal or gastric varices, as well as hepatic encephalopathy (HE), hyponatremia, acute kidney injury (AKI), and spontaneous bacterial peritonitis (SBP)[4].

Several studies have demonstrated hospital readmissions in DC place a large financial burden on the United State healthcare system. The 30-d readmission rate has been reported to be 20%-37%[5-14]. We have recently published on early readmission rates up to 27% in patients with DC and developed the Mumtaz readmission risk score based on United States data[15]. We also reported that nearly one-third of patients with HE were readmitted within 30 d, and early readmission adversely impacted healthcare utilization and calendar-year mortality[16].

Interventions to reduce readmissions have been shown to be safe and effective. For instance, Morales *et al*[17] developed a program including a hepatologist follow-up exam within 7 d after discharge. This program resulted in a reduction in 30-d readmissions, 60-d mortality, emergency department visits and associated costs[17]. Similarly, another group demonstrated that follow-up with a "care management check-up" as opposed to "standard outpatient care" reduced 30-d readmission, 12-mo mortality and saved 1500 euros per patient month of life[18].

There is a paucity of prospective studies on interventions to reduce early readmission rates in patients with DC. Therefore, we prospectively studied 30-d readmission rates in patients with DC and compared various interventions (INT) with standard of care (SOC) to reduce early readmission rates. We hypothesized that DC patients in the INT arm would have decreased 30-d readmission *vs* the SOC arm.

MATERIALS AND METHODS

This study was conducted at the Ohio State University Wexner Medical Center (OSUWMC), Columbus, Ohio from July 2019 to December 2020. Our study was approved by OSUWMC Institutional Review Board. All aspects of the studying involving human participants including informed consent for enrollment were in accordance with the ethical standards of our Institutional Review Board and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Screening

All patients admitted with DC to the hepatology (inpatient or consult) service were screened for enrollment. Patients meeting inclusion criteria were approached for study consent. Of note, due to the global coronavirus disease 2019 (COVID-19) pandemic, beginning March 2020, only COVID negative patients were approached for informed consent. Elective readmissions for inpatient procedures including endoscopy, trans-arterial chemoembolization, transjugular intrahepatic portosystemic shunt (TIPS), paracentesis or readmissions unrelated to DC such as motor vehicle accidents were excluded.

Randomization and data collection

Study data were collected and managed using research electronic data capture (REDCap) hosted at The Ohio State University Wexner Medical Center[19,20]. Informed consent was obtained from all individual participants included in the study. Consented patients were randomly assigned to either the INT arm or the SOC arm in a 1:1 ratio using the REDCap randomization tool. The following data were collected on all patients *via* REDCap software including demographics (age, sex, insurance type, income based on the zip code), hospitalization data [date of index admission defined as initial admission during which patient consented for study, reason for admission, length of stay (LOS) defined as difference in days between index admission date and index admission discharge date, discharge disposition, associated cost of care of admission as obtained through medical record billing tab], etiology of cirrhosis (alcoholic and non-alcoholic including viral, non-alcoholic fatty liver disease, autoimmune, primary biliary cirrhosis, primary sclerosing cholangitis or cryptogenic), complications of cirrhosis (HE, AKI, ascites, variceal bleeding, SBP, hepatorenal syndrome, coagulopathy, portal hypertension, hepato-pulmonary syndrome, hepatocellular carcinoma), and procedures performed during admission [esophago-gastro-duodenoscopy, colonoscopy or flexible sigmoidoscopy, paracentesis, TIPS and hemodialysis (HD) on admission and discharge]. We also collected data including Elixhauser comorbidity index, discharge medications, and laboratory data (complete blood counts, serum creatinine, liver function tests including total bilirubin, INR, and sodium). Child Turcotte Pugh (CTP) and Sodium-model for end stage liver disease (MELD-Na) score were calculated from the data. The nurse case manager (CM) also recorded labs & medications at readmission and discharge and associated cost of readmission. Status of early readmission, liver transplantation, and mortality at one year were also collected.

Follow-up

The CM phoned each patient enrolled in either arm weekly for 30 d after index discharge to find out if the patient has been readmitted to OSUWMC or another hospital. In the INT arm, during the call CM also ensured i) early (defined as within 30 d from index admission discharge) outpatient hepatology follow-up ii) compliance of medication, iii) arrangement of outpatient paracentesis if needed, and reviewed outpatient hepatology clinic follow-up records. SOC arm as per our center's protocol had to be taken care of by the primary inpatient team. This included arranging early outpatient clinic follow-up, providing list of medications, and advice for outpatient paracentesis if needed at the time of discharge. Due to the nature of intervention, the study could not be blinded.

Definition of outcomes

Early readmission was defined as admission within 30 d of index admission discharge. Reasons for readmission were gathered by CM by reviewing the electronic medical record (EMR) of all enrolled patients readmitted at OSUWMC or outside hospital within 30 d of index admission. Predictors of early readmission were also compared in the two arms.

Sample size

Based on the sample size calculation, target of recruitment for the study was 848 patients, admitted to the hospital with DC under the hepatology (inpatient and consult) services. Patients were randomly assigned in a 1:1 ratio into INT or SOC arms. Based on our previous study using the National Readmissions Administrative Database, we expected a 30-d readmission rate of 27% among patients meeting inclusion criteria, which yield 114/424 patients with 30-d readmission events, thus meeting the target sample size. Based on this calculation, a total sample size of 848 (424 per group) provided 80% power to detect a 30% decrease in 30-d readmission rate (from 27% to 19%) with a type I error rate of 0.05. However, planned sample size could not be achieved due to the COVID-19 pandemic related restriction started in our center in March 2020. Therefore, we end up with available sample size of a total

of 240 patients. The modified consort flow diagram for enrollment in our study trial is illustrated in Figure 1.

Statistical analysis

Means of continuous response variables between two groups were compared using robust t-test (Welch test). Proportions were compared using χ-test or Fisher's exact test as applicable. Logarithmic transformation was used for comparing the LOS and admission cost across groups. Level of significance was kept at 0.05 for each comparison. JMP Version 15 (SAS Institute, NC) was used for all the analyses.

RESULTS

Initial screening data

From July 1, 2019, to December 1, 2020, 1392 patients were screened. Due to the COVID-19 pandemic, recruitment was held from March 2020 to July 2020 and subsequently resumed until December 2020. Out of the patients screened, only 499 (35.85%) were eligible for inclusion; however, 240 patients consented and randomized: 120 each into the INT and SOC arm (Figure 1).

Patient demographics and clinical characteristics

The mean age of patients was 56.34 ± 11.19 years, majority were males (135, 56.25%), belonged to White race (n = 202, 84.17%) and non-Hispanic or Latino ethnicity (n = 227, 94.58%). Almost two-thirds of the patients had public insurance (n = 76, 31.67% on Medicare and n = 70, 29.17% on Medicaid); 73 (30.42%) had private insurance. At admission, the mean MELD-Na score and mean CTP Score were 21.89 ± 8.03 and 9.36 ± 1.96, respectively. Major etiology of cirrhosis was alcohol (n = 121, 50.42%) followed by non-alcoholic fatty liver disease (n = 79, 32.92%) and viral hepatitis (n = 43, 17.92%). Furthermore, 116 (48.33%) patients were actively under evaluation for liver transplantation.

Characteristics of index admissions

The index admission mean LOS was 8.13 ± 5.83 d (median 6, range 1-43 d). The mean cost of index admission was $60595 ± $47174 ($n$ = 225, median $42932, range $1630-251991). The top five reasons for index admission included volume overload (n = 111, 46.25%), AKI (n = 65, 27.08%), hepatic encephalopathy (n = 45, 18.75%), variceal bleed (n = 42, 17.50%), lower GI bleed (n = 19, 7.92%) and hyponatremia (n = 16, 6.67%). The top five interventions performed were esophago-gastro-duodenoscopy (n = 136, 56.67%), paracentesis (n = 115, 47.92%), colonoscopy/flexible sigmoidoscopy (n = 24, 10 %), HD (n = 15, 6.25%) and TIPS (n = 10, 4.17%). Most patients were discharged from index admission to home (n = 159, 66.25%) followed by home with health care (n = 42, 17.50%) and skilled nursing facility (n = 32, 13.33 %, Table 1).

Characteristics and reasons for early readmissions

Overall, 81 (33.75%) patients were readmitted within 30 d of discharge. The major reasons for first readmission included hepatic encephalopathy (n = 26, 32.10%) followed by volume overload (n = 22, 27.16%), AKI (n = 16, 19.75%), variceal bleed (n = 12, 14.82%) and hyponatremia (n = 10, 12.35%). 14 patients were readmitted twice, 3 admitted thrice and one admitted 5 times within 30 d. The mean time to first readmission was 12.65 ± 7.55 d (median 12 d, range 1-30 d). The mean LOS of first readmission was 8.11 ± 8.98 days. The mean cost of stay of first readmission was $55548.29 ± $65164.91 (Table 2). Those readmitted had a higher MELD-Na score on index admission (23.54 ± 7.80 *vs* 21.05 ± 8.03, P = 0.02) and index discharge (21.67 ± 7.95 *vs* 19.39 ± 6.89, P = 0.03) than those not readmitted. Similarly, those readmitted had a higher index admission creatinine (1.80 ± 1.53 *vs* 1.39 ± 1.16, P = 0.03), index discharge creatinine (1.61 ± 1.34 *vs* 1.20 ± 0.97, P = 0.02), and higher index admission INR (1.80 ± 0.64 *vs* 1.63 ± 0.50, P = 0.05) than those not readmitted.

Comparison of demographics and clinical characteristics in two intervention arms

Demographics including age, race, ethnicity, income, and insurance were comparable in two groups, as well as etiology of cirrhosis, MELD-Na score, CTP score, status of evaluation for liver transplant. There were majority females in the INT arm (60/120, 50% *vs* 45/120, 32.50%) and males in SOC arm (75/120, 62.50% *vs* 60/120, 50%, P = 0.03, Table 3). Index admission characteristics, disposition and index admission were also comparative in two arms (Tables 4 and 5).

Comparison of reasons of 1st readmission and outcomes in the INT vs SOC arm

There was no difference in the readmission rates for patients in the INT (n = 4, 35.83%) *vs* SOC arm (n = 38, 31.67%, P = 0.59, Table 6). Other outcomes including number of readmissions within 30 d (P = 0.65), index admission cost (P = 0.49), index admission LOS (P = 0.63), 1st readmission LOS (P = 0.58), all readmissions' LOS (P = 0.82) and waiting time for 1st readmission (P = 0.06) were comparable in two arms.

Table 1 Characteristic features of index admission by readmission status, *n* (%)

	Total	Not readmitted (*n* = 159)	Readmitted (*n* = 81)	*P* value
Index admission characteristics				
Reasons for admission[1]				
Acute kidney injury	65, 27.08	41, 25.79	24, 29.63	0.54
Hyponatremia	16, 6.67	11, 6.92	5, 6.17	1.00
Hepatic encephalopathy	45, 18.75	26, 16.35	19, 23.46	0.22
Volume overload	111, 46.25	81, 50.94	30, 37.04	0.06
Variceal bleed	42, 17.50	31, 19.50	11, 13.58	0.29
Lower GI bleed	19, 7.92	11, 6.92	8, 9.88	0.45
SBP	21, 8.75	14, 8.81	7, 8.64	1.00
Complications of cirrhosis during admission[1]				
Presence of AKI	80, 33.33	50, 31.45	30, 37.04	0.39
HE	49, 20.42	31, 19.50	18, 22.22	0.62
Ascites	139, 57.92	95, 59.75	44, 54.32	0.49
Variceal bleeding	37, 15.42	26, 16.35	11, 13.58	0.71
SBP	16, 6.67	12, 7.55	4, 4.94	0.59
HRS	14, 5.83	8, 5.03	6, 7.41	0.56
Coagulopathy	56, 23.33	36, 22.64	20, 24.69	0.75
Portal hypertension	46, 19.17	34, 21.38	12, 14.81	0.30
HPS	15, 6.25	8, 5.03	7, 8.64	0.27
HCC	11, 4.58	6, 3.77	5, 6.17	0.51
Procedures performed during admission[1]				
EGD	136, 56.67	92, 57.86	44, 54.32	0.68
Paracentesis	115, 47.92	73, 45.91	42, 51.85	0.41
Emergent TIPS	10, 4.17	9, 5.66	1, 1.23	0.17
HD	15, 6.25	7, 4.40	8, 9.88	0.16
Colonoscopy/flex sig	24, 10.00	18, 11.32	6, 7.41	0.37
Disposition[1]				
Home	159, 66.25	107, 67.30	52, 64.20	0.66
Home with Home Health Newly Arranged	39, 16.25	24, 15.09	15, 18.52	
Home with Home Health Previously Arranged	3, 1.25	2, 1.26	1, 1.23	
SNF newly Arranged	21, 8.75	16, 10.06	5, 6.17	
SNF Previously Arranged	11, 4.58	5, 3.14	6, 7.41	
Left Against Medical Advice	2, 0.83	1, 0.63	1, 1.23	
Transfer (long term acute care hospital)	3, 1.25	2, 1.26	1, 1.23	
Homeless	2, 0.83	2, 1.26	0, 0.00	

[1]Patient can have more than one of variable listed.

SBP: Spontaneous bacterial peritonitis; AKI: Acute kidney injury; HE: Hepatic encephalopathy; HRS: Hepatorenal syndrome; HPS: Hepato-pulmonary syndrome; HCC: Hepatocellular carcinoma; EGD: Esophago-gastro-duodenoscopy; GI: Gastrointestinal; TIPS: Transjugular intrahepatic portosystemic shunt; HD: Hemodialysis; SNF: Skilled Nursing Facility.

Table 2 Characteristics and reasons for readmission

Readmission status	*n*	%
No	159	66.25
Yes	81	33.75
Number of readmissions within 30 d		
0	159	66.25
1	63	26.25
2	14	5.83
3	3	1.25
5	1	0.42
Location of 1st readmission		
OSUWMC	59	72.84
Outside hospital	22	27.16
Reason for 1st readmission[1]		
Hepatic encephalopathy	26	32.10
Volume overload	22	27.16
Acute kidney injury	16	19.75
Variceal bleed	12	14.82
Hyponatremia	10	12.35
Lower GI bleed	4	4.94
Spontaneous Bacterial Peritonitis	3	3.70
LOS of first readmission (*n* = 81, mean ± SD), median = 5, range = 1 to 69	8.11 ± 8.98	
LOS of all readmissions (*n* = 105, mean ± SD), median = 4, range = 0 to 124	9.03 ± 14.42	
Cost of first readmission (*n* = 45, mean ± SD), median= $31848.95, range $765-325656.38	$55548.29 ± 65164.91	
Waiting time for first readmission (*n* = 81, mean ± SD), median = 12, range = 1-30	12.65 ± 7.55	

[1]Patient can have more than one of variable listed.
OSUWMC: The Ohio State University Wexner Medical Center; GI: Gastrointestinal; LOS: Length of stay.

Statistically significant differences were noticed in INT arm in location of 1st readmission (*n* = 36, 83.72% at OSU as compared to *n* = 23, 60.5% outside hospital, *P* = 0.03), and lesser 1st readmission with HE in the INT arm (*n* = 9, 20.9%) *vs* SOC (*n* = 17, 44.7%, *P* = 0.03). Finally, contingency analysis of readmission data showed fewer readmissions in patients who attended outpatient follow-up within 30 days of discharge from index admission (*n* = 17, 23.61% *vs n* = 55, 76.39%, *P* = 0.04).

At the end of our study, 47 (19.58%) patients received a liver transplant and 62 (25.83%) died; among those who died, 5 patients were post-transplant and 22 died in hospice. Due to the COVID-19 pandemic we were unable to achieve the anticipated sample size. Therefore, multivariate analysis was not performed.

DISCUSSION

This prospective randomized study investigated early readmission rates and healthcare utilization in patients with DC. Our readmission rate of 33.75% is higher than the United States national average (27%). While our nurse CM interventions did not reduce told readmissions, we found that HE was the top reason for readmission and such interventions were helpful in reducing early readmissions in patients with HE. This is an important lesson learned given increased burden of HE on hospitalizations, falls, mortality, impaired quality of life and caregiver burden[21]. In the validation of readmission using the liver-renal-risk score or "LIRER score", Freitas *et al*[22] showed that HE was not only a predictor of

Table 3 Comparison of patient demographics and clinical characteristics by randomization arm, *n* (%)

	Intervention (*n* = 120)	Standard of care (*n* = 120)	*P* value
Patient demographics			
Age (mean ± SD)	56.54 ± 11.21	56.14 ± 11.21	0.78
Age group			
65+	32, 26.67	28, 23.33	0.79
40-64	75, 62.50	80, 66.67	
18-39	13, 10.83	12, 10.00	
Gender			
Male	60, 50.00	75, 62.50	0.03
Female	60, 50.00	45, 32.50	
Race			
White	105, 87.50	97, 80.83	0.22
Other	15, 12.50	23, 19.17	
Ethnicity			
Not Hispanic or latino	113, 94.17	114, 95.00	0.81
Hispanic or latino	3, 2.50	1, 0.83	
Unknown / Not reported	4, 3.33	5, 4.17	
Zip code income (mean ± SD)	$68045 ± $21370	$68455 ± $21651	0.88
Employment status			
Unemployed	33, 27.50	30, 25.00	0.78
Disabled	24, 20.00	24, 20.00	
Retired	26, 21.67	30, 20.00	
Employed, part time	5, 4.17	3, 2.50	
Employed, full time	23, 19.17	28, 23.33	
Other / Unknown	9, 7.50	14, 11.67	
Insurance type			
Self-pay	4, 3.33	3, 2.50	0.54
No Charge / Other / Unknown	7, 5.83	7, 5.83	
Private insurance	38, 31.67	35, 29.17	
Medicare	32, 26.67	44, 36.67	
Medicaid	39, 32.50	31, 25.83	
Number of admissions at OSU for DC in last 1 year (mean ± SD)	1.99 ± 1.61	1.84 ± 1.48	0.45
MELD-Na score admit (mean ± SD)	21.32 ± 8.19	22.47 ± 7.85	0.27
MELD-Na score discharge (mean ± SD, *n* = 117+118)	20.07 ± 7.74	20.25 ± 6.93	0.84
CTP score admit (mean ± SD)	9.31 ± 2.02	9.41 ± 1.89	0.69
CTP score discharge (mean ± SD)	8.44 ± 1.86	8.73 ± 1.89	0.24
Etiology of cirrhosis (Index admission[1])			
Alcoholic	61, 50.83	60, 50.00	1.00
Non-alcoholic fatty liver	42, 35.00	37, 30.83	0.58
Viral	21, 17.50	22, 18.33	1.00
Hep B	1, 4.76	3, 13.64	0.80
Hep C	19, 90.48	18, 81.82	

Hep B and C	1, 4.76	1, 4.55	
Cryptogenic	6, 5.00	7, 5.83	1.00
Autoimmune	1, 0.83	1, 0.83	1.00
Primary sclerosing cholangitis	2, 1.67	2, 1.67	1.00
Hemochromatosis	0, 0.0	3, 2.5	0.25
Alpha 1 anti-trypsin deficiency	3, 2.5	0, 0.0	0.25
Under evaluation for liver transplant			
No	45, 37.50	61, 50.83	0.08
Yes	63, 52.50	53, 44.17	
Unknown	12, 10.00	6, 5.00	

[1]Patient can have more than one of variable listed.

OSUWMC: The Ohio State University Wexner Medical Center; DC: Decompensated cirrhosis; MELD-Na: Model of End Stage Liver Disease Score; CTP: Child Pugh Score.

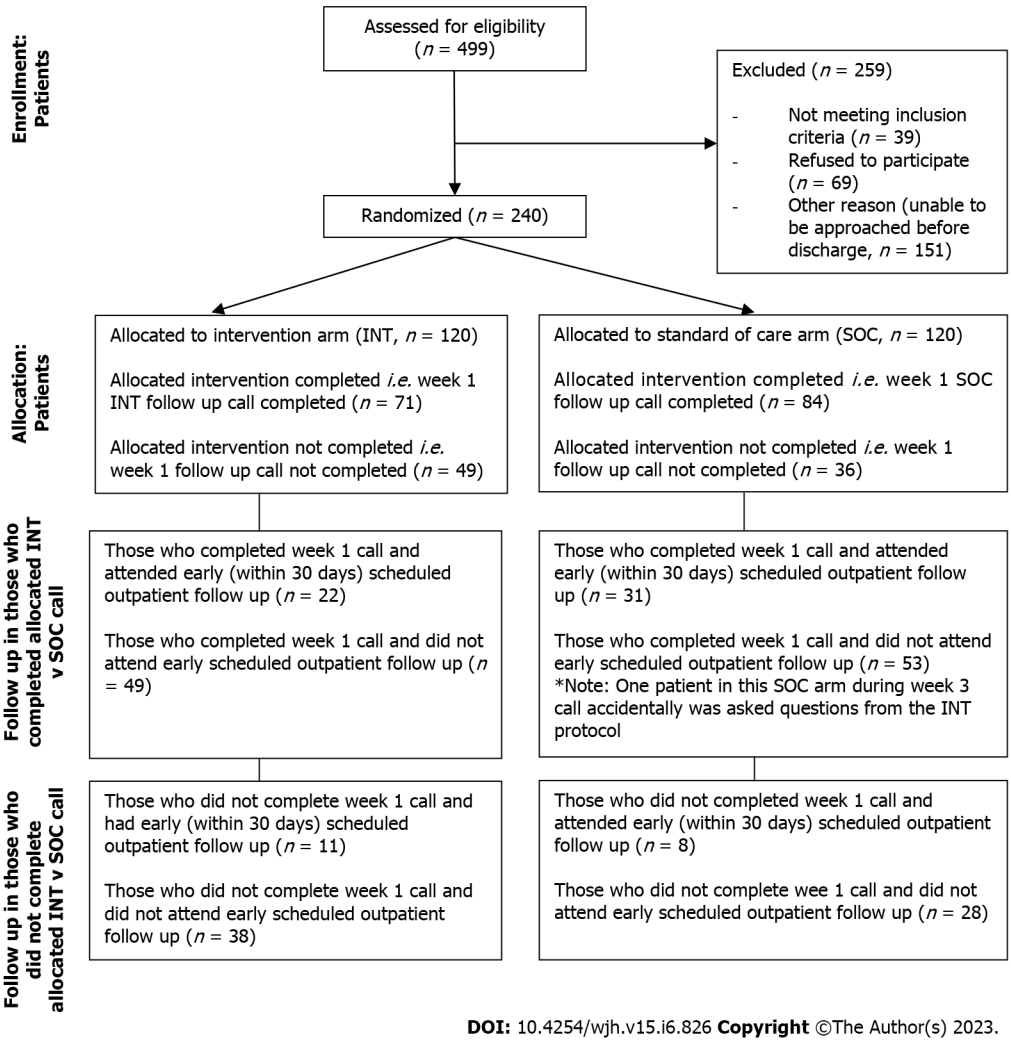

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Figure 1 Modified consort flow diagram of patients eligible for enrollment in study trial. INT: Intervention; SOC: Standard of care.

30 d readmission independent of MELD score, index, first-year, two-years and overall mortality, but also HE at admission had significantly higher mean LIRER scores. Furthermore HE patients on Medicare and geographically from the South or Midwest have higher in-hospital mortality[23]. Considerable research has been done to address HE readmissions. Bajaj *et al*[24] found that efforts to reduce medication-precipitated HE, prevent aspiration pneumonia and optimize HE medications on hospital

Table 4 Characteristic features during index admission in two randomization arms, *n* (%)

Index admission characteristics	Intervention (*n* = 120)	Standard of care (*n* = 120)	*P* value
Reasons for admission[1]			
Acute kidney injury	30, 25.00	35, 29.17	0.56
Hyponatremia	10, 8.33	6, 5.00	0.44
Hepatic encephalopathy	22, 18.33	23, 19.17	1
Volume overload	59, 49.17	52, 43.33	0.44
Variceal bleed	21, 17.50	21, 17.50	1
Lower GI bleed	8, 6.67	11, 9.17	0.63
SBP	9, 7.50	12, 10.00	0.65
Complications of cirrhosis during admission[1]			
Presence of AKI	39, 32.50	41, 34.17	0.89
HE	25, 20.83	24, 20.00	1
Ascites	70, 58.33	69, 57.50	1
Variceal bleeding	21, 17.50	16, 13.33	0.48
SBP	10, 8.33	6, 5.00	0.44
HRS	7, 5.83	7, 5.83	1
Coagulopathy	32, 26.67	24, 20.00	0.29
Portal hypertension	19, 15.83	27, 22.50	0.25
HPS	10, 8.33	5, 4.17	0.29
HCC	6, 5.00	5, 4.17	1
Procedures performed during admission[1]			
EGD	68, 56.67	68, 56.67	1
Paracentesis	60, 50.00	55, 45.83	0.61
TIPS	7, 5.83	3, 2.50	0.33
HD	5, 4.17	10, 8.33	0.29
Colonoscopy/flex sig	13, 10.83	11, 9.17	0.83
Disposition			
Home	83, 69.17	76, 63.33	0.44
Home with home health newly arranged	17, 14.17	22, 18.33	
Home with home health previously arranged	2, 1.67	1, 0.83	
SNF newly arranged	7, 5.83	14, 11.67	
SNF previously arranged	6, 5.00	5, 4.17	
Left against medical advice	1, 0.83	1, 0.83	
Transfer (Long term acute care hospital)	3, 2.50	0, 0.00	
Homeless	1, 0.83	1, 0.83	

[1]Patient can have more than one of variable listed.

SBP: Spontaneous Bacterial Peritonitis; AKI: Acute kidney injury; HE: Hepatic encephalopathy; HRS: Hepatorenal syndrome; HPS: Hepato-pulmonary syndrome; HCC: Hepatocellular carcinoma; EGD: Esophago-gastro-duodenoscopy; TIPS: Transjugular intrahepatic portosystemic shunt; HD: Hemodialysis; SNF: Skilled Nursing Facility; GI: Gastrointestinal.

discharge should be areas of focus to decrease HE readmissions. Tapper *et al*[25] demonstrated that development of a checklist for HE protocols integrated into the EMR and order entry system reduced odds of 30-d readmission for patients with HE (from 39.2% to 27.6%). Thus, our results are congruent with existing evidence that interventions should be invested in post-discharge education and

Table 5 Clinical and laboratory features during index admission and discharge in two randomization arms, *n* (%)

	Intervention (*n* = 120)	Standard of care (*n* = 120)	*P* value
Index admission labs (mean ± SD)			
Sodium	132.59 ± 5.58	132.28 ± 6.28	0.68
Serum creatinine (mg/dL)	1.42 ± 1.11	1.64 ± 1.47	0.19
Total bilirubin (mg/dL)	5.90 ± 9.10	6.19 ± 7.80	0.79
Albumin (g/dL)	2.83 ± 0.59	2.85 ± 0.55	0.72
INR	1.68 ± 0.52	1.70 ± 0.59	0.80
Hemoglobin (g/dL)	10.22 ± 2.34	10.02 ± 2.04	0.48
Ascites			
Absent	35, 29.17	35, 29.17	0.44
Slight	26, 21.67	34, 28.33	
Moderate	59, 49.17	51, 42.50	
Encephalopathy			
None	91, 75.83	96, 80.00	0.78
Grade 1-2	22, 18.33	18, 15.00	
Grade 3-4	7, 5.83	6, 5.00	
Dialysis at least twice in last week			
No	117, 97.50	115, 95.83	0.72
Yes	3, 2.50	5, 4.17	
Index admission discharge labs (mean ± SD)			
Sodium (mmol/L)	134.72 ± 4.14	134.95 ± 3.57	0.64
Serum creatinine (mg/dL)	1.31 ± 1.06	1.37 ± 1.18	0.69
Total bilirubin (mg/dL, *n* = 237)	5.50 ± 8.80	5.39 ± 6.96	0.92
Albumin (g/dL, *n* = 237)	2.98 ± 0.64	2.94 ± 0.61	0.65
INR (*n* = 238)	1.71 ± 0.49	1.69 ± 0.45	0.65
Hemoglobin (g/dL)	9.30 ± 1.69	9.21 ± 1.68	0.68
Ascites			
Absent	42, 35.00	39, 32.50	0.35
Slight	56, 46.67	66, 55.00	
Moderate	22, 18.33	15, 12.50	
Encephalopathy			
None	117, 97.50	112. 93.33	0.10
Grade 1-2	2, 1.67	8, 6.67	
Grade 3-4	1, 0.83	0, 0.00	
Dialysis at least twice in last week			
No	114, 95.00	110, 91.67	0.44
Yes	6, 5.00	10, 8.33	

communication for all patients with cirrhosis, especially with HE.

One of the components of intervention in our study was to arrange appointment of patients in the clinic within a week with their hepatologist. Patients with DC who attended their follow up appointment within 30 d of discharge from index admission had fewer readmissions. This suggests that overall, in our cohort, outpatient linkage with a hepatologist should be a priority to reduce readmission rates [26]. Morales *et al*[17] in their retrospective program looked at the impact of follow-up of cirrhotics within 7 d after discharge with a hepatologist. They reported reduced 30-d readmission, 60-d mortality

Table 6 Outcomes and reasons of readmission characteristics by randomization arms, *n* (%)

	Intervention (*n* = 120)	Standard of care (*n* = 120)	*P* value
Readmission			
No	77, 64.17	82, 68.33	0.59
Yes	43, 35.83	38, 31.67	
Number of readmissions within 30 d			
0	77, 64.17	82, 68.33	0.65
1	31, 25.83	32, 26.67	
2	9, 7.50	5, 4.17	
3	2, 1.67	1, 0.83	
5	1, 0.83	0, 0.00	
Location of 1st readmission			
Our institution	36, 83.72	23, 60.53	0.03
Outside hospital	7, 16.28	15, 39.47	
Reason for 1st readmission[1]			
Acute kidney injury	10, 23.26	6, 15.79	0.58
Hyponatremia	4, 9.30	6, 15.79	0.50
Hepatic encephalopathy	9, 20.93	17, 44.74	0.03
Volume overload	13, 30.23	9, 23.68	0.62
Variceal bleed	6, 13.95	6, 15.79	1.00
Lower GI bleed	1, 2.33	3, 7.89	0.34
Spontaneous bacterial peritonitis	2, 4.65	1, 2.63	1.00
Other	20, 46.51	22, 57.89	0.37
Index admission cost (mean ± SD, *n* = 116 + 109)	61581 ± 47825	59547 ± 46669	0.46
Index admission LOS (mean ± SD)	8.17 ± 5.56	8.08 ± 6.11	0.63
First readmission LOS (*n* = 43 + 38, mean ± SD)	7.58 ± 7.57	8.71 ± 10.41	0.58
All readmissions LOS (*n* = 60 + 45, mean ± SD)	9.28 ± 16.88	8.69 ± 10.44	0.82
Waiting time for first readmission (*n* = 43 + 38, mean ± SD)	11.16 ± 7.10	14.34 ± 7.77	0.06

[1]Patient can have more than one of variable listed.
GI: Gastrointestinal; LOS: Length of stay.

and rate of emergency department visits and associated costs in those who followed up within 7 d. Morando *et al*[18] demonstrated that follow up with a "care management check-up" group as opposed to "standard outpatient care" reduced 30-d readmission, reduced 12-mo mortality, and saved almost 1500 euros per patient month of life. While Kanwal *et al*[9] found early outpatient follow-up after discharge was associated with a small increase in readmissions, they found an lower overall mortality in their patients with cirrhosis admitted to Veterans Affairs hospitals. Thus our results are also consistent with the current evidence that patients with DC likely benefit from early post-hospitalization follow up with specialty providers[27,28].

One of the major limitations of our study was inability to enroll patients according to the proposed sample size due to the COVID-19 pandemic. Our study was underpowered to perform multiple regression analysis to detect differences in readmission rates in INT *vs* SOC arm. From March 2020 to July 2020 our recruitment process was put on hold due to hospital regulations to reduce patient and staff exposure. Despite this major limitation, we were able to enroll 80.17% (279 consented out of 348 approached) of patients in our study.

This study was also performed in the setting of a large academic medical center and a high-volume liver transplant center. While our methods and results may be applicable to the clinical practice of other such centers, the same impact may not be appreciated by smaller, community hospitals that are not liver transplant centers.

Future work in patients with DC should continue to focus on prospective intervention strategies to reduce early readmissions and educate patients and providers. To achieve desired sample size, we would suggest collaborations with various centers to identify and recruit patients with DC into a multicenter prospective cohort. Given our finding that there were fewer readmissions in patients with follow-up within 30 d, studies should evaluate the use of telehealth visits for follows up, especially in the COVID19 era, as outlined by Stotts *et al*[29].

CONCLUSION

In conclusion, this prospective randomized study investigated the impact of various pragmatic interventions to reduce early readmission and healthcare utilization in patients with DC. Our study was underpowered to detect statistically significant differences in readmission rates in INT *vs* SOC arm. We reported that readmission rate of our medical center was 33.75% and HE was the top reason for readmission. We found a reduction in early readmission in patients with HE in the INT arm and those who attended their follow up appointment within 30 d of discharge from index admission. We demonstrated that simple interventions in patients with DC are pragmatic and there is need for more prospective multicenter trials in this area of research.

ARTICLE HIGHLIGHTS

Research background

Decompensated cirrhosis (DC) is a leading cause of morbidity and mortality in the United States often requiring multiple hospitalizations to manage. Studies show 20%-37% of patients with DC are readmitted within 30 d of index admission, which has significant burden on patients, their families and the healthcare system.

Research motivation

We were motivated to study and reduce readmissions as we see the physical, mental and emotional toll repeated hospitalizations for DC take on our patients and their families.

Research objectives

We sought to enroll patients in a randomized trial seeing if a nurse case manager (CM) ensuring early outpatient follow up, medication compliance and outpatient paracentesis if needed reduced readmissions in patients with DC.

Research methods

We sought to enroll patients in a randomized trial seeing if a nurse CM ensuring early outpatient follow up, medication compliance and outpatient paracentesis if needed reduced readmissions in patients with DC.

Research results

While our calculated sample size was not achieved due to the coronavirus disease 2019 pandemic, we found a 33.75% 30 d readmission rate in our patients admitted with DC. There was no difference in readmission between intervention and standard of care arms. Most patients were re-admitted with hepatic encephalopathy. There was a lower 30 d readmission rate in patients with hepatic encephalopathy in the intervention arm and those who attended early outpatient follow up.

Research conclusions

Our 30 d readmission was higher than the national rate. Further efforts should explore the positive impact of a nurse CM and early outpatient follow up in reducing readmissions for patients with cirrhosis, especially with hepatic encephalopathy.

Research perspectives

Further development of strategies to predict and reduce readmissions for patients with DC should be done.

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FOOTNOTES

Author contributions: Pusateri A and Mumtaz K study design, team administration, training team members for recruiting, recruiting patients for study, interpreting data, drafting manuscript; both approved the final submitted version of this manuscript; Litzenberg K, Griffiths C, Hayes C, Gnyawali B and Manious M recruiting patients for study, drafting manuscript, approved the final submitted version of manuscript; Jalil S, Kelly S and Conteh L reviewed and edited the final draft of the manuscript; Nagaraja K analyzed data, edited manuscript, and approved the final submitted version of this manuscript.

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Institutional review board statement: This study was conducted at the Ohio State University Wexner Medical Center (OSUWMC), Columbus, Ohio from July 2019 to December 2020. Our study was approved by OSUWMC Institutional Review Board. All aspects of the studying involving human participants including informed consent for enrollment were in accordance with the ethical standards of our Institutional Review Board and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Clinical trial registration statement: Since our randomized trial was of a nursing intervention only, it was not an official randomized control clinical trial that needed registering on a national level.

Informed consent statement: All study participants, or their legal guardian, provided informed written consent prior to study enrollment.

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SYSTEMATIC REVIEWS

Liver injury from direct oral anticoagulants

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Abstract

BACKGROUND

Drug-induced liver injury (DILI) can be caused by any prescribed drug and is a significant reason for the withdrawal of newly launched drugs. Direct-acting oral anticoagulants (DOACs) are non-vitamin K-based antagonists recently introduced and increasingly used for various clinical conditions. A meta-analysis of 29 randomised controlled trials and 152116 patients reported no increased risk of DILI with DOACs. However, it is challenging to predict the risk factors for DILI in individual patients with exclusion of patients with pre-existing liver disease from these studies.

AIM

To determine the risk factors and outcomes of patients who developed DILI secondary to DOACs by systematic review and meta-summary of recent case reports and series.

METHODS

A systematic search was conducted on multiple databases including PubMed, Science Direct, *Reference Citation Analysis*, and Google Scholar. The search terms included "Acute Liver Failure" OR "Acute-On-Chronic Liver Failure" OR "Acute Chemical and Drug Induced Liver Injury" OR "Chronic Chemical and Drug Induced Liver Injury" AND "Factor Xa Inhibitors" OR "Dabigatran" OR "Rivaroxaban" OR "apixaban" OR "betrixaban" OR "edoxaban" OR "Otamixaban". The results were filtered for literature published in English and on adult patients. Only case reports and case studies reporting cases of DILI secondary to

DOACs were included. Data on demographics, comorbidities, medication history, laboratory investigations, imaging, histology, management, and outcomes were extracted.

RESULTS

A total of 15 studies (13 case reports and 2 case series) were included in the analysis, comprising 27 patients who developed DILI secondary to DOACs. Rivaroxaban was the most commonly implicated DOAC (n = 20, 74.1%). The mean time to onset of DILI was 40.6 d. The most common symptoms were jaundice (n = 15, 55.6%), malaise (n = 9, 33.3%), and vomiting (n = 9, 33.3%). Laboratory investigations showed elevated liver enzymes and bilirubin levels. Imaging studies and liver biopsies revealed features of acute hepatitis and cholestatic injury. Most patients had a favourable outcome, and only 1 patient (3.7%) died due to liver failure.

CONCLUSION

DOACs are increasingly used for various clinical conditions, and DILI secondary to DOACs is a rare but potentially serious complication. Prompt identification and cessation of the offending drug are crucial for the management of DILI. Most patients with DILI secondary to DOACs have a favourable outcome, but a small proportion may progress to liver failure and death. Further research, including post-marketing population-based studies, is needed to better understand the incidence and risk factors for DILI secondary to DOACs.

Key Words: Anticoagulants; Direct-acting oral anticoagulants; Drug induced liver injury; Drug reactions; Hepatotoxicity; Novel oral anticoagulants

Core Tip: Drug-induced liver injury (DILI) can be ascribed to practically any prescribed drug. The side effect profile of relatively newer direct-acting oral anticoagulants (DOACs) is yet to be completely determined. Even though the data from earlier clinical trials suggested no significant liver toxicity, several case reports and series describing DOAC-induced DILI have been recently published. Most of these cases have been reported in elderly patients, not on concomitant hepatotoxic drugs. However, these patients may have good clinical outcomes, with complete recovery of liver function, if an early diagnosis is made and the offending agent is stopped.

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INTRODUCTION

In general, drug-induced liver injury (DILI) can be ascribed to any sort of prescribed drug. DALI remains a major reason behind the premature termination of the development of a promising therapeutic agent or even the withdrawal of a newly launched drug in the market. On the contrary, the true incidence of DILI remains unknown because of under-reporting, missed diagnosis and the usage of different criteria to define DILI. The incidence of DILI varies from 2.3 and 2.7 per 100000 exposed individuals in the United Kingdom and the United States, respectively[1,2]. The incidence of the drug-induced acute liver failure (ALF) has been reported to be 1.61/1000000 persons-years[3]. However, the incidence varies with geographic, socio-economic, and cultural status of the population. For example, a recent retrospective study from China reported an incidence of 23.8 per 100000 persons. Moreover, DILI is the most common cause of ALF in the United States and Europe, accounting for almost 10% of all the causes of hepatitis. In these countries, the over-the-counter medicines and herbal or dietary supplements are the leading causes of DILI[3,4]. In comparison, the traditional Chinese and herbal medicines, dietary supplements and anti-tubercular drugs remain the major reasons for DILI in China[5]. In case of India, both Ayurvedic medicines as well as anti-tubercular drugs are responsible for most of the DILI cases[6].

Traditionally, the mechanism of DILI can be divided into direct (dose-dependent, predictable) and indirect (idiosyncratic, non-predictable) causes. Further, the idiosyncratic reactions may be immune-mediated (*e.g.*, phenytoin) or metabolic (*e.g.*, isoniazid). Acetaminophen is the best example for dose-dependent DILI. However, the recent studies demonstrated a dose-dependent mechanism in case of idiosyncratic type of DILI. While the exact pathogenesis of DILI remains unknown, genetic risk factors

(including human leucocyte antigen and its associations) have been identified in the literature[7,8]. Female gender, race (higher need for liver transplantation among Asians and chronicity among African Americans), age (elderly or young based on the type of drug), pre-existing liver disease, alcohol abuse, malnutrition, and mutations in the P450 gene are the main risk factors identified for DILI. However, it is challenging to predict the risk factors for DILI in individual patients. High index of suspicion is recommended for DILI, especially among those patients with large number of risk factors and when no other etiology is identified. A detailed review of the drug prescriptions should be performed for at least 3-6 mo. Prompt cessation of the suspected drug(s) is the first step in the management of DILI.

Direct-acting oral anticoagulants (DOACs) are non-vitamin K-based antagonists that have been recently introduced and are increasingly used for varied clinical conditions. Ximelagatran, a direct thrombin inhibitor, was withdrawn from the market because of its hepatoxicity[9]. There was no signal of hepatoxicity recorded during the randomised controlled trials (RCTs) of other novel DOACs. However, the patients with significant pre-existing liver diseases were excluded from the pre-approved RCTs for DOACs. Further, these RCTs remain inadequately powered to detect any difference in rare events such as DILI. A meta-analysis conducted by Caldeira *et al*[10], included 29 RCTs and 152116 patients and the study did not find any increased risk of DILI with DOACs. On the contrary, it reported a "protective effect" against hepatoxicity, when compared with the low molecular weight heparin. Though the meta-analysis overcame the limitations of inadequate power of individual studies, the absence of the individual patient data, variable follow-up among the individual studies, usage of DOACs for different indications and hepatotoxicity being measured as a secondary outcome measure cannot reliably prove the safety of the DOACs. Hence, the post-marketing population-based, case-control, real-world data, and pharmacovigilance reports are required to assess the risk of hepatoxicity of the DOACs.

More recently, multiple case reports and series have reported DILI secondary to the use of these drugs. However, the incidence, probability, and the risk factors for the development of DILI are not entirely elucidated and may also vary with different DOACs. Hence, the aim of the current study is to collate the data from recent case reports and series and analyse them to determine the risk factors and the outcomes of patients, who developed DILI secondary to DOACs.

MATERIALS AND METHODS

The authors conducted a systematic search for this meta-summary from multiple databases such as PubMed, Science Direct, *Reference Citation Analysis* and Google Scholar. The search terms included were "Acute Liver Failure" OR "Acute-On-Chronic Liver Failure" OR "Acute Chemical and Drug Induced Liver Injury" OR "Chronic Chemical and Drug Induced Liver Injury" AND "Factor Xa Inhibitors" OR "Dabigatran" OR "Rivaroxaban" OR "Apixaban" OR "Betrixaban" OR "Edoxaban" OR "Otamixaban".

The results were filtered for the literature published in the English language and on adult (> 18 years) humans. All the search results were manually screened by the authors while only those relevant literature for DOAC-induced-DILI was analysed. Duplicate articles from different search databases were excluded (Figure 1). All the case reports and case series were evaluated and the data in terms of patient demographics, clinical symptomatology, type, dose and duration of the DOACs, clinical interventions, intensive care unit course, need for organ support and outcomes was extracted. The concomitant usage of the hepatotoxic drugs was also noted down. A datasheet was prepared for further evaluation.

Statistical analysis

The prepared datasheet was evaluated using MS Excel and Microsoft office, 2019. The categorical variables were presented as frequency and percentage. Median (interquartile range) or mean ± SD was used for continuous variables. Tabulation and final documentation were done using the MS Office software (MS office 2019, Microsoft Corp, WA, United States).

RESULTS

From the current study search, 27 cases of acute liver damage published between 2011 and 2021[11-25] following DOAC exposure were retrieved (Table 1). There was an equal distribution of males and females (48.1%) while most of the cases were reported from Europe (77.8%) and North America (18.5%). At the time of presentation, the age of the patients was in the range of 41 to 91 years. Out of the total population, 20 (74.1%) patients were aged ≥ 65 years or above. The time between the initiation of DOACs and the onset of liver injury ranged from 6 d to 6 mo, while one patient presented with acute condition after accidental ingestion. The major indications for DOACs were prevention of venous thromboembolism (VTE) in patients who were undergoing elective knee surgery and prevention of stroke and systemic embolism in patients with nonvalvular atrial fibrillation (AF). However, three

Table 1 The baseline patient parameters, *n* (%)

Variables	Number of patients (*n* = 27)
Age (± SD), yr	72.7 (± 11.4)
Sex	Females, 13 (48.1)
	Males, 13 (48.1)
	Not mentioned, 1 (3.7)
Country of origin	Switzerland, 12 (44.4)
	Italy, 4 (14.8)
	United States of America, 4 (14.8)
	France, 3 (11.1)
	Australia, 1 (3.7)
	Canada, 1 (3.7)
	Ireland, 1 (3.7)
	Spain, 1 (3.7)
Clinical presentation	Jaundice, 15 (55.6)
	Malaise, 9 (33.3)
	Vomiting, 9 (33.3)
	Abdominal pain, 3 (11.1)
	Itching, 3 (11.1)
	Anorexia, 2 (7.4)
	Fatigue, 2 (7.4)
	Dyspepsia, 1 (3.7)
	Breathlessness, 1 (3.7)
	Pruritus, 1 (3.7)
	Weight loss, 1 (3.7)
Comorbidities	Hypertension, 6 (22.2)
	Diabetes, 3 (11.1)
	Coronary artery disease, 3 (11.1)
	Others, 6 (22.2)
Other drugs causing liver injury	None, 11 (40.7)
	Statins, 4 (14.8)
	Not mentioned, 12 (44.4)
DOACs implicated	Rivaroxaban, 20 (74.1)
	Apixaban, 4 (14.8)
	Dabigatran, 3 (11.1)
Indications of DOACs	Atrial fibrillation, 13 (48.1)
	Pulmonary embolism, 2 (7.4)
	Transient ischemic attack, 1 (3.7)
	Anti-phospholipid antibody syndrome, 1 (3.7)
	Deep vein thrombosis, 1 (3.7)
	Major lower limb surgery, 1 (3.7)
	Sinus node dysfunction, 1 (3.7)
	Accidental, 1 (3.7)

Time to presentation after initiation of DOACs (d)	40.6 ± 42.8
Need for organ support	Renal replacement therapy, 1 (3.7)
	Vasopressors, 1 (3.7)
	Invasive mechanical ventilation, 1 (3.7)
Need for specific antidote	Idarucizumab, 1 (3.7)
Days in hospital (d)	8 ± 9.3
Days in ICU (d)	7 ± 12.1
Outcome	Alive, 26 (96.3)
	Death, 1 (3.7)

SD: Standard deviation; DOAC: Direct-acting oral anticoagulants; ICU: Intensive care unit.

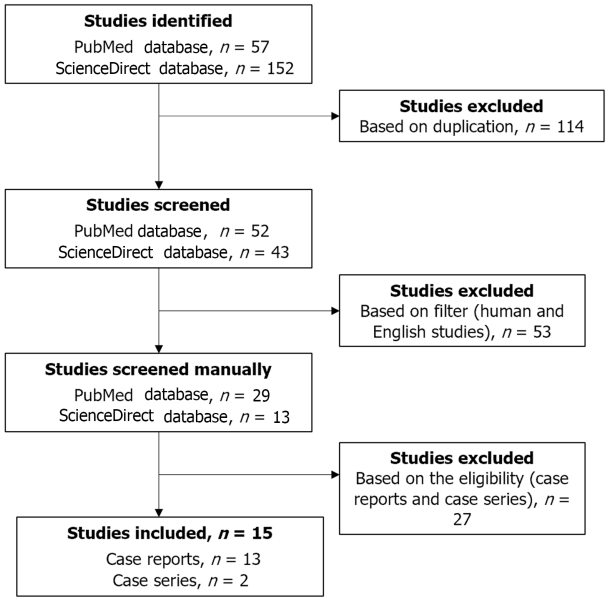

Figure 1 PRISMA flow diagram of the selected literature for the current meta summary.

patients received DOACs for the management of deep vein thrombosis (DVT) and pulmonary embolism (PE). A total of 20 patients (74.1%) received rivaroxaban, four (14.8%) received apixaban, and three (11.1%) received dabigatran. In four cases (14.8%), the consumption of other potential hepatotoxic medications (statins) was reported. The most reported symptom was jaundice in 15 (57.7%) patients, followed by malaise and vomiting in 9 (34.6%) patients. Extracorporeal therapy was initiated in one patient, whereas only one patient with dabigatran-induced DILI received idarucizumab therapy. Table 2 shows the liver function parameters at the time of presentation. Though most of the patients showed complete recovery of the liver function, only 3 (11.1%) patients were reported to have persistent liver dysfunction at the time of discharge, and only one (3.7%) death was reported.

DISCUSSION

Prolonged anticoagulant therapy translates to varied clinical conditions for which vitamin K antagonists are the only therapeutic option. However, the introduction of DOACs has provided the physicians, a safer and an effective alternative. They possess several inherent advantages, including predictable pharmacokinetics and immediate activity after the first dose. This pattern helps in using a fixed dose and obliterate the need for routine laboratory monitoring in most of the patients. However, their side effect profile needs to be fully understood. The first DOAC *i.e.*, ximelagatran was approved for clinical use based on the data from short-term trials. However, the long-term data found DALI in up to 8% of the patients, who received the drug[26]. The fact came into light after its prolonged clinical use. Hence, ximelagatran was withdrawn from the market because of its unfavourable side effect profile. Similarly,

Table 2 Liver function test parameters

Parameter	Mean	Standard deviation	Range
SGOT, at presentation (units/L)	575.5	672.2	90-2304
SGPT, at presentation (units/L)	800.2	1159.6	90-4000
Total bilirubin, at presentation (mg/dl)	6.3	6	0.64-21.8
Direct bilirubin, at presentation (mg/dl)	4.8	4.9	0.3-10.3
Alkaline phosphatase, at presentation (units/L)	269.2	279.5	60-1039

SGOT: Serum glutamic oxaloacetic transaminase; SGPT: Serum glutamic pyruvic transaminase.

significant hepatotoxicity was not reported in the initial trials of other DOACs. However, several case reports have been published in the recent years, describing their hepatotoxic potential.

It is challenging to diagnose DILI with the absence of any specific clinical or biochemical marker. The clinical features of DILI are usually non-specific and may vary from asymptomatic liver enzyme elevation to ALF. Even liver histology is not specific and may also be found in other liver disorders[27]. Hence, a high index of suspicion is warranted to enable early diagnosis. Even though jaundice was present in 55.6% of the study patients, all others presented with non-specific symptoms like malaise, vomiting, anorexia and fatigue. The latency from the exposure to DILI, especially in case of metabolic idiosyncrasies, varies from days to weeks and at times, even months too. The data, from the meta-analysis study that assessed the hepatotoxic potential of DOACs, also reported that the mean age of the patients was in the range of 55 to 71 years. Further, the time to develop DILI after the initiation of DOACs ranged from 2 wk to 2 years[10].

The diagnosis of DILI requires the exclusion of other liver dysfunction etiologies associated with a similar clinical and biochemical picture, along with the documentation of temporal association related to the exposure of the offending agent[28]. The accepted definition for hepatocellular liver injury has been given by an international DILI expert working group; fivefold or greater elevation above the upper limit of normal (ULN) for alanine aminotransferase/serum glutamic pyruvic transaminase (ALT/SGPT)[29]. The cholestatic picture is defined to be more than twofold elevation above the ULN for alkaline phosphatase with a concomitant elevation of the activity of 5'-nucleotidase or γ-glutamyltranspeptidase [29]. However, when the ratio of elevation, above the ULN for ALT/SGPT and alkaline phosphatase, is more than two-fold yet less than five-fold, it is defined as a mixed pattern of liver injury[29,30]. The use of DOACs has been associated with both hepatocellular and cholestatic patterns of liver injury[11].

As per the clinical studies, DOACs are rarely associated with the development of DILI with the frequency of developing DILI ranging between 0.1%-1%[10]. However, a higher incidence of 2.3% was reported when using rivaroxaban[31]. In the current study analysis, rivaroxaban was reported to be the offending agent in 74% of the cases. This may also be explained by the fact that it is arguably the most prescribed DOAC[32]. However, these clinical trials were neither designed nor powered to detect the risks of rare idiosyncratic adverse reactions like DILI. Moreover, the patients at high risk of developing these complications and with pre-existing liver disease were often not included in these clinical trials.

Several DOACs are approved and prescribed for both prevention and the treatment of DVT or PE. Further, they are also prescribed to prevent stroke and systemic embolism in adults with non-valvular AF and other risk factors. DOACs are also routinely used in the prevention of VTE after hip or knee surgeries. The current study analytical outcomes too depict a similar usage pattern. The patients, who developed DILI, were prescribed DOACs for similar indications, among which the non-valvular AF was the most common indication. The data from the systematic analysis has also shown that the most common indication for initiating DOACs was non-valvular AF[10].

Except for dabigatran and its glucuronide metabolite, which are predominantly excreted through kidneys, all the DOACs are metabolised in the liver. Moreover, dabigatran is also a substrate for P-glycoprotein (P-gp), and hence, dose adjustment needs to be done for patients with renal dysfunction and those on P-gp inhibitors like azole antifungals, amiodarone, macrolides, atorvastatin, digoxin *etc* [33]. Nearly one-third of the administered rivaroxaban is excreted unchanged in the urine, whereas the rest undergoes metabolic degradation. In this context too, half gets eliminated by the kidneys and the other half is eliminated through hepatobiliary route. In the liver, it is mainly metabolised *via* the cytochrome P450 (CYP) 3A4 and some CYP-independent mechanisms. It is also a substrate of P-gp. Apixaban is excreted through multiple pathways such as the renal (25%), intestinal (55%) and the rest through hepatobiliary route (CYP3A4-dependent mechanisms)[34]. Concomitant use of hepatotoxic drugs, or CYP3A4/P-gp inhibitors may increase the risk of hepatotoxicity with DOACs. Hence, these patients require a close monitoring of their liver function. Further, patients may develop hepatoxicity even in the absence of these drugs. As per the current study analysis, the concomitant use of potentially hepatotoxic drugs was reported in only 14.8% of the cases.

The current study has several strengths to its credit. The authors included DILI associated with all the currently prescribed DOACs and included all the latest case reports and series. However, there exists some limitations to the meta-summary. The studies included, were only case reports and case series without a control arm. Additionally, these studies were heterogeneous, with a high risk of bias and missing data. So, it may affect the generalisability of the results. Further, the authors might have missed some relevant cases too, because those case reports or series that did not have any individual biochemical data were excluded.

CONCLUSION

DOACs may rarely lead to DILI. Most of the cases have been reported among elderly patients, who may be at higher risk of developing DILI. It may develop without the concomitant use of other hepatotoxic drugs. The outcomes of these patients are generally favourable and liver dysfunction is mainly reversible if it is recognised early and the intake of offending agent is stopped. Hence, physicians prescribing these drugs must be aware of such rare complications to ensure early diagnosis and prompt management.

ARTICLE HIGHLIGHTS

Research background

Drug-induced liver injury (DILI) can be caused by any prescribed drug and is a significant reason for the withdrawal of newly launched drugs. Direct-acting oral anticoagulants (DOACs) are non-vitamin K-based antagonists recently introduced and increasingly used for various clinical conditions. It is challenging to predict the risk factors for DILI in individual patients with exclusion of patients with pre-existing liver disease from these studies.

Research motivation

To determine the risk factors and outcomes of DILI in patients taking DOACs and provide clinicians with essential information for the management of DILI secondary to DOACs.

Research objectives

To determine the incidence, probability, and risk factors for developing DILI secondary to DOACs and its outcomes in affected patients.

Research methods

The authors conducted a systematic search of multiple databases for the literature published in English using specific search terms. The results were filtered and analysed to determine the risk factors and outcomes of DILI in patients taking DOACs.

Research results

The analysis of recent case reports and series showed that DOACs can rarely cause DILI, and the incidence, probability, and risk factors for developing DILI varied among different DOACs.

Research conclusions

DOACs can cause DILI, and the incidence, probability, and risk factors for developing DILI vary among different DOACs. Clinicians should have a high index of suspicion for DILI in patients taking DOACs, especially those with multiple risk factors. Prompt cessation of the suspected drug is recommended as the first step in managing DILI.

Research perspectives

The findings of this study provide essential information for clinicians to manage DILI secondary to DOACs. However, further research is required to identify the true incidence of DILI and its risk factors, including genetic associations. Post-marketing pharmacovigilance reports can help to assess the risk of hepatoxicity associated with DOACs.

FOOTNOTES

Author contributions: Nasa P conceptualized and designed the article; Juneja D, Nasa P, and Jain R performed acquisition of data, analysis and interpretation of data, and drafted the article; Juneja D and Jain R revised the article; and all authors have read and approve the final manuscript.

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SYSTEMATIC REVIEWS

Management of sepsis in a cirrhotic patient admitted to the intensive care unit: A systematic literature review

Nkola Ndomba, Jonathan Soldera

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Abstract

BACKGROUND

Sepsis is a severe medical condition that occurs when the body's immune system overreacts to an infection, leading to life-threatening organ dysfunction. The "Third international consensus definitions for sepsis and septic shock (Sepsis-3)" defines sepsis as an increase in sequential organ failure assessment score of 2 points or more, with a mortality rate above 10%. Sepsis is a leading cause of intensive care unit (ICU) admissions, and patients with underlying conditions such as cirrhosis have a higher risk of poor outcomes. Therefore, it is critical to recognize and manage sepsis promptly by administering fluids, vasopressors, steroids, and antibiotics, and identifying and treating the source of infection.

AIM

To conduct a systematic review and meta-analysis of existing literature on the management of sepsis in cirrhotic patients admitted to the ICU and compare the management of sepsis between cirrhotic and non-cirrhotic patients in the ICU.

METHODS

This study is a systematic literature review that followed the PRISMA statement's standardized search method. The search for relevant studies was conducted across multiple databases, including PubMed, Embase, Base, and Cochrane, using predefined search terms. One reviewer conducted the initial search, and the eligibility criteria were applied to the titles and abstracts of the retrieved articles. The selected articles were then evaluated based on the research objectives to ensure relevance to the study's aims.

RESULTS

The study findings indicate that cirrhotic patients are more susceptible to infections, resulting in higher mortality rates ranging from 18% to 60%. Early identification of the infection source followed by timely administration of antibiotics, vasopressors, and corticosteroids has been shown to improve patient outcomes. Procalcitonin is a useful biomarker for diagnosing infections in

cirrhotic patients. Moreover, presepsin and resistin have been found to be reliable markers of bacterial infection in patients with decompensated liver cirrhosis, with similar diagnostic performance compared to procalcitonin.

CONCLUSION

This review highlights the importance of early detection and management of infections in cirrhosis patients to reduce mortality. Therefore, early detection of infection using procalcitonin test and other biomarker as presepsin and resistin, associated with early management with antibiotics, fluids, vasopressors and low dose corticosteroids might reduce the mortality associated with sepsis in cirrhotic patients.

Key Words: Sepsis; Septic shock; Cirrhosis; Sequential organ failure assessment score; Mean arterial pressure; Intensive care unit

Core Tip: Sepsis is a severe condition encountered in the intensive care unit (ICU), and when it occurs in cirrhotic patients, it often leads to high mortality due to impaired immunity and multiorgan failure. To diagnose and monitor sepsis in cirrhotic patients, various scoring systems have been developed, including the Sequential Organ Failure Assessment (SOFA) score, the Chronic Liver Failure-Sequential Organ Failure Assessment (CLIF-SOFA) score, quick SOFA (qSOFA), Model for End-Stage Liver Disease (MELD), and MELD-Na score. Although the proposed current management of sepsis in cirrhotic patients might follow the guidelines proposed by the Surviving Sepsis Campaign, this approach has might not cause significant improvement in patient outcomes. Therefore, early recognition of infection and its source is critical, followed by timely initiation of antibiotic therapy, fluid resuscitation with albumin (5% or 20%), vasopressors, and low-dose corticosteroids such as hydrocortisone. Studies have shown that this approach reduces mortality in the ICU. In addition to pharmacological interventions, interventions to control the source of infection, such as surgical drainage, may also be necessary. Finally, procalcitonin levels can be used as a diagnostic biomarker in cirrhotic patients with sepsis, helping to guide antibiotic therapy and improve patient outcomes. In conclusion, timely recognition and management of sepsis in cirrhotic patients in the ICU is crucial, and early initiation of appropriate interventions, including antibiotics, fluids, and corticosteroids, may improve patient outcomes.

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INTRODUCTION

Physiologically, sepsis is viewed as a proinflammatory and procoagulant response to invading pathogens with three recognized stages in the inflammatory response, with a progressively increased risk of end-organ failure and death[1]. Evidence shows that sepsis in cirrhotic patients causes a marked imbalance of cytokine response, known as a "cytokine storm," which converts responses that are normally beneficial for fighting infections into excessive, damaging inflammation. Therefore, the three recognized stages are sepsis, severe sepsis, and septic shock, and cirrhotic patients are prone to developing sepsis-induced organ failure and death[1]. Severe sepsis in cirrhotic patients is associated with high production of proinflammatory cytokines that play a role in the worsening of liver function and development of organ or system failure such as shock, acute lung injury, acute respiratory distress syndrome, coagulopathy, renal failure, or hepatic encephalopathy[1]. Furthermore, cirrhotic patients with severe sepsis can develop sepsis-induced hyperglycemia, defective arginine-vasopressin secretion, adrenal insufficiency, or compartmental syndrome[2].

Sepsis is a severe condition characterized by a deregulation of the body's response to infection and can lead to life-threatening organ dysfunction. As one of the leading causes of admission to intensive care units (ICUs), sepsis has been found to have poorer outcomes in patients with comorbidities such as cirrhosis, as stated in the "Third International Consensus Definitions for Sepsis and Septic Shock"[1-3]. Organ dysfunction in sepsis is measured by an increase of two points or more in the Sequential Organ Failure Assessment (SOFA) score, which is associated with a mortality rate greater than 10%[4-5]. The SOFA score comprises six sub-scores, including liver failure, which has been found to be associated with

higher mortality. The other sub-scores include respiratory, coagulation, cardiovascular, central nervous system, and renal. Each sub-score is rated on a scale of 0 to 4 and summed up to a final score from 0 to 24. Systematic Inflammatory Response Syndrome occurs when two or more criteria, such as body temperature > 38 ℃ or < 36 ℃, tachycardia > 90/min, hyperventilation, and abnormal white blood cell count, are met[2].

Septic shock is a subset of sepsis that leads to profound circulatory and cellular metabolism abnormalities, resulting in substantially increased mortality[4]. To identify septic shock, one should look for hypotension that requires vasopressor therapy and a mean arterial pressure (MAP) of less than 65 mmHg despite adequate fluid resuscitation and systolic blood pressure. Additionally, signs of tissue hypoperfusion such as low urinary output, acidosis, and worsening mental status, along with evidence of systemic inflammatory response syndrome, including a body temperature above 38 or below 36 ºC, tachycardia, tachypnea, leukocytosis, and documented infection, are also considered[5].

Elevated lactate levels reflect cellular dysfunction in sepsis, and multiple factors contribute to their elevation, including insufficient tissue oxygen delivery, impaired aerobic respiration, acceleration of aerobic glycolysis, and reduced hepatic clearance[1]. However, defining sepsis and septic shock poses inherent challenges[3].

The acute change in total SOFA score of more than 2 points due to an infection is identified as organ dysfunction. In patients with a SOFA score of 2 or more, the overall mortality risk is approximately 10%, which is higher than the overall mortality rate of ST-segment elevation myocardial infarction. This score also identifies a 2-25 fold increased risk of dying compared to patients with a SOFA score less than 2. However, this score is not used as a tool for managing septic patients in the ICU but rather to characterize them clinically. SOFA has greater predictive validity in patients suspected of sepsis in an ICU[3,4].

There are several risk factors associated with sepsis, including patient factors such as immunosuppression, comorbidity, or therapy, microbe factors such as the presence of multi-resistant or virulent bacteria, and procedural risks such as surgery, indwelling catheters, or implantable devices[6]. Cirrhosis, which is the end-stage of most chronic liver diseases, has two clinical phases: Compensated and decompensated. The compensated phase is defined as the period between the onset of cirrhosis with minor or no symptoms and the first major complication, while the decompensated phase is when the patient first presents with ascites, variceal hemorrhage, hepatic encephalopathy, spontaneous bacterial peritonitis, and hepatorenal syndrome. This period is associated with a short survival time. Cirrhosis may be diagnosed by liver biopsy or by signs of chronic liver disease with documented portal hypertension. Cirrhotic patients have a reduced capacity of the reticuloendothelial system to clear bacteria from the gut, resulting in a higher rate of infections and a worse prognosis[7].

Cirrhosis is an irreversible condition caused by several factors or conditions, such as viral hepatitis, alcoholic liver disease, and non-alcoholic fatty liver disease. According to the World Health Organization, cirrhosis was the 9th leading cause of death in the west in 2015[8]. Studies have shown that mortality among cirrhotic patients with sepsis in the ICU ranges from 18%-66%, with mechanical ventilation being an independent predictor of mortality. The MELD and MELD-Na scores are used for the prediction of 90-day mortality and for organ allocation in liver transplantation. A cohort study by Baudry *et al*[9] found that mortality of cirrhotic patients with sepsis ranges from 18%-66%. WHO estimates cirrhosis as the 12th cause of mortality in the world, with deaths exceeding 1 million per year. ICUs provide specialized treatment and monitoring for critically ill patients.

The aim of the present paper is to determine the optimal current management of sepsis in cirrhotic patients admitted to the ICU.

MATERIALS AND METHODS

This systematic review follows the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA-P) protocol[10] and examines published papers on the management of sepsis in cirrhotic patients admitted to the ICU.

Inclusion criteria

Inclusion criteria were cirrhotic patients over 18 years old, admitted to the ICU with sepsis. The study analyzed the management and prognosis of cirrhotic patients with sepsis, as well as compared the management of sepsis in cirrhotic patients to those without cirrhosis. Only English-language randomized controlled trials (RCTs), retrospective cohort studies, and prospective cohort studies were included.

Outcomes

The analyzed outcomes include survival, length of ICU stay, and the overall prognosis of cirrhotic patients with sepsis admitted to the ICU.

Search strategies

Searches were conducted on PubMed, Google Scholar, Embase, and Cochrane databases. Retrieved papers were initially filtered based on their titles and abstracts, and the full text of selected papers were then retrieved and analyzed. Only papers that met the inclusion criteria were included and analyzed. The search strategy is described in Appendix 1 and the critical appraisal of the papers is presented in Appendix 2.

RESULTS

Study selection

Figure 1 illustrates the selection process following the PRISMA-P protocol. Initially, 351 search results were retrieved, out of which 284 were excluded after screening the titles, 46 were excluded after the abstract, and 3 were excluded after full articles. A total of 19 papers met the inclusion criteria and were included for full-text review. The primary outcome of all reviewed papers was the survival of cirrhosis patients with sepsis in the ICU. The review also analyzed the prognostic value of scores such as Child-Turcotte-Pugh, Model for End-Stage Liver Disease (MELD), Model for End-Stage Liver Disease Sodium (MELD-Na), and SOFA scores for cirrhotic patients with sepsis. The summarized data is available on Table 1 for randomized controlled trials, Table 2 for prospective cohort studies, Table 3 for retrospective cohort studies and Table 4 for selected studies.

Findings of the review

Albumin: Philips *et al*[11] found that 5% human albumin corrected hypotension in sepsis with cirrhosis (Table 1). Maimone *et al*[12] found that albumin 20% increased MAP above 65 mmHg 3 h after infusion compared to plasmolyte, but with a risk of inducing pulmonary edema (Table 3).

Corticosteroids: Arabi *et al*[13] concluded that corticosteroids improved the hemodynamic status of the patient but did not change mortality (Table 1). Rinaldi *et al*[5] and Piccolo Serafim *et al*[14] found similar results to Arabi *et al*[13] (Tables 2 and 3).

Infection diagnosis: Villarreal *et al*[15] concluded that procalcitonin as a biomarker helped with infection diagnosis in cirrhotic patients. Fischer *et al*[16] found that both presepsin and resistin may be reliable markers of bacterial infection in patients with decompensated liver cirrhosis and have similar diagnostic performance compared to procalcitonin (Table 3).

Prognosis: Baudry *et al*[9], Fischer *et al*[16], Chang *et al*[17], and Sauneuf *et al*[18], found that the prognosis is poor in ICU for cirrhosis patients with sepsis (Table 3). Sasso *et al*[19] found that mechanically ventilated cirrhotic patients with sepsis have an extremely poor prognosis, and vasopressor use was strongly a predictor of mortality (Table 3).

Vasopressors: Durst *et al*[20] found that norepinephrine is the best vasopressor to use in cirrhotic patients with sepsis to maintain MAP above 60 mmHg. Umgelter *et al*[21] concluded that terlipressin is effective as a vasopressor in septic cirrhotic patients in combination with norepinephrine to correct hypotension. Chebl *et al*[22] recommend starting vasopressors early to avoid aggressive fluid resuscitation and maintain MAP > 65 mmHg (Table 3).

Hyperdynamic syndrome: Thierry *et al*[23] found that echocardiography helps diagnose hyperdynamic syndrome with high LVEF in septic patients (Table 3).

Mortality: Bal *et al*[24] found 50-day mortality to be about 43.11%. Baudry *et al*[9] found that the mortality of cirrhotic patients with sepsis ranges from 18%-66%, which is close to the WHO finding that estimates cirrhosis as the 12th cause of mortality in the world, with death exceeding 1 million a year (Table 3).

Scoring system: Chen *et al*[25] concluded that the qSOFA (Quick SOFA) criteria, consisting of 3 variables, are a better predictor of adverse outcomes associated with sepsis. The presence of two or more abnormalities in patients with suspected infection identifies a higher risk of developing adverse outcomes.

Hemodynamic monitoring: Administer antibiotics within the first hour and monitor physiological parameters like urine output and lactate clearance to prevent end-organ dysfunction[7]. In advanced cirrhosis, elevated cardiac index, low systemic vascular resistance, low MAP, and higher central venous oxygen saturation may be present. Lactate levels should be carefully evaluated as they may take a while to lower down to normal levels. Serum lactate measurement is still recommended in these patients[7]. Skin mottling score and tissue oxygenation saturation assessed with laser Doppler can also be used as hypoxia of the tissue markers in cirrhosis[7].

Table 1 Summary of randomized controlled trials

Ref.	Purpose	Type of study	Sample size	Conclusion	Setting
Philips *et al*[11]	Assessed the use of 5% human fluid for the resuscitation of cirrhotic patients with sepsis		Three hundred-eight patients were divided into two groups	5% human albumin was safe and more beneficial in correcting hypotension than normal saline	ICU
Arabi *et al*[13]	Assess the use of a low dose of hydrocortisone in cirrhotic patients with sepsis	RCT	140 patients were 65 excluded, and 39 received hydrocortisone and 36 placeboes	That study did not find mortality improvement with corticosteroids despite hemodynamic improvement. The treatment proposed: Hemodynamic monitoring and management, laboratory test culture, stress ulcer prophylaxis as histamine H2 receptor antagonists, norepinephrine as vasopressors, and empiric antibiotic. The outcome was 28 days of all-cause mortality	ICU

RCT: Randomized controlled trial; ICU: Intensive care unit.

Table 2 Prospective cohort studies

Ref.	Purpose	Type of study	Sample size	Conclusion	Setting
Rinaldi *et al*[5]	The aim was to evaluate the effect of adherence to evidence-based guidelines of the Surviving Sepsis Campaign (SSC) on the outcome of cirrhotic patients with shock admitted to the ICU. Resuscitation of sepsis with hydrocortisone	Prospective cohort	38 patients		ICU
Thierry *et al*[23]	Assess the use of echocardiography in assessing the LVEF on cirrhotic patients with septic shock	The prospective cohort single-center study	34 patients compared	Echocardiography in a cirrhotic patient with septic shock show hyperdynamic syndrome with high LVEF	ICU

SSC: Surviving sepsis campaign; ICU: Intensive care unit; LVEF: Left ventricle ejection function.

Fluid resuscitation: Aggressive intravenous fluid resuscitation is recommended in any patient with hypotension or elevated serum lactate. However, the choice of fluid between crystalloid or colloid remains controversial[6,11,12]. The SAFE study concluded that albumin improves hemodynamic status and may reduce mortality, while the VISEP study found that pentastarch colloids can cause acute kidney injury in sepsis and increase 90-day mortality[6,11]. Human albumin is the fluid of choice in cirrhotic patients with sepsis, as it corrects hypotension more effectively than crystalloid[12]. Early goal-directed therapy can help reduce mortality, but the methodology of the Rivers study has been questioned. The recommended fluid should be one that sustains an increase in intravascular volume and contains a chemical composition similar to that of extracellular fluid[6]. Hydroxyethyl starch is not recommended in cirrhosis patients as it increases nephrotoxicity, while albumin is associated with dose-dependent acute kidney injury[6]. An albumin dose of 50-100 g/day is used over crystalloid for initial fluid resuscitation in cirrhosis patients, but no strong evidence exists[6].

Sepsis bundle protocol: According to the Surviving Sepsis Campaign (SSC) guidelines, the sepsis bundle protocol did not improve survival in cirrhotic patients with sepsis[7].

Vasopressors: Vasopressors are frequently indicated to maintain a MAP of at least 65 mmHg in persistently hypotensive patients. Norepinephrine is widely used in distributive shock for its predominantly alpha-adrenoceptor agonism and vasoconstrictive effect[21]. Cirrhotic patients with sepsis and cirrhosis needing vasopressors should have a goal of maintaining the MAP above 60 mmHg. Blood culture and antibiotics should be started as early as possible according to the SCC guidelines[20]. SSC international guideline does not recommend vasopressors as monotherapy or the first line for septic shock treatment, and a randomized trial shows the benefit of angiotensin II for refractory vasodilatory shock treatment[7].

Corticosteroids: Corticosteroids are commonly used for unsatisfactory responses to vasopressors. It helps hasten shock resolution, decreases the required dose of vasopressors, and improves the 90-day survival in septic shock patients, and it might increase shock recurrence. Nevertheless, its use in liver cirrhosis remains controversial[7]. Hydrocortisone improves the hemodynamic status of the patient without a relevant change in mortality[13,14,18]. Hydrocortisone is associated with better shock resolution, although without an impact on survival[5]. Low-dose corticosteroid is recommended to be administered early in patients with severe septic shock to patients who are not responding to

Table 3 Retrospective cohort studies

Ref.	Purpose	Type of study	Sample size	Conclusion	Setting
Guo *et al* [26]	Assessment of VCS parameter for evaluation of sepsis in cirrhotic patients	Retrospective analysis of prospective data	257 patients	Proposed management was collection of blood culture, white cell volume determination, procalcitonin, and interleukin -6, sCD163 laboratory tests. Conclusion VCS parameters have the potential to be used to evaluate and predict early infections in patients with cirrhosis, and VCS can increase sensitivity and specificity in the diagnosis of sepsis and cirrhosis patients	ICU
Villarreal *et al*[15]	Assessing the usefulness of procalcitonin for diagnosing infection in cirrhotic patients	Retrospective cohort study	66 patients of 255 admitted had procalcitonin tests. Patients with infection suspicion had a serum procal-citonin (PCT) test within the first 12 h	Septic patients with cirrhosis had elevated procalcitonin. As PCT has a sensitivity of 83% and specificity of 75% is an effective tool for diagnosing infection in patients with liver cirrhosis. Excellent tool for differentiating infectious disease in cirrhotic patients	ICU
Galbois *et al*[27]	Assess whether the mottling score and tissue oxygen saturation (StO_2) may be used as early death predictors on cirrhotic patients with septic shock. Hemodynamic parameters at 6 h in patients with liver cirrhosis according to their survival status at 14 days	42 out of 46 patients admitted with cirrhosis and septic shock were analyzed		There is systemic vasodilation and increased mortality in cirrhosis patients with sepsis. Patients with increased mottling died, and those with decreased survived. Mottling score and knee StO_2 measures 6 h after starting vasopressors are excellent predictors of 14-day mortality	ICU
Piccolo Serafim *et al*[14]	The study evaluates the use of steroids in a patient with septic shock and cirrhosis	A retrospective cohort study (2007-2017)	56 patients out of 179 admitted with septic shock received steroids during ICU	The use of steroids did not show significant differences in mortality. Vasopressor requirement and is not associated with decreased mortality	ICU
Chang *et al*[17]	aimed to determine whether septic patients with liver cirrhosis had worse survival than patients without liver cirrhosis	Retrospective cohort	776 patients, 64 had sepsis with cirrhosis, 712 sepsis without cirrhosis	Cirrhotic patients with sepsis had a poor outcome, and the survival of sepsis and cirrhosis after matching was not inferior to those without cirrhosis	ICU
Sauneuf *et al*[18]	Assess the use of albumin as an adjuvant to vasopressors in managing septic shock in cirrhotic patients	Retrospective cohort single center and observational overdone over 14 years studied done from 1997 to 2004 and 2005 to 2010	During the period 2005 to 2010, 42, cirrhotic patients with septic shock in ICU were included	In conclusion, the survival rate of septic shock in cirrhosis remains low, and current shock management could benefit cirrhotic patients. Treatment use is: Vasopressors used is norepinephrine, epinephrine, and dobutamine; mechanical ventilation was used in the case of ARDS, and a protective strategy with a low tidal volume of 6 m/kg of body weight, and the plateau was kept below 30 cmH2O, small -dose of corticosteroids (200 mg hydrocortisone per day, insulin therapy, The main sites of infections were: Pneumonia, spontaneous or secondary peritonitis, and urinary tract infection. There were gram-positive and negative. Septic shock represent a severe complication of cirrhosis with very low survival rates. Sepsis in a cirrhotic patient has a poor prognosis. Hydrocortisone did not reduce mortality and was associated with adverse effects such as shock relapse and gastrointestinal bleeding. Cirrhotic patients are commonly perceived as poor candidates for `ICU admission because of the very high mortality	ICU
Umgelter *et al*[21]	Assess the outcome of the continuous low dose of TP in a septic shock patient	Small cohort study	2004-2007: 12 patients, 8 males, and 4 females were included with sepsis due to spontaneous bacterial peritonitis, pneumonia, and cholangitis	TP is currently used in treating cirrhotic patients with hepatorenal syndrome and as an adjunct to NE in a cirrhotic patient with septic shock and kidney failure; TP dose 2 ug/kg if a patient was started NE in the first 24 h. 11 patients had RRT, TP increased SVR index and NE doses needed to obtain target MAP decreased while the CI remained stable. Despite hemodynamic improvement, 11 out of 12 patients died. The author concluded that TP was effective as a vasopressor in septic cirrhotic patients at a low dose in combination with NE, and there was no dramatic decrease in CI. TP has a role in the early treatment of septic shock, and the author recommends a controlled study with TP in a cirrhotic patient with sepsis	ICU

Durst *et al* [20]	The study aimed to evaluate the use of vasopressor in septic shock with cirrhosis and without cirrhosis	single-center, retrospective cohort, 18 years	122 patients included were 61 with cirrhosis and 61 non-cirrhosis with sepsis, and septic with cirrhosis		ICU
Maimone *et al*[12]	Compare the 20% albumin to plasmolytes in managing cirrhosis and sepsis in the intensive care unit	Retrospective cohort study	100 patients with cirrhosis and sepsis-induced hypotension		ICU
Bal *et al* [24]	The aim is to predict 50 days in hospital mortality in decomposed cirrhosis patients with SBP	A single-centre study prospective study	218 were admitted to ICU from 2013-2014 with cirrhosis and spontaneous bacterial peritonitis		ICU
Chebl *et al*[22]	Assess the outcome and mortality predictor of cirrhosis patients with sepsis	A single-center retrospective cohort study	200 patients	The study revealed an increased risk of sepsis in cirrhotic patients and sepsis-induced organ failure and related death in cirrhosis. The management of shock is to keep MAP above 65 mmgh with vasopressors; the aggressive fluid hydration may worsen the outcome as there is low oncotic pressure in a cirrhotic patient, which may lead to oedema with aggressive fluid hydration, so it is good to start with vasopressors early in the treatment of septic cirrhosis patients to avoid complications, a cirrhotic patient has higher lactate than the non-cirrhotic because of decreased lactate clearance by the liver	ICU
Chen *et al* [25]		A single-center, retrospective cohort study from 2015 to 2018	104 patients with cirrhosis and bacteremia were subdivided into afebrile (55) and febrile (49)	The cirrhotic patient is prone to infection. Cirrhotic patients with bacterial infections present with atypical manifest-ations such as normothermia. Scoring systems focused on organ dysfunction, such as quick sequential organ failure assessment (qSOFA) score or chronic liver failure sequential organ failure assessment (CLIF-SOFA) score, have better predictor ability	In the emergency department
Sasso *et al* [19]	Assess the prediction of mortality in a cirrhotic patient	Prospective cohort	113 patients mechanically ventilator cirrhotic from 2014-2018	Conclude that cirrhotic patients requiring mechanical ventilation have an extremely poor prognosis, and the vasopressor requirement was strongly a predictor of mortality in mechanical ventilation cirrhosis with sepsis	ICU
Fischer *et al*[12]	Assess the use of presepsin and resistin as markers of bacterial infections in cirrhotic patients with sepsis			Conclusion: Both presepsin and resistin may be reliable markers of bacterial infections in patients with decompensated liver cirrhosis and have similar diagnostic performance for bacterial infection and sepsis compared to C-reactive protein (CRP) and PCT. The best cut-off level of presepsin for diagnosis of sepsis was 1444 pg/mL. Conclusion PCT, CRP, Presepsin, and resistin had similar accuracy in diagnosing infection and sepsis in decompensated cirrhosis	ICU
Baudry *et al*[9]	Assess the prognosis of sepsis in cirrhotic patients	A Retrospective cohort study from 2002-2013	7644 patients were admitted, where 149 were		ICU

VCS: Value of volume conductivity and scattering; PCT: Procalcitonin; STO_2: Tissue oxygen saturation; ARDS: Acute respiratory distress syndrome; TP: Telipressin; NE: Norepinephrine; SVR: Systemic vascular resistance; RRT: Renal replacement therapy MAP: Mean arterial pressure; CI: Cardiac index, ATB: Antibiotic; MELD: Model for End-Stage Liver Disease; AKI: Acute kidney injury; SBP: Systolic blood pressure; qSOFA: Quick sequential organ failure assessment; ICU: Intensive care unit.

vasopressors, but this is still controversial[6].

Antibiotics: Broad-spectrum empirical antimicrobial therapy should be commenced early after obtaining blood for culture and microscopy. Many studies have shown mortality improvement when the antibiotic is administered within 1 h of the recognition of sepsis and hypotension[6]. The selection of the antimicrobial agents considers antifungal, antiviral, or antiparasitic agents that are directed by the clinical finding, knowledge of the common local pathogens and their antibiotic resistance profiles, and consideration of the patient's potential predisposition to a specific infection, for example, immunosuppression as for Cirrhosis[6]. Avoid prolonged therapy with broad-spectrum antimicrobials because it promotes the evolution of resistant organisms, which can lead to the failure of the treatment[6]. Sepsis in cirrhotic patients requires a high grade of suspicion so that empiric antibiotics might be started as early

Table 4 Summary of selected studies

Ref.	Year	Aim	Setting	Results	Conclusion
Philips *et al*[11]	2021	Assessed the use of 5% human fluid for the resuscitation of cirrhotic patients with sepsis	ICU	Found that primary, the two groups were different, with *P* values of less than 0.05, which is statistically significant. Study was done among 300 patients with sepsis with hypotension and cirrhosis 123 (n = 154, 79.8% receive albumin, and 131 (154, 85.1%) receive normal saline. Outcome related to MAP. measurement only 7.5 (n = 23) show reversal hypotension MAP > 65 at the end of the first hours of the resuscitation period; after 3 h, it was 11.7% (n = 18) and 32% (n = 5) in albumin and saline groups respectively (P = 0.008); Secondary outcome related to MAP, in the first hours while the study group, 62 patients (20.1%, n = 308) fluid resuscitation and sustained at 2 h in 42 (13.6%) patients improved MAP more than 65 mmHgf was seen in 25.3 (n = 39). In the albumin group at the end of the first hour compared to 14.9% (n = 23) patients in the saline group (P = 0.03). In second hour 17.5% (n = 27) in albumin group compare 9.7% (in = 15, P = 0.06) in saline group 5% albumin showed better improvement of MAP hemodynamic response compared to saline with P < 0.001 that is statistically significant. First hour, HA *vs* NS: 99.5 ± 7.9 *vs* 101.7 ± 8.8, P = 0.02; Second hour 97.9 ± 5.5 *vs* 103.4 ± 6.7, P < 0.001); Third hour 96.6 ± 3.6 *vs* 103.1 ± 5.9, P < 0.001	Found 5% human albumin correcting hypotension in sepsis with cirrhosis patients. Data were analyzed using IBM SPSS 22.0 statistic window. Quantitative variables were presented as mean ± SD and presented as number and percentage, the Chi-square or Fischer's exact test was used for categorical data, and continuous data were analyzed using the students' test or Mann-Whitney *U* test, Kaplan-Meier used for survival curves
Arabi *et al*[13]	2010	Assess the use of a low dose of hydrocortisone in cirrhotic patients with sepsis	ICU	140 patients screened 75 enrolled in the study. 60 (80%) with shock within 24 h and 71 (95%) with shock within 48 h. Twenty-eight days mortality with hydrocortisone treatment compared to its placebo 33 (85%) *vs* 26 (72%) relative risk (RR) 1.17, 95% confidence interval 0.92-1.49, P = 0.19). There was relative adrenal insufficiency in cirrhosis patients presenting with septic shock. Hydrocortisone show significant hemodynamic improvement. The 28-day mortality 33 (85) P = 0.19 and ICU mortality 24 (62) P = 0.64. Hemodynamic response was shock reversal 24 (62) P = 0.05 statistically significant	Found that corticosteroids improve hemodynamic status of the patient but do not change mortality
Rinaldi *et al*[5]	2013	The aim was to evaluate the effect of adherence to evidence-based guidelines of the surviving sepsis campaign (SSC) on the outcome of cirrhotic patients with shock admitted to the ICU	ICU	30 day-mortality of cirrhotic patients with septic shock in ICU is extremely high, and the application of SSC guidelines did not seem to improve the survival rate in this population. In addition, approximately 40% of cirrhotic patients developed an infection. 30 days mortality of 31 (81.6%) patients, 13 (86.6) with the bundle completed and 18 (78.2%) with the bundle not completed. This difference was not statistically significant	Hydrocortisone was associated with shock resolution but no survival modification. Chang *et al*[17], (2022) show that sepsis in cirrhotic patients has poor outcome than sepsis without Cirrhosis. And Sauneuf *et al*[18], (2013) found also that sepsis in cirrhotic patient survival remain low despite current management. Bal *et al*[24], 2016 found that mortality in 50 days in septic with cirrhosis patients was 43%
Thierry *et al*[23]	2007	Assess the use of echocardiography in assessing the LVEF on cirrhotic patients with septic shock	ICU	Show clinical and echocardiographic hemodynamic parameters between patients with Cirrhosis and without Cirrhosis; Cirrhosis had higher. Without Cirrhosis, Cirrhosis had higher values for the CI (3.69+/-1 *vs* 2.86+/-0.81/min/m2, P = 0.02. SI (37.5 ± 8 *vs* 32.4 ± 7 mL/m2, P = 0.04); LVEF (67 ± 7 *vs* 55.9 ± 12%, P = 0.005 and lower value for the SVR (1636.1 ± 523 *vs* 2136.6+/-633 dynes/cm5 m2, P = 0.04). The MELD score was not significantly correlated with the CI (R = 0.20, P = 0.49, or S (r = 0.15, P = 0.6). Mortality in ICU was 53% overall (64% *vs* 45%, P = 0.27), not statistically different from the patient without Cirrhosis	Show that echocardiography is of important help in the management of Cirrhosis with sepsis, showing hyperdynamic syndrome with high LVEF
Guo *et al*[26]	2019	Assessment of VCS parameter for evaluation of sepsis in cirrhotic patients	ICU	52% of positive culture in septic patients with Cirrhosis, with traditional infection markers (PCT, IL-6) and sCD163 between the two groups significantly different (P < 0.001). VCS parameters WBC range from 1.4 to 18.3 in sepsis, and leucocytes range from 1.6 to 19.2 in patients with infection no difference in the two groups for WBC. Test sensitivity was 75.9%, and a specificity of 73% was achieved	Reviewed the management of cirrhosis patients with sepsis and proposed: Blood vulture collection, white cell volume determination, procalcitonin and interleukin -6 and sCD163 test, and he concluded that VCS parameters predict the presence of infection early in cirrhotic patients
Villareal *et al*[15]	2016	Assessing the usefulness of procal-citonin for diagnosing infection in cirrhotic patients	ICU	Found that the mean scores as mean child-Pugh score 9.5 ± 2 and MELD score 23 ± 8 with P = 0.14 and P = 0.33, respectively, and there were not statistically significant for Cirrhosis with and without infection, and the mortality was high 62.9%. Procalcitonin (PCT) as biomarkers was found to be higher in a patient with infection than those without infection 4.20 (1.4-	Procalcitonin as biomarker might help with infection diagnosis in cirrhotic patients, and P. Fischer *et al*. (2019) found that both presepsin and resistin may be reliable markers of

				10.2) *vs* 0.16 (0.1-0.23) through statistically significant differences were not reached $P = 0.53$, severe sepsis or septic shock was associated with higher PCT	bacterial infection in patients with decompensated liver cirrhosis and have similar diagnostic performance compared to PCT
Chen *et al* [25]	2019		ICU	Find that the mean time of initiation of the antibiotic treatment was 3.5 h in the patient (afebrile: 4.3 h, febrile 2.8 h $P = 0.23$ high incidence of the afebrile group admitted in ICU (43.6% *vs* 20.4% $P = 0.01$) and higher 30 days mortality in afebrile group 40% *vs* 18.4%) $P = 0.02$) and endotracheal intubation 27.3% *vs* 10.2%, $P = 0.03$) infection	Found that the cirrhotic patient has an atypical presentation, and the qSOFA score or CLIF-SOFA score has a better predictor ability
Umgelter *et al*[21]	2008	Assess the outcome of the continuous low dose of terlipressin (TP) in a septic shock patient	ICU	Find that ICU admission patients had a mean age of 58 ± 85 mean Child-Pugh score of 13.8 ± 0.8, and a mean APACHE ii score of 31 ± 6 where TP decreases systemic vascular resistance index and norepinephrine (NE) doses needed to obtain the target MAP decreased, while cardiac index CI remained stable, median survival after initiating TP was ranging 5-15 days	Found that TP at a dose of 2 ug/kg can be used as an adjunct to NE in a cirrhotic patient with sepsis for hemodynamic improvement
Durst *et al*[20]	2021	The study aimed to evaluate the use of vasopressor in septic shock with cirrhosis and without cirrhosis	ICU	state that sepsis in cirrhosis was more likely to occur than in non-cirrhotic 34 (55.7%) versus 23(37.8%), $P = 0.046$, and received steroid 38.3% and 19.7%, respectively $P = 0.024$. The cirrhosis group requires increased median (IQR) total vasopressor dosage when compared to non-cirrhotic [71.5 (15.5-239.5)] *vs* 24.7 (5.3-77.9) mg NE equivalent, $P = 0.003$ and required a significantly higher total number of vasopressor agents 3 (1-4) *vs* 2 (1-3) agents $P = 0.03$. The length of ICU stays 7.0 (3.6-11.4) *vs* 5.0 (2.6-10.4) days $P = 0.146$ no statistically significant and MAP goal greater than baseline BP was 3 (4.9%)	Found that for sepsis and Cirrhosis needing vasopressors, MAP should be maintained above 60 mmgh, and blood culture and antibiotic should be started early as a survival campaign guideline
Galbois *et al*[27]	2015	Assessment of VCS parameter for evaluation of sepsis in cirrhotic patients	ICU	Found that cirrhosis patients with sepsis admitted to ICU were child-Pugh c without mottling, and mortality at 14 days was 71% (at day 28:78% in ICU: 76% in hospital: 82%). Hemodynamic parameters at 6 h were: MAP more than 65mmgh that was 88%, CVP more than 8mmgh: was 90%, $ScvO_2$ more than 81%, Urine output more than 0.5 mL/kg/h: 24%. Thenar and knee Sto2 at H6 to predict the outcome. Thenar Sto2 levels measured at H0 and H6 were not different in survivors and non-survivors. [H0: 77% (72-87) *vs* 84% (79-90), $P = 0.11$, H6:84% (79-89) *vs* 83% (71-92), $P = 0.89$]. Mottling score changes during the first 24 h of septic shock in a patient with and without Cirrhosis; in survivors, the proportion of patients with a mottling score of more than 2 decreased over time in both groups. in non-survivors, the proportion of patients with severe mottling score (4-5) increased over time in both groups. In non-survivors, the proportion of patients with a mottling score (0-1) was higher in patients with Cirrhosis than in patients without at H0 $P = 0.001$) and at H6 ($P = 0.02$), but was not significantly later	Described that mottling score and knee StO_2 measurement at 6 h after vasopressors have excellent 14 days mortality prediction
Piccolo Serafim *et al*[14]	2021	The study evaluates the use of steroids in a patient with septic shock and Cirrhosis	ICU	Found that patients who received steroids received a higher total of vasopressors (91.2 mg *vs* 39.1 mg, $P = 0.04$) and lower of lactate (1.8 mmol/L *vs* 2.6 mmol/L, $P = 0.007$)	Show that steroids did not improve mortality despite hemodynamic changes
Chebl *et al*[22]	2021	Assess the outcome and mortality predictor of cirrhosis patients with sepsis	ICU	found that cirrhotic patients were more likely to get intubated than non-cirrhotic patients (72.49% *vs* 61.62%, $P = 0.001$), and there was no statistically significant difference in mechanical ventilation duration or ICU LOS among survivors. Cirrhotic patients have higher hospital mortality than non-cirrhotic patients (64.79% *vs* 31.54% $P = 0.001$) and higher ICU mortality (47.47% *vs* 18.05% $P = 0.001$)	proposed as management of cirrhotic patient with sepsis to keep MAP > 65 mmgh with vasopressors, and start vasopressors early because of reduced oncotic pressure and risk of pulmonary oedema, avoid aggressive fluid resuscitation, cirrhotic patients have higher lactate levels
Maimone *et al*[12]	2022	Compare the 20% albumin to plasmolytes in managing Cirrhosis and sepsis in the intensive care unit.	ICU	Found that sepsis and septic shock in cirrhotic patient was the leading cause of acute decompensation or acute, chronic liver failure and had a poor prognosis and increased mortality	Show that albumin 20% increases MAP above 65 mmgh 3 h after infusion compared with plasmolyte and restores hemodynamic status rapidly but induce pulmonary oedema; why is it important to close monitoring with ultrasound so early detection of pulmonary oedema and management
Bal *et al* [24]	2013	The aim is to predict 50 days in hospital mortality in decomposed cirrhosis	ICU	Bal *et al*[24] study show that 50 days mortality in ICU was 43.11% of the patient admitted	Show that patients admitted to intensive care units with sepsis and Cirrhosis have poor prognoses and are a poor

		patients with SBP			candidate for ICU
Baudry *et al*[9]	2019	Assess the prognosis of sepsis in cirrhotic patients.	ICU		
Sauneuf *et al*[18]	2013	Assess the use of albumin as an adjuvant to vasopressors in managing septic shock in cirrhotic patients.	ICU	Find from 2005 to 2010, 40.5% were discharged from ICU, and 26% were alive six months after discharge. IV albumin was frequently given (57.1% *vs* 8.5%, $P < 0.001$), and crystalloid infusion was reduced at the same time [3 (1.7-4.5) L *vs* 6 (3-8,9) L, $P = 0.08$]. The ventilatory management with a smaller tidal volume 8.6 *vs* 7ml/kg, $P = 0.001$). Intensive insulin therapy and low-dose glucocorticoids were also used frequently during the second period, 83.3% *vs* 31.9% $P < 0.001$ and 81% *vs* 44.7, $P < 0.001$, respectively. Marked survival improvement in ICU as compared 1997-2004 period (40% *vs* 17%, $P = 0.02$, and 29% *vs* 6%, $P = 0.009$, respectively)	
Sasso *et al* [19]	2020	Assess the Prediction of mortality in a cirrhotic patient	ICU	Study shows changes in SOFA score median (IQR) in a cirrhotic patient. 24 h post admission 2.5 (0.75 to 5, $P = 0.122$ and 48 h post admission 1(0 to 4) $P = 0.269$. End of vasopressor therapy 0 (-3.5 to 21, $P = 0.963$, that is not statistically significant. But the duration of vasopressor in (hour) median (IQR) 86 (42.0-164.5) $P = 0.003$. MAP goal decreased during vasopressor course *n* (%) 13 (21.3) $P = 0.041$ statistically significant	Concluded that mechanically ventilated cirrhotic patients with sepsis have an extremely poor prognosis, and vasopressor use was strongly a predictor of mortality
Chang *et al*[17]	(2022)	The study aimed to determine whether septic patients with liver cirrhosis had worse survival than patients without liver cirrhosis	ICU	Found that liver cirrhosis was more common in male patients with 48% median range APACHE II was 25.5%, 27% of ICU mortality, sepsis with compensated liver cirrhosis mechanical ventilation 24% *P* value 0.179 and 4% ($P = 0.842$) needed for renal replacement therapy	
Fischer *et al*[16]	2019	Assess the use of presepsin and resistin as markers of bacterial infections in cirrhotic patients with sepsis	ICU	Found that 63% of the aetiology of Cirrhosis was alcoholism, 46% was bacterial infection (SBP), as infection markers presepsin, resistin, CRP, and PCT for predicting 28 days survival were AUROC = 0.74 (95% VI: 0.64-0.84) ($P < 0.001$), 0.68 (95%CI: 0.57-0.82) ($P = 0.006$, 0.74 (95%CI: 0.64-0.84)($P < 0.001$) and 0.70 (95%CI: 0.59-0.81) ($P = 0.001$) respectively	

CI: Confidence interval; ICU: Intensive care unit; IL-6: Interleukin 6; LVEF: Left ventricle ejection function; MAP: Mean arterial pressure; MELD: Model for End-Stage Liver Disease; NE: Norepinephrine; PCT: Procalcitonin; qSOFA: Quick sequential organ failure assessment; RR: Relative risk; SBP: Systolic blood pressure; SSC: Surviving sepsis campaign; TP: Terlipressin; VCS: Value of volume conductivity and scattering; WBC: White blood cell.

as possible. Each hour delay in the starting the antimicrobial increases mortality by 1.86 times. Broad-spectrum antibiotics should be considered in patients at risk for resistant bacteria[6,7]. Early antibiotic start and intravenous administration of albumin 5% or 20% decrease the risk of renal failure development and improve survival in a cirrhotic patient with sepsis[2].

Procalcitonin: Procalcitonin is used as a biomarker for the risk of severe bacterial infection and for stopping antimicrobial therapy, but its role in cirrhotic patients has not been established yet[7]. In contrast, Villarreal *et al*[15] found that procalcitonin might be helpful in identifying bacterial infections in cirrhotic patients. Fischer *et al*[16] by Fischer *et al*[16] concludes that both presepsin and resistin are reliable markers of bacterial infection in patients with decompensated liver cirrhosis and have similar diagnostic performance to procalcitonin.

Liver transplantation: Liver transplantation is the definitive treatment for cirrhotic patients[7]. Cirrhotic patients are prone to bacterial infections and have higher mortality. Early therapeutic management of sepsis in the cirrhotic patient is crucial, and treatment should focus on correcting hypotension and avoiding aggressive fluid resuscitation[22]. Echocardiography can help diagnose hyperdynamic syndrome with high LVEF in cirrhotic patients with sepsis. Blood tests and VCS parameters can predict the presence of infection early in cirrhotic patients[23,26]. Mottling score and knee score and tissue oxygen saturation measurement six hours after vasopressors have an excellent 14-day mortality prediction[27]. Sepsis in cirrhotic patients has a poor outcome compared to sepsis without cirrhosis. Vasopressors, mechanical ventilation, and corticosteroids are suggested treatments, but mortality in 50 days in cirrhosis patients with sepsis was 43%. Mechanically ventilated cirrhotic patients with sepsis have an extremely poor prognosis, and vasopressor use was a predictor of mortality[17,18,19,24]. Cirrhotic patients have atypical presentations, and the qSOFA score or CLIF-SOFA score has better predictive ability[25].

Renal-replacement therapy and liver-support system: The use of hemofiltration in patients with sepsis

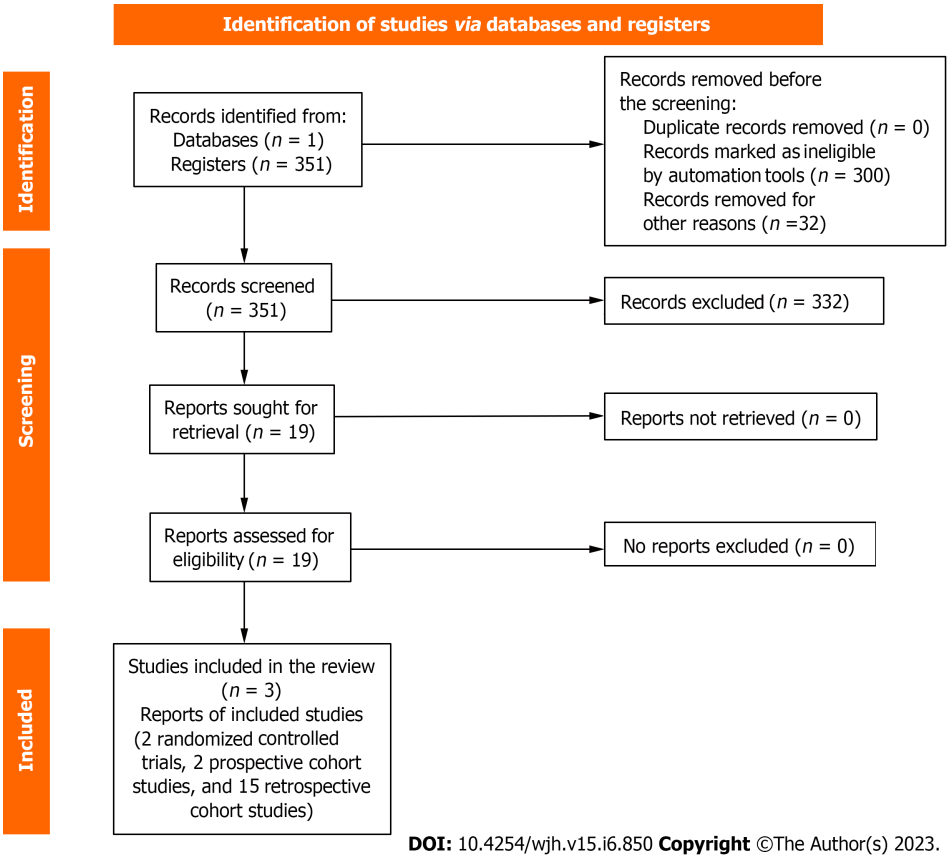

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Figure 1 PRISMA-P protocol for the systematic review.

has the potential benefit of alleviating the systemic inflammation of sepsis by removing circulating inflammatory mediators. However, two RCTs did not demonstrate significant reduction in inflammatory mediators nor patients' outcomes. Therefore, hemofiltration should not be recommended for routine management of patients with severe sepsis[1,6]. For liver support in the management of cirrhosis, it is recommended to treat the grade of ascites that are grade1 (mild) or grade 2 (moderate) where it is managed out of the ICU with restricted dietary sodium intake, start antidiuretic and monitor urea and electrolyte. For grade 3 that have a large volume of ascites with respiratory implication, paracentesis is recommended followed by dietary sodium restriction and diuretic therapy. Antibiotic prophylaxis should be used to prevent severe sepsis in a cirrhotic patient with ascites, gastrointestinal bleeding, or with more than one episode of spontaneous bacterial infection[4].

Glucose control: Hyperglycemia and insulin resistance are common in sepsis, and hyperglycemia may act as a procoagulant, impair neutrophil function, and increase the risk of death. Therefore, it is recommended to monitor and control glucose levels in patients with sepsis[1].

Infection source control: Source control in sepsis involves physical measures for removing the focus of infection. It is essential to identify and manage the source of infection promptly in the ICU[6].

DISCUSSION

The management of sepsis in cirrhosis patients is crucial to decrease the high mortality rate associated with this condition. In recent years, research has aimed to find the most effective therapeutic management for sepsis in cirrhosis patients. Interestingly, current therapeutic strategies for sepsis in cirrhosis patients are similar to the SSC international guidelines accepted for the general population.

Despite current management strategies, mortality remains high in cirrhosis patients with sepsis. Mortality rates are currently around 38%, with 30% of deaths due to infection[28]. Liver-specific scores, such as the CLIF-SOFA, CLIF-C Acute-on-Chronic Liver Failure (ACLF), and CLIF-C acute decompensation, have been developed to predict mortality in severely decompensated cirrhosis patients[29].

As the cirrhotic liver patient is prone to bacterial infection and impaired immunity status, which triggers complications related to cirrhosis such as hepatic encephalopathy, ascites, variceal bleeding, or hepatorenal syndrome[30-33] that further impaired prognosis[34]. The SSC guideline recommends early

detection of the source of infection, early initiation of antibiotics, fluid resuscitation, vasopressors, and corticosteroids[11,12].

Several studies have investigated the effectiveness of different therapeutic strategies for sepsis in cirrhosis patients. The use of human albumin 5% and 20% has been found to be beneficial for correcting hypotension and maintaining MAP above 65 instead of crystalloid[11,12]. Furthermore, norepinephrine has been found to be the best vasopressor for correcting hypotension in cirrhosis patients with sepsis, and combination therapy with terlipressin and norepinephrine has also been found to be effective[20, 22].

One interesting finding is that early vasopressor administration may be more beneficial than aggressive fluid administration in cirrhotic patients with sepsis. Chebl *et al*[22] found that early use of vasopressors was associated with better outcomes in cirrhosis patients. However, the use of corticosteroids did not show a decrease in mortality in cirrhotic patients with sepsis[9,18,19].

The management of sepsis in cirrhosis patients requires early detection and intervention with antibiotics, fluid resuscitation, vasopressors, and corticosteroids. While current management strategies are similar to those recommended in the SSC international guideline[36], studies have shown that the use of human albumin and norepinephrine or combination therapy with terlipressin and norepinephrine may be more effective. Choudhury *et al*[35] found that terlipressin is as effective as noradrenaline in increasing the MAP of more than 65 mmHg at 6 h and 48 h, and has a potential role in treating and preventing variceal bleeding as well as acute kidney injury[36]. Despite these efforts, mortality remains high, emphasizing the need for further research in this area to improve outcomes in cirrhosis patients with sepsis[37].

The use of EASL-CLIF criteria on ACLF and CLIF-SOFA for prognostication of sepsis in cirrhotic patients admitted to the ICU has gained significant attention[8,38]. These scoring systems have been developed to assess the severity of liver disease and predict mortality in severely decompensated cirrhosis patients[39-42]. By incorporating organ failure parameters, such as cardiovascular, renal, respiratory, neurological, hematological, and hepatic dysfunction, these criteria provide a comprehensive evaluation of the patient's condition. In the context of sepsis, the EASL-CLIF criteria can help identify cirrhotic patients at higher risk of poor outcomes and guide clinicians in making informed decisions regarding treatment strategies and resource allocation[8,38]. The CLIF-SOFA score, in particular, has shown promise in predicting short-term mortality and facilitating risk stratification in this vulnerable population[29,43-45]. By utilizing these criteria, healthcare professionals can enhance their ability to prognosticate sepsis in cirrhotic patients, thereby improving patient care and potentially reducing mortality rates. Further research and validation studies are warranted to optimize the use of EASL-CLIF criteria for prognostication and guide personalized interventions in this challenging clinical scenario[8,38].

The studies included in this systematic review provide valuable insights into the management of sepsis in patients with cirrhosis. However, these studies also have several limitations that need to be acknowledged. One of the major limitations of these studies is the absence of complete guidelines on the management of sepsis in patients with cirrhosis[37]. Although different therapeutic steps were proposed, these studies do not provide a comprehensive guide for managing these patients.

Moreover, most of the studies included in this systematic review were RCTs and cohort prospective and retrospective studies. While these studies provide strong and moderate evidence, they also have limitations in terms of generalizability. This is because most of these studies were conducted on single centers with small sample sizes. For instance, studies by Rinaldi *et al*[5], Philips *et al*[11], Maimone *et al*[12], Arabi *et al*[13], Sauneuf *et al*[18], Durst *et al*[20], Thierry *et al*[23] Bal *et al*[24], Chen *et al*[25], Galbois *et al*[27] were conducted on small sample sizes, which limits the generalizability of their findings.

Furthermore, the prospective nature of some studies can also affect the results due to missing information. For example, studies by Rinaldi *et al*[5], Baudry *et al*[9], Philips *et al*[11], Maimone *et al*[12], Arabi *et al*[13], Piccolo Serafim *et al*[14], Chang *et al*[17], Sauneuf *et al*[18] Sasso *et al*[19] Durst *et al*[20], Chebl *et al*[22], Thierry *et al*[23], Bal *et al*[24], and Chen *et al*[25], and Galbois *et al*[27] were conducted prospectively and some information was missing, which can affect the accuracy of the results.

Moreover, retrospective studies have their limitations as well, as not all information was present. For instance, Rinaldi *et al*[5], Baudry *et al*[9], Philips *et al*[11], Maimone *et al*[12], Arabi *et al*[13], Piccolo Serafim *et al*[14], Villarreal *et al*[15], Fischer *et al*[16] Chang *et al*[17], Sauneuf *et al*[18], Durst *et al*[20], Umgelter *et al* [21] Chebl *et al*[22], Thierry *et al*[23], Bal *et al*[24], Chen *et al*[25], Guo *et al*[26] and Galbois *et al*[27], all suffered from selection bias and missing information bias.

In addition, it is important to acknowledge that this review has certain limitations. Although we made efforts to gather relevant sources, we were unable to conduct an exhaustive search, leading to some sources remaining unexplored. This constraint resulted from the time limitations imposed during the review process. Consequently, the review may not encompass the full breadth and depth of available literature on the management of cirrhosis patients with sepsis admitted to the ICU. Furthermore, it is worth noting that a substantial proportion of the included research papers were retrospective studies with occasional missing information. To enhance the understanding and enhance outcomes in cirrhotic patients with sepsis, further research endeavors are warranted.

CONCLUSION

In conclusion, sepsis in cirrhotic patients is a complex and challenging clinical scenario. Our systematic review of the literature revealed that there is no standardized approach to the management of sepsis in cirrhotic patients admitted to the ICU. Although there is evidence to support early identification of infection, prompt administration of antibiotics, and aggressive resuscitation with fluids and vasopressors, the optimal management of these patients remains unclear. Furthermore, the studies included in this review were limited by small sample sizes, single-center designs, and missing data, highlighting the need for larger, multicenter trials to establish best practices for managing sepsis in cirrhotic patients.

Despite these limitations, our review suggests that using prognostic scores such as SOFA, MELD, and MELD-Na can help identify high-risk patients and guide clinical decision-making. Furthermore, improving outcomes in septic cirrhotic patients will require a multidisciplinary approach, including collaboration between intensivists, hepatologists, infectious disease specialists, and other healthcare providers. With the growing burden of cirrhosis and sepsis worldwide, further research is urgently needed to clarify the optimal management of this complex patient population and improve outcomes for these critically ill patients.

ARTICLE HIGHLIGHTS

Research background

The background of the study lies in the physiological response of sepsis, characterized by a dysregulated inflammatory reaction to infection, which can progress to organ failure and death. Cirrhotic patients are particularly susceptible to sepsis-induced organ failure and have higher mortality rates. The imbalance of cytokine response, known as a "cytokine storm," plays a significant role in the worsening of liver function and the development of organ/system failure in severe sepsis. The severity of sepsis in cirrhotic patients is associated with increased production of proinflammatory cytokines. Additionally, cirrhotic patients with severe sepsis can experience complications such as shock, acute lung injury, coagulopathy, renal failure, or hepatic encephalopathy. Understanding the background and significance of sepsis in cirrhosis is crucial for effective management and improved outcomes.

Research motivation

The motivation behind this research is to address the impact of sepsis in cirrhotic patients and the associated challenges in managing this complex condition. Sepsis is a major cause of admission to intensive care units (ICUs), and its outcomes are worse in patients with comorbidities like cirrhosis. Organ dysfunction in sepsis, measured by the Sequential Organ Failure Assessment (SOFA) score, including liver failure, is linked to higher mortality rates. Defining sepsis and septic shock accurately remains challenging. Given the high mortality and complexity of sepsis in cirrhosis, understanding the key problems and finding effective solutions is crucial. Solving these problems not only improves patient outcomes but also contributes to future research in this field by providing insights into personalized interventions, risk stratification, and resource allocation.

Research objectives

The main objectives of this study are to determine the optimal management of sepsis in cirrhotic patients admitted to the ICU and to explore strategies for improving outcomes in this population. Realizing these objectives has significant implications for future research in this field. By identifying effective management approaches, personalized interventions can be developed to address the specific needs of cirrhotic patients with sepsis. Furthermore, understanding the impact of different interventions on mortality and organ failure rates provides valuable insights for risk stratification and resource allocation. The successful realization of these objectives contributes to the advancement of knowledge and practices in managing sepsis in cirrhotic patients, ultimately improving patient care and outcomes in this challenging clinical scenario.

Research methods

This study utilized a systematic review methodology following the PRISMA-P protocol to investigate the management of sepsis in cirrhotic patients admitted to the ICU. The inclusion criteria comprised cirrhotic patients over 18 years old with sepsis in the ICU, and the analysis focused on sepsis management and prognosis in this population. English-language randomized controlled trials, retrospective cohort studies, and prospective cohort studies were considered. The outcomes assessed included survival, ICU length of stay, and overall prognosis. Searches were conducted on PubMed, Google Scholar, Embase, and Cochrane databases, with filtering based on titles and abstracts. Relevant papers underwent full-text analysis, and only those meeting the inclusion criteria were included. This systematic review offers valuable insights into sepsis management and prognosis in cirrhotic patients

admitted to the ICU, utilizing a comprehensive approach to assess existing literature.

Research results

The study conducted a systematic review to investigate the management of sepsis in cirrhotic patients admitted to the ICU. The researchers selected 19 papers that met the inclusion criteria, focusing on survival and prognostic factors for this patient population. The findings indicated that albumin administration corrected hypotension in sepsis with cirrhosis, while corticosteroids improved hemodynamic status without affecting mortality. Procalcitonin was found to be helpful in diagnosing bacterial infections in cirrhotic patients, and vasopressors such as norepinephrine and terlipressin were recommended to maintain mean arterial pressure above specific thresholds. The prognosis was generally poor for cirrhotic patients with sepsis, especially for mechanically ventilated patients or those requiring vasopressors. The use of fluid resuscitation, particularly with human albumin, was recommended, and early antibiotic administration within the first hour showed improved outcomes. The qSOFA criteria were identified as a better predictor of adverse outcomes in sepsis, and echocardiography aided in diagnosing hyperdynamic syndrome. Liver transplantation was highlighted as the definitive treatment for cirrhotic patients. The study also mentioned the potential benefits and limitations of renal replacement therapy and liver support systems in sepsis management. Source control and glucose control were emphasized as essential aspects of sepsis management.

Research conclusions

The study proposes that the current therapeutic strategies for sepsis in cirrhosis patients, which are similar to the Surviving Sepsis Campaign guidelines for the general population, may not be sufficient in reducing mortality rates in this specific patient group. It highlights the need for further research and development of comprehensive management guidelines for sepsis in cirrhosis patients. The study suggests that the use of human albumin and norepinephrine, as well as combination therapy with terlipressin and norepinephrine, may be effective in correcting hypotension and improving outcomes in cirrhosis patients with sepsis. Additionally, it indicates that early administration of vasopressors could be more beneficial than aggressive fluid administration in this patient population. However, the use of corticosteroids did not show a decrease in mortality.

Research perspectives

Future research should focus on developing standardized management guidelines specifically tailored for sepsis in cirrhosis patients. These guidelines should encompass early detection of infection, appropriate antibiotic therapy, fluid resuscitation, vasopressor selection, and corticosteroid use. There is a need for larger, multicenter trials to validate the findings of existing studies and establish best practices for managing sepsis in cirrhosis patients. These studies should have larger sample sizes and address the limitations of previous research, such as single-center designs and missing data. Prognostic scores, such as SOFA, Model for End-Stage Liver Disease (MELD), and MELD-Na, should be further evaluated and incorporated into the management of sepsis in cirrhosis patients to identify high-risk individuals and guide treatment decisions. A multidisciplinary approach involving intensivists, hepatologists, infectious disease specialists, and other healthcare providers is essential for improving outcomes in septic cirrhotic patients. Collaboration and coordination among these specialties should be emphasized in future research and clinical practice.

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