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REVIEW

Role of microRNA-regulated cancer stem cells in recurrent hepatocellular carcinoma

Lei Li, Chen Xun, Chun-Hong Yu

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Abstract

Among the most common cancers, hepatocellular carcinoma (HCC) has a high rate of tumor recurrence, tumor dormancy, and drug resistance after initial successful chemotherapy or radiotherapy. A small subset of cancer cells, cancer stem cells (CSCs), exhibit stem cell characteristics and are present in various cancers, including HCC. The dysregulation of microRNAs (miRNAs) often accompanies the occurrence and development of HCC. miRNAs can influence tumorigenesis, progression, recurrence, and drug resistance by regulating CSCs properties, which supports their clinical utility in managing and treating HCC. This review summarizes the regulatory effects of miRNAs on CSCs in HCC with a special focus on their impact on HCC recurrence.

Key Words: Hepatocellular carcinoma; Cancer stem cells; MicroRNAs; Recurrence.

Core Tip: The liver cancer stem cells (LCSCs) play a crucial role in the development of hepatocellular carcinomas (HCCs) and play a significant role in the development of drug resistance and cancer recurrence. LCSCs are regulated by many factors, of which microRNAs (miRNAs) are an important part. miRNAs can influence the development of HCC by regulating the stem cell properties of LCSCs.

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INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the most prevalent cancers in the liver, accounting for about 75% of all liver cancers, with a poor clinical prognosis, resulting in 500000-600000 deaths each year[1-4]. In recent years, there has been substantial progress in the diagnosis and treatment of HCC, but the high recurrence and metastasis rates of HCC still pose a headache for doctors and patients. The proposal of cancer stem cell (CSC) theory provides us with a direction. CSCs are considered one of the very small cell types in tumor cells with unlimited proliferative potential, which can drive tumorigenesis, and development. They can confer unique drug resistance, recurrence, and metastasis capabilities to tumors [5-8]. Conventional cancer treatments only kill common cancer cells, but CSCs remain. When in the right microenvironment, CSCs begin to proliferate and differentiate, leading to cancer recurrence. In recent years, many studies have focused on liver cancer stem cells (LCSCs) and achieved satisfactory results. Therefore, targeting CSCs is considered a more promising approach to improving the outcomes of conventional treatments (Figure 1).

An example of a microRNA (miRNA) is a small, non-coding RNA that is produced by endogenous cells and can be used to regulate gene expression by binding to the 3' untranslated region (UTR) of genes to inhibit their translation[9,10]. It has been shown that miRNAs can regulate tumorigenesis, progression, invasion, and even tumor recurrence in HCC by acting as tumor promoters or suppressors [11,12]. Another important finding is that miRNAs can modulate the stemness profile of LCSCs to combat conventional therapy further. Pollutri *et al*[13] reported that miR-494 induces sorafenib resistance in HCC and is associated with stem cell phenotypes. Further research has demonstrated that miR-181 family members play a critical role in maintaining the stem cell characteristics of HCC cells in a study by Ai *et al*[14]. Therefore, we believe miRNAs play a key role in LCSCs, and understanding this information will help our further research and development of HCC therapies. This review summarizes recent years' research findings and reports, outlines the role of miRNAs in LCSCs, and discusses potential therapeutic strategies for HCC recurrence, intending to provide clinical practitioners with information about how to treat HCC patients effectively.

SURFACE MARKERS OF LCSCS AND THEIR ROLE IN HCC

A number of characteristics of LCSCs are similar to those of normal stem cells, including their ability to self-renew and differentiate. LCSCs are more prevalent *in vivo* than other tumor cell types. They can promote the growth of primary cancer cells and facilitate the metastasis of transplanted secondary tumors, and they are crucial in the recurrence of HCC. In order to identify and isolate CSCs effectively, it is mostly necessary to take advantage of surface markers. Common LCSCs are CD133, CD90, CD44, CD13, CD47, *etc*. During the past few decades, a growing body of evidence has been generated concerning the properties of specific surface markers on LCSCs, which has provided opportunities for investigating potential biological functions, signaling pathways, and therapeutic approaches for HCC (Figure 2). Table 1 summarizes the major surface molecular markers of LCSCs and their potential roles in HCC.

CD133

In 1997, CD133 was discovered as the first protein on the surface of neuroepithelial stem cells[15]. A transmembrane glycoprotein consisting of five transmembrane domains, two extracellular glycosylation chains, and three transmembrane domains is an important surface glycoprotein that serves as a cell surface marker. CD133 is expressed in embryonic epithelial stem cells, colon cancer, prostate cancer, pancreatic cancer, brain tumor, HCC, hematopoietic stem cells, and the like. CD133 was identified as a liver CSC marker in 2007[16-18]. According to studies conducted by our laboratory, the expression of CD133 in HCC cells is negatively related to the overall survival rate of patients with HCC and the rate of recurrence[19]. HCC patients with higher CD133 expression levels in the primary lesion tend to live shorter and have a higher recurrence rate postoperatively than those with lower CD133 expression levels[20]. HCC patients with higher CD133 expression levels also responded poorly to the conventional chemotherapy drug sorafenib. Several molecular mechanisms have been involved in the action of CD133 on tumors, including angiogenesis, self-renewal, growth, invasion, and chemoresistance. $CD133^+$ cells in HCC contribute to chemoresistance by preferentially activating the Akt/PKB and Bcl-2 cell survival receptors during the chemoresistance response[21]. As a result of the interaction between

Table 1 Hepatic cancer stem cell markers and their roles in hepatocellular carcinoma recurrence

Markers	Biological functions in LCSCs	Signaling pathways	Recurrence	Ref.
CD133	Tumor angiogenesis, growth, self-renewal, invasion, and chemoresistance	AKT/PKB, IL-8/CXCL1, Notch	High recurrence	[16-23]
CD90	Preferably in poorly differentiated HCC, inflammation, circulation, drug resistance, and lipid metabolism	TGF-β/Smad	A shorter time to recurrence	[25-28]
CD44	Extensive proliferation, self-renewal, invasion, and tunorigenicity	TGF-β, AKT/GSK-3β/β-catenin, AKT/ERK/CXCR4	The significant risk factors of recurrence	[30-36]
CD24	Cell surface glycoprotein, drives CSC genesis	Stat3/Notch	A prognostic predictor for recurrence-free survival	[37-41]
CD13	Tumorigenicity, cell proliferation, cell cycle, self-renewal, and chemoresistance	ERK1/2	Early recurrence	[42-44]
CD47	Tumor initiation, self-renewal, and metastasis	CTSS/PAR2, NF-κB, IL-6	Shorter recurrence-free survival	[45-47]
OV6	Invasive and metastatic potential, form tumors, invasiveness, metastasis, substantial chemoresistance	Wnt/β-catenin, CXCL12/CXCR4/β-catenin		[48, 49]
EpCAM	An early biomarker for HCC, self-renewal, differentiation, chemoresistance, highly invasion and tumorigenisis	Wnt/β-catenin	High recurrence	[50-54]

HCC: Hepatocellular carcinoma; CSC: Cancer stem cell; LCSCs: Liver cancer stem cells.

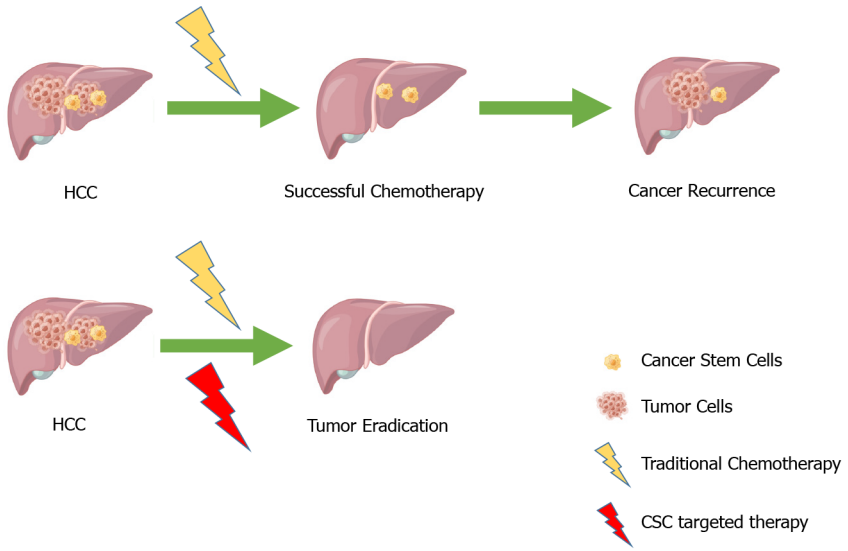

Figure 1 Combination therapy for hepatocellular carcinoma. Top: Conventional treatment may lead to tumour recurrence due to cancer stem cell reactivation. Bottom: Combination therapy leads to increased efficacy of tumour eradication. Adapted from Dzobo *et al*[8]. HCC: Hepatocellular carcinoma; CSC: Cancer stem cell.

neurotensin and interleukin-8 and CXCL1 signals in the liver, CD133 controls tumorigenesis, growth, and self-renewal of liver tumor-initiating cells[22]. The expression of iNOS in $CD24^{+}CD133^{+}$ LCSCs, but not $CD24^{-}CD133^{-}$ LCSCs, enhanced Notch1 signaling, and accelerated HCC initiation in the mouse xenograft tumor model[23].

CD90

$CD90^{+}$ cells from HCC Cell Lines were reported to have higher tumorigenic and metastatic potential than $CD90^{-}$ cells in 2008, suggesting that $CD90^{+}$ cells can be used as a marker of metastatic HCC[24,25]. Consistent with these findings, CD90 expression is positively correlated with HCC progression and poor prognosis[26-28]. CD90 is involved in varies molecular mechanisms, including inflammation, circulation, drug resistance, and lipid metabolism. In HCC 97H cells, the cyclin D1-mediated activation of Smad2/3 and Smad4 is an important regulatory mechanism in enhancing single sphere formation, enhancing the $CD90^{+}$ population, increasing stemness gene expression, and increasing chemoresistance [29]. Therefore, CD90 may also be a surface marker for poor prognosis of HCC and a potential therapeutic target.

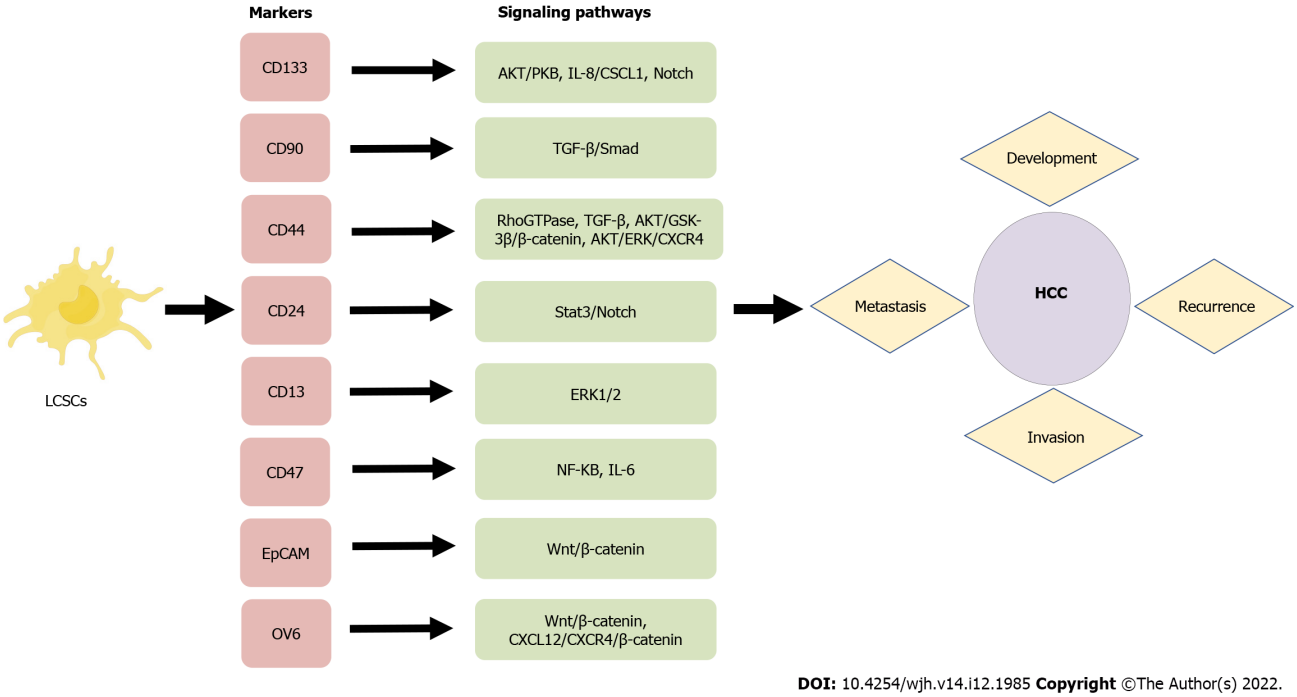

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Figure 2 Liver cancer stem cells markers and their potential related functional pathways in hepatocellular carcinoma. HCC: Hepatocellular carcinoma; LCSCs: Liver cancer stem cells.

CD44

A transmembrane glycoprotein named CD44 has been found to be expressed on numerous cells, including hepatocytes, endothelial cells, lymphocytes, and mesenchymal stem cells. It plays a role in extensive proliferation, self-renewal, invasion, and tumorigenicity[30]. It is possible to isolate cancer cells with stem cell markers by using CD44 alone or in combination with other markers. CD44v6, a variant of CD44, participates in the proliferation of HCC cells by stimulating the Ras/MAPK signaling cascade through interaction with c-Met[31]. Several studies have indicated that CD44s are associated with poor prognoses in hepatocellular carcinoma patients and regulate the TGF-β-mediated mesenchymal phenotype[32]. TGF-β1 and CD44 are synergistic in that they contribute to epithelial mesenchymal transition (EMT) induction and the development of CSC properties in tumor cells by interacting *via* the AKT/GSK-3β/β-catenin pathway in HCC cells[33]. In addition, CD44 is known to enhance HCC migration and local metastases by triggering the AKT/ERK pathway *via* the CXCR4 receptor[34]. Therefore, CD44 may be a potential treatment target for HCC and a marker of poor prognosis for HCC[35,36].

CD24

It is known that CD24 is a glycoprotein that is expressed on the surface of stem cells, mature granulocytes, and B cells, as well as in malignant tumors, such as HCC, breast cancer, colon cancer, and small cell lung carcinoma[37,38]. As well as driving CSC development, CD24 is involved in the differentiation of progenitor and stem cells in the liver and in metastatic development, self-renewal, and chemotherapy resistance of HCC cells[39]. $CD24^+$ liver tumor-initiating cells are driven to self-renew and initiate tumors *via* STAT3-mediated NANOG signaling[40]. An IL-6/STAT3 axis regulates CD24 and epithelial cell adhesion molecule (EpCAM) expression in liver cancer stem cells through long non-coding RNA DILC[41].

CD13

A membranous glycoprotein called CD13 is associated with the progression of cancer and drug resistance. Cell cycle, self-renewal, and tumorigenicity are all regulated by CD13, which is involved in tumorigenesis, cell proliferation, and chemoresistance[42]. The combination of CD13 with other surface markers could lead to prostate cancer tumorigenesis. The CD13 gene is expressed in LCSCs that are slow-growing or semi-quiescent, which contributes to the formation of HCC tumors[43]. Quiescent $CD13^+$ CSCs accumulate after chemotherapy in HCCs, serving as a source of recurrence[44].

CD47

CD47 is a transmembrane member of immunoglobulin associated with immune evasion, tumor apoptosis, metastasis, tumor-initiating ability, chemoresistance, and proliferation in various cancers. In

addition to tumor initiation and self-renewal, CD47 also plays an important role in metastasis in HCC. HCC growth can be inhibited by suppression of CD47, which inhibits CTSS/PAR2 signaling *in vivo* and causes chemosensitization[45]. There is a positive correlation between CD47 and NF-κB expression in HCC samples from clinical trials[46]. Patients with HCC with upregulated CD47 expression had poor overall survival and recurrence-free survival, and IL-6 derived from macrophages infiltrating the tumor was shown to activate STAT3 and upregulate CD47 expression on hepatoma cells[47].

OV6

OV6, a monoclonal antibody raised against hepatic progenitor cells isolated from rat livers treated with carcinogens, was shown to be a marker for such cells. An HCC cell line expressing $OV6^+$ tumor-initiating cells has a greater potential for invasiveness and metastatic spread, both *in vitro* and *in vivo*, which promotes the metastasis and progression of HCC[48]. There was an association between higher levels of $OV6^+$ tumor cells, aggressive clinicopathologic features, and a poor prognosis. Inhibition of β-catenin signaling leads to a decrease in the proportion of $OV6^+$ cells in HCC cell lines and primary HCC tissues, which indicates the role of Wnt/β-catenin signaling in $OV6^+$ HCC cells[49].

EpCAM

As another transmembrane glycoprotein found in most epithelial tissues, the EpCAM plays a role in signal transduction, cell adhesion, migration, proliferation, and differentiation[50-54]. EpCAM was discovered as a biomarker early in the diagnosis of HCC. A strong correlation was found between EpCAM expression in LCSCs and differentiation, chemoresistance, high invasion, and tumorigenesis in HCC. EpCAM is a target gene for Wnt-beta-catenin signaling that may help improve HCC prognosis.

MIRNAS IN HEPATOCELLULAR CARCINOMA

Dysregulated miRNAs contribute to many critical processes in HCC, ranging from growth, proliferation, apoptosis, and differentiation to migration, invasion, and progress. Moreover, miRNAs are important in tumor recurrence and metastasis. Understanding miRNAs' biological roles and specific targets will help further research and development of HCC therapies. Table 2 summarizes the major miRNAs in HCC and their potential roles in HCC.

The upregulated miRNAs in HCC

Cells from HCC cell lines and patients express high levels of miR-21. There is a positive correlation between miR-21 expression and HCC migration and invasion. As a result of silencing miR-21, the protein levels of PTEN, RECK, PDCD4, and KLF5, as well as the protein and mRNA levels of KLF5, increase, leading to a reduction in HCC cell migration and invasion[55,56]. Hepatocellular carcinoma growth is promoted by exosomal miR-21 regulation of the TETs/PTENp1/PTEN pathway, and three novels predicted miR-21 targets (CAMSAP1, DDX1, and MARCKSL1) correlate with HCC patient survival[57,58]. There is an association between miR-130b-3p up-regulation in HCC and a poor prognosis[59]. Patients who undergo HCC resection are at an increased risk of recurrence if their miR-135a expression is high[60]. A direct target of TP53INP1 is MiR-155, which regulates the migration and invasion of liver cancer cells, EMT, and CSC acquisition (which is positively correlated with CD90 and CD133)[61,62]. Patients with HCC who express MiR-182-5p in tumor tissues are more likely to experience poor prognosis and recurrence of the disease at an earlier stage. miR-182-5p activates AKT/FOXO3a pathway and Wnt/β-catenin signaling by targeting FOXO3a, enhancing HCC proliferation, motility, and invasion both *in vitro* and *in vivo*[63]. As miR-221 targets PTEN and TIMP3 tumor suppressors through activation of the AKT pathway, liver cancer cells express high levels of miR-221 [64]. Upon Fas-induced fulminant liver failure, miR-221 is upregulated, which regulates liver expression of the p53 upregulated modulator of apoptosis[65].

The downregulated miRNAs in HCC

Several miRNAs like miR-9-3p, miR-26, miR-30a, miR-122, miR-125b, miR-142, miR-142-3p, miR-199b-5p, miR-200a, miR-203, miR-449a, and miR-541 showed lower levels in HCC than in healthy donors. HBGF-5 expression is significantly downregulated by miR-9-3p overexpression, HCC viability and proliferation are reduced, and ERK1/2 is strongly downregulated[66]. Apoptosis is promoted by MiR-26 by targeting ULK1, EphA2, TAK1, and TAB3, which enhance chemosensitivity and radiosensitivity in HCC cells[67-69]. MiR-30a inhibits HCC cell proliferation by targeting FOXA1 *via* the Ras/Raf/MEK/ERK signaling pathway, suppressing autophagy-mediated resistance and metastasis[70-72]. It facilitates tumor cell invasion, migration, and EMT when miR-30a is downregulated[73]. By downregulating miR-122, HCC cells proliferate, colonize, migrate, invade, metastasize, and activate IGF-1R and RAS/RAF/ERK pathways[74-77]. When miR-122 expression levels are elevated in HCC cells, it inhibits the EMT process by upregulating the expression of E-cadherin and downregulating ZEB1/2, Snail1/2, N-cadherin, and Vimentin[78]. miR-125b is correlated with cell proliferation, differentiation, metastasis, apoptosis migration, and EMT[79-81]. miR-125b overexpression attenuates EMT-associated chemores-

Table 2 The regulatory roles of miRNAs in hepatocellular carcinoma

miRNA	Target genes/pathways	Effects	Expression	Clinical relevance	Ref.
miR-9-3p	HBGF5, lncRNA SAMMSON, ERK1/2 pathway	Cell proliferation, migration, and invasion	Down	Lower levels in HCC than in healthy donors	[66]
miR-21	KLF5, CAMSAP1, DDX1, MARCKSL1, PTEN, AKT, D24 RECK, PDCD4, TETs/PTENp1/PTEN pathway, TGF-β1/smad3 pathway	Cell proliferation, migration, invasion, and metastasis	Up	Higher in HCC than in CHB and in healthy volunteers, early diagnosis	[55-58]
miR-26	ULK1, EphA2, TAK1, TAB3, NF-κB pathway	Apoptosis	Down	Poor survival	[67-69]
miR-30a	Beclin1, Atg5, Snail1, FOXA1, ADAMTS14, Ras/Raf/MEK/ERK pathway	Proliferation, apoptosis, metastasis, migration, invasion, and EMT	Down	Prevention of HCC recurrence	[70-73]
miR-122	ADAM10, ADAM17, IGF1R, SRF, SNAI1, SNAI2, WNT1, CREB1, BCL9, Cyclin G1, NMPDK4, LDHA, and CD133, Wnt/β-catenin pathway, IGF-1R pathway	Cell growth, proliferation, differentiation, metabolism, invasion, and EMT	Down	More sensitive to chemotherapeutic agents and improves the anti-tumor effect of sorafenib on HCC *in vivo*	[74-78]
miR-125b	MCL1, BCLw, IL-6R, SIRT7, SMAD2, SMAD4	Proliferation, metastasis, migration, and apoptosis	Down	A significantly longer time to recurrence and longer overall survival time	[79-82]
miR-130b-3p	HOXA5		Up	Poor prognosis, higher in patients with recurrence	[59]
miR-142	TGFβ, THBS4, LDHA, CD-133, HMGB1	Cell growth, metastasis, migration, and invasion	Down		[83-85]
miR-155	ZHX2, TP53INP1, TGF-β1 pathway	Cell proliferation, migration, invasion, and EMT	Up	Diagnostic biomarkers for HCC	[62, 62]
miR-182-5p	FOXO3, AKT, Wnt/β-catenin	Proliferation, motility, invasion, and metastasis	Up	Poor prognosis and early recurrence	[63]
miR-199b-5p	TGFβ, MAP4K3, DDR1	Metastasis, migration, invasion, and EMT	Down		[86, 87]
miR-200a	GAB1, FOXA2	Proliferation, invasion, migration, and EMT	Down	Biomarkers for early-stage HCC	[87, 89]
miR-203	Ki67, CAPNS1	Proliferation, invasion, migration, and metastasis	Down	Tumor recurrence and poor survival of patients with early-stage HCC	[90, 91]
miR-221	p53, PUMA, NF-kB, STAT3, AAV8, PTEN, TIMP3, TRAIL, RAS/RAF/ERK, AKT	Apoptosis, and proliferation	Up		[64, 65]
miR-449a	Notch1, FOS, Met, Calpain6, POU2F1, Notch pathway	Metastasis, apoptosis, proliferation, migration, invasion, and EMT	Down	Short-term recurrence	[92-94]
miR-541	ATG2A, RAB1B	Inhibited the growth, metastasis, and autophagy	Down	Associated with malignant clinicopathologic phenotypes, recurrence and survival	[95]

HCC: Hepatocellular carcinoma; EMT: Epithelial mesenchymal transition.

istance, migration, and stemness and negatively correlated with CSC marker, EpCAM and CD13 expressions in HCC specimens by targeting SMAD2 and SMAD4[82]. Increasing the amount of miR-142 in the cells results in a decrease in vitality, proliferation, and EMT outcomes, as well as an increase in THBS4 which is overexpressed by cancer cells, resulting in more rapid migration and vascular invasion [83,84]. As a result of miR-142-3p inhibiting self-renewal, initiating tumor growth, invasion, migration, inducing angiogenesis and resisting chemotherapy in HCC cells, miR-142-3p is directly targeting CD133 to control the ability to confer cancer and stem cell-like characteristics[85]. It was found that overexpression of miR-19b-5p increases cell aggregation, suppresses migration and invasion in HCC cells, and inhibits the metastasis of xenograft tumors in nude mice. Akt phosphorylation is inhibited by miR-199b-5p overexpression, and N-cadherin and DDR1 are directly targeted and inhibited by miR-199b-5p overexpression[86,87]. In HCC, microRNA-200a directly targets GAB1 and FOXA2 to suppress cell invasion, migration, and metastasis[88,89]. MiR-203 expression is significantly associated with tumor recurrence and poor survival in HCC patients with early-stage tumors. In contrast, miR-203 overexpression suppresses Ki67 and CAPNS1 expression to inhibit proliferation, invasion, and metastasis of hepatic residual HCC[90,91]. Activating EMT *via* the Notch pathway promotes invasiveness *in vitro* by

downregulating Calpain 6 and POU2F1; mir-449a induces apoptosis in liver cancer cells by downregulating Calpain 6 and POU2F1, it inhibits Met signaling and Snail accumulation in cells by targeting its 3'-UTR, and miR-449a contributes to short-term HCC recurrence[92-94]. HCC cells *in vitro* and *in vivo* are inhibited by miR-541 by inhibiting growth, metastasis, and autophagy, and the target genes are ATG2A and RAB1B[95].

Therapeutic potential of miRNAs targeting CSC

It has been demonstrated that miRNAs could be therapeutic targets for HCC, but miRNA-based therapies have not been well developed for clinical applications. CSC therapies targeting miRNA are considered to be one of the most promising cancer treatments. In this way, miRNAs can regulate multiple genes at once, contributing to the regulation of CSC-related pathways. For example, miR-365 can regulate LCSCs through the RAC1 pathway[96]; miR-520f-3p is involved in altering the sensitivity of HCC cells to sorafenib treatment under hypoxic conditions by increasing stem cell phenotype[97]; miR-4320 inhibited epithelial-mesenchymal transition and reduced stemness characteristics in HCC cells by targeting FOXQ1 expression[98]; miR-206 inhibited LCSCs expansion by regulating EGFR expression [99]; Li *et al*[100] found that miR-613 inhibits LCSC proliferation and differentiation through regulation of SOX9; therapeutic delivery of miR-125b in a mouse model reduces the expression of CSC markers and inhibits HCC metastasis[82]. The findings of these studies suggest that miRNA therapy combined with targeting CSCs can treat HCC. However, the development of miRNA therapy remains challenging. The development of miRNA delivery systems *in vivo* has always been an area of interest for clinical treatment research. A specific, stable, low toxicity and durable delivery system is our hope, but currently, in the clinical treatment of HCC, there is still no very suitable *in vivo* delivery system. Furthermore, CSCs have great heterogeneity between patients, and how to accurately target CSCs is also a problem that needs to be addressed further.

CONCLUSION

In recent years, although research focusing on CSC has entered a trend of rapid growth, there are still many problems to be solved in clinical translation and practical application, especially in HCC patients. Targeting CSCs is considered as a potential therapeutic approach that can overcome the shortcomings of traditional treatments and significantly inhibit tumor recurrence. miRNAs play key roles in the post-transcriptional regulation of genes, and miRNAs are involved in various biological processes, including tumorigenesis. miRNA therapy has been used in some tumors and has entered the clinical stage, such as miR-34a has been used in a phase 1 study in patients with advanced solid tumors[101]. In clinical treatment, miRNAs can enhance the sensitivity of LCSCs to treatment, and targeting the deregulated key miRNAs in LCSCs can effectively reduce the role of LCSCs in metastasis and recurrence[102-104]. El-Mahdy *et al*[105] summarized the key signaling pathways associated with miRNAs (such as TP53, PI3k/AKT/mTOR, JAK/STAT, Wnt/β-catenin, and MAPK pathways), through which miRNAs can further affect the cellular processes and responses of HCC to clinical treatment. Therefore, investigating the role of miRNAs in LCSCs can help improve the prognosis of HCC patients and inform the development of new therapies.

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Immunological classification of hepatitis B virus-positive hepatocellular carcinoma by transcriptome analysis

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Abstract

BACKGROUND

Hepatitis B virus (HBV) infection is a major factor responsible for HBV+ hepatocellular carcinoma (HCC).

AIM

An immunological classification of HBV+ HCC may provide both biological insights and clinical implications for this disease.

METHODS

Based on the enrichment of 23 immune signatures, we identified two immune-specific subtypes (Imm-H and Imm-L) of HBV+ HCC by unsupervised clustering. We showed that this subtyping method was reproducible and predictable by analyzing three different datasets.

RESULTS

Compared to Imm-L, Imm-H displayed stronger immunity, more stromal components, lower tumor purity, lower stemness and intratumor heterogeneity, lower-level copy number alterations, higher global methylation level, and better overall and disease-free survival prognosis. Besides immune-related pathways, stromal pathways (ECM receptor interaction, focal adhesion, and regulation of actin cytoskeleton) and neuro-related pathways (neuroactive ligand-receptor interaction, and prion diseases) were more highly enriched in Imm-H than in Imm-L. We identified nine proteins differentially expressed between Imm-H and Imm-L, of which MYH11, PDCD4, Dvl3, and Syk were upregulated in Imm-H, while PCNA, Acetyl-a-Tubulin-Lys40, ER-α_pS118, Cyclin E2, and β-Catenin were upregulated in Imm-L.

CONCLUSION

Our data suggest that "hot" tumors have a better prognosis than "cold" tumors in HBV+ HCC and that "hot" tumors respond better to immunotherapy.

Key Words: Hepatitis B virus; Hepatocellular carcinoma; Immunological classification; Transcriptomics; Tumor immunity; Cancer immunotherapy

Core Tip: First, for the first time, we identified immune-specific subtypes of hepatitis B virus (HBV) + hepatocellular carcinoma (HCC) based on immune signature scores and demonstrated that this new subtyping method was reproducible in three different datasets. Second, our subtyping method captures the comprehensive heterogeneity of HBV+ HCC in the tumor microenvironment, genomic integrity, protein expression profiles, DNA methylation profiles, tumor stemness, intratumor heterogeneity, and clinical outcomes. Third, our data suggest that it is copy number alterations but not tumor mutations responsible for the different immunity between the "hot" and "cold" tumor subtypes in HBV+ HCC. Finally, our identification of the immune-specific subtypes of HBV+ HCC may provide new insights into the tumor biology and identify the HBV+ HCC patients beneficial from immunotherapy.

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INTRODUCTION

Hepatocellular carcinoma (HCC) is a major cancer of the liver that constitutes around 90% of liver cancer cases[1]. Although traditional therapeutic approaches, including surgery, chemotherapy, radiotherapy, and targeted therapy, are effective in improving the survival of HCC patients, the overall survival prognosis of HCC patients is generally unfavorable[2]. More recently, immunotherapy, such as immune checkpoint blockade (ICB), has shown success in the treatment of various cancers, including HCC[3]. However, only a small proportion of cancer patients respond well to immunotherapies to date [4]. To this end, certain predictive markers for cancer immunotherapy responses have been uncovered, *e.g.*, PD-L1 expression[5], tumor mutation burden (TMB)[6], and mismatch repair deficiency[7]. In addition, the tumor immune microenvironment (TIME) plays an important role in immunotherapy responses[8]. Overall, the "hot" tumors infiltrated by a substantial number of tumor-infiltrating lymphocytes (TILs) are more responsive to immunotherapies, compared to the "cold" tumors lacking TILs[9]. Hence, an investigation of the TIME in HCC would aid in the prediction of immunotherapy responses.

With the recent emergence of large-scale cancer genomics data, such as the Cancer Genome Atlas (TCGA) (https://portal.gdc.cancer.gov/) and International Cancer Genome Consortium (ICGC) (https://dcc.icgc.org/), many studies have investigated the TIME in HCC based on these data[10-12]. For example, Gao *et al*[10] identified four immune-relevant subtypes of HCC based on the enrichment of 13 signatures and revealed significantly different molecular and clinical characteristics among these subtypes. Sia *et al*[11] uncovered an immune subclass of HCC representing nearly 25% of HCC cases, based on gene expression profiles in tumor, stromal, and immune cells. Based on the enrichment of immune cell subpopulations, Farha *et al*[12] identified two immune clusters of HCC, and found that the cluster enriched with M0 macrophages had a worse prognosis.

Despite these previous studies[10-12], the discovery of immune-specific subtypes of hepatitis B virus-positive (HBV+) HCC is worth investigating, considering that HBV infection is a major cause of HCC [13]. In this study, to characterize the immunological landscape of HBV+ HCC, we identified its immune-specific subtypes by the unsupervised machine learning in transcriptomic data. Furthermore, we comprehensively compared the clinical and molecular features of these subtypes. Our analysis would provide new insights into the HBV+ HCC immunity and its associated clinical and molecular features, as well as potential clinical implications for the management of this disease.

MATERIALS AND METHODS

Datasets

We obtained the TCGA Hepatocellular Carcinoma (TCGA-LIHC) dataset, including transcriptomes (RSEM-normalized RNA-Seq gene expression profiles), somatic mutations ("maf" file), somatic copy number alterations (SCNAs) ("SNP6" files), normalized protein expression profiles by Reverse Phase

Protein Array (RPPA), and clinical data, from the Genomic Data Commons (GDC) Data Portal (https://portal.gdc.cancer.gov/). We obtained other two HCC transcriptomic datasets (GSE14520 and GSE121248) from the Gene Expression Omnibus (GEO) (https://www.ncbi.nlm.nih.gov/geo/). A description of these datasets is provided in Supplementary Table 1.

Single-sample gene set enrichment analysis

We evaluated the enrichment of an immune signature or pathway in a tumor by the single-sample gene-set enrichment analysis (ssGSEA)[14]. This method extends the GSEA method[15] to obtain the enrichment scores of input gene sets in specimens with input of gene expression matrices and marker or pathway gene sets. The marker or pathway gene sets of immune signatures or pathways are presented in Supplementary Table 2.

Identification of immune-specific subtypes of HBV+ HCC

By hierarchical clustering, we identified immune-specific subtypes of HBV+ HCC based on the enrichment scores of 23 immune signatures. The 23 immune cell types included Pro-inflammatory cytokines, APC co-inhibition, APC co-stimulation, Cytolytic activity, Immune cell infiltrate, Inflammation-promoting, Interferon, M1 macrophage, MHC Class I, Myeloid-derived suppressor cell, T cell co-inhibition, T cell exhaustion, Th1 cell, Th2 cell, TILs, Activated dendritic cell, Eosinophil, Immature dendritic cell, Macrophage, Monocyte, Natural killer cell, Plasmacytoid dendritic cell, Activated B cell. Before clustering, we performed the Z-score normalization of the ssGSEA scores and converted them into distance matrices using the R function "dist" with the following parameter: Method = "euclidean." We performed the hierarchical clustering using the function "hclust()" in the R package "Stats" with the following parameters: method = "ward.D2" and members = NULL.

Class prediction

We conducted classification with the random forest (RF) algorithm. In the RF, the size of trees was 500, and the features were the 23 immune signatures. The prediction performance, namely the accuracy and weighted F-score, were reported. We performed this procedure using the R package "randomForest".

Survival analysis

We compared overall survival (OS) and disease-free survival (DFS) rates between two classes of samples with the Kaplan-Meier (K-M) method[16]. K-M curves were used to show the differences in survival rates, and log-rank tests were utilized to evaluate their significance.

Calculation of TMB, SCNAs, Stemness scores, intratumor heterogeneity scores, immune scores, and tumor purity in tumors

A tumor's TMB was defined as its total count of somatic mutations. We used GISTIC2[17] to calculate SCNA frequencies and amplitudes in the immune-specific subtypes of HBV+ HCC with the input of "SNP6" files. A tumor's stemness score was its ssGSEA score of the stemness marker genes, as shown in Supplementary Table 1. We measured intratumor heterogeneity (ITH) levels with the DEPTH algorithm [18], which evaluates ITH levels based on gene expression profiles. We assessed immune scores and tumor purity of bulk tumors using ESTIMATE[19]. The immune scores indicate the levels of tumor immune infiltration, and tumor purity represents the fraction of tumor cells within a tumor bulk.

Pathway and gene ontology enrichment analysis

To identify pathways that are more enriched in one group compared to another group, we first uncovered the genes significantly upregulated in the group versus another group by Student's *t* tests using thresholds of false discovery rate (FDR) < 0.05 and mean gene expression levels' fold change (FC) > 2. We then input the upregulated genes into the GSEA web tool[15] to obtain the Kyoto Encyclopedia of Genes and Genomes (KEGG)[20] pathways using a threshold of FDR < 0.05, which were more enriched in that group versus another class. Besides, we used the weighted gene co-expression network analysis (WGCNA)[21] to identify the gene modules of co-expressed genes. Based on the expression correlations between the hub genes in gene modules, we identified the gene ontology (GO) terms showing significant correlations with specific traits. The WGCNA analysis was performed with the R package "WGCNA" (version 1.68).

Statistical analysis

In comparisons of two classes of normally distributed data, including gene expression levels, protein expression levels, and the ratios of immune-stimulatory to immune-inhibitory signatures, we used two-tailed Student's *t* tests. In comparisons of two classes of data that were not normally distributed, including immune scores, stemness scores, ITH scores, TMB, and global methylation levels, we performed one-tailed Mann–Whitney *U* tests. We utilized the Spearman method to calculate correlations between immune scores and protein expression levels or pathways' enrichment scores, and reported correlation coefficients (ρ) and *P* values. We employed the Benjamini–Hochberg method[22] to

calculate FDR for adjusting for multiple tests. We performed all statistical analyses in the R programming environment (version 4.1.3).

RESULTS

Clustering analysis identifies two immune-specific subtypes of HBV+ HCC

This analysis identified two subtypes of HBV+ HCC according to the enrichment scores of 23 immune signatures by hierarchical clustering, consistently in three transcriptomic datasets (TCGA-LIHC, GSE14520, and GSE121248) (Figure 1A). We termed the subtypes Imm-H and Imm-L, respectively, which showed high and low immune signature enrichment, respectively (Figure 1A). To explore whether this classification is predictable, we took one of the three datasets as the training set and the rest as test sets, in turn, to predict the subtypes by RF based on the attribute values (ssGSEA scores of the 23 immune signatures). The 10-fold CV accuracies and weighted F-scores in the training sets were all above 90%. The prediction accuracies and weighted F-scores in test sets with TCGA-LIHC or GSE121248 as the training set were not less than 90% (Figure 1B). These results demonstrate that the subtyping is predictable.

We further compared the expression levels of 25 genes encoding human leukocyte antigens between the subtypes. Of note, in TCGA-LIHC, all 25 genes were expressed at significantly higher levels in Imm-H than in Imm-L (FDR < 0.01; FC > 1.5) (Figure 2A). The immune scores were significantly higher in Imm-H than in Imm-L, consistently in the three datasets ($P < 0.001$) (Figure 2B). Furthermore, *PD-L1*, an antitumor immunosuppressive signature, was more highly expressed in Imm-H than in Imm-L ($P < 0.01$) (Figure 2C). Nevertheless, the ratios of immunostimulatory to immunosuppressive signatures (CD8+/CD4+ regulatory T cells), the base-2 Log-transformed values of the geometric mean expression levels of all marker genes of CD8+ T cells divided by those of CD4+ regulatory T cells, were significantly higher in Imm-H than in Imm-L ($P < 0.05$) (Figure 2D). Taken together, these results support that Imm-H has stronger anti-tumor immunity compared to Imm-L.

The immune-specific subtypes of HBV+ HCC have different clinical and phenotypic features

We compared 5-year OS and DFS prognosis between the immune-specific subtypes of HBV+ HCC in TCGA-LIHC, which had survival-related data available. Notably, Imm-H displayed significantly higher OS and DFS rates than Imm-L ($P < 0.05$) (Figure 3A). It supports the positive association between antitumor immune responses and survival prognosis in HBV+ HCC. We further compared several tumor progression-associated phenotypic features, including tumor stemness and ITH. We found that both stemness and ITH scores were markedly higher in Imm-L than in Imm-H in two of the three datasets (TCGA-LIHC and GSE121248) ($P < 0.05$) (Figure 3B). As expected, tumor purity was consistently higher in Imm-L than in Imm-H in the three datasets (Figure 3C). Altogether, these results indicate more favorable clinical outcomes in Imm-H than in Imm-L.

Comparisons of genomic and epigenomic features between the immune-specific subtypes of HBV+ HCC

Tumor aneuploidy, also known as copy number alteration (CNA), is a typical genomic feature in tumors [23]. We found that Imm-L had higher frequencies of arm-level copy number amplification and deletion across chromosomes (Figure 4A). Moreover, Imm-L likely had higher amplitudes of copy number amplification and deletion across chromosomes as compared to Imm-H (Figure 4B). It suggested a higher level of genomic instability in Imm-L *vs* Imm-H, supporting a negative correlation between tumor aneuploidy and antitumor immunity[24]. Nevertheless, TMB showed no significant difference between Imm-H and Imm-L (Mann–Whitney *U* test, $P = 0.86$). Furthermore, we found that Imm-H had significantly higher global methylation levels[25] than Imm-L ($P = 0.006$) (Figure 4C). It conforms to a previous study showing that reduced DNA methylation levels promote antitumor immunosuppression [25].

Pathways and GO enriched in the immune-specific subtypes of HBV+ HCC

Based on the DEGs between Imm-H and Imm-L, we identified the KEGG pathways enriched in Imm-H and Imm-L common across the three datasets. Because there were no pathways enriched in Imm-L overlapping among the three datasets, we only attained the pathways enriched in Imm-H. We identified a total of 39 pathways highly enriched in Imm-H common in the three datasets. As expected, many immune-related pathways were on the list, including allograft rejection, antigen processing and presentation, apoptosis, asthma, autoimmune thyroid disease, B cell receptor signaling, cell adhesion molecules, chemokine signaling, complement and coagulation cascades, cytokine-cytokine receptor interaction, cytosolic DNA sensing, Fc epsilon RI signaling, Fc gamma R-mediated phagocytosis, graft *vs* host disease, hematopoietic cell lineage, intestinal immune network for IgA production, Jak-STAT signaling, leishmania infection, leukocyte transendothelial migration, natural killer cell-mediated cytotoxicity, NOD-like receptor signaling, primary immunodeficiency, T cell receptor signaling,

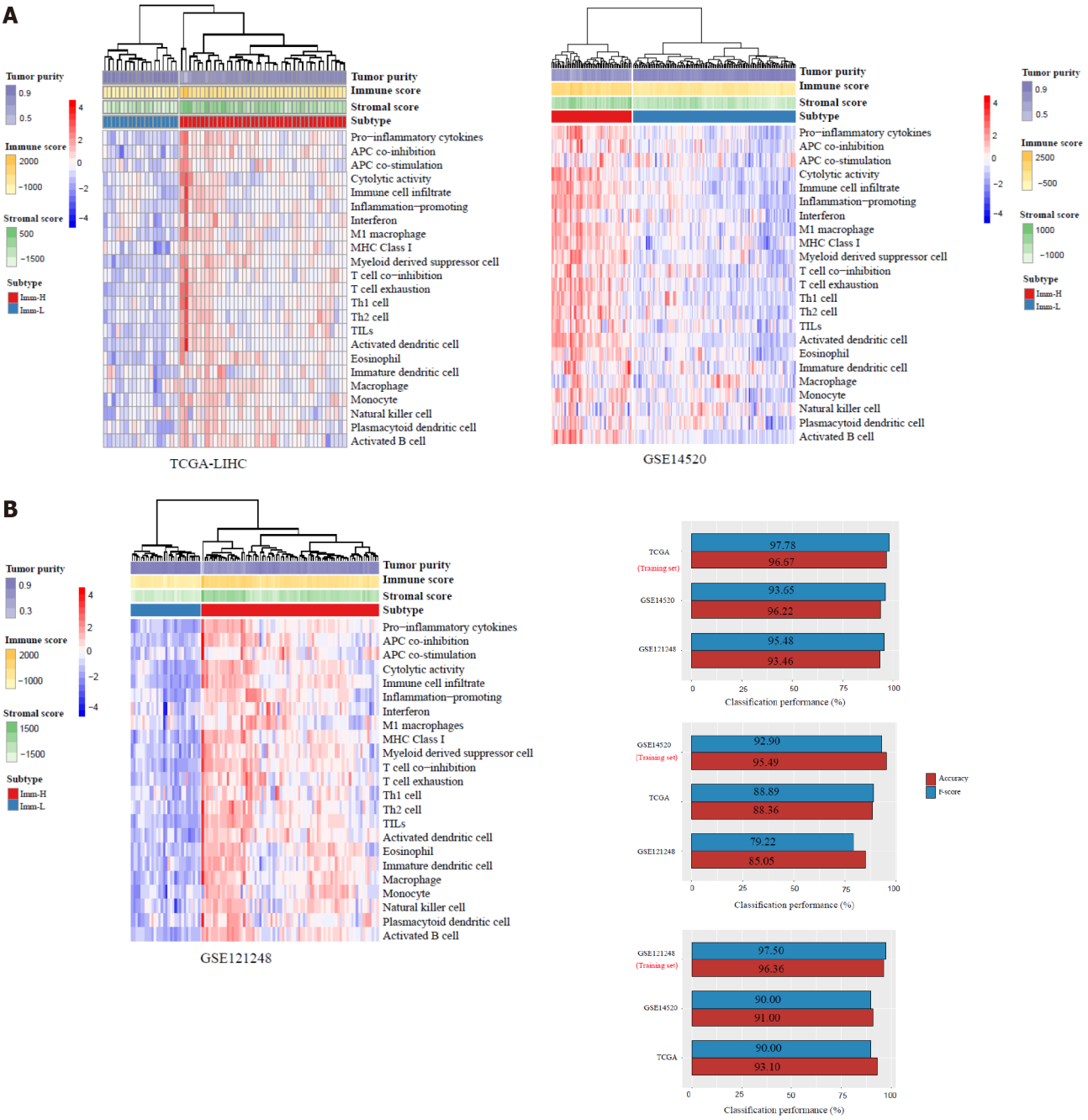

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Figure 1 Identification of immune-specific subtypes of hepatitis B virus + hepatocellular carcinoma by clustering analysis. A: Based on the enrichment scores of 23 immune signatures, hierarchical clustering identifies two immune-specific subtypes of hepatitis B virus (HBV) + hepatocellular carcinoma (HCC): Imm-H and Imm-L, with high and low immunity, respectively, consistently in three datasets; B: Prediction of the immune-specific subtypes of HBV+ HCC by the Random Forest algorithm using the 23 immune signatures as attributes. The 10-fold cross-validation results in the training set and classification results in test sets are shown.

systemic lupus erythematosus, Toll-like receptor signaling, and viral myocarditis (Figure 5A). Besides, several stromal pathways were included in the list, such as ECM receptor interaction, focal adhesion, and regulation of actin cytoskeleton. Interestingly, we found two neuro-associated pathways included in the pathway list: neuroactive ligand-receptor interaction, and prion diseases. It indicates that the activities of these neuro-associated pathways are positively correlated with antitumor immunity. Indeed, the enrichment scores of these pathways correlated positively with immune scores in these datasets (Spearman correlation, $P < 0.05$) (Figure 5B).

WGCNA[21] identified nine gene modules significantly differentiating HBV+ HCC by the immune-specific subtypes, OS, and/or DFS (Figure 5C). The gene modules upregulated in Imm-H while downregulated in Imm-L included the green module (with the representative GO term of immune response) and the yellow module (with the representative GO term of extracellular matrix) ($P < 0.001$). It is consistent with the results from the prior pathway analysis showing more highly enriched immune

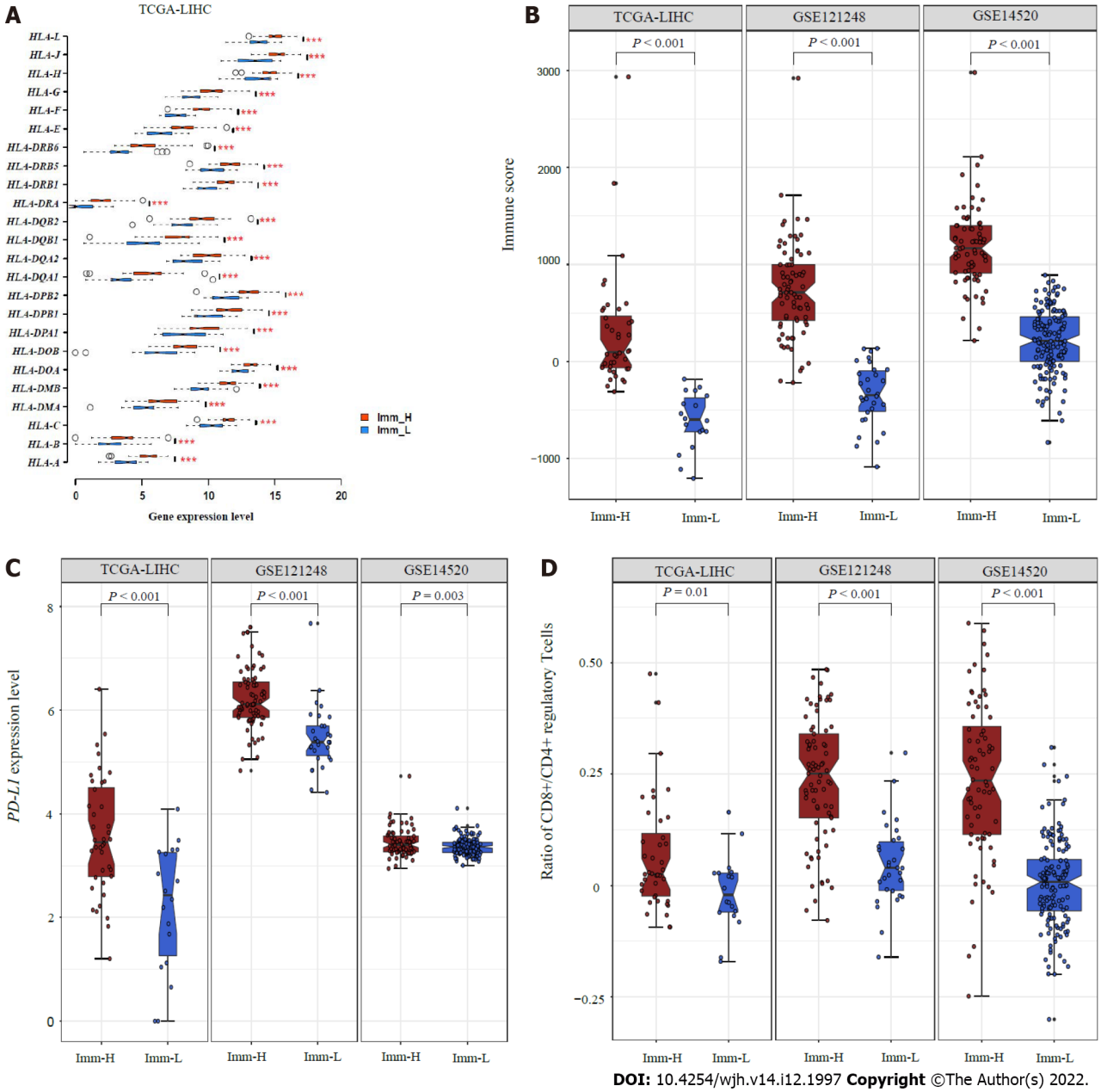

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Figure 2 Comparisons of immune features between the immune-specific subtypes of hepatitis B virus + hepatocellular carcinoma. A: Imm-H shows significantly higher expression levels of HLA genes; B: Immune scores; C: *PD-L1* expression levels; D: Ratios of immunostimulatory to immunosuppressive signatures (CD8+/CD4+ regulatory T cells) than Imm-L. The two-tailed Student's *t* test *P* values are shown in (A, C, D), and the one-tailed Mann–Whitney *U* test *P* values are shown in (B).

and stromal pathways in Imm-H versus Imm-L. Moreover, both modules were positively correlated with the OS prognosis ($P < 0.05$), consistent with the better OS in Imm-H relative to Imm-L. Besides, the blue module (with the GO term of homophilic cell adhesion *via* plasma membrane adhesion molecules) was significantly and positively correlated with the OS prognosis ($P = 0.02$), although it showed no significant enrichment difference between Imm-H versus Imm-L. It is reasonable that the positive association between the blue module and OS since reduced homophilic cell adhesion can promote tumor progression[26]. In contrast, the brown module had significant negative correlations with both OS and DFS time. The representative GO term for this module was cell cycle. It is justified since elevated cell cycle activity suggests tumor progression.

Proteins enriched in the immune-specific subtypes of HBV+ HCC

We compared the expression levels of 219 proteins between the immune-specific subtypes of HBV+ HCC in TCGA-LIHC. We identified nine proteins differentially expressed between Imm-H and Imm-L ($P < 0.05$) (Figure 6A). Among them, MYH11, PDCD4, Dvl3, and Syk were more highly expressed in Imm-H, while PCNA, Acetyl-a-Tubulin-Lys40, ER-α_pS118, Cyclin E2, and β-Catenin were more highly expressed in Imm-L. As expected, the proteins more enriched in Imm-H showed significantly positive

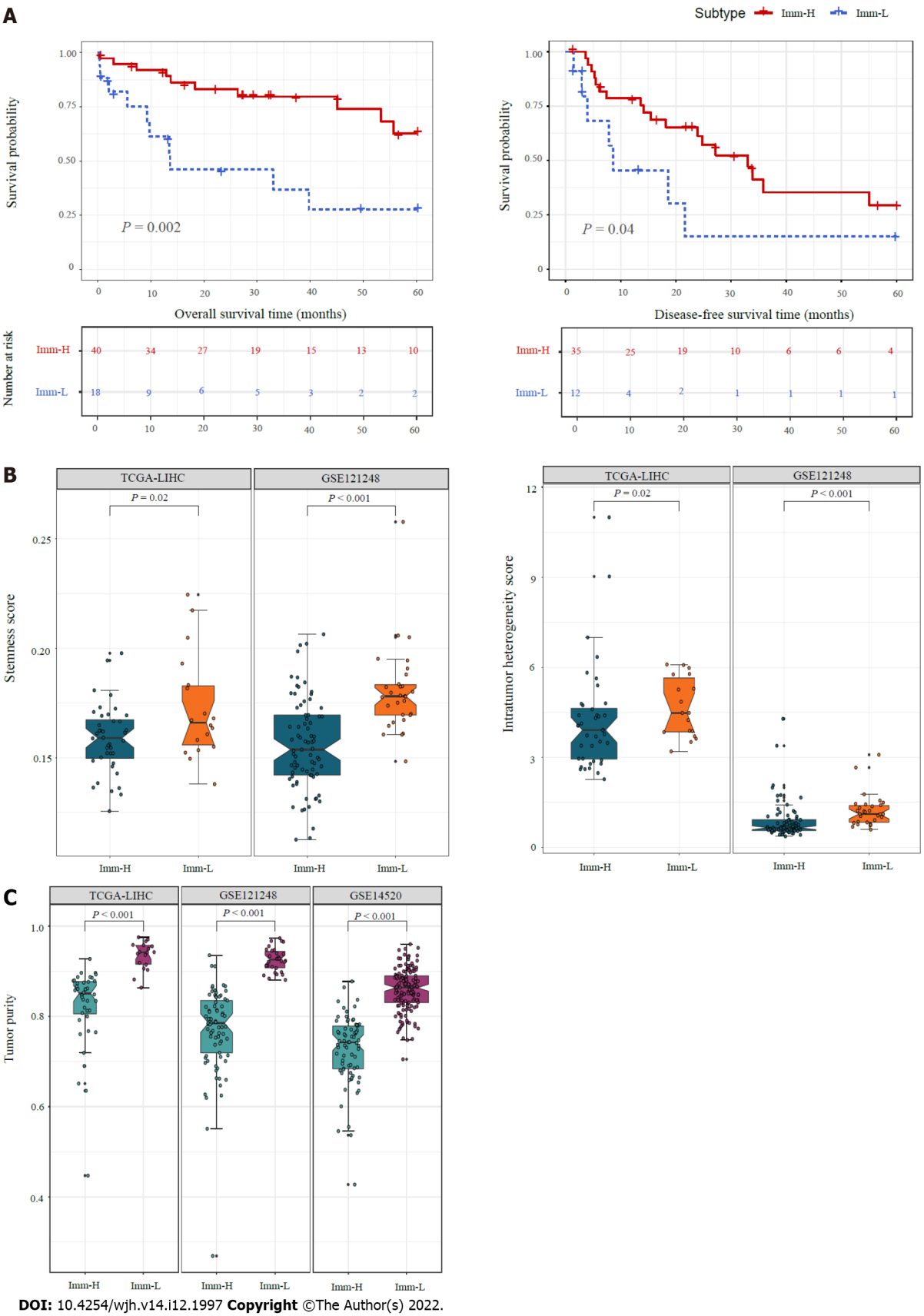

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Figure 3 Comparisons of clinical and phenotypic features between the immune-specific subtypes of hepatitis B virus + hepatocellular carcinoma. A: K–M curves showing that Imm-H has significantly higher 5-year overall survival and disease-free survival rates than Imm-L. The log-rank test *P* values are shown; B: Imm-H has significantly lower stemness and intratumor heterogeneity than Imm-L; C: Imm-H has significantly lower tumor purity than Imm-L. The one-tailed Mann–Whitney *U* test *P* values are shown in (B, C).

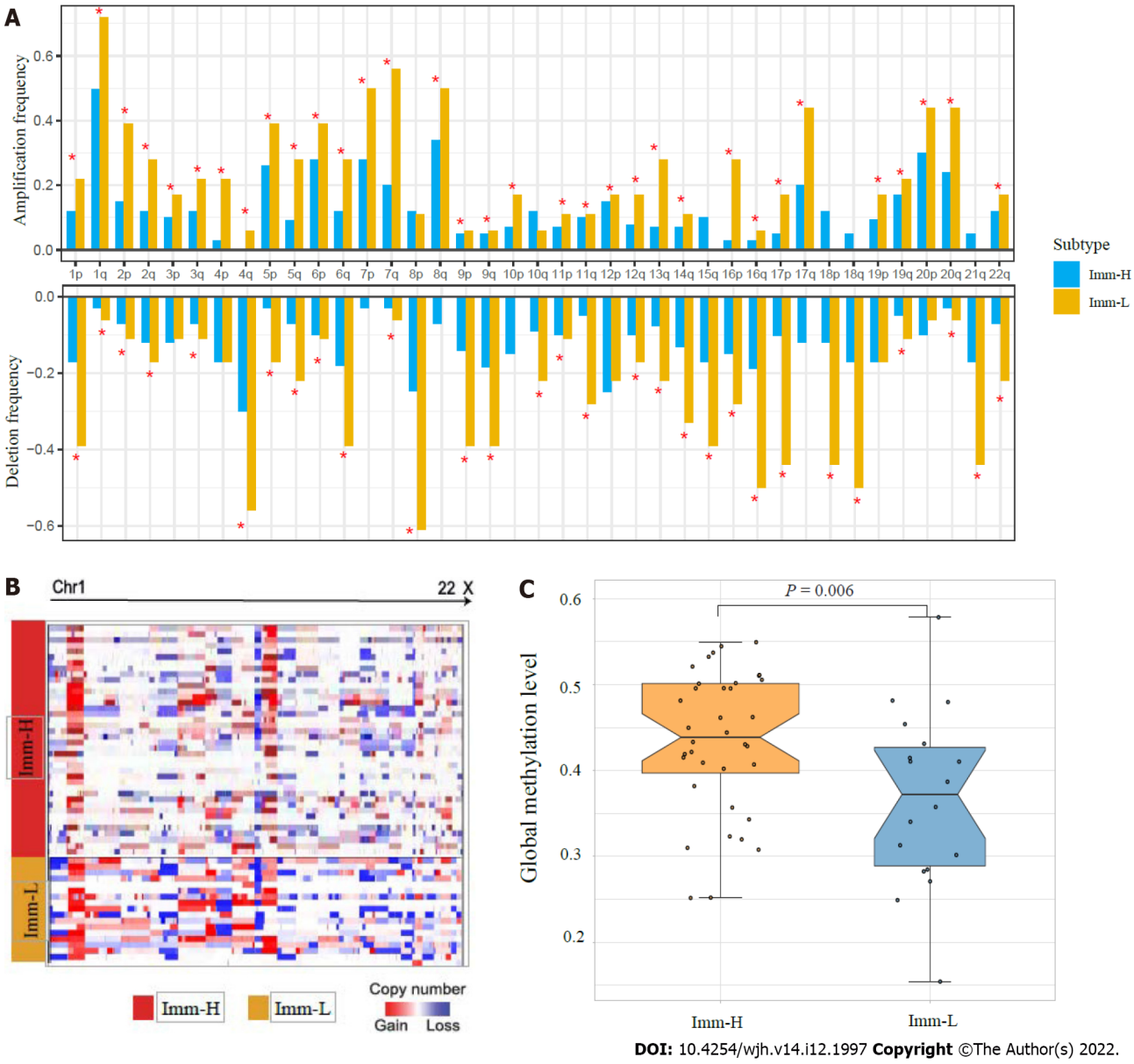

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Figure 4 Comparisons of genomic and epigenomic features between the immune-specific subtypes of hepatitis B virus + hepatocellular carcinoma in TCGA-LIHC. A: Comparison of the arm-level SCNAs between Imm-H and Imm-L. The red asterisks indicate the chromosome arms in which Imm-L has higher amplification or deletion frequency than Imm-H; B: Heatmap showing that Imm-L likely has higher amplitudes of copy number amplification and deletion across chromosomes than Imm-H; C: Imm-H has significantly higher global methylation levels than Imm-L. The one-tailed Mann–Whitney *U* test *P* values are shown.

expression correlations with immune scores in HBV+ HCC, and the proteins more enriched in Imm-L showed negative expression correlations with them (Spearman correlation, $P < 0.05$) (Figure 6B). Previous studies have shown that most of these proteins have associations with tumor immune regulation. For example, MYH11 is a smooth muscle myosin of the myosin heavy chain family, whose expression has been associated with antitumor immune infiltration in cancer[27]. PDCD4 is a tumor suppressor, whose expression in the tumor microenvironment is correlated with increased immune infiltration[28,29]. Syk is also a tumor suppressor and has a role in tumor immune regulation[30]. PCNA is involved in the DNA repair pathway in response to DNA damage, whose upregulation may promote tumor immune evasion[31,32]. This is consistent with its upregulation in Imm-L versus Imm-H. ER-α has been shown to induce antitumor immunosuppression[33], supporting our finding. Cyclin E2 is a positive regulator of cell cycle, which may inhibit antitumor immune responses and immunotherapy responses[34]. Again, this is consistent with our result. β-Catenin is an activator of the Wnt/β-catenin signaling pathway, whose overexpression suppresses antitumor immune responses[35], in line with our findings.

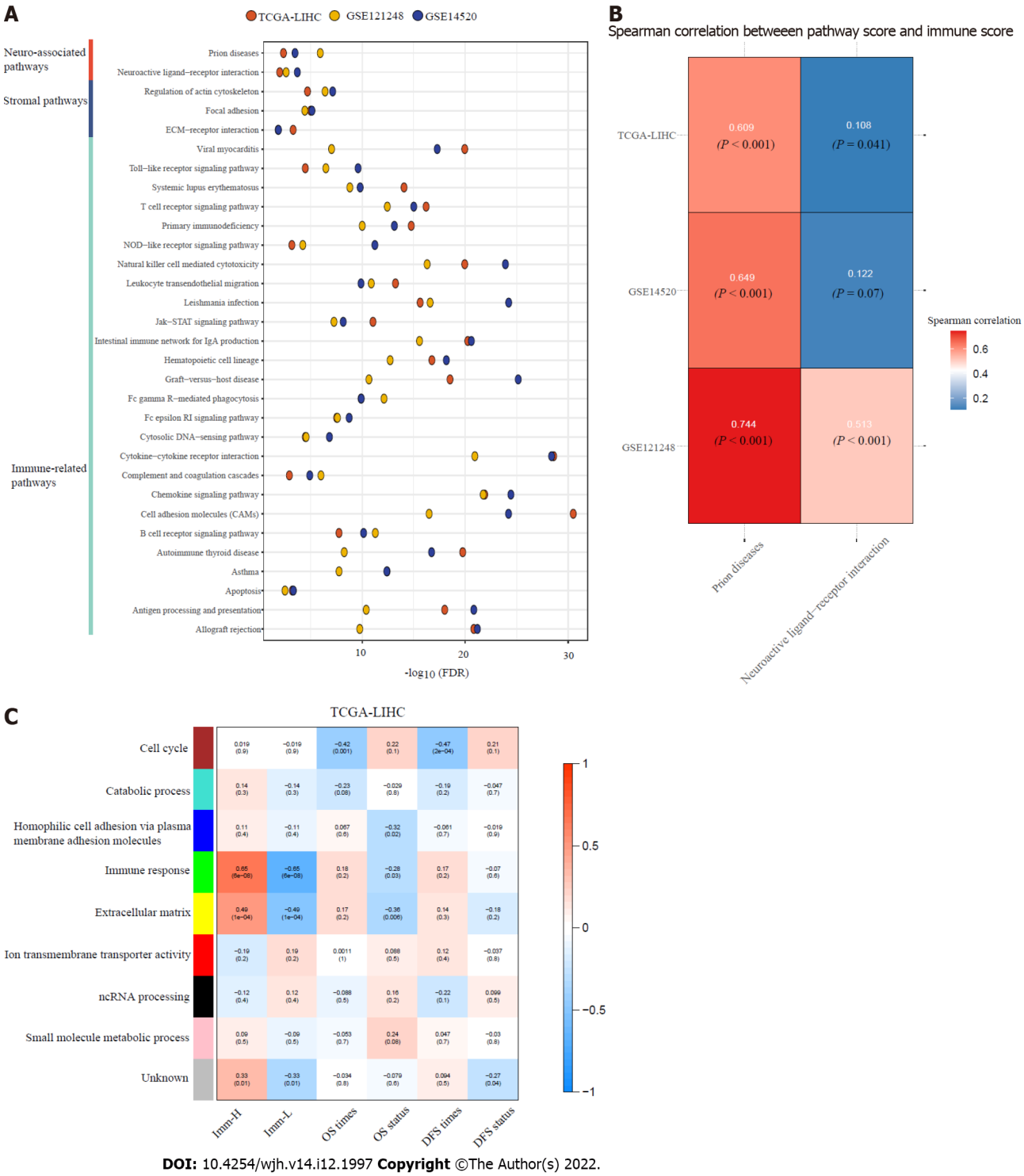

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Figure 5 Pathways and gene ontology enriched in the immune-specific subtypes of hepatitis B virus + hepatocellular carcinoma. A: The immune, stroma, and neuro-associated pathways more highly enriched in Imm-H versus Imm-L (false discovery rate < 0.05); B: Spearman correlations between the enrichment scores of the two neuro-associated pathways upregulated in Imm-H and immune scores in hepatitis B virus (HBV) + hepatocellular carcinoma (HCC); C: The gene modules and their representative gene ontology terms significantly differentiating HBV+ HCC by the immune-specific subtypes, overall survival, and/or disease-free survival. The correlation coefficients and *P* values (in parenthesis) are shown.

DISCUSSION

This study identified two immune-specific subtypes (Imm-H and Imm-L) of HBV+ HCC based on the enrichment of 23 immune signatures by unsupervised clustering. We showed that this subtyping method was reproducible as well as predictable by analyzing three different datasets. Furthermore, we demonstrated that both subtypes had significantly different clinical and molecular features. Compared to Imm-L, Imm-H displayed stronger immunity, more stromal components, lower tumor purity, lower stemness and ITH, lower-level CNAs, higher global methylation level, and better overall and disease-

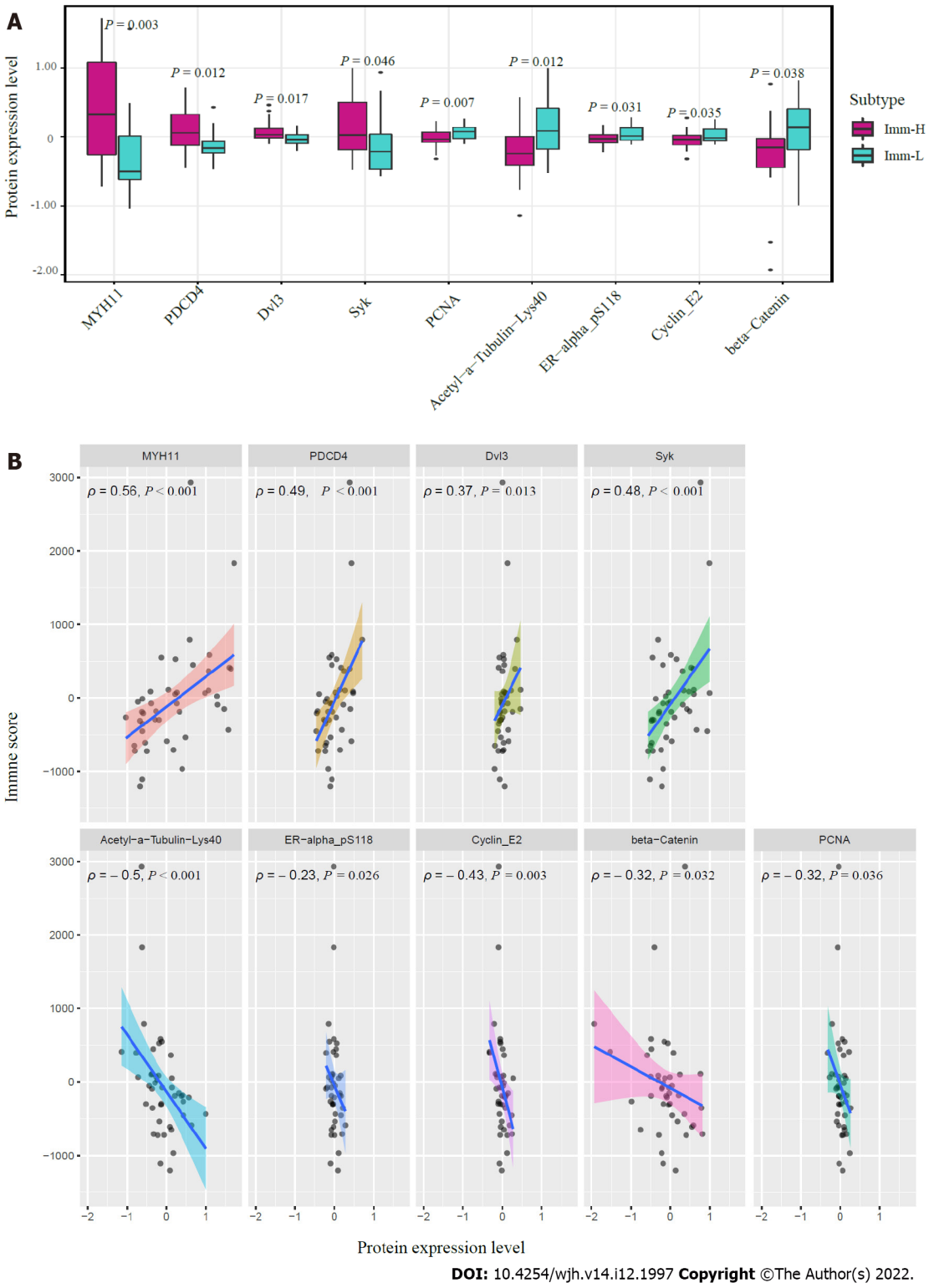

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Figure 6 Proteins enriched in the immune-specific subtypes of hepatitis B virus + hepatocellular carcinoma in TCGA-LIHC. A: Nine proteins having significantly different expression levels between Imm-H and Imm-L. The two-tailed Student's *t* test *P* values are shown; B: Spearman correlations between the expression levels of the nine proteins and immune scores in hepatitis B virus + hepatocellular carcinoma. The Spearman correlation coefficients (ρ) and *P* values are shown.

free survival prognosis (Figure 7). Our data support that "hot" tumors have a better prognosis than "cold" tumors in HBV+ HCC for their stronger antitumor immune responses. Similar findings were also observed in other cancers[36-38]. Intriguingly, although continual inflammatory responses in the *liver* caused by HBV infection is a major etiology for HBV+ HCC[39], higher immune/inflammatory responses are associated with a better prognosis in HBV+ HCC patients, as demonstrated by this analysis. It indicates that the relationship between immune/inflammatory responses and clinical outcomes in cancer is complex. Indeed, in some cancer types, such as glioma[40] and prostate cancer

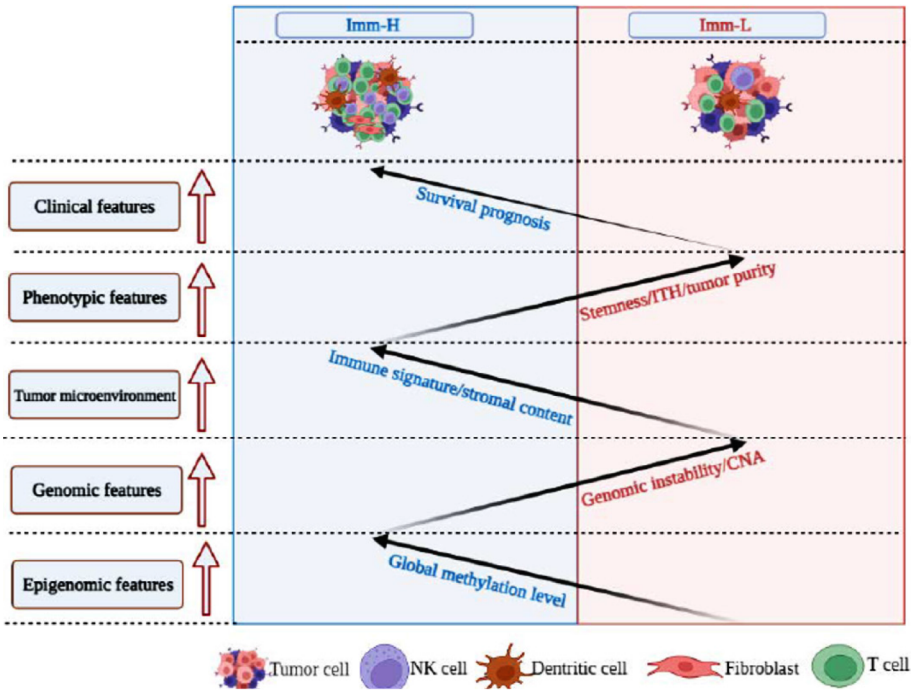

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Figure 7 Schematic comparisons of clinical and molecular features between the immune-specific subtypes of hepatitis B virus + hepatocellular carcinoma. The figure was created with BioRender.com.

[41], the relationship between immune/inflammatory responses and clinical outcomes is negative. Thus, the relationship between immune responses and clinical outcomes in cancers is dependent on their tissue or cellular origins, the tumor microenvironment, the ratio of immunostimulatory over immunosuppressive signatures, as well as whether the immune response is the tumor progression-promoting inflammation or immune cell-mediated elimination of tumor cells.

Prior studies have shown that TMB and CNAs have a positive and negative association with antitumor immune responses, respectively[24]. However, our analysis suggests that TMB has no a significant association with antitumor immunity in HBV+ HCC, although CNAs have a negative association with antitumor immune responses. It suggests that it is CNAs but not TMB responsible for the significantly different immunity between the "hot" and "cold" tumor subtypes in HBV+ HCC. Furthermore, the significantly lower stemness and ITH of Imm-H compared to Imm-L suggest that stemness and ITH may lead to antitumor immunosuppression, consistent with previous findings[18,42,43].

Pathway analysis showed that two neuro-associated pathways (neuroactive ligand receptor interaction, and prion diseases) had higher enrichment in Imm-H than in Imm-L and that their upregulation was associated with increased tumor immune infiltration levels. The positive association between neuro-related pathways and antitumor immunity has been revealed in prior studies[44]. Interestingly, many studies have demonstrated the inverse relationship between cancer and Alzheimer's disease (AD)[45]. AD is known as a progressive neurodegenerative disease as well as neuroinflammation disease[46]. A recent study has proposed that AD is an autoimmune disorder of innate immunity[47]. The present and prior data stimulate our imagination that the immune and inflammation could bridge the relationship between cancer and AD, such as hyperactivation of the immune system in AD patients reducing the risk of cancer.

Interestingly, besides the antitumor immune signatures, the immunosuppressive signature PD-L1, was also upregulated in Imm-H *vs* Imm-L. Because both PD-L1 expression[48] and ample TILs[9] are positive predictors of the response to ICB, Imm-H would respond better to immunotherapy than Imm-L. Thus, our subtyping method may stratify HBV+ HCC patients for immunotherapy. That is, immunotherapy may yield more *propitious* efficacy for Imm-H than for Imm-L HBV+ HCC patients.

CONCLUSION

HBV+ HCCs can be classified into two immune-specific subtypes in terms of their immune signature enrichment. Both subtypes have significantly different immunity, stromal contents, tumor purity, stemness, ITH, CNAs, methylation profiles, and survival prognosis. The immune-specific subtyping of HBV+ HCC may provide new biological insights as well as clinical implications for the management of

this disease.

ARTICLE HIGHLIGHTS

Research background

Hepatocellular carcinoma (HCC) is a major cancer of the liver that constitutes around 90% of liver cancer cases. Although traditional therapeutic approaches, including surgery, chemotherapy, radiotherapy, and targeted therapy, are effective in improving the survival of HCC patients, the overall survival prognosis of HCC patients is generally unfavorable. More recently, immunotherapy, such as immune checkpoint blockade, has achieved success in the treatment of various cancers, including HCC. However, only a small proportion of cancer patients respond well to immunotherapies to date.

Research motivation

Certain predictive markers for cancer immunotherapy responses have been uncovered, *e.g.*, PD-L1 expression, tumor mutation burden (TMB), and mismatch repair deficiency. In addition, the tumor immune microenvironment (TIME) plays an important role in immunotherapy responses. Overall, the "hot" tumors infiltrated by a substantial number of tumor-infiltrating lymphocytes (TILs) are more responsive to immunotherapies, compared to the "cold" tumors lacking TILs. Hence, an investigation of the TIME in HCC would aid in the prediction of immunotherapy responses.

Research objectives

Despite these previous studies, the discovery of immune-specific subtypes of hepatitis B virus-positive (HBV+) HCC is worth investigating, considering that HBV infection is a major cause of HCC.

Research methods

In this study, to characterize the immunological landscape of HBV+ HCC, we identified its immune-specific subtypes by the unsupervised machine learning in transcriptomic data. Furthermore, we comprehensively compared the clinical and molecular features of these subtypes.

Research results

Compared to Imm-L, Imm-H displayed stronger immunity, more stromal components, lower tumor purity, lower stemness and intratumor heterogeneity, lower-level copy number alterations, higher global methylation level, and better overall and disease-free survival prognosis.

Research conclusions

Our immune-specific subtyping of HBV+ HCC may provide new biological insights as well as clinical implications for the management of this disease.

Research perspectives

This study is interesting for several reasons. First, for the first time, we identified immune-specific subtypes of HBV+ HCC based on immune signature scores and demonstrated that this new subtyping method was reproducible in three different datasets. Second, our subtyping method captures the comprehensive heterogeneity of HBV+ HCC in the tumor microenvironment, genomic integrity, protein expression profiles, DNA methylation profiles, tumor stemness, intratumor heterogeneity, and clinical outcomes. Third, our data suggest that it is copy number alterations but not tumor mutations responsible for the different immunity between the "hot" and "cold" tumor subtypes in HBV+ HCC. Finally, our identification of the immune-specific subtypes of HBV+ HCC may provide new insights into the tumor biology and identify the HBV+ HCC patients beneficial from immunotherapy.

FOOTNOTES

Author contributions: Li SW contributed to software, validation, formal analysis, investigation, data curation, visualization, writing - review & editing; Han LF contributed to software, formal analysis, data curation; He Y contributed to data curation; Wang XS contributed to conceptualization, methodology, resources, investigation, writing - original draft, supervision, project administration, funding acquisition.

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SYSTEMATIC REVIEWS

Liver chemistries in severe or non-severe cases of COVID-19: A systematic review and meta-analysis

Xuan Dong, Dan-Yi Zeng, Qing-Qing Xing, Mei-Zhu Hong, Jin-Shui Pan

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Abstract

BACKGROUND

Coronavirus disease (COVID-19) patients exhibit different patterns of liver impairment, according to growing evidence.

AIM

In this study, we sought to provide a comprehensive analysis of liver test parameters in patients with severe and non-severe COVID-19.

METHODS

We performed a meta-analysis of published liver manifestations and described the liver damage in COVID-19. We searched PubMed, Google Scholar, Embase, Cochrane Library, medRxiv, bioRxiv, and three Chinese electronic databases through April 18, 2020, in accordance with the Preferred Reporting Items for Meta-Analyses. We analyzed pooled data on liver chemistries stratified by COVID-19 severity using a fixed or random-effects model.

RESULTS

A meta-analysis of 56 studies, including 11052 patients, found that the pooled mean alanine aminotransferase (ALT) in severe COVID-19 cases was 35.9 IU/L whereas in non-severe COVID-19 cases was 27.3 IU/L. Average aspa-rtate aminotransferase (AST) levels were 44.3 IU/L in severe cases compared to 27.9 IU/L in non-severe cases. In addition, AST levels are often higher than ALT levels regardless of disease severity. The severe cases tended to have a higher gamma-glutamyltransferase level but a lower albumin level than the non-severe cases.

CONCLUSION

Severe COVID-19 was more likely to be associated with abnormal liver test results. Monitoring liver chemistry closely can help detect disease progression

early.

Key Words: Systematic reviews and Meta-Analyses; COVID-19; SARS-CoV-2; Meta-analysis; Liver chemistries; Severe

Core Tip: Data on abnormal liver chemistries related to coronavirus disease (COVID-19) are cumulating but are potentially confusing. We performed a meta-analysis of 56 studies that included a total of 11052 patients with COVID-19. We noted that patients with abnormal liver test results are at higher risk of progression to severe disease and close monitoring of liver chemistries provides early warning against disease progression.

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DOI: https://dx.doi.org/10.4254/wjh.v14.i12.2012

INTRODUCTION

According to World Health Organization, as of April 18, 2020, 2160207 coronavirus disease (COVID-19) cases were confirmed globally, of which 146088 led to deaths[1]. Although effectively controlled in mainland China, COVID-19 has spread and risen dramatically in most other countries. Similarly, the other two previously identified coronaviruses, namely severe acute respiratory syndrome coronavirus (SARS-CoV) and Middle East Respiratory Syndrome-CoV, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) causes severe viral pneumonia in humans. As no specific acquired immunity exists in the general population, SARS-CoV-2 has high infectivity, which has resulted in an ongoing global health crisis.

Apart from the respiratory system, gastrointestinal tract, the urinary system, and even the central nervous system are the probable target organs of SARS-CoV-2, which utilizes the angiotensin-converting enzyme 2 (ACE2) receptors located in the respiratory and gastrointestinal tracts as the entry point for epithelial cells[2]. Among patients' common complaints related to COVID-19 are gastrointestinal symptoms, including nausea/vomiting, diarrhea, and abdominal pain[3-6]. Abundant ACE2 protein expression in the glandular cells of gastrointestinal tract supports the entry of SARS-CoV-2 into the host epithelial cells[7]. Single-cell RNA sequencing has revealed a specific ACE2 expression in cholangiocytes[8]. Thus, performing liver chemistry tests for a number of patients with COVID-19 seems reasonable. In fact, several studies have found liver injury in patients with COVID-19[9-12]. In Cai's study 76.3% had abnormal liver test results, total bilirubin (TBIL), alanine aminotransferase (ALT), aspartate aminotransferase (AST), and γ-glutamyltransferase (GGT) levels elevated to more than 3× the upper limit of normal.

Furthermore, there are differences in liver chemistry between patients with severe and non-severe COVID-19 based on cumulative observations. Although liver manifestations of COVID-19 pose an immense diagnostic challenge to clinicians when treating patients with symptoms related to COVID-19, these are potentially useful for recognizing severe cases of COVID-19 in the early stage.

Considering the diverse clinical manifestations and increasing number of reported COVID-19 cases, a systematic summary of the liver manifestations of COVID-19 is urgently needed. Liver chemistries generally consist of hepatocellular injury-related indexes, including ALT and AST; cholestatic injury-related indexes, comprised of alkaline phosphatase (ALP) and GGT; and hepatocellular function-related indexes such as albumin (ALB) level and prothrombin time (PT)[13]. In general, international standardized ratio (INR), TBIL, direct bilirubin (DBIL), and globulin (GLB) levels, and are also assessed in clinical practice. However, there are few observations to comprehensively analyze liver chemistries in patients with COVID-19 patients. We therefore aimed to provide a comprehensive overview of liver test parameters in patients with severe and non-severe COVID-19. It is possible to develop more effective therapies and holistic approaches to care with a better understanding of the disease.

MATERIALS AND METHODS

Studies selection

The following databases were searched from December 1, 2019, through April 18, 2020: PubMed, Google Scholar, Embase, Cochrane Library, medRxiv, bioRxiv, and three Chinese electronic databases (CQVIP, Wanfang Data, and Chinese National Knowledge Infrastructure). "Coronavirus," "COVID-19," "2019-nCoV-2," "SARS-CoV-2," or novel coronavirus were used as search keywords. Potential studies were retrieved in accordance with the Systematic reviews and Meta-Analyses guideline[14]. Details of the database search are listed in the Supplementary file. The retrieved articles were imported to Endnote X9.3 (Thompson and Reuters, Philadelphia, Pennsylvania), and duplicates were removed. The Reference Citation Analysis had be used to further improve the manuscript content when revised the manuscript (https://www.referencecitationanalysis.com/).

Selection criteria

The eligibility of the potential studies was determined independently by two authors (XD and DYZ), and dissonance was arbitrated by the third author (JSP). The inclusion criteria were as follows: (1) Study population: adult COVID-19 patients; (2) study design: case series, case report, prospective cohort study, retrospective cohort study, case-control study, and randomized controlled trial; and (3) language: Studies published in English or Chinese. The exclusion criteria were as follows: (1) Pediatric patients or pregnant women; (2) patients without nucleic acid data or serology evidence of SARS-CoV2 infection; (3) asymptomatic patients with SARS-CoV2 infection; and (3) study design: Review article, meta-analysis, editorial, or commentary. Studies that only reported the percentages of the indexes related to liver chemistries rather than the mean or median values of the corresponding indexes were also excluded.

Data extraction

For the eligible articles, we recorded the following items: first author, study location, sample size, patient age and sex, and liver chemistry-related indexes such as ALT, AST, TBIL, DBIL, GGT, ALP, and ALB levels. The severity of COVID-19 was also recorded. Severe disease was defined according to the American Thoracic Society and Infectious Disease Society of America guidelines for community-acquired pneumonia, and the guidelines for diagnosis and management of COVID-19 released by National Health Commission of China, need of intensive care unit admission, mechanical ventilation[15, 16].

Data analysis

The statistical analyses were performed using the R version 3.2.3 statistical software (R Foundation for Statistical Computing). The continuous variables that showed a normal distribution were expressed as mean ± SD, while those that conformed to a skewed distribution were expressed as median [interquartile range (IQR)]. For the studies that provided summary data of median, minimum, and maximum values, we used the method developed by Luo *et al*[17] to estimate the sample mean and SD for the continuous outcomes. The online tool used is provided at http://www.math.hkbu.edu.hk/~tongt/papers/median2mean.html. The 95% confidence interval (CI) was presented as a Forest plot. The Cochran Q test was used to detect the heterogeneity among studies, with a p value of < 0.10 indicating significant heterogeneity. The I^2 statistics was calculated to measure the proportion of total variation among the studies to which the heterogeneity was attributed. I^2 values of < 25%, 25%-75%, and > 75% represent low, moderate, and high heterogeneity, respectively[18]. Publication bias was evaluated using a funnel plot. A subgroup analysis was performed according to disease severity.

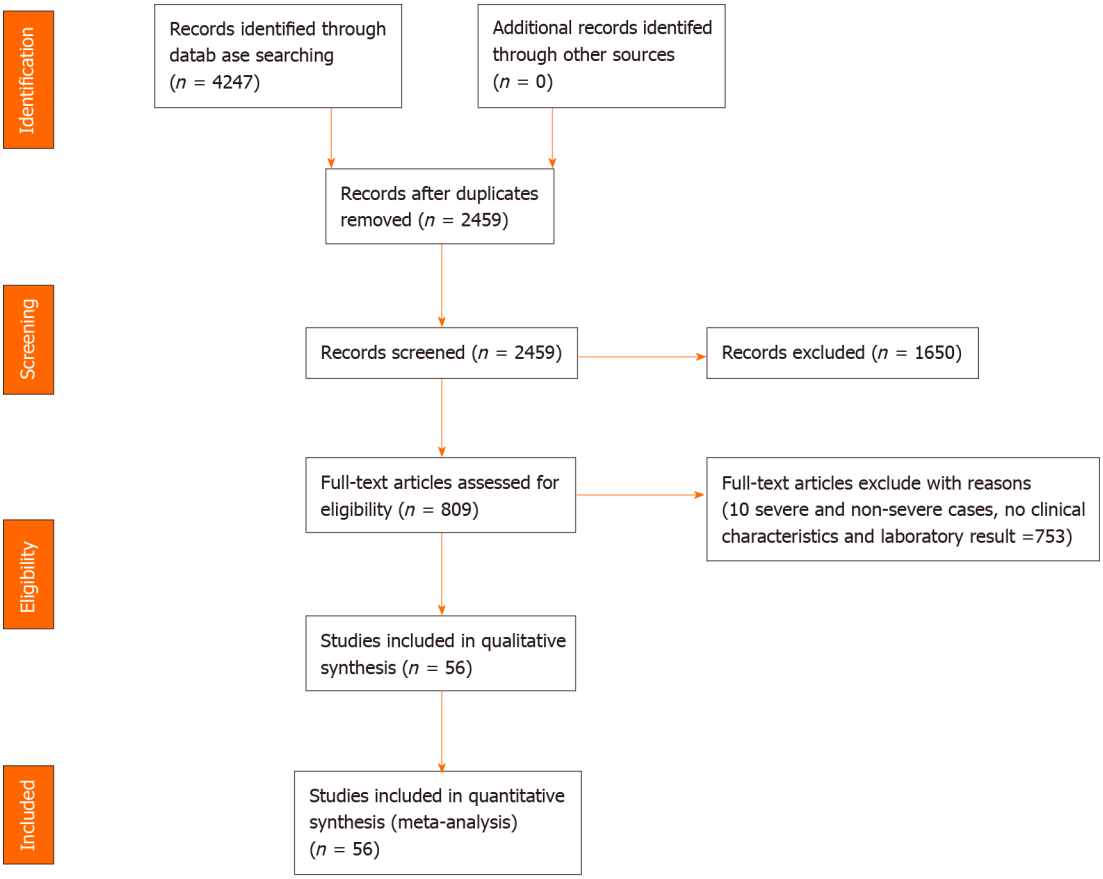
RESULTS

Characteristics of the enrolled studies

Screening process of the potential studies was shown in Figure 1. The meta-analysis consisted 56 studies, whose characteristics were listed in Supplementary Table 1. Information, including the study location, sample size, patient age and sex, disease severity, TBIL, DBIL, ALB, GLB, ALT, AST, GGT, ALP, INR, and PT levels, was recorded. The mean ages of patients with non-severe and severe COVID-19 were 50.1 and 63.2 years, respectively (Supplementary Figure 1). Male patients accounted for 50.7% in the enrolled studies. Among the studies that reported disease severity, severe disease accounted for 25.3% of the cases.

Hepatocellular injury-related abnormalities in liver chemistries

Of the enrolled studies, 56 reported assays of ALT or AST in a total of 6235 patients with COVID-19. The pooled mean ALT level was 35.9 IU/L in the patients with severe COVID-19 and 27.3 IU/L in the patients with non-severe COVID-19 (95%CI: -9.7 to -5.9, P < 0.0001; Figure 2A), with significant hetero-



DOI: 10.4254/wjh.v14.i12.2012

Figure 1 Study selection flow diagram. If all the liver chemistry indexes were not reported, these were regarded as "not available" and excluded from the meta-analysis. However, this study still enrolled studies that reported individual liver chemistry indexes if the severity of coronavirus disease 2019 was reported in relation with the index.

geneity among the studies (I^2 = 70%, P < 0.01). Similarly, the pooled mean AST level was 44.3 IU/L in the severe cases and 27.9 IU/L in the non-severe cases (95%CI: -13.9 to -9.9, P < 0.0001; Figure 2B). Among the studies, significant heterogeneity for the AST levels was observed (I^2 = 74%, P < 0.01). Using a funnel plot, potential publication bias was evaluated (Supplementary Figure 2). Average AST level tended to be higher than average ALT level in both the severe and non-severe groups. Furthermore, the severe group showed an even greater difference between levels of AST and ALT (44.3 and 36.1 IU/L, respectively; Figure 3). Supplementary Figure 3 presented the evaluation of publication bias.

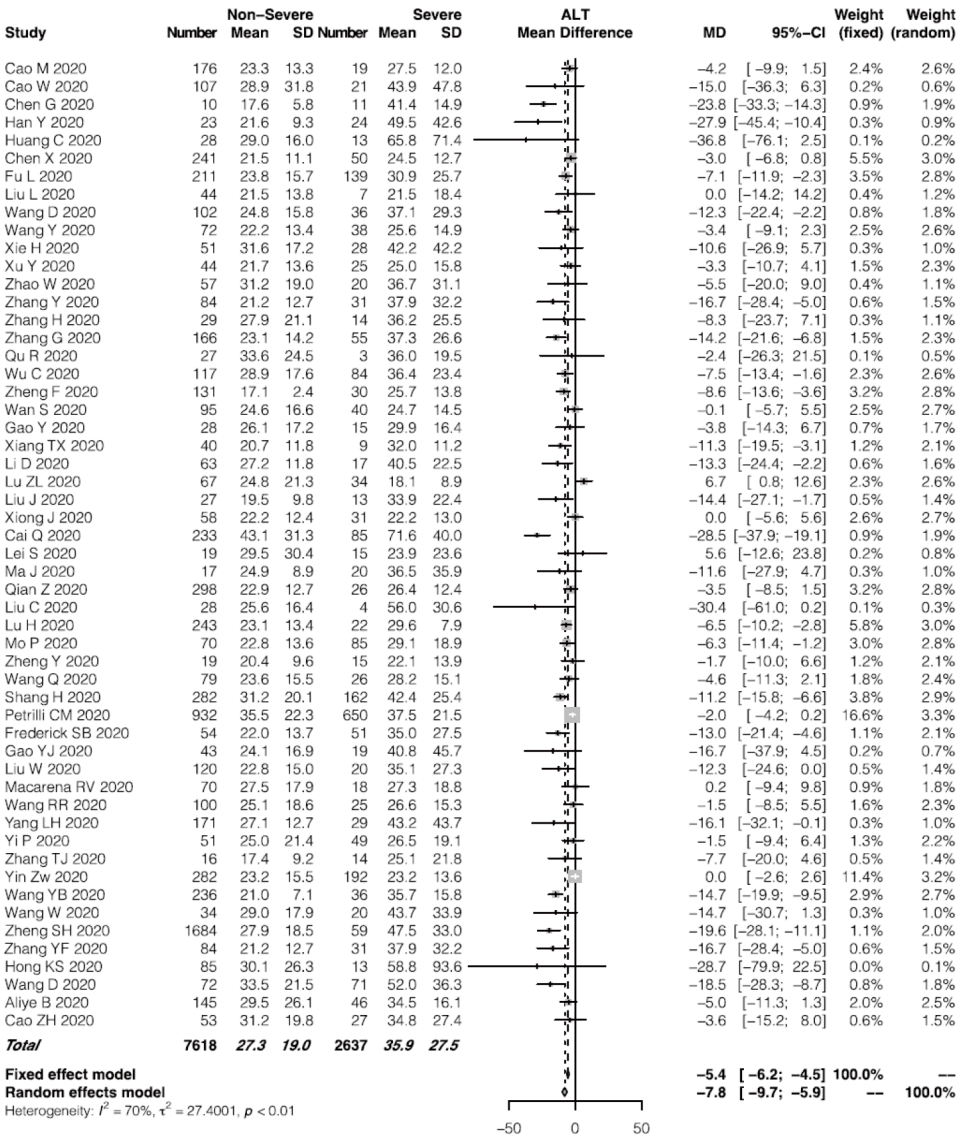
Cholestasis-related abnormalities in liver chemistries

Compared with the studies that frequently reported ALT and AST levels, cholestasis-related indexes such as ALP, GGT, and DBIL levels were presented in rather fewer studies. Among the enrolled studies, 10 reported ALP assays and 6 studies reported GGT measurements. The pooled mean ALP level was 67.8 IU/L in the patients with severe COVID-19 and 61.8 IU/L in those with non-severe COVID-19 (95%CI: -11.2 to 0.9, P = 0.02; Figure 4A). Figure 4B showed that the pooled mean GGT level was 44.2 IU/L in the severe group while 30.5 IU/L in the non-severe group. As compared with the non-severe group, the severe group had a slightly higher pooled mean TBIL level. However, TBIL levels remained within normal ranges in both groups (Figure 4C). Even fewer studies reported DBIL values in patients with COVID-19. In fact, no significant difference in mean DBIL level was found between the 2 groups (Figure 4D). In terms of TBIL levels, the studies showed low heterogeneity (I^2 = 29%, P = 0.06). Supplementary Figure 4 showed a funnel plot of TBIL levels.

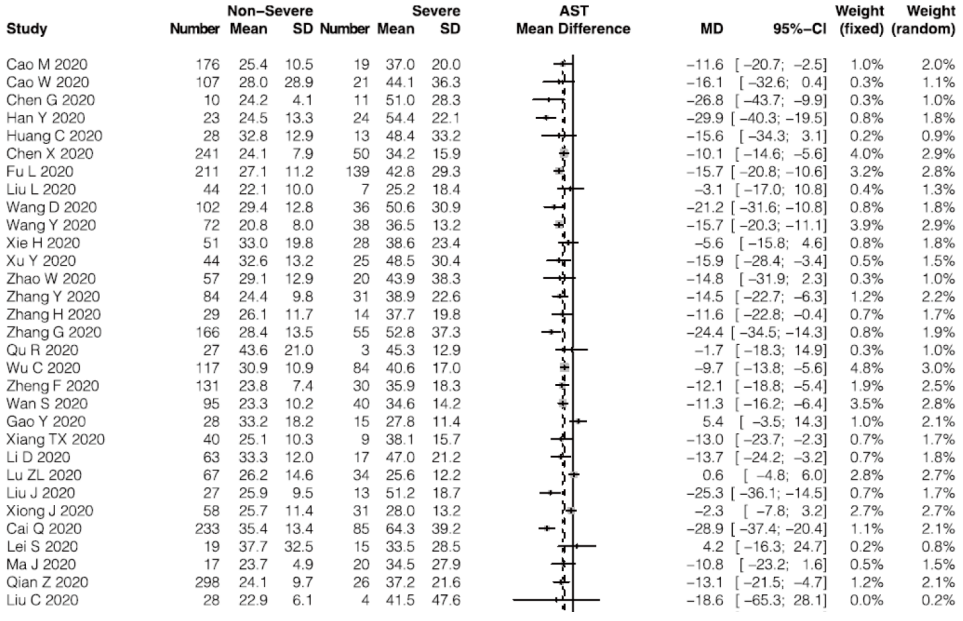
Hepatocellular function-related abnormalities in liver chemistries

27 studies compared the mean ALB levels according to COVID-19 severity, between 1232 and 4475 severe and non-severe cases, respectively (Figure 5A). Across the studies, a significant heterogeneity was observed (I^2 = 96%, P < 0.01). Average ALB level in the patients with severe disease was significantly lower than that in the patients with non-severe disease. No significant difference in GLB level was found between the groups (P = 0.14; Figure 5B). However, PT and INR, the coagulation-related indexes, showed no significant differences were found between the severity groups. The patients in the severe group tended to have longer PT or higher INR (Figure 5C and D). An evaluation of

A


Study
Non–Severe Number Mean SD
Severe Number Mean SD
ALT Mean Difference
MD 95%–CI
Weight (fixed) Weight (random)
Cao M 2020 176 23.3 13.3 19 27.5 12.0 −4.2 [−9.9; 1.5] 2.4% 2.6%
Cao W 2020 107 28.9 31.8 21 43.9 47.8 −15.0 [−36.3; 6.3] 0.2% 0.6%
Chen G 2020 10 17.6 5.8 11 41.4 14.9 −23.8 [−33.3; −14.3] 0.9% 1.9%
Han Y 2020 23 21.6 9.3 24 49.5 42.6 −27.9 [−45.4; −10.4] 0.3% 0.9%
Huang C 2020 28 29.0 16.0 13 65.8 71.4 −36.8 [−76.1; 2.5] 0.1% 0.2%
Chen X 2020 241 21.5 11.1 50 24.5 12.7 −3.0 [−6.8; 0.8] 5.5% 3.0%
Fu L 2020 211 23.8 15.7 139 30.9 25.7 −7.1 [−11.9; −2.3] 3.5% 2.8%
Liu L 2020 44 21.5 13.8 7 21.5 18.4 0.0 [−14.2; 14.2] 0.4% 1.2%
Wang D 2020 102 24.8 15.8 36 37.1 29.3 −12.3 [−22.4; −2.2] 0.8% 1.8%
Wang Y 2020 72 22.2 13.4 38 25.6 14.9 −3.4 [−9.1; 2.3] 2.5% 2.6%
Xie H 2020 51 31.6 17.2 28 42.2 42.2 −10.6 [−26.9; 5.7] 0.3% 1.0%
Xu Y 2020 44 21.7 13.6 25 25.0 15.8 −3.3 [−10.7; 4.1] 1.5% 2.3%
Zhao W 2020 57 31.2 19.0 20 36.7 31.1 −5.5 [−20.0; 9.0] 0.4% 1.1%
Zhang Y 2020 84 21.2 12.7 31 37.9 32.2 −16.7 [−28.4; −5.0] 0.6% 1.5%
Zhang H 2020 29 27.9 21.1 14 36.2 25.5 −8.3 [−23.7; 7.1] 0.3% 1.1%
Zhang G 2020 166 23.1 14.2 55 37.3 26.6 −14.2 [−21.6; −6.8] 1.5% 2.3%
Qu R 2020 27 33.6 24.5 3 36.0 19.5 −2.4 [−26.3; 21.5] 0.1% 0.5%
Wu C 2020 117 28.9 17.6 84 36.4 23.4 −7.5 [−13.4; −1.6] 2.3% 2.6%
Zheng F 2020 131 17.1 2.4 30 25.7 13.8 −8.6 [−13.6; −3.6] 3.2% 2.8%
Wan S 2020 95 24.6 16.6 40 24.7 14.5 −0.1 [−5.7; 5.5] 2.5% 2.7%
Gao Y 2020 28 26.1 17.2 15 29.9 16.4 −3.8 [−14.3; 6.7] 0.7% 1.7%
Xiang TX 2020 40 20.7 11.8 9 32.0 11.2 −11.3 [−19.5; −3.1] 1.2% 2.1%
Li D 2020 63 27.2 11.8 17 40.5 22.5 −13.3 [−24.4; −2.2] 0.6% 1.6%
Lu ZL 2020 67 24.8 21.3 34 18.1 8.9 6.7 [0.8; 12.6] 2.3% 2.6%
Liu J 2020 27 19.5 9.8 13 33.9 22.4 −14.4 [−27.1; −1.7] 0.5% 1.4%
Xiong J 2020 58 22.2 12.4 31 22.2 13.0 0.0 [−5.6; 5.6] 2.6% 2.7%
Cai Q 2020 233 43.1 31.3 85 71.6 40.0 −28.5 [−37.9; −19.1] 0.9% 1.9%
Lei S 2020 19 29.5 30.4 15 23.9 23.6 5.6 [−12.6; 23.8] 0.2% 0.8%
Ma J 2020 17 24.9 8.9 20 36.5 35.9 −11.6 [−27.9; 4.7] 0.3% 1.0%
Qian Z 2020 298 22.9 12.7 26 26.4 12.4 −3.5 [−8.5; 1.5] 3.2% 2.8%
Liu C 2020 28 25.6 16.4 4 56.0 30.6 −30.4 [−61.0; 0.2] 0.1% 0.3%
Lu H 2020 243 23.1 13.4 22 29.6 7.9 −6.5 [−10.2; −2.8] 5.8% 3.0%
Mo P 2020 70 22.8 13.6 85 29.1 18.9 −6.3 [−11.4; −1.2] 3.0% 2.8%
Zheng Y 2020 19 20.4 9.6 15 22.1 13.9 −1.7 [−10.0; 6.6] 1.2% 2.1%
Wang Q 2020 79 23.6 15.5 26 28.2 15.1 −4.6 [−11.3; 2.1] 1.8% 2.4%
Shang H 2020 282 31.2 20.1 162 42.4 25.4 −11.2 [−15.8; −6.6] 3.8% 2.9%
Petrilli CM 2020 932 35.5 22.3 650 37.5 21.5 −2.0 [−4.2; 0.2] 16.6% 3.3%
Frederick SB 2020 54 22.0 13.7 51 35.0 27.5 −13.0 [−21.4; −4.6] 1.1% 2.1%
Gao YJ 2020 43 24.1 16.9 19 40.8 45.7 −16.7 [−37.9; 4.5] 0.2% 0.7%
Liu W 2020 120 22.8 15.0 20 35.1 27.3 −12.3 [−24.6; 0.0] 0.5% 1.4%
Macarena RV 2020 70 27.5 17.9 18 27.3 18.8 0.2 [−9.4; 9.8] 0.9% 1.8%
Wang RR 2020 100 25.1 18.6 25 26.6 15.3 −1.5 [−8.5; 5.5] 1.6% 2.3%
Yang LH 2020 171 27.1 12.7 29 43.2 43.7 −16.1 [−32.1; −0.1] 0.3% 1.0%
Yi P 2020 51 25.0 21.4 49 26.5 19.1 −1.5 [−9.4; 6.4] 1.3% 2.2%
Zhang TJ 2020 16 17.4 9.2 14 25.1 21.8 −7.7 [−20.0; 4.6] 0.5% 1.4%
Yin Zw 2020 282 23.2 15.5 192 23.2 13.6 0.0 [−2.6; 2.6] 11.4% 3.2%
Wang YB 2020 236 21.0 7.1 36 35.7 15.8 −14.7 [−19.9; −9.5] 2.9% 2.7%
Wang W 2020 34 29.0 17.9 20 43.7 33.9 −14.7 [−30.7; 1.3] 0.3% 1.0%
Zheng SH 2020 1684 27.9 18.5 59 47.5 33.0 −19.6 [−28.1; −11.1] 1.1% 2.0%
Zhang YF 2020 84 21.2 12.7 31 37.9 32.2 −16.7 [−28.4; −5.0] 0.6% 1.5%
Hong KS 2020 85 30.1 26.3 13 58.8 93.6 −28.7 [−79.9; 22.5] 0.0% 0.1%
Wang D 2020 72 33.5 21.5 71 52.0 36.3 −18.5 [−28.3; −8.7] 0.8% 1.8%
Aliye B 2020 145 29.5 26.1 46 34.5 16.1 −5.0 [−11.3; 1.3] 2.0% 2.5%
Cao ZH 2020 53 31.2 19.8 27 34.8 27.4 −3.6 [−15.2; 8.0] 0.6% 1.5%
Total 7618 27.3 19.0 2637 35.9 27.5
Fixed effect model −5.4 [−6.2; −4.5] 100.0% —
Random effects model −7.8 [−9.7; −5.9] — 100.0%
Heterogeneity: I^2 = 70%, τ^2 = 27.4001, p < 0.01
−50 0 50

B


Study
Non–Severe Number Mean SD
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AST Mean Difference
MD 95%–CI
Weight (fixed) Weight (random)
Cao M 2020 176 25.4 10.5 19 37.0 20.0 −11.6 [−20.7; −2.5] 1.0% 2.0%
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Chen G 2020 10 24.2 4.1 11 51.0 28.3 −26.8 [−43.7; −9.9] 0.3% 1.0%
Han Y 2020 23 24.5 13.3 24 54.4 22.1 −29.9 [−40.3; −19.5] 0.8% 1.8%
Huang C 2020 28 32.8 12.9 13 48.4 33.2 −15.6 [−34.3; 3.1] 0.2% 0.9%
Chen X 2020 241 24.1 7.9 50 34.2 15.9 −10.1 [−14.6; −5.6] 4.0% 2.9%
Fu L 2020 211 27.1 11.2 139 42.8 29.3 −15.7 [−20.8; −10.6] 3.2% 2.8%
Liu L 2020 44 22.1 10.0 7 25.2 18.4 −3.1 [−17.0; 10.8] 0.4% 1.3%
Wang D 2020 102 29.4 12.8 36 50.6 30.9 −21.2 [−31.6; −10.8] 0.8% 1.8%
Wang Y 2020 72 20.8 8.0 38 36.5 13.2 −15.7 [−20.3; −11.1] 3.9% 2.9%
Xie H 2020 51 33.0 19.8 28 38.6 23.4 −5.6 [−15.8; 4.6] 0.8% 1.8%
Xu Y 2020 44 32.6 13.2 25 48.5 30.4 −15.9 [−28.4; −3.4] 0.5% 1.5%
Zhao W 2020 57 29.1 12.9 20 43.9 38.3 −14.8 [−31.9; 2.3] 0.3% 1.0%
Zhang Y 2020 84 24.4 9.8 31 38.9 22.6 −14.5 [−22.7; −6.3] 1.2% 2.2%
Zhang H 2020 29 26.1 11.7 14 37.7 19.8 −11.6 [−22.8; −0.4] 0.7% 1.7%
Zhang G 2020 166 28.4 13.5 55 52.8 37.3 −24.4 [−34.5; −14.3] 0.8% 1.9%
Qu R 2020 27 43.6 21.0 3 45.3 12.9 −1.7 [−18.3; 14.9] 0.3% 1.0%
Wu C 2020 117 30.9 10.9 84 40.6 17.0 −9.7 [−13.8; −5.6] 4.8% 3.0%
Zheng F 2020 131 23.8 7.4 30 35.9 18.3 −12.1 [−18.8; −5.4] 1.9% 2.5%
Wan S 2020 95 23.3 10.2 40 34.6 14.2 −11.3 [−16.2; −6.4] 3.5% 2.8%
Gao Y 2020 28 33.2 18.2 15 27.8 11.4 5.4 [−3.5; 14.3] 1.0% 2.1%
Xiang TX 2020 40 25.1 10.3 9 38.1 15.7 −13.0 [−23.7; −2.3] 0.7% 1.7%
Li D 2020 63 33.3 12.0 17 47.0 21.2 −13.7 [−24.2; −3.2] 0.7% 1.8%
Lu ZL 2020 67 26.2 14.6 34 25.6 12.2 0.6 [−4.8; 6.0] 2.8% 2.7%
Liu J 2020 27 25.9 9.5 13 51.2 18.7 −25.3 [−36.1; −14.5] 0.7% 1.7%
Xiong J 2020 58 25.7 11.4 31 28.0 13.2 −2.3 [−7.8; 3.2] 2.7% 2.7%
Cai Q 2020 233 35.4 13.4 85 64.3 39.2 −28.9 [−37.4; −20.4] 1.1% 2.1%
Lei S 2020 19 37.7 32.5 15 33.5 28.5 4.2 [−16.3; 24.7] 0.2% 0.8%
Ma J 2020 17 23.7 4.9 20 34.5 27.9 −10.8 [−23.2; 1.6] 0.5% 1.5%
Qian Z 2020 298 24.1 9.7 26 37.2 21.6 −13.1 [−21.5; −4.7] 1.2% 2.1%
Liu C 2020 28 22.9 6.1 4 41.5 47.6 −18.6 [−65.3; 28.1] 0.0% 0.2%

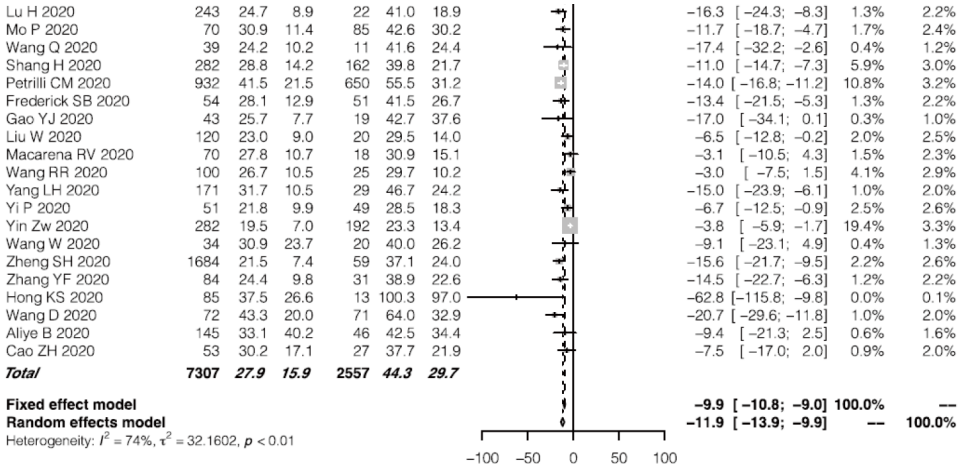

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Figure 2 Forest plot of the association between serum alanine aminotransferase/aspartate aminotransferase level and disease severity. A: Pooled levels of alanine aminotransferase; B: Pooled levels of aspartate aminotransferase in the patients with coronavirus disease 2019. ALT: Alanine aminotransferase; AST: Aspartate aminotransferase.

publication bias in relation to ALB level and PT is shown in Supplementary Figure 5. Supplementary Figure 5 illustrated an evaluation of publication bias related to ALB level and PT.

DISCUSSION

In this meta-analysis, 56 studies that consisted a total of 11052 patients with COVID-19 from China, United States, Chile, Iran and South Korea were enrolled. According to the pooled analysis, hepatocellular injury, hepatocellular dysfunction, and cholestasis, three patterns of liver impairment, can develop in quite a part of patients with COVID-19 at variable severity. In brief, the patients with severe COVID-19 tended to have higher ALT/AST, ALP/GGT, and TBIL levels; higher INR; and prolonged PT. However, the severe cases had lower ALB levels than the non-severe cases. Particularly in severe cases of COVID-19, the AST levels were often higher than the ALT levels. We also observed a tendency of the severe cases to arise in the elderly.

Although the liver may act as the latent target of SARS-CoV-2, the actual prevalence of abnormal liver chemistries could be underestimated since many studies did not report cholestasis-related indexes such as ALP and GGT levels, and synthetic function-related indexes such as ALB level and INR. Moreover, most studies reported ALT/AST levels on the day of admission while not the entire disease course. This issue further compromises the role of liver chemistries in disease monitoring and provides an early warning against severe cases. As SARS-CoV-2 can lead to bile duct damage by conquering the ACE2 expressed on cholangiocytes and induce a subsequent cholestatic liver injury[8], cholestasis-related abnormalities could be overlooked.

Cumulating studies have linked abnormal liver chemistries to the severity of COVID-19[9]. It is more likely that patients with abnormal liver test results will progress to severe cases[9]. In fact, coronavirus infection can cause direct damage to liver cells[11]. Moreover, several underlying diseases, comorbidities, and complications that develop in the course of the disease, such as sepsis and multiple-organ failure, and drugs that can cause potential liver damage also increase the risk of liver injury. Lopinavir/ritonavir use during hospitalization has been reported to possible lead to liver damage[9,19]. The liver chemistry tests in the enrolled studies were all performed on admission, which suggests that the influences of the drugs on the liver tests, if any, should be minor.

ALB level and PT are known to reflect hepatocellular function. Albumin, which has a circulating half-life of 3 weeks, is a plasma protein exclusively synthesized by the liver[20]. Hypoalbuminemia results from and reflects the inflammatory state, which leads to inflammatory exudate. Effective nutrition support helps to correct hypoalbuminemia[21]. Our meta-analysis revealed that ALB level was lower in the severe cases than in the non-severe cases, which indicated that the severe cases tended to have more intense inflammation and require more solid nutrition support. PT is a far more sensitive measure of hepatocellular function than ALB level because PT may be prolonged in patients with severe liver disease duration of < 24 h[13]. In accordance with the alteration of the ALB level, PT was prolonged in the severe cases, which further indicated impairment of hepatocellular function in the severe cases[20].

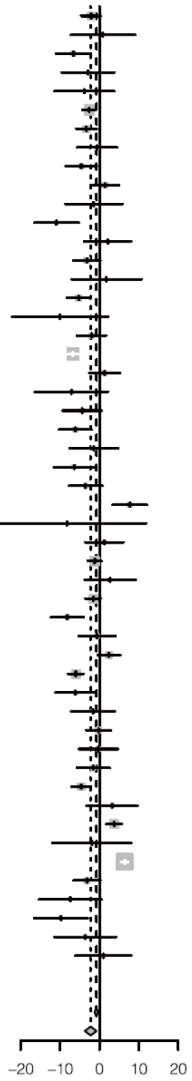
According to our meta-analysis, another interesting feature of liver impairment related to COVID-19 is that the AST level often overrides the ALT level, especially in severe cases. By contrast, in patients with chronic hepatitis B or nonalcoholic fatty liver disease, the ALT level is generally higher than the

A

Study	ALT Number	ALT Mean	ALT SD	AST Number	AST Mean	AST SD	MD	95%–CI	Weight (fixed)	Weight (random)
Cao M 2020	176	23.3	13.3	176	25.4	10.5	−2.1	[−4.6; 0.4]	3.0%	2.4%
Cao W 2020	107	28.9	31.8	107	28.0	28.9	0.9	[−7.2; 9.0]	0.3%	1.4%
Chen G 2020	10	17.6	5.8	10	24.2	4.1	−6.6	[−11.0; −2.2]	1.0%	2.1%
Han Y 2020	23	21.6	9.3	23	24.5	13.3	−2.9	[−9.5; 3.7]	0.4%	1.7%
Huang C 2020	28	29.0	16.0	28	32.8	12.9	−3.8	[−11.4; 3.8]	0.3%	1.5%
Chen X 2020	241	21.5	11.1	241	24.1	7.9	−2.6	[−4.3; −0.9]	6.4%	2.5%
Fu L 2020	211	23.8	15.7	211	27.1	11.2	−3.3	[−5.9; −0.7]	2.8%	2.4%
Liu L 2020	44	21.5	13.8	44	22.1	10.0	−0.6	[−5.6; 4.4]	0.7%	2.0%
Wang D 2020	102	24.8	15.8	102	29.4	12.8	−4.6	[−8.5; −0.7]	1.2%	2.2%
Wang Y 2020	72	22.2	13.4	72	20.8	8.0	1.4	[−2.2; 5.0]	1.5%	2.2%
Xie H 2020	51	31.6	17.2	51	33.0	19.8	−1.4	[−8.6; 5.8]	0.4%	1.6%
Xu Y 2020	44	21.7	13.6	44	32.6	13.2	−10.9	[−16.5; −5.3]	0.6%	1.9%
Zhao W 2020	57	31.2	19.0	57	29.1	12.9	2.1	[−3.9; 8.1]	0.5%	1.8%
Zhang Y 2020	84	21.2	12.7	84	24.4	9.8	−3.2	[−6.6; 0.2]	1.6%	2.3%
Zhang H 2020	29	27.9	21.1	29	26.1	11.7	1.8	[−7.0; 10.6]	0.2%	1.3%
Zhang G 2020	166	23.1	14.2	166	28.4	13.5	−5.3	[−8.3; −2.3]	2.1%	2.3%
Qu R 2020	27	33.6	24.5	27	43.6	21.0	−10.0	[−22.2; 2.2]	0.1%	0.9%
Wu C 2020	117	28.9	17.6	117	30.9	10.9	−2.0	[−5.8; 1.8]	1.3%	2.2%
Zheng F 2020	131	17.1	2.4	131	23.8	7.4	−6.7	[−8.0; −5.4]	10.6%	2.5%
Wan S 2020	95	24.6	16.6	95	23.3	10.2	1.3	[−2.6; 5.2]	1.2%	2.2%
Gao Y 2020	28	26.1	17.2	28	33.2	18.2	−7.1	[−16.4; 2.2]	0.2%	1.3%
Xiang TX 2020	40	20.7	11.8	40	25.1	10.3	−4.4	[−9.3; 0.5]	0.8%	2.0%
Li D 2020	63	27.2	11.8	63	33.3	12.0	−6.1	[−10.3; −1.9]	1.1%	2.1%
Lu ZL 2020	67	24.8	21.3	67	26.2	14.6	−1.4	[−7.6; 4.8]	0.5%	1.8%
Liu J 2020	27	19.5	9.8	27	25.9	9.5	−6.4	[−11.5; −1.3]	0.7%	2.0%
Xiong J 2020	58	22.2	12.4	58	25.7	11.4	−3.5	[−7.8; 0.8]	1.0%	2.1%
Cai Q 2020	233	43.1	31.3	233	35.4	13.4	7.7	[3.3; 12.1]	1.0%	2.1%
Lei S 2020	19	29.5	30.4	19	37.7	32.5	−8.2	[−28.2; 11.8]	0.0%	0.5%
Ma J 2020	17	24.9	8.9	17	23.7	4.9	1.2	[−3.6; 6.0]	0.8%	2.0%
Qian Z 2020	298	22.9	12.7	298	24.1	9.7	−1.2	[−3.0; 0.6]	5.7%	2.5%
Liu C 2020	28	25.6	16.4	28	22.9	6.1	2.7	[−3.8; 9.2]	0.4%	1.7%
Lu H 2020	243	23.1	13.4	243	24.7	8.9	−1.6	[−3.6; 0.4]	4.6%	2.4%
Mo P 2020	70	22.8	13.6	70	30.9	11.4	−8.1	[−12.3; −3.9]	1.1%	2.1%
Wang Q 2020	79	23.6	15.5	39	24.2	10.2	−0.6	[−5.3; 4.1]	0.9%	2.0%
Shang H 2020	282	31.2	20.1	282	28.8	14.2	2.4	[−0.5; 5.3]	2.3%	2.3%
Petrilli CM 2020	932	35.5	22.3	932	41.5	21.5	−6.0	[−8.0; −4.0]	4.8%	2.5%
Frederick SB 2020	54	22.0	13.7	54	28.1	12.9	−6.1	[−11.1; −1.1]	0.7%	2.0%
Gao YJ 2020	43	24.1	16.9	43	25.7	7.7	−1.6	[−7.2; 4.0]	0.6%	1.9%
Liu W 2020	120	22.8	15.0	120	23.0	9.0	−0.2	[−3.3; 2.9]	1.9%	2.3%
Macarena RV 2020	70	27.5	17.9	70	27.8	10.7	−0.3	[−5.2; 4.6]	0.8%	2.0%
Wang RR 2020	100	25.1	18.6	100	26.7	10.5	−1.6	[−5.8; 2.6]	1.1%	2.1%
Yang LH 2020	171	27.1	12.7	171	31.7	10.5	−4.6	[−7.1; −2.1]	3.1%	2.4%
Yi P 2020	51	25.0	21.4	51	21.8	9.9	3.2	[−3.3; 9.7]	0.5%	1.7%
Yin Zw 2020	282	23.2	15.5	282	19.5	7.0	3.7	[1.7; 5.7]	4.8%	2.5%
Wang W 2020	34	29.0	17.9	34	30.9	23.7	−1.9	[−11.9; 8.1]	0.2%	1.2%
Zheng SH 2020	1684	27.9	18.5	1684	21.5	7.4	6.4	[5.4; 7.4]	20.9%	2.5%
Zhang YF 2020	84	21.2	12.7	84	24.4	9.8	−3.2	[−6.6; 0.2]	1.6%	2.3%
Hong KS 2020	85	30.1	26.3	85	37.5	26.6	−7.4	[−15.4; 0.6]	0.3%	1.5%
Wang D 2020	72	33.5	21.5	72	43.3	20.0	−9.8	[−16.6; −3.0]	0.4%	1.7%
Aliye B 2020	145	29.5	26.1	145	33.1	40.2	−3.6	[−11.4; 4.2]	0.3%	1.5%
Cao ZH 2020	53	31.2	19.8	53	30.2	17.1	1.0	[−6.0; 8.0]	0.4%	1.6%
Total	**7347**	***27.5***	***19.2***	**7307**	***27.9***	***15.9***				
Fixed effect model							**−0.8**	**[−1.2; −0.4]**	**100.0%**	—
Random effects model							**−2.2**	**[−3.7; −0.7]**	—	**100.0%**

Heterogeneity: $I^2 = 89\%$, $\tau^2 = 22.2736$, $p < 0.01$

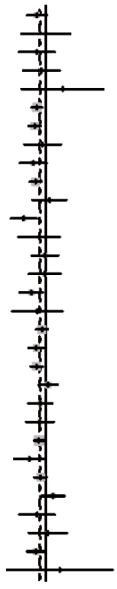
Non-Severe Mean Difference

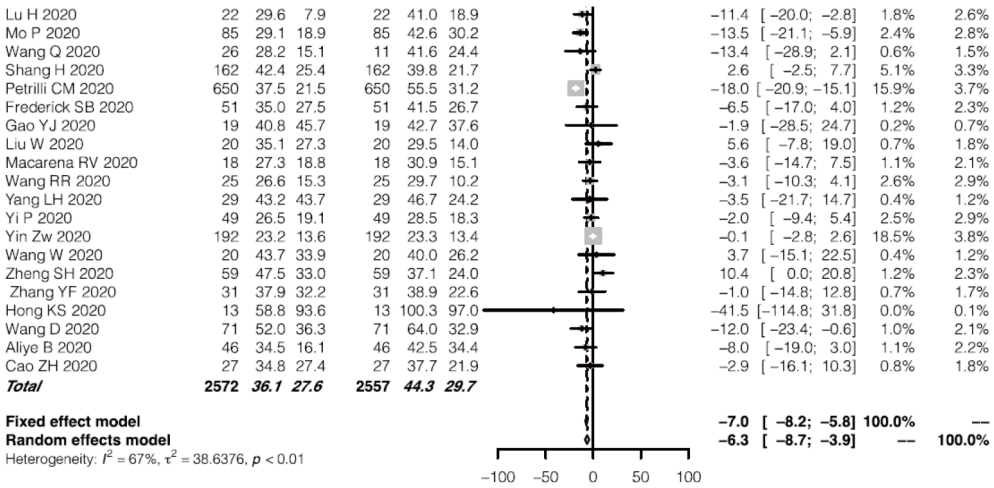


B

Study	ALT Number	ALT Mean	ALT SD	AST Number	AST Mean	AST SD	MD	95%–CI	Weight (fixed)	Weight (random)
Cao M 2020	19	27.5	12.0	19	37.0	20.0	−9.5	[−20.0; 1.0]	1.2%	2.3%
Cao W 2020	21	43.9	47.8	21	44.1	36.3	−0.2	[−25.9; 25.5]	0.2%	0.7%
Chen G 2020	11	41.4	14.9	11	51.0	28.3	−9.6	[−28.5; 9.3]	0.4%	1.2%
Han Y 2020	24	49.5	42.6	24	54.4	22.1	−4.9	[−24.1; 14.3]	0.4%	1.1%
Huang C 2020	13	65.8	71.4	13	48.4	33.2	17.4	[−25.4; 60.2]	0.1%	0.3%
Chen X 2020	50	24.5	12.7	50	34.2	15.9	−9.7	[−15.3; −4.1]	4.2%	3.2%
Fu L 2020	139	30.9	25.7	139	42.8	29.3	−11.9	[−18.4; −5.4]	3.2%	3.1%
Liu L 2020	7	21.5	18.4	7	25.2	18.4	−3.7	[−23.0; 15.6]	0.4%	1.1%
Wang D 2020	36	37.1	29.3	36	50.6	30.9	−13.5	[−27.4; 0.4]	0.7%	1.7%
Wang Y 2020	38	25.6	14.9	38	36.5	13.2	−10.9	[−17.2; −4.6]	3.4%	3.1%
Xie H 2020	28	42.2	42.2	28	38.6	23.4	3.6	[−14.3; 21.5]	0.4%	1.2%
Xu Y 2020	25	25.0	15.8	25	48.5	30.4	−23.5	[−36.9; −10.1]	0.7%	1.8%
Zhao W 2020	20	36.7	31.1	20	43.9	38.3	−7.2	[−28.8; 14.4]	0.3%	0.9%
Zhang Y 2020	31	37.9	32.2	31	38.9	22.6	−1.0	[−14.8; 12.8]	0.7%	1.7%
Zhang H 2020	14	36.2	25.5	14	37.7	19.8	−1.5	[−18.4; 15.4]	0.5%	1.3%
Zhang G 2020	55	37.3	26.6	55	52.8	37.3	−15.5	[−27.6; −3.4]	0.9%	2.0%
Qu R 2020	3	36.0	19.5	3	45.3	12.9	−9.3	[−35.8; 17.2]	0.2%	0.7%
Wu C 2020	84	36.4	23.4	84	40.6	17.0	−4.2	[−10.4; 2.0]	3.5%	3.1%
Zheng F 2020	30	25.7	13.8	30	35.9	18.3	−10.2	[−18.4; −2.0]	2.0%	2.7%
Wan S 2020	40	24.7	14.5	40	34.6	14.2	−9.9	[−16.2; −3.6]	3.4%	3.1%
Gao Y 2020	15	29.9	16.4	15	27.8	11.4	2.1	[−8.0; 12.2]	1.3%	2.3%
Xiang TX 2020	9	32.0	11.2	9	38.1	15.7	−6.1	[−18.7; 6.5]	0.8%	1.9%
Li D 2020	17	40.5	22.5	17	47.0	21.2	−6.5	[−21.2; 8.2]	0.6%	1.6%
Lu ZL 2020	34	18.1	8.9	34	25.6	12.2	−7.5	[−12.6; −2.4]	5.2%	3.4%
Liu J 2020	13	33.9	22.4	13	51.2	18.7	−17.3	[−33.2; −1.4]	0.5%	1.5%
Xiong J 2020	31	22.2	13.0	31	28.0	13.2	−5.8	[−12.3; 0.7]	3.2%	3.1%
Cai Q 2020	85	71.6	40.0	85	64.3	39.2	7.3	[−4.6; 19.2]	1.0%	2.0%
Lei S 2020	15	23.9	23.6	15	33.5	28.5	−9.6	[−28.3; 9.1]	0.4%	1.2%
Ma J 2020	20	36.5	35.9	20	34.5	27.9	2.0	[−17.9; 21.9]	0.3%	1.1%
Qian Z 2020	26	26.4	12.4	26	37.2	21.6	−10.8	[−20.4; −1.2]	1.5%	2.4%
Liu C 2020	4	56.0	30.6	4	41.5	47.6	14.5	[−41.0; 70.0]	0.0%	0.2%

Severe Mean Difference





DOI: 10.4254/wjh.v14.i12.2012

Figure 3 Forest plot for the comparison of alanine aminotransferase and aspartate aminotransferase levels in the patients with coronavirus disease 2019 stratified by disease severity. A: Forest plot for the comparison of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels in the non-severe cases of coronavirus disease 2019 (COVID-19); B: Forest plot for the comparison of ALT and AST levels in the severe cases of COVID-19. ALT: Alanine aminotransferase; AST: Aspartate aminotransferase.

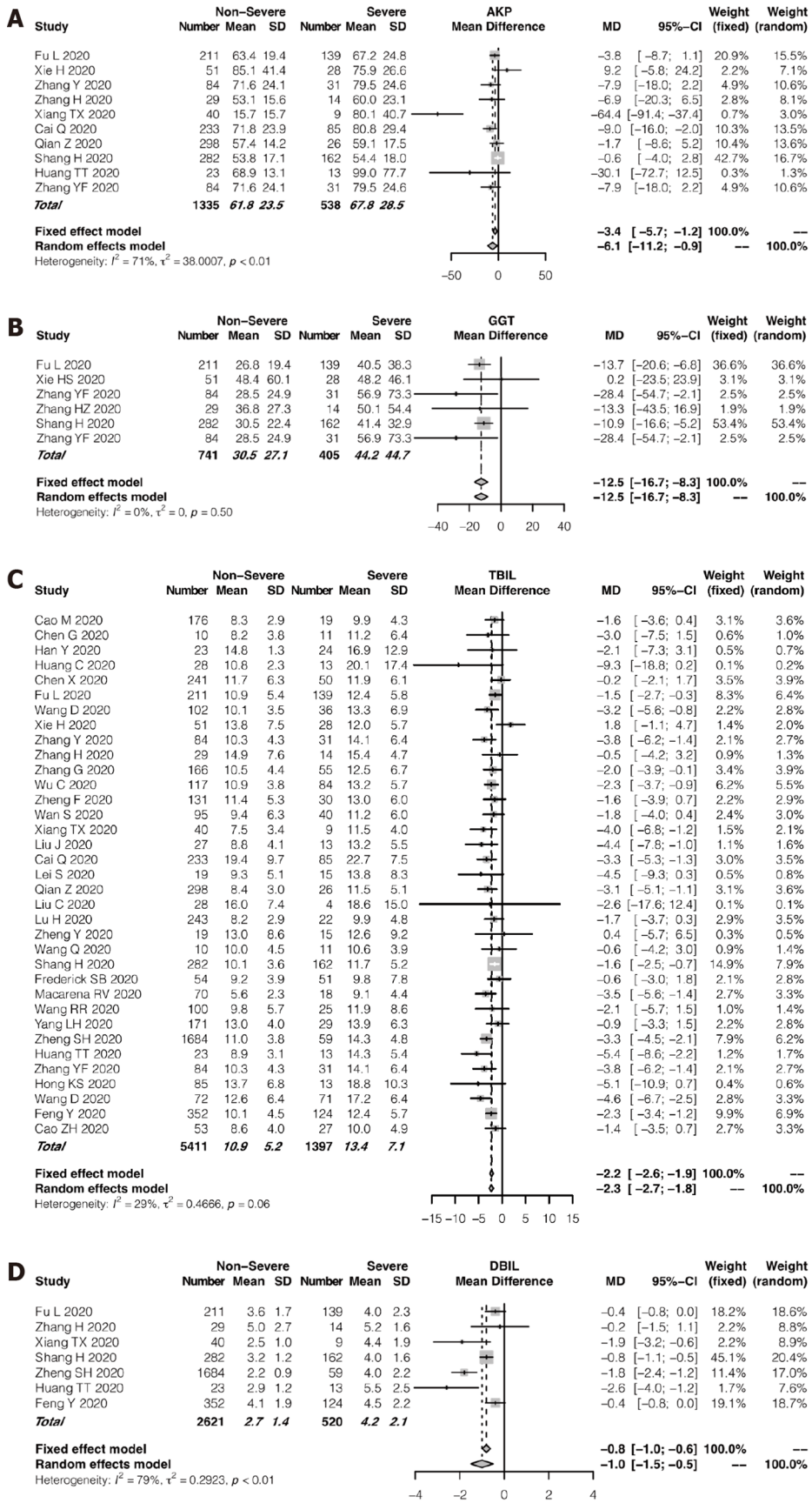
AST level. While ALT is primarily present in the liver and is a more specific indicator for hepatocellular injury, the distribution of AST is far wider than that of ALT, including the cardiac muscle, skeletal muscle, kidney, and brain[13]. An elevated AST level accompanied by a normal ALT level often suggests cardiac or muscle disease. In fact, cardiac injury is frequent in severe cases of COVID-19, especially in deceased patients[22,23].

This study has some substantial merits. First, acomprehensive review of COVID-19 literature, which is rapidly developing and sometimes confusing, was presented in this meta-analysis regarding the manifestation of liver chemistries. The extensive coverage of 37 studies allowed a more precise evaluation of the abnormalities of liver chemistries. Our subgroup analysis revealed that the abnormal liver chemistries were associated with a more severe disease course. It is imperative that liver chemistries should be monitored more closely for diagnostic and prognostic purposes.

Second, this analysis extensively covered hepatocellular injury, hepatocellular dysfunction, and cholestasis, three patterns of liver impairment. Most observations focused on ALT, AST, and ALB levels. However, cholestasis-related impairment (*e.g.*, abnormal ALP and GGT levels) tended to be inadvertently ignored. Moreover, we also compared hepatocellular dysfunction between the severe and non-severe cases. The alarmingly high prevalence of hypoalbuminemia in the severe cases prompts further nutrition support in severe cases. In addition, coagulation dysfunction in severe cases requires vigilance. Third, the enrolled studies included multiple observations not only from mainland China but also from other ethnic groups. This facilitates the assessment of abnormal liver chemistries related to COVID-19 in a broader ethnic context. Fourth, eligible studies preprinted in medRxiv and bioRxiv were also covered. As a result, our analysis has a clear leading position. However, our study has a few limitations. As mentioned earlier, cholestasis-related indexes such as ALP/GGT level may be under-reported in quite a number of studies, which may lead to less precise pooled data. Second, most studies were conducted in mainland China. It was difficult to determine if liver chemistry was abnormal in other ethnic groups. Most of the studies that came from mainland China seem to have an adverse impact. On the contrary, this helps to abate the heterogeneity caused by the disease grouping, as some potential discrepancies may exist in the definition of severe and non-severe cases of COVID-19 between different countries.

CONCLUSION

In this meta-analysis, we comprehensively described hepatocellular injury, hepatocellular dysfunction, cholestasis, three patterns of liver impairment, related to COVID-19. Severe COVID-19 was more likely to be associated with abnormal liver test results. A close monitoring of liver chemistries can provide an early warning of disease progression.



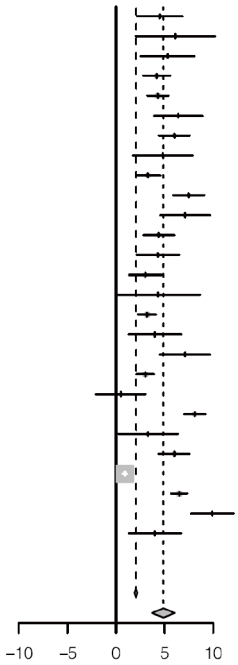
DOI: 10.4254/wjh.v14.i12.2012

Figure 4 Forest plot for the association of the cholestasis-related indexes and disease severity. A: Pooled levels of alkaline phosphatase; B: Pooled levels of γ-Glutamyltransferase; C: Pooled levels of total bilirubin; D: Pooled levels of direct bilirubin in the patients with coronavirus disease 2019. ALP: Alkaline phosphatase; GGT: γ-Glutamyltransferase; TBIL: Total bilirubin; DBIL: Direct bilirubin.

A

Study	Non-Severe Number	Mean	SD	Severe Number	Mean	SD	MD	95%-CI	Weight (fixed)	Weight (random)
Cao M 2020	176	41.1	3.4	19	36.6	5.1	4.5	[2.2; 6.8]	0.5%	3.6%
Chen G 2020	10	37.6	4.0	11	31.5	5.5	6.1	[2.0; 10.2]	0.2%	2.8%
Huang C 2020	28	33.7	4.9	13	28.4	3.8	5.3	[2.6; 8.0]	0.4%	3.4%
Chen X 2020	241	38.1	4.5	50	33.9	4.6	4.2	[2.8; 5.6]	1.4%	4.0%
Fu L 2020	211	40.4	5.4	139	36.1	4.7	4.3	[3.2; 5.4]	2.4%	4.1%
Liu L 2020	44	41.0	4.6	7	34.6	2.8	6.4	[3.9; 8.9]	0.4%	3.6%
Zhang Y 2020	84	40.4	3.2	31	34.4	4.1	6.0	[4.4; 7.6]	1.1%	3.9%
Zhang H 2020	29	42.9	4.3	14	38.1	5.0	4.8	[1.7; 7.9]	0.3%	3.3%
Wu C 2020	117	33.6	4.0	84	30.3	4.7	3.3	[2.1; 4.5]	1.8%	4.0%
Wan S 2020	95	43.3	4.7	40	35.8	4.2	7.5	[5.9; 9.1]	1.0%	3.9%
Xiang TX 2020	40	44.3	4.5	9	37.2	3.3	7.1	[4.5; 9.7]	0.4%	3.5%
Li D 2020	63	38.8	2.9	17	34.4	3.0	4.4	[2.8; 6.0]	1.1%	3.9%
Lu H 2020	243	40.7	4.2	22	36.4	5.1	4.3	[2.1; 6.5]	0.6%	3.7%
Mo P 2020	70	39.0	4.5	85	36.0	6.0	3.0	[1.3; 4.7]	1.0%	3.9%
Wang Q 2020	39	42.1	5.2	10	37.8	6.5	4.3	[0.0; 8.6]	0.1%	2.7%
Shang H 2020	282	32.0	5.1	162	28.8	4.3	3.2	[2.3; 4.1]	3.4%	4.1%
Macarena RV 2020	70	41.4	3.8	18	37.4	5.6	4.0	[1.3; 6.7]	0.4%	3.4%
Yang LH 2020	171	38.2	3.9	29	31.1	6.9	7.1	[4.5; 9.7]	0.4%	3.5%
Yin Zw 2020	282	40.1	5.1	192	37.1	5.0	3.0	[2.1; 3.9]	3.2%	4.1%
Wang W 2020	34	32.6	3.1	20	32.1	5.3	0.5	[-2.0; 3.0]	0.4%	3.5%
Zheng SH 2020	1684	38.8	4.1	59	30.7	4.2	8.1	[7.0; 9.2]	2.3%	4.1%
Huang TT 2020	23	41.6	3.7	13	38.3	4.9	3.3	[0.2; 6.4]	0.3%	3.3%
Zhang YF 2020	84	40.4	3.2	31	34.4	4.1	6.0	[4.4; 7.6]	1.1%	3.9%
Hong KS 2020	85	3.9	0.5	13	3.0	0.3	0.9	[0.7; 1.1]	71.4%	4.2%
Wang D 2020	72	38.6	2.3	71	32.1	2.9	6.5	[5.6; 7.4]	3.7%	4.1%
Aliye B 2020	145	45.8	5.1	46	35.9	7.0	9.9	[7.7; 12.1]	0.6%	3.7%
Cao ZH 2020	53	36.8	4.3	27	32.8	6.5	4.0	[1.3; 6.7]	0.4%	3.5%
Total	**4475**	***38.3***	***7.0***	**1232**	***33.6***	***6.5***				
Fixed effect model							**2.1**	**[1.9; 2.2]**	**100.0%**	---
Random effects model							**4.9**	**[3.7; 6.1]**	---	**100.0%**

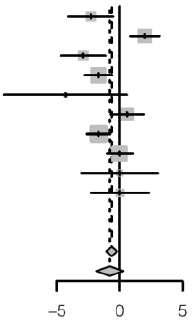
Heterogeneity: $I^2 = 96\%$, $\tau^2 = 8.5888$, $p < 0.01$



B

Study	Non-Severe Number	Mean	SD	Severe Number	Mean	SD	MD	95%-CI	Weight (fixed)	Weight (random)
Chen X 2020	241	26.8	4.3	50	29.1	6.1	-2.3	[-4.1; -0.5]	6.0%	10.1%
Fu L 2020	211	29.4	5.2	139	27.4	5.5	2.0	[0.8; 3.2]	14.3%	12.1%
Zhang Y 2020	84	28.4	3.5	31	31.3	4.6	-2.9	[-4.7; -1.1]	6.0%	10.1%
Wu C 2020	117	30.3	3.2	84	32.0	4.3	-1.7	[-2.8; -0.6]	16.1%	12.3%
Xiang TX 2020	40	27.0	3.4	9	31.3	7.3	-4.3	[-9.2; 0.6]	0.8%	3.6%
Mo P 2020	70	29.0	4.5	85	28.4	3.8	0.6	[-0.7; 1.9]	10.8%	11.5%
Shang H 2020	282	30.8	4.3	162	32.5	5.1	-1.7	[-2.6; -0.8]	21.9%	12.7%
Yin Zw 2020	192	27.1	5.1	192	27.1	5.1	0.0	[-1.0; 1.0]	18.3%	12.4%
Wang W 2020	20	30.2	4.9	20	30.2	4.9	0.0	[-3.0; 3.0]	2.1%	6.6%
Zhang YF 2020	31	31.3	4.6	31	31.3	4.6	0.0	[-2.3; 2.3]	3.6%	8.6%
Total	**1288**	***28.9***	***4.7***	**803**	***29.5***	***5.5***				
Fixed effect model							**-0.6**	**[-1.1; -0.2]**	**100.0%**	---
Random effects model							**-0.8**	**[-1.9; 0.3]**	---	**100.0%**

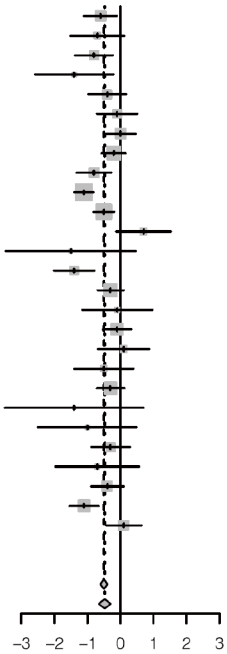
Heterogeneity: $I^2 = 80\%$, $\tau^2 = 2.1428$, $p < 0.01$



C

Study	Non-Severe Number	Mean	SD	Severe Number	Mean	SD	MD	95%-CI	Weight (fixed)	Weight (random)
Cao M 2020	176	13.3	0.6	19	13.9	1.1	-0.6	[-1.1; -0.1]	4.2%	4.6%
Chen G 2020	10	13.4	1.0	11	14.1	0.9	-0.7	[-1.5; 0.1]	1.6%	2.9%
Han Y 2020	23	11.9	0.9	24	12.7	1.1	-0.8	[-1.4; -0.2]	3.2%	4.1%
Huang C 2020	28	10.9	1.8	13	12.3	1.8	-1.4	[-2.6; -0.2]	0.7%	1.8%
Wang D 2020	102	12.9	0.8	36	13.3	1.7	-0.4	[-1.0; 0.2]	3.2%	4.1%
Xu Y 2020	44	12.1	1.7	25	12.2	0.9	-0.1	[-0.7; 0.5]	2.8%	3.9%
Zhao W 2020	57	12.7	0.7	20	12.7	1.0	0.0	[-0.5; 0.5]	4.7%	4.7%
Zhang H 2020	29	10.4	0.5	14	10.6	0.6	-0.2	[-0.6; 0.2]	7.9%	5.5%
Zhang G 2020	166	12.7	1.0	55	13.5	1.9	-0.8	[-1.3; -0.3]	3.8%	4.4%
Wu C 2020	117	10.7	1.1	84	11.8	1.0	-1.1	[-1.4; -0.8]	12.3%	5.9%
Wan S 2020	95	10.8	0.7	40	11.3	0.9	-0.5	[-0.8; -0.2]	10.7%	5.8%
Gao Y 2020	28	12.0	1.2	15	11.3	1.4	0.7	[-0.1; 1.5]	1.5%	2.8%
Xiang TX 2020	40	12.5	0.7	9	14.0	3.0	-1.5	[-3.5; 0.5]	0.3%	0.8%
Li D 2020	63	13.6	0.9	17	15.0	1.2	-1.4	[-2.0; -0.8]	2.8%	3.9%
Liu J 2020	27	13.1	0.6	13	13.4	0.6	-0.3	[-0.7; 0.1]	6.7%	5.2%
Lei S 2020	19	11.5	1.4	15	11.6	1.7	-0.1	[-1.2; 1.0]	0.9%	2.1%
Lu H 2020	243	13.4	0.6	22	13.5	1.0	-0.1	[-0.5; 0.3]	5.8%	5.1%
Frederick SB 2020	54	14.5	2.4	51	14.4	1.6	0.1	[-0.7; 0.9]	1.7%	3.1%
Gao YJ 2020	43	12.1	0.8	19	12.6	1.9	-0.5	[-1.4; 0.4]	1.3%	2.6%
Yang LH 2020	171	11.0	0.7	29	11.3	1.1	-0.3	[-0.7; 0.1]	6.1%	5.1%
Zhang TJ 2020	16	11.3	1.0	14	12.7	3.9	-1.4	[-3.5; 0.7]	0.2%	0.7%
Reza S 2020	102	15.3	2.4	11	16.3	2.4	-1.0	[-2.5; 0.5]	0.5%	1.2%
Wang W 2020	34	13.3	1.0	20	13.6	1.1	-0.3	[-0.9; 0.3]	3.0%	4.0%
Huang TT 2020	23	11.4	1.6	13	12.1	2.0	-0.7	[-2.0; 0.6]	0.7%	1.6%
Wang D 2020	72	11.3	1.4	71	11.7	1.6	-0.4	[-0.9; 0.1]	4.3%	4.6%
Aliye B 2020	145	12.4	1.1	46	13.5	1.4	-1.1	[-1.5; -0.7]	5.4%	4.9%
Cao ZH 2020	53	12.7	0.7	27	12.6	1.3	0.1	[-0.4; 0.6]	3.8%	4.4%
Total	**1980**	***12.5***	***1.6***	**733**	***12.7***	***1.9***				
Fixed effect model							**-0.5**	**[-0.6; -0.4]**	**100.0%**	---
Random effects model							**-0.5**	**[-0.7; -0.3]**	---	**100.0%**

Heterogeneity: $I^2 = 62\%$, $\tau^2 = 0.1249$, $p < 0.01$



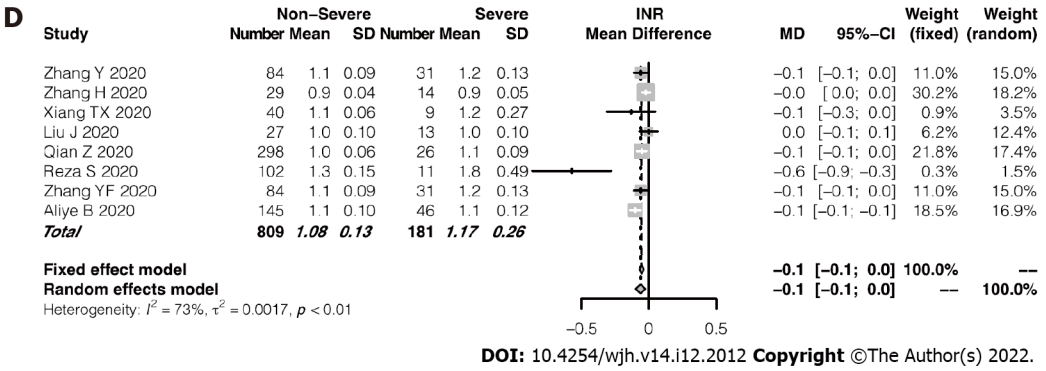


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Figure 5 Forest plot for the association of the synthetic function-related indexes and disease severity. A: Pooled albumin levels; B: Globulin levels; C: Prothrombin times; D: International standardized ratios in the patients with coronavirus disease 2019. ALB: Albumin; GLB: Globulin; PT: Prothrombin time; INR: International standardized ratio.

ARTICLE HIGHLIGHTS

Research background

According to the World Health Organization released situation report, many of people were confirmed coronavirus disease (COVID-19) globally.

Research motivation

Severe COVID-19 was more likely to be associated with abnormal liver test results.

Research objectives

A close monitoring of liver chemistries can provide an early warning of disease progression.

Research methods

We used 56 studies, which included a total of 11052 patients for Meta-Analyses to explored the difference of liver chemistries from severe cases of COVID-19 to non-severe cases.

Research results

This article showed that severe cases of COVID-19 tended to have higher alanine aminotransferase or aspartate aminotransferase, alkaline phosphatase/γ-glutamyltransferase, and total bilirubin levels; prolonged prothrombin time; and higher international standardized ratio. However, the severe cases had lower albumin levels than the non-severe cases.

Research conclusions

Severe COVID-19 was more likely to be associated with abnormal liver test results.

Research perspectives

In the future, more targeted therapies and holistic care approaches may be developed as a result of better knowledge.

FOOTNOTES

Author contributions: Pan JS and Hong MZ were involved with the study conceptualization and design; analysis and interpretation of data; drafting of the manuscript; and approval of the final version of the manuscript; Dong X, Zeng DY, and Xing QQ were involved in data retrieval; All authors read and approved the final manuscript; Dong X and Zeng DY contributed equally to this work.

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PRISMA 2009 Checklist statement: The study only utilizes publically available published aggregated anonymous data, not a human subject research. Potential studies were retrieved in accordance with the PRISMA guideline.

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SYSTEMATIC REVIEWS

CLIF-SOFA and CLIF-C scores for the prognostication of acute-on-chronic liver failure and acute decompensation of cirrhosis: A systematic review

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Abstract

BACKGROUND

Acute-on-chronic liver failure (ACLF) is a syndrome characterized by decompensation in individuals with chronic liver disease, generally secondary to one or more extra-hepatic organ failures, implying an elevated mortality rate. Acute decompensation (AD) is the term used for one or more significant consequences of liver disease in a short time and is the most common reason for hospital admission in cirrhotic patients. The European Association for the Study of Liver-Chronic-Liver Failure (EASL-CLIF) Group modified the intensive care Sequential Organ Failure Assessment score into CLIF-SOFA, which detects the presence of ACLF in patients with or without AD, classifying it into three grades.

AIM

To investigate the role of the EASL-CLIF definition for ACLF and the ability of CLIF-SOFA, CLIF-C ACLF, and CLIF-C AD scores for prognosticating ACLF or AD.

METHODS

This study is a literature review using a standardized search method, conducted using the steps following the guidelines for reporting systematic reviews set out by the PRISMA statement. For specific keywords, relevant articles were found by searching PubMed, ScienceDirect, and BioMed Central-BMC. The databases were searched using the search terms by one reviewer, and a list of potentially eligible studies was generated based on the titles and abstracts screened. The data were then extracted and assessed on the basis of the Reference Citation Analysis (https://www.referencecitationanalysis.com/).

RESULTS

Most of the included studies used the EASL-CLIF definition for ACLF to identify cirrhotic patients with a significant risk of short-term mortality. The primary

outcome in all reviewed studies was mortality. Most of the study findings were based on an area under the receiver operating characteristic curve (AUROC) analysis, which revealed that CLIF-SOFA, CLIF-C ACLF, and CLIF-C AD scores were preferable to other models predicting 28-d mortality. Their AUROC scores were higher and able to predict all-cause mortality at 90, 180, and 365 d. A total of 50 articles were included in this study, which found that the CLIF-SOFA, CLIF-C ACLF and CLIF-C AD scores in more than half of the articles were able to predict short-term and long-term mortality in patients with either ACLF or AD.

CONCLUSION

CLIF-SOFA score surpasses other models in predicting mortality in ACLF patients, especially in the short-term. CLIF-SOFA, CLIF-C ACLF, and CLIF-C AD are accurate short-term and long-term mortality prognosticating scores.

Key Words: End-stage liver disease; Acute-on-chronic liver failure; CLIF-SOFA; CLIF-C ACLF; CLIF-C AD

Core Tip: Acute-on-chronic liver failure (ACLF) is a serious medical challenge worldwide, and its occurrence is a difficult clinical incident due to its severe presentation, quick disease course, and elevated short-term mortality. The European Association for the Study of Liver-Chronic-Liver Failure (EASL-CLIF) Consortium proposal has gained considerable acceptance as a diagnostic criteria for ACLF. CLIF-SOFA has increased the ability to detect patients with ACLF. Unless presenting with renal impairment and/or mild to moderate hepatic encephalopathy, cirrhotic patients with acute decompensation and single liver failure (or any other single "non-renal" organ failure) had a minimum mortality risk. These results suggest that CLIF-SOFA score surpasses other models in predicting mortality in ACLF patients, especially in the short-term.

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INTRODUCTION

Acute-on-chronic liver failure (ACLF) is a syndrome characterized by liver decompensation in individuals with chronic liver disease. It is associated with one or more extra-hepatic organ failures and an elevated mortality rate[1-4].

Acute decompensation (AD) is the term used for the occurrence of one or more significant complications of liver disease in a short period of time (*i.e.*, bacterial infection, gastrointestinal haemorrhage, ascites, encephalopathy)[5-9]. It is the most common reason for hospital admission in cirrhotic patients. Most of these patients will develop AD without any other significant features, while others will develop AD associated with multiple organ failures (*i.e.*, kidney failure, declining liver function, and/or other organ failures). Nevertheless, AD patients with extra-hepatic organ failures are at greater risk for short-term mortality[10-12].

In Europe and America, the primary cause of ACLF is alcohol, while viral hepatitis infection is the main cause of ACLF in Asia, particularly in China[13]. Despite procedures such as haemodialysis and liver transplantation significantly increasing short-term survival, they are not widely available in medical care due to their high cost, the requirement for hospital admission, and the limited availability of liver resources[14]. ACLF places a significant financial burden on patients and on the healthcare system.

A European prospective multi-centric study named CANONIC developed and published in 2013 definitions and a classification and grading of ACLF. The most common reasons for cirrhosis were alcoholic liver disease, chronic hepatitis C, and/or both[15]. Hepatic (alcoholic liver injury) and extra-hepatic disorders (gastrointestinal bleeding or bacterial infection) were the most common precipitating disorders for decompensation of cirrhosis, with or without ACLF. The most common organ failures (OFs) were kidney (55.8% of ACLF patients) and liver failure (43.6%), then coagulation (27.7%) and cerebral failure (24.1%). Heart and respiratory failures were the least common, around 16.8% and 9.2%, respectively[15]. Twenty-eight-day transplant-free mortality rate in ACLF patients was 32.8%, while in

patients without ACLF, it was 1.9%[15].

Ascites, a higher model for end-stage liver disease (MELD) score, low haemoglobin (Hb) levels, and low mean arterial pressure were defined as predictive factors for ACLF development in a large single-centre Italian prospective cohort of cirrhotic outpatients[16]. The European Association for the Study of Liver-Chronic-Liver Failure (EASL-CLIF) consortium has stated that today's global mortality rate of ACLF ranges from 30% to 50%.

The aim of the current study is to provide an overview of research into the role of the EASL-CLIF definition for ACLF, as well as the ability of CLIF-Sequential Organ Failure Assessment (SOFA), CLIF-C ACLF and CLIF-C AD scores to predict adverse outcomes associated with chronic liver disease.

Prognostic scoring systems

Various predictive scores have previously been developed. Nearly fifty years ago, the Child-Turcotte-Pugh (CTP) (Table 1) score was established as the most relevant liver-specific score[17]. Wiesner's study evaluated data to develop the MELD score that outperformed the CTP score in predicting 90-d death in individuals with chronic end-stage liver disease[18]. The MELD-Na score (Table 2), which combines the MELD score with serum sodium content, has enhanced predictive accuracy in patients with cirrhosis awaiting liver transplantation[19]. The CLIF-SOFA score, a new scoring system that is an adaptation of the original SOFA score, was used to describe ACLF in the EASL-CLIF CANONIC study of ACLF in cirrhotic patients (Table 3). It has been used to distinguish AD from ACLF, classifying it into three grades[15]. The EASL-CLIF consortium also established the CLIF consortium organ failure (CLIF-C OF) score.

Jalan *et al*[20], described that age and white blood cell (WBC) counts are independent risk factors for death in subsequent investigations and developed the CLIF-C ACLF score. The EASL-CLIF Group created an online calculator for calculating CLIF-SOFA and either CLIF-C ACLF or CLIF-C AD (https://www.clifresearch.com/ToolsCalculators.aspx).

CLIF-C ACLF Score Formula: The CLIF-C ACLF Score Formula[21] combines (CLIF-C OF score, age, and WBC) with the following formula: CLIF-C ACLF = 10 × [0.33 × CLIF-OFs + 0.04 × Age + 0.63 × Ln (WBC)] – 2.

CLIF-C AD Score Formula: The CLIF-C AD Score Formula (non-ACLF patients with AD) combines (Age, Creatinine, international normalized ratio (INR), WBC, and Sodium) with the following formula [22,23]: CLIF-C AD = 10 × [0.03 × Age + 0.66 × Ln (Creatinine mg/dL) + 1.71 × Ln (INR) + 0.88 × Ln (WBC 10^9 cells/L) – 0.05 × (Sodium mmol/L) + 8].

ACLF Grades[15]: Grade I ACLF: Only kidney failure. [According to Shah *et al*[24], grade 1 could be with one of the following: Liver failure, kidney failure, coagulation, circulatory, or lung failure, with creatinine (1.5 - 1.9 mg/dL), or hepatic encephalopathy (grade 1 or 2), or brain failure with creatinine (1.5 - 1.9 mg/dL)]. Grade II ACLF: Two organ failures. Grade III ACLF: Three organ failures.

MATERIALS AND METHODS

This study is a literature review using a standardized search method, conducted using the steps following the guidelines for reporting systematic reviews set out by the PRISMA statement (Preferred Reporting Items for Systematic Reviews and Meta-analyses)[25].

Search strategy

For relevant original studies, a literature search was conducted using PubMed, ScienceDirect, and BioMed Central-BMC databases. The search command used was a combination of words and Boolean characters: ("CLIF-SOFA" OR "CLIF-C ACLF" OR "CLIF-C AD") AND ("acute-on-chronic liver failure"). Reference Citation Analysis (https://www.referencecitationanalysis.com/) was used to supplement the search.

Study selection

Studies were included if they analyzed data of patients more than 18 years old from the emergency department or inpatient settings. They needed to report data using ACLF definitions and scores published by the EASL-CLIF group and had a full text available. Studies were excluded if they used only scores other than CLIF-SOFA and CLIF-C AD or CLIF-C ACLF, if they were not written in English or if they were reviews, letters, editorials, opinion articles, conference abstracts, and *in-vitro* studies.

Data extraction and synthesis

The databases were searched using the above search terms by one reviewer, and a list of potentially eligible studies was generated based on the titles and abstracts screened. Then, a full-text review was conducted, using the inclusion and exclusion criteria.

Table 1 Child-Turcotte-Pugh scores

Points	1	2	3
Ascites	Absent	Slight	Moderate
Serum Bilirubin (mg/dL)	< 2	2-3	> 3
Serum Albumin (g/dL)	> 3.5	2.8-3.5	< 2.8
PT ratio or	< 4	4-6	> 6
INR	< 1.7	1.7–2.3	> 2.3
HE	None	Grade I-II	Grade III-IV

PT: Prothrombin time; INR: International normalized ratio; HE: Hepatic encephalopathy.

Table 2 MELD and MELD-Na[62,63]: Model for end-stage liver disease–sodium

MELD	Mortality rate (%)	MELD-Na	Mortality rate (%) (90-d)
≤ 9	1.9	< 17	< 2
10-19	6	17-20	3-4
20-29	19.6	21-22	7-10
30-39	52.6	23-26	14-15
≥ 40	71.3	27-31	27-32
		≥ 32	65-66

MELD: End-stage liver disease.

Table 3 CLIF-SOFA score[64]

Points	0	1	2
Liver Bilirubin (mg/dL)	< 1.2	≥ 1.2 - < 2.0	≥ 2.0 - < 6.0
Renal Creatinine (mg/dL)	< 1.2	≥ 1.2 - < 2.0	≥ 2.0 - < 3.5
Neurological HE grade	-	1	2
Haematological INR	< 1.1	≥ 1.1 - < 1.25	≥ 1.25 - < 1.5
Circulation MAP (mmHg)	≥ 70	< 70	Dopamine ≤ 5 or Dobutamine or Terlipressin
Respiratory PaO_2/FiO_2 or SpO_2/FiO_2	> 400; > 512	> 300-≤ 400; > 357 - ≤ 512	> 200 - ≤ 300; > 214 - ≤ 357

RRT: Renal Replacement Therapy; HE: Hepatic encephalopathy; INR: International Normalized Ratio; PaO_2: Partial pressure of arterial oxygen; MAP: Mean Arterial Pressure; FiO_2: Fraction of inspired oxygen; SpO_2: Pulse oximetric saturation.

RESULTS

Study selection

Figure 1 shows the study search and the selection process, including the reasons for exclusion after a full-text review. A total of 50 related articles were included in the final review.

Study quality

Most of the included studies used the EASL-CLIF definition for ACLF to identify patients with cirrhosis who had a significant risk of short-term mortality. Some articles used the Asian Pacific Association for the Study of the Liver and Chinese Group on the Study of Severe Hepatitis B-ACLF (COSSH-ACLF) prognostic criteria. The included studies were not assessed using a quality assessment tool, although they were considered to be good quality.

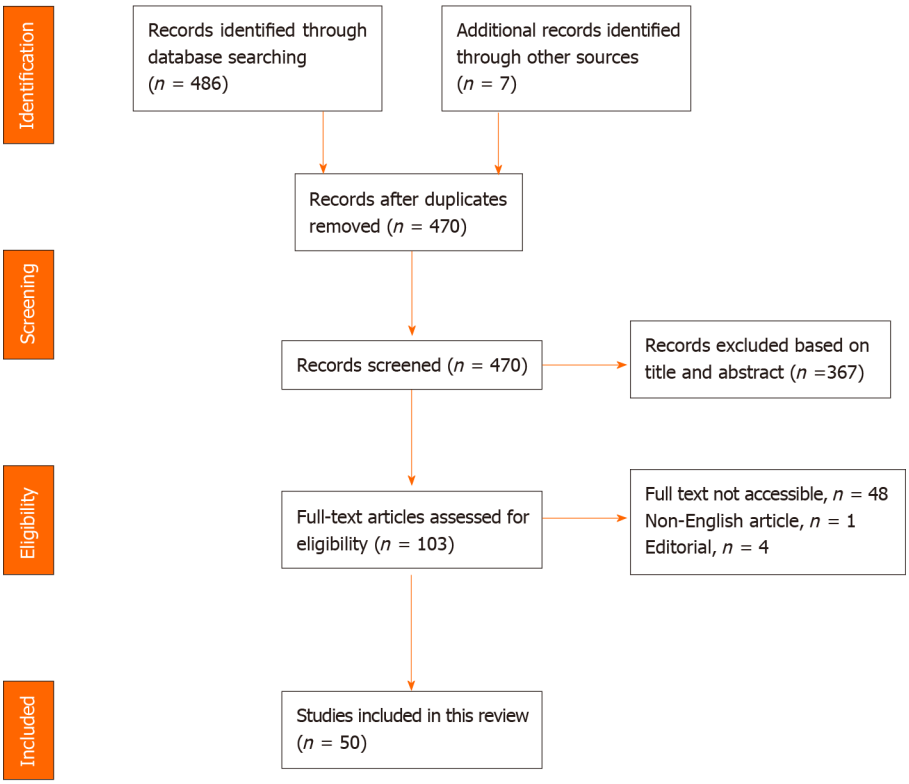

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Figure 1 PRISMA diagram of the study selection process.

Study outcome

The primary outcome in all reviewed studies was mortality. Most of the studies' findings were based on an area under the receiver operating characteristic curve (AUROC) analysis, which revealed that CLIF-SOFA, CLIF-C ACLF, and CLIF-C AD scores were preferable to other models predicting 28-d mortality (Table 4). They had the greatest AUROC scores predicting overall mortality at 90, 180, and 365 d.

DISCUSSION

ACLF has become a serious medical challenge, and it remains a complex clinical scenario for hepatologists and specialists in different related departments due to its severe presentation, and quick disease course with high short-term mortality. Regional differences when defining ACLF and understanding its diagnostic methods has led to many clinical phenotypes. The current therapeutic management of ACLF patients primarily focuses on treating and supporting multiple organ failures[26].

The CANONIC study introduced accurate criteria for the diagnosis of this condition. The CLIF-SOFA score was developed and evaluated for the prognosis of ACLF in the CANONIC research[15]. This development has increased the ability to distinguish patients with ACLF from those with AD using the CLIF-SOFA parameters[15].

Every scoring system has advantages and disadvantages. Even though the CLIF-SOFA score has a significant prognosticative accuracy, its calculation is challenging due to the combination of many indicators[14]. The CTP score is calculated by the ascites, serum bilirubin, albumin, prothrombin time, and hepatic encephalopathy (HE) levels[17]. The presence of HE and ascites is a component of the CTP score; nevertheless, these are subjective, without a defined cut-off value. The MELD score includes three laboratory markers: INR, bilirubin, and creatinine; nevertheless, it is susceptible to confounding factors such as haemorrhage, ascites, and diuretic treatment, and there are no obviously defined cut-off levels for identifying patients with cirrhosis[27]. The MELD score does not include subjective indicators, which may diminish evaluating reliability[28].

Hyponatraemia is strongly associated with the prognosis of cirrhotic patients, especially those with ascites; thus, the MELD-Na score was developed to improve on the MELD score[29].

Jalan *et al*[20] in 2014, showed that the CLIF-C OF accuracy is similar to the CLIF-SOFA score in predicting mortality. The CLIF-C ACLF score does not consider only the role of extra-hepatic organ injuries, circulatory system failure, and coagulation impairment on prognosis, but also includes the WBC count, in order to assess the level of inflammation. In this study, the CLIF-C ACLF score outper-

Table 4 Summary of selected studies

Ref.	Year	Country	Aim	Setting	Results	Conclusions
Kuo *et al*[65]	2021	Taiwan	Assess the predictive value and clinical reliability of three different scores	ACLF patients admitted to the ICU	Non-survivor: CLIF-C ACLF, CLIF-C ACLF lactate, and CLIF-C ACLF-D were 58.85 ± 11.40, 60.88 ± 13.71, and 34.03 ± 1.57, respectively. Survivor: 44.55 ± 9.14, 46.91 ± 11.66, and 32.29 ± 1.17, respectively, (all *P* values < 0.01)	The CLIF-C ACLF-D score may be a better predictor of short- and long-term mortality
Li *et al*[66]	2017	China	Assess various prognostic scores, such as the CLIF-C OFs, CLIF-SOFAs, CLIF-C ACLFs, ACLF grade, and MELD, predicted short-term (28-d) mortality	CHB patients with ACLF	Scores in no ACLF group and for ACLF group grades 1, 2, and 3, respectively: CLIF-C OFs: 7, 9, 10, and 13; CLIF-C ACLFs: 29, 37, 44, and 60; CLIF-SOFAs: 5, 7, 9, and 13; MELDs: 16, 22, 30, and 37	CLIF-C OF score outperforms other scores
Dong *et al*[67]	2020	China	Determine the characteristics and outcomes of ACLF	ACLF patients who have or do not have cirrhosis	COSSH ACLF score (AUROC = 0.778 or 0.792, 95%CI 0.706-0.839 or 0.721–0.851) displayed the better prognostic ability for EASL ACLF patients with non-cirrhosis. CLIF-C ACLF score (AUROC = 0.757 or 0.796, 95%CI 0.701–0.807 or 0.743-0.843) still was the best prognostic scoring system in EASL ACLF patients with cirrhosis	CLIF-C ACLF score was better at predicting short-term mortality in ACLF patients with cirrhosis, while the COSSH ACLF score was better for ACLF patients without cirrhosis
Grochot *et al*[68]	2020	Brazil	Determine the accuracy of the presence of ACLF in predicting mortality.	Patients with cirrhosis	CLIF-SOFA score at 28-, 90-, and 365-d was 1.32, 1.3, and 1.2, respectively. CLIF-C AD/ACLF score was 1.0, 1.0, and 1.0, respectively	CLIF-SOFA score increased mortality by 1.3 times for each point
Jacques *et al*[41]	2020	Brazil	Assess and compare the liver-specific scores ability to predict mortality	Cirrhotic patients with SBP	CLIF-SOFA was able to predict mortality at 30-, 90-, and 365-d, with an AUROC of 0.75, 0.64, and 0.64, respectively. CLIF-C AD or CLIF ACLF scores 0.59, 0.51, and 0.52, respectively	CLIF-SOFA outperformed other liver-specific measures
Terres *et al*[39]	2022	Brazil	Assess and compare the significance of liver-specific scores in predicting mortality	HRS patients who received terlipressin	CTP at 30-, 90- and 365-d mortality 0.76, 0.75 and 0.72, respectively. CLIF-SOFA 0.66, 0.63, and 0.57. CLIF-C ACLF 0.60, 0.55, and 0.53. MELD 0.67, 0.64, and 0.5. MELD-Na 0.65, 0.63, and 0.52	CTP was able to predict increased mortality at 30-, 90- and 365-d
Terres *et al*[40]	2021	Brazil	Evaluate the liver-specific scores to predict mortality	AOVH patients who received terlipressin	AUROC at 30- and 90-d**:** MELD-Na 0.77 and 0.78. CLIF-SOFA 0.76 and 0.75. CLIF-C AD or ACLF 0.64 and 0.60. MELD 0.75 and 0.77. CTP 0.75 and 0.76	CLIF-SOFA was better in ACLF patients. CTP performed better in AD patients
Grochot *et al*[56]	2019	Brazil	Assess the validity of CLIF SOFA in predicting mortality and compare it to other liver-specific scores	AD and ACLF patients	AUROC **at** 28-, 90- and 365-d**,** respectively: CLIF-SOFA 0.71, 0.75 and 0.66. CLIF-C AD/ACLF 0.52, 0.51, and 0.56. MELD 0.54, 0.50, and 0.52. MELD-Na 0.57, 0.54, and 0.55	CLIF-SOFA predicted 90-d mortality better than other scores
Jacques *et al*[69]	2021	Brazil	Evaluate the relation between ACLF and mortality	Cirrhotic patients with SBP	Scores for 28- and 90-d mortality, respectively: MELD 0.83 and 0.87. CLIF-SOFA 1.1 and 1.1. CTP 31 and 8.3	Elevated CLIF-SOFA scores and the presence of ACLF were related to higher 28- and 90-d mortality
Engelmann *et al*[21]	2018	United Kingdom	Assess if the currently available scores can identify patients with ACLF	Patients with ACLF	AUROC of 28-d mortality prediction: CLIF-C ACLF 0.8. CLIF-C OF 0.75. MELD, 0.68. CP 0.66	CLIF-C ACLF accurately predicted 28-d mortality
Barosa *et al*[70]	2017	Portugal	Evaluate CLIF-C ACLF, MELD, MELD-Na, and CTP scores for short/medium-term mortality, to identify ACLF frequency and to compare mortality between non-ACLF and ACLF patients	Patients admitted for AD of cirrhosis	Cut-off point in 28- and 90-d mortality, respectively: CLIF-C ACLF 50 and 50. CTP 10 and 10. MELD 17 and 14. MELD-Na 22 and 22	CLIF-C ACLF score outperformed other scores
Ferreira Cardoso *et al*[71]	2019	Portugal	Validate the EASL-CLIF C scores	Patients with and without ACLF	AUROC for CLIF-C ACLF score for 28-d mortality was (0.856 ± 0.071)	CLIF-C AD score of 60 was related to an increased risk of

						developing ACLF
Maipang *et al*[57]	2019	Thailand	Assess ACLF prognostic models and investigation of their discriminative capacities in ACLF patients	Cirrhotic patients with AD and ACLF	Scores for 28-d, 90-d, 6-mo, and 1-yr mortality, respectively: CLIF-SOFA: 0.84, 0.85, 0.80, 0.80. CLIF-C OF: 0.83, 0.82, 0.78, and 0.78. CLIF-C ACLF: 0.79, 0.80, 0.77, and 0.77. CTP: 0.7, 0.67, 0.64, and 0.63. MELD: 0.63, 0.60, 0.56, and 0.56. MELD-Na: 0.63, 0.59, 0.56, and 0.56. iMELD: 0.73, 0.71, 0.67, and 0.68. APACHE II: 0.69, 0.65, 0.63, and 0.63	The CLIF-SOFA had similar predictive accuracy for 28-d mortality as the CLIF-C OF
Li *et al*[36]	2016	China	Assess if CLIF-C OFs criteria can be used to identify patients and if the CLIF-C ACLF score can be used to predict prognosis	HBV cirrhotic patients with ACLF	Assess patients with ACLF for 28-, 90-, 180-, and 360-d mortality, respectively: HBV-ACLF: 0.654, 0.645, 0.644, and 0.640. CLIF-C ACLF: 0.704, 0.685, 0.687, and 0.682. MELD: 0.554, 0.543, 0.543, and 0.540. MELD-Na: 0.549, 0.541, 0.541, and 0.537. Patients without ACLF: for 28-, 90-, 180-, and 360-d mortality, respectively: HBV-AD: 0.737, 0.716, 0.720, and 0.721. CLIF-C AD: 0.733, 0.724, 0.728, and 0.728. MELD: 0.667, 0.653, 0.657, and 0.639. MELD-Na: 0.719, 0.710, 0.701, and 0.682	CLIF-C ACLFs were found to be more accurate in predicting short-term mortality
Chirapongsathorn *et al*[49]	2022	Thailand	Collect epidemiological data and assess a scoring system for predicting mortality	ACLF patients.	AUROC of prognostic scores for 30- and 90-d mortality, respectively: CLIF-SOFA: 0.64 and 0.61 (95%CI: 0.585-0.704). CLIF-OF: 0.62 and 0.59. CLIF-C: 0.62 and 0.61. MELD: 0.60 and 0.56. MELD-Na: 0.60 and 0.57	CLIF-SOFA score had a higher AUROC than the other scores
Zhang *et al*[31]	2018	China	Assess bacterial infection and predictors of mortality	ACLF patients with autoimmune liver disease	CLIF-SOFA score for 28-d mortality was 1.362 and 1.093, respectively.Scores for 90-d mortality were, respectively: CLIF-SOFA 2.936 and 1.578. MELD 1.232 and 0.664. CP 2.003 and 0.595	All scores of ACLF patients with bacterial infection were high
Shin *et al*[72]	2020	South Korea	To look into the risk factors for mortality in cirrhotic patients and to see how ACLF affected their prognosis	Cirrhotic patients with variceal bleeding	Prediction of mortality at 28- and 90-d with AUROC were, respectively: CTP 0.842 and 0.846. MELD 0.857 and 0.867. MELD-Na 0.828 and 0.834. CLIF-SOFA 0.895 (95%CI, 0.829-0.962) and 0.897 (95%CI, 0.842-0.951)	CLIF-SOFA model well predicted 28-d or 90-d mortality
Gao *et al*[73]	2018	China	Investigate the CLIF-SOFA lung score's predictive value and determine the best voriconazole regimen	ACLF patients with IPA	CLIF-SOFA 10 (P = 0.083). CLIF-C ACLF 46.8 (P = 0.028). MELD 27.2 (P = 0.145). MELD-Na 28.6 (P = 0.064)	Patients with a CLIF-SOFA lung score of less than 2 had a superior 28-d survival rate than those with a lung score of more than 1 (P = 0.001)
Chen *et al*[74]	2021	China	Create a predictive nomogram	HBV-ACLF patients undergoing LT	CP score (0.626), MELD (0.627), MELD-Na (0.583), CLIF-C OF (0.674), and CLIF-C ACLF (0.684)	The nomogram's concordance index for predicting 1-yr survival was 0.707, which was significantly greater than that of other prognostic models. The nomogram could be helpful in determining which HBV-ACLF patients may improve after LT
Yu *et al*[75]	2021	China	Multicenter study to develop and evaluate a novel scoring system that uses baseline and dynamic data to predict short-term prognosis	ACLF patients	For 90-d prognosis: DP-ACLF with an AUC value of 0.907, CTP (0.601/74.6%), MELD (0.721/76.2%), MELD-Na (0.740/73.8%), CLIF-SOFA (0.701/76.9%), CLIF-C ACLF (0.694/74.6%), and COSSH-ACLF (0.724/77.7%) (P < 0.001)	The validation group had a higher predictive accuracy of DP-ACLF on ACLF prognosis and an accuracy rate of 85.4%, according to ROC analysis
Liu *et al*[35]	2020	China	Assess different prognostic models to predict short-term mortality	ACLF patients	The AUROCS of the CLIF-SOFA score, PWR, ALBI score, and MELD score was 0.804, 0.759, 0.710, and 0.670, respectively	CLIF-SOFA was the best model for predicting 28-d mortality
Zhang *et al*[76]	2015	China	Examine and contrast the various ACLF diagnostic criteria currently in use. Also,	Selected patients were cirrhotic, fulfilling at	CTP 12 and 11 (P = 0.53). MELD 17.8 and 16.0 (P = 0.02). MELD-Na 20.1 and 18.7 (P = 0.02). CLIF-SOFA 7 and 7 (P = 0.01)	The maximum rise in the CLIF-SOFA score, MELD-Na score, and total bilirubin were all

			to identify predictors of the progress from ACLF at enrolment defined by APASL alone or by both APASL and CMA	least APASL criteria for ACLF		independent predictors of progression into post-enrollment EASL-CLIF ACLF from ACLF at enrollment
Li *et al*[77]	2020	China	Randomized study to assess the scoring systems for predicting short-term results	HBV-ACLF patients	ALBI score (30-d mortality: HR = 3.452; 90-d mortality: HR = 3.822), MELD (30-d mortality: HR = 1.073; 90-d mortality: HR = 1.082), CLIF-C ACLF score (30-d mortality: HR = 1.061; 90-d mortality: HR = 1.065)	All scores accurately predicted 30-d and 90-d mortality. A higher CLIF-C ACLF score was linked to a lower overall survival rate
Zhang *et al*[14]	2020	China	Find prognostic scores that can be used to predict short- and long-term outcomes	ACLF patients with cirrhosis	Scores for survivors and [non-survivors] at 28-d, 3- and 6-mo, respectively: CTP 10 [12] (P = 0.001), 10 [11] (P = 0.028) and 10 [11] (P = 0.033). MELD 16 [24] (P = 0.004), 15 [23] (P = 0.001) and 15 [23] (p=0.002). MELD-Na 18 [24] (P = 0.081), 16.54 [23.27] (P = 0.011) and 17.27 [23] (P = 0.020). CLIF-C OF 9 [11] (P = < 0.001), 9 [10.00] (P = 0.001) and 9 [10] (P = 0.001). CLIF-SOFA 8 [12] (P ≤ 0.001), 8.55 [11.46] (P ≤ 0.001) and 8.53 [11.33] (P ≤ 0.001). CLIF-C ACLF 45.01 [53.98] (P ≤ 0.001), 44.39 [52.85] (P ≤ 0.001) and 44.11 [52.56] (P = 0.001)	The CLIF-SOFA score was particularly useful for assessing 28-d mortality
Kim *et al*[42]	2016	South Korea	A comparative study to evaluate the performance of suggested ACLF-specific scores in predicting short-term mortality	Alcoholic hepatitis patients	The AUROC of CLIF-SOFA, CLIF-C OFs, DF, ABIC, GAHS, MELD, and MELD-Na was 0.86 (0.81-0.90), 0.89 (0.84-0.92), 0.79 (0.74-0.84), 0.78 (0.72-0.83), 0.81 (0.76-0.86), 0.83 (0.78-0.88), and 0.83 (0.78-0.88), respectively, for 28-d mortality. CLIF-SOFA score of 8 had (78.1% Sn and 79.7% Sp), and CLIF-C OFs of 10 had (68.8% Sn and 91.4% Sp) for predicting 28-d mortality	CLIF-SOFA and CLIF-C OF scores performed well for short-term mortality
Costa E Silva *et al*[78]	2021	Brazil	Assess how well prognostic scores predict mortality	Cirrhotic patients admitted to the ICU	AUC revealed in all patients: CTP 0.701, APACHE II 0.695, MELD 0.727, MELD-Na 0.729, MESO index 0.723, iMELD 0.640, SOFA 0.753, CLIF-SOFA 0.776, CLIF-C OF 0.807 and CCI 0.627. CLIF-C OF in ACLF patients (0.749). CLIF-SOFA in AD patients (0.716) and CLIF-C AD (0.695)	CLIF-C OF and CLIF-SOFA had the best ability to predict mortality in all patients
Chen *et al*[38]	2020	Taiwan	Compare the eight prognostic scores	Cirrhotic patients with ACLF	Score on admission to ICU median (IQR) (P ≤ 0.001): CTP 9.0, MELD 23.0, CLIF-C OF 10.0, CLIF-C ACLF 49.2, SAP III 51.0, MPM0-III 0.0 (P = 0.001), APACHE II 16.0, and APACHE III 81.0. Predict overall mortality by AUROC: CTP 0.719, MELD 0.702, CLIF-C OF 0.721, CLIF-C ACLF 0.772, MPM0-III 0.607, SAP III 0.739, APACHE II 0.756 and APACHE III 0.817	APACHE III and CLIF-C ACLF scores were superior to other models for predicting overall mortality
Sheng *et al*[79]	2021	China	Create a new and effective prognosis model and identify new prognostic factors	HRS with AD patients	AUROC in derivation and validation, respectively: GIMNS (0.830 and 0.732), MELD (0.759 and 0.623), CLIF-SOFA (0.767 and 0.661), COSSH-ACLF (0.759 and 0.674). Mortality at 28-d according to the developed GIMNS score: (GIMNS ≥ 2) 100.0%, (GIMNS 1-2) 73.8%, (GIMNS 0-1) 57.1% and (GIMNS < 0) 30.3%	GIMNS had a higher accuracy AUROC and outperformed MELD and CLIF-SOFA
Hong *et al*[80]	2016	South Korea	Evaluate the features and outcomes of ACLF patients	ACLF patients with underlying liver disease	Scores in Type A (non-cirrhosis), B (cirrhosis), and C (cirrhosis with the previous decompensation), respectively: MELD 29, 27 and 26. Hepatic CLIF-SOFA 19, 34 and 21. Extra-hepatic CLIF-SOFA 7, 11 and 31	The 30-d overall survival rate for types A, B, and C, respectively, was 85.3%, 81.1%, and 83.7%
Sy *et al*[54]	2016	Canada	Assess if the CLIF-SOFA score could predict survival	Severely ill patients with ACLF	APACHE II 23; MELD 26; CTP 12; SOFA 15 and CLIF-SOFA 17. The CLIF-SOFA (AUROC 0.865). SOFA (AUROC 0.935)	CLIF-SOFA outperformed the other scores
Cai *et al*[2]	2019	China	Evaluate prognostic scoring models and create prediction models	Various causes of AD in cirrhotic patients	Hepatitis B group, AUROC for 28-d mortality for MELD, CLIF-C-AD, MELD-Na, AARC-ACLF, and the newly developed AD scores was 0.663, 0.673, 0.657, 0.662, and 0.773, respectively.	In predicting the prognosis of AD cirrhosis, the newly developed scoring models for short-term

					Alcoholic liver disease group, 0.731, 0.737, 0.735, 0.689, and 0.778, respectively. Others group 0.765, 0.767, 0.814, 0.720, and 0.814, respectively	mortality outperformed the other models
Marciano *et al*[81]	2017	Argentina	Compare the predictive accuracy for 28- and 90-d transplant-free mortality of a modified CLIF-SOFA score with that of the classic CLIF-SOFA and KDIGO scores	AKI in cirrhotic patients with AD	Classic CLIF-SOFA and modified CLIF-SOFA by AUCROC: In 28-d transplant-free, 0.93 and 0.92 (P = 0.34), respectively. In 90-d transplant-free, 0.79 and 0.78 (P = 0.78), respectively. In AKI 28-d and 90-d transplant-free mortality by AUCROC, 0.67 (P = 0.002) and 0.63 (P = 0.02)	Both CLIF-SOFA scores were extremely accurate in predicting 28-d and 90-d transplant-free mortality
Xu *et al*[82]	2018	China	Recognizing mortality risk variables and optimizing stratification are crucial for increasing survival rates	Cirrhotic patients with pneumonia	Scores by AUROC for predicting mortality in 30-d and 90-d respectively: CLIF-SOFA 0.890 and 0.900. MELD 0.853 and 0.889. MELD-Na 0.801 and 0.849, qSOFA 0.854 and 0.777, PSI 0.867 and 0.831. CTP 0.726 and 0.768	CLIF-SOFA outperformed the other models in predicting mortality
Silva *et al*[83]	2021	Brazil	Assess the prognostic scores predicting mortality	Cirrhotic patients who were admitted to the ICU without being pre-screened	ROC curves SOFA 0.88, MELD-Na 0.76, MELD 0.75, CPS 0.71 and SAPS 3 (0.51). In patients with ACLF, CLIF-ACLF 0.74, CLIF-OF 0.70, MELD-Na 0.73 and MELD 0.69, SAPS 3 (0.55), SOFA 0.63 and CLIF-SOFA 0.66	In patients with and without ACLF, CLIF-ACLF and SOFA had higher accuracy in predicting mortality
McPhail *et al*[46]	2015	United Kingdom	Compare the capabilities of SOFA and CLIF-SOFA scores to predict patient survival and evaluate CLIF-SOFA	Cirrhotic patients	At the time of admission, with AUROC values, CLIF-SOFA and SOFA scores were 0.813 and 0.799, respectively. At 48 h after admission were 0.853 and 0.840, respectively. After 1 wk were 0.842 and 0.844, respectively	SOFA and CLIF-SOFA scores appear to have equal ability to predict patient survival
Yang *et al*[52]	2022	China	Estimate the short-term prognosis of ACLF patients	ACLF patients who had undergone LT	AUROC of MELDs 0.704, ABIC: 0.607, CLIF-C OFs 0.606, CLIF-C ACLFs 0.653 and CLIF-SOFAs 0.633 of the 90-d outcome	MELDs had a higher AUROC than others for predicting the 90-d outcome in ACLF patients after LT
Moreau *et al*[15]	2013	12 European countries	Multicenter study to establish ACLF diagnostic criteria and characterize the progression of the disease	Cirrhotic patients with AD	The increased 28-d mortality rate was linked to three risk variables identified from the CLIF-SOFA score at enrollment: ≥ 2 organ failures, kidney failure alone, a combination of renal dysfunction, and a single organ failure other than kidney and/or hepatic encephalopathy (mild-moderate)	In patients with ACLF, higher CLIF-SOFA scores and leukocyte counts were predictors of mortality. The mortality rates at 28-d and 90-d, respectively: No ACLF 4.7% and 14%. ACLF g1: 22.1% and 40.7%. ACLF g2: 32% and 52.3%. ACLF g3: 76.7% and 79.1%
Li *et al*[37]	2021	China	Create a new simple prognostic score that can accurately predict outcomes	HBV-ACLF patients	The C-indices of the new score for 28- and 90-d mortality (0.826 and 0.809), COSSH-ACLF 0.793 and 0.784; CLIF-C ACLF 0.792 and 0.770; MELD 0.731 and 0.727; MELD-Na 0.730 and 0.726 (all P < 0.05)	The C-indices of the new score were significantly higher than other existing scores for 28-d and 90-d mortality
Perdigoto *et al*[58]	2019		Identify and characterize ACLF, and compare the CLIF-C OF score to the MELD-Na and the CP score. Also, to assess the CLIF-C ACLF and CLIF-C AD scores	Patients with ACLF	In the whole study group, the AUC: For 28-d mortality, the scores MELD, CLIF-C OF, and CP were 0.908, 0.844, and 0.753, respectively. For 90-d mortality 0.902, 0.814, and 0.724, respectively (P < 0.0001 for AUC in all scores)	CLIF-C OF shows good accuracy and diagnoses ACLF. MELD performed better in terms of 90-d mortality prediction
Ramzan *et al*[84]	2020		Evaluate the CLIF-C CLF score and compare it to the MELD score	ACLF patients in ICU	MELD scores 30, 40 and 50 at 48 h were 0.532, 0.594 and 0.529, respectively. CLIF-C ACLF ≥ 70 at 0 h, 24 h, and 48 h were 0.498, 0.605, and 0.643, respectively	CLIF-C ACLF score of 70 or higher accurately predicts mortality
Verma *et al*[85]	2021		Assess the prognostic models	ACLF patients	Day-7 AARC model had the numerically highest c-index, 0.872, best accuracy of 84.0%, Day-7 NACSELD-ACLF sensitivity (100%) but with a lower PPV (70%) for mortality	Patients having an AARC score of > 12 on day 7 had the lowest 30-d survival rate. All model performance parameters were better on day 7
Picon *et al*[59]	2017	Brazil	Assess prognostic	Patients with	Patients with ACLF, at 28-d from the	The CLIF-C ACLF score

			scores	AD of cirrhosis and ACLF	diagnosis: CLIF-C ACLF with an AUC of 0.71. Patients with AD, regarding 28-d mortality: CLIF-C AD 0.75; CP 0.72; MELD 0.75; MELD-Na 0.76; CLIF-C OF 0.74. Patients with AD regarding 90-d mortality: CLIF-C AD 0.70; CP 0.73; MELD 0.7; MELD-Na 0.73; CLIF-C OF 0.65	is the most accurate for predicting 28-d death in patients with ACLF. The CLIF-C AD score was also good in predicting death in cirrhosis with AD
Gupta *et al*[44]	2017	India	Assess the variations in mortality outcomes and predictors	Patients admitted with AD and ACLF caused by hepatic or extra-hepatic insults	AUROC for 28-d mortality in the extrahepatic ACLF group for CLIF-SOFA, MELD, iMELD, APACHE-11, and CTP was 0.788, 0.724, 0.718, 0.634, and 0.726, respectively. AUROC for 28-d mortality in the hepatic ACLF group for CLIF-SOFA, MELD, iMELD, APACHE-11, and CTP was 0.786, 0.625, 0.802, 0.761, and 0.648, respectively	iMELD and CLIF-SOFA were the best for predicting 28-d mortality
Niewiński *et al*[45]	2020	Poland	Use the available prognostic scores to find the best mortality risk factor(s)	Critically unwell ACLF patients	Predictive 90-d mortality: MELD 1.10, SOFA 1.33, CLIF-SOFA 1.40, and CLIF-C OF 1.64	SOFA score surpassed the CLIF-C values
Kulkarni *et al*[55]	2018	India	Determine the in-hospital predictors of 28-d mortality	ACLF patients admitted to the Medical ICU	MELD 0.783 (Sn 75% and Sp 82.1%). CLIF-SOFA 0.947 (Sn 83.3% and Sp 96.4%). CTP 0.795 (Sn 94.4% and Sp 57.1%). APACHE-II 0.876 (Sn 91.6% and Sp 78.5%)	CLIF-SOFA and APACHE-II scores had a superior ability to predict mortality
Dhiman *et al*[86]	2014	India	Assess the efficacy of the CLIF-SOFA and APASL definitions of ACLF in predicting the short-term prognosis of ACLF patients	Patients selected were cirrhotic with AD	AUROCs for 28-d mortality were 0.795, 0.787, 0.739, and 0.710 for CLIF-SOFA, APACHE-II, CTP, and MELD, respectively	The strongest predictor of short-term mortality was the CLIF-SOFA score
Safi *et al*[87]	2018	Germany	Evaluate how infection detected at the time of admission, as well as other clinical baseline factors, affected the mortality	Cirrhotic patients with emergency admissions	Predictors of mortality up to 90 d (all patients): HR, 95%Cl, and *P*, respectively: SOFA 0.15, 0.03-0.69 and 0.015. CLIF C ACLF 1.09, 1.06-1.13 and < 0.001. Infection and CLIF-SOFA and infection and CLIF-C-ACLF: HR, 95%CI and *P*, respectively: CLIF-SOFA 1.33, 1.17- 1.51 and < 0.001 CLIF-SOFA: Infection 0.85, 0.71-1.02 and 0.074. CLIF-C-ACLF 1.09, 1.06-1.12 and < 0.001 CLIF-C-ACLF: Infection 0.96, 0.92-1.01 and 0.082	Infection reduced the significant relation between mortality and CLIF-C-ACLF or CLIF-SOFA-score
Leão *et al*[88]	2019	Brazil	Assess how different ACLF diagnostic criteria performed in terms of predicting mortality	Cirrhotic patients with AD	AUROC at 28-d for CLIF-C, AARC and NACSELD criteria were 0.710, 0.560 and 0.561 ($P = 0.002$), respectively. AUROC at 90-d mortality were 0.760, 0.554 and 0.555 respectively ($P < 0.001$)	CLIF-C performed better in predicting mortality at 28-d and 90-d
Bartoletti *et al*[89]	2018	Different European countries	Summarize the current epidemiology of BSI, and assess predictors of 30-d mortality and antibiotic resistance risk factors	Cirrhotic patients	In a Cox regression model, CLIF-SOFA scores were (HR 1.35; 95%CI 1.28-1.43; $P < 0.001$)	The SOFA and CLIF-SOFA scores were the best predictors of 30-d mortality
Mendizabal *et al*[47]	2021	11 Latin American countries	Evaluate whether SARS-CoV-2 infection affects the outcome and assess the effectiveness of the different prognostic models in predicting mortality	Hospitalized cirrhotic patients	AUROC for performance evaluation in predicting 28-d mortality for CLIF-C, NACSELD, CTP score and MELD-Na were 0.85, 0.75, 0.69, 0.67; respectively ($P < 0.0001$)	In patients with cirrhosis and SARS-CoV-2 infection, CLIF-C performed better than other models

ACLF: Acute-on-chronic liver failure; AD: Acute decompensation; AUC: Area under the curve; AOVH: Acute oesophageal variceal haemorrhage; HRS: Hepatorenal syndrome; CTP: Child-Turcotte-Pugh; SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2; MELD: Model of End-Stage Liver Disease; iMELD: integrated MELD; MELD-Na: sodium MELD; CPC: Child-Pugh class; SOFA: Sequential Organ Failure Assessment; CLIF-SOFA: CLIF-Consortium modification of Sequential Organ Failure Assessment; CLIF-C OF: Organ Failure score; ICU: Intensive care unit; CHB: Chronic hepatitis B; IPA: Invasive pulmonary aspergillosis; CMA: Cow milk induced allergies; APASL: Asian Pacific Association for the Study of the Liver; CPS: Complex Problem Solving; LT: Liver transplantation; PPV: Pulse pressure variation; BSI: Bronchiectasis severity index.

formed the CTP, MELD and MELD-Na scores[30].

This was also true of the CANONIC study data, which demonstrated that CLIF-SOFA, CLIF-C OF and CLIF-C ACLF scores were able to outperform CTP, MELD, and MELD-Na scores when predicting short- and long-term mortality in ACLF patients[15,20].

ACLF and infection

Zhang *et al*[31] in 2018, assessed the relationship between bacterial infection and predictors of mortality in ACLF patients with autoimmune liver disease. No significant association was found between 28-d and 90-d transplant-free mortality and any predictor. The CTP, MELD, and CLIF-SOFA scores of ACLF patients with bacterial infection were all high[31].

ACLF and ascites

Ascites at admission were a potential risk for post-enrollment development of ACLF in the study by Moreau *et al*, as it is an independent prognostic factor of renal failure following bacterial infection[15,32,33]. CLIF-SOFA scores at enrollment and ACLF diagnosis were significant independent predictors for post-enrollment ACLF development and ACLF-associated death, respectively[15].

ACLF and albumin-bilirubin

The albumin-bilirubin (ALBI) score, which uses albumin and bilirubin values to indicate liver injury, effectively predicts the outcome of hepatocellular carcinoma[34]. The ALBI score and the CLIF-SOFA score had a comparable effect in predicting the outcome of ACLF patients, according to the findings of Liu *et al*[35].

ACLF and hepatitis B virus

Hepatitis B virus (HBV) is the most common etiology of ACLF in the East, which differed from patients in Western societies. HBV-ACLF is a pan-Asian and African condition associated with excessively elevated short-term mortality[36]. In 2021, Li *et al*[37] created a new simple prognostic score that can accurately predict outcomes in HBV-ACLF patients. The C-indices of the new score were significantly higher than the C-indices of four existing scores (COSSH-ACLF, CLIF-C ACLF, MELD, and MELD-Na) for 28- and 90-d mortality. Without assessing organ failure, the novel prognostic score can correctly predict short-term mortality in patients with HBV-ACLF and could be used to guide clinical care[37]. In Taiwan, a viral hepatitis endemic country[38], a study demonstrated that APACHE III, CLIF-OF and CLIF-C ACLF scores have outperformed other models for predicting 28-d overall mortality[38].

ACLF and HRS

Terres *et al*[39] assessed and compared the significance of liver-specific scores in predicting mortality in hepatorenal syndrome (HRS) patients who received terlipressin. CTP was superior to CLIF-SOFA, CLIF-ACLF, MELD, and MELD-Na in estimating 30-d, 90-d, and 365-d mortality[39].

ACLF and AOVH

CTP was superior to CLIF-SOFA, CLIF-ACLF, MELD, and MELD-Na in estimating 30-d and 90-d mortality in AD patients, while CLIF-SOFA was better in ACLF patients with acute oesophageal variceal haemorrhage (AOVH) who received terlipressin[40].

ACLF and SBP

CLIF-SOFA has demonstrated superior performance in spontaneous bacterial peritonitis (SBP)[41] and alcoholic hepatitis[42].

ACLF and AKI

Both the standard and the modified CLIF-SOFA scores demonstrated remarkable accuracy for the prognostication of 28-d transplant free-mortality evaluation (AUC-ROC greater than 0.9) in acute kidney injury (AKI) patients with cirrhosis and AD. Nevertheless, it presents a reduced effectiveness in 90-d mortality assessment (AUC-ROC 0.78). These results are comparable to the results reported by Angeli *et al*[43] in 2015.

Hepatic and extra-hepatic injury

A study by Gupta *et al*[44] in 2017, that included hepatic and extra-hepatic ACLF patients showed that, in the hepatic group, iMELD was the best indicator of 28-d mortality. On the other hand, CLIF-SOFA was the strongest predictor of death in the extra-hepatic ACLF cohort. The majority of patients in this cohort were decompensated, and infection was the most frequent extra-hepatic event, leading to systemic inflammation and extra-hepatic organ involvement with fewer liver failures[44].

Critically unwell conditions

In predicting 90-d mortality, the SOFA score surpassed the more commonly used prognostic liver-specific scores (MELD, SOFA, CLIF-SOFA, CLIF-C OF, and CLIF-C ACLF/CLIF-C AD) in a study conducted to describe the best mortality risk factor(s) in critically unwell ACLF patients[45]. The CLIF-C ACLF, CLIF-C OF and ACLF grades varied widely between ACLF patients who underwent liver transplantation and those who died waiting for an organ. At the time of admission, those with two or three organ failures had survival rates ranging from 30% to 55%, whereas patients with more than three

organ failures had mortality rates approaching 80%[46].

AD and SARS-CoV-2

Mendizabal *et al*[47] performed a study to evaluate whether severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection affects the outcome of hospitalized cirrhotic patients and to assess the effectiveness of the different prognostic models in predicting mortality. CLIF-C scores performed better than North American Consortium for the Study of End-Stage Liver Disease (NACSELD)–ACLF score, CTP, and MELD-Na.

ACLF and alcohol intake

Aggressive alcohol intake, alcoholic hepatitis, and bacterial infection were the most common causes of ACLF in alcohol liver disease[48]. The AUROCs of the CLIF-SOFA, CLIF-OF, and CLIF-C scores showed a slight superior effect in estimating short-term mortality; however, they were equivalent to MELD and MELD-Na[49]. To clarify this finding, Chirapongsathorn *et al*[49] had elevated short- and long-term mortality rates. In patients with ACLF, as per the CLIF-C definition, the prediction accuracy of the CLIF-SOFA, CLIF-OF and CLIF-C scoring tools were no better than the accuracy of MELD and MELD-Na scores. In a retrospective investigation by Lee *et al*[50] the CLIF-SOFA score surpassed other scoring systems in estimating short-term mortality in alcoholic cirrhotic patients with AD.

Prognostic scores and liver transplantation

The MELD score is commonly used in liver transplantation (LT) as a scoring method for organ allocation and is the standard model prognostic tool for predicting 3-mo to 6-mo survival in patients with liver failure[51]. Nevertheless, ACLF has a distinct clinical characteristic (Table 5); therefore, the MELD score for patients with ACLF is not expected to be optimal[52].

The MELD score was associated with post-transplant survival but is considered to have poor prediction accuracy[53]. No more trials demonstrated that CLIF-SOFA, CLIF-C ACLF, or CLIF-C OF had good prognostic value for short-term survival after LT[52].

General comparison of prognostic scores

Despite the excellent predictive accuracy of CLIF-C ACLF and CLIF-C OF scores, they were developed analyzing data from patients generally with alcohol-related liver disease from Europe and the United States, and more research is necessary to confirm whether this is appropriate for Asian populations. However, according to the study by Zhang *et al*[14], the scores were also applicable in Asian populations.

A higher CLIF-SOFA was separately associated with higher mortality; this is consistent with previous research, which found that the CLIF-SOFA was better than other liver-specific scores in predicting mortality[42,54,55]. It has been shown by other researchers that CLIF-C ACLF or CLIF-C AD, MELD, and MELD-Na are preferred, even for extra-hepatic injuries[56,57].

In the study by Zhang *et al*[14], the prognostication accuracy and power of the six scores (CTP score, MELD score, MELD-Na, CLIF-ACLF score, CLIF-C OF score and CLIF-SOFA score) were analyzed and compared for 28-, 90- and 180-d overall mortality. The AUROC of CLIF-SOFA was superior to other predictive scores at 28-, 90-, and 180-d mortality, particularly at 28 d. The CLIF-SOFA score provides an overall and efficient evaluation of the severity of multi-organ failure in patients with ACLF by considering various systems, including the hepatic, respiratory, coagulation, circulatory, nervous, and renal systems. Zhang *et al*[14] and other researchers found that at all times, the CLIF-SOFA scores AUROCs were higher than those of other scores. A study performed by Perdigoto *et al*[58] showed that when ACLF is present, the CLIF-C OF score has good accuracy and is able to diagnose ACLF. MELD, on the other hand, performed better in terms of 90-d mortality prediction.

The CLIF-C ACLF score is the most accurate way to predict 28-d mortality in patients with ACLF. The CLIF-C AD score was also beneficial in predicting death in cirrhotic individuals with AD who did not meet diagnostic criteria for ACLF, although it did not outperform other well-established prognostication measures[59].

The CANONIC study found that 28-d mortality was 33.9%, while two Brazilian studies found that mortality rates in ACLF patients were 39%[56,60].

Within the included articles in this study from 2013 to 2022 (Figure 2), CLIF-SOFA was superior to other scores for predicting mortality (mostly in the short-term) in ACLF patients in more than 50% of the included articles, followed by CLIF-C ACLF and CLIF-C AD (30% of the articles)[61-89]. CLIF-C OF was more accurate at 10%. CTP accurately prognosticated ACLF patients with HRS and AOVH patients with AD. The MELD score accurately predicted short-term mortality in ACLF patients who underwent LT (Figure 3).

Table 5 Acute-on-chronic liver failure *vs* acute decompensation liver transplantation[45]

	Liver transplantation ACLF	Liver transplantation AD	*P* value
Total	22 (73.3%)	7 (26.7%)	-
Age (yr)	57.0 (IQR 11.0)	54.0 (IQR 5.0)	n.s.
MELD	30.7 (IQR 5.0)	12.9 (IQR 7.3)	< 0.001
iMELD	53.1 (IQR 8.7)	36.5 (IQR 15.6)	< 0.001
MELD-Na	34.4 (IQR 18.7)	14.3 (IQR 17.6)	0.002
CPC	13.0 (IQR 1.0)	9.0 (IQR 3.0)	< 0.001
SOFA	8.0 (IQR 3.0)	4.0 (IQR 3.0)	< 0.001
CLIF-SOFA	12.0 (IQR 3.0)	5.0 (IQR 3.0)	< 0.001
CLIF-C OF	11.5 (IQR 2.0)	7.0 (IQR 1.0)	< 0.001

ACLF: Acute-on-chronic liver failure; AD: Acute decompensation; MELD: Model of End-Stage Liver Disease; iMELD: integrated MELD; MELD-Na: sodium MELD; CPC: Child-Pugh class; SOFA: Sequential Organ Failure Assessment; CLIF-SOFA: CLIF-Consortium modification of Sequential Organ Failure Assessment; CLIF-C OF: Organ Failure score.

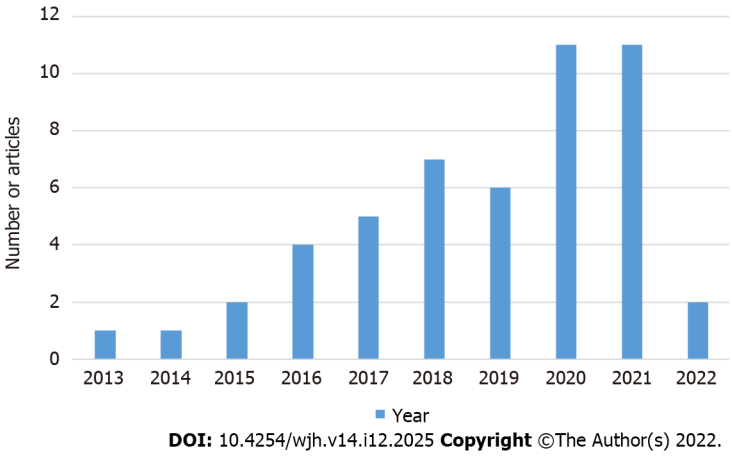

Figure 2 Year of publication.

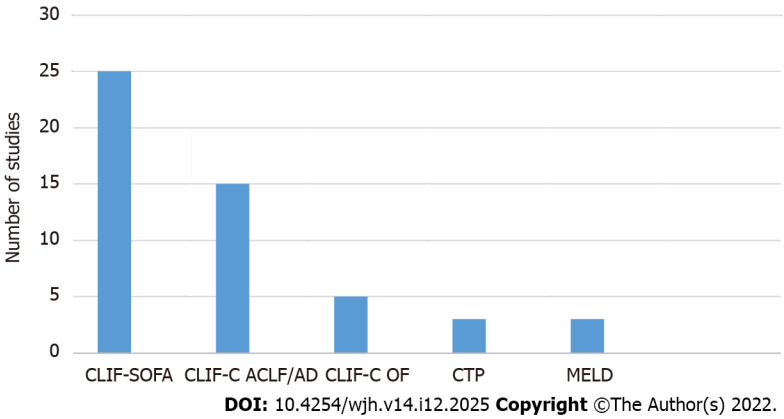

Figure 3 Predicting scores accuracy according to studies. ACLF: Acute-on-chronic liver failure; AD: Acute decompensation; CTP: Child-Turcotte-Pugh; SOFA: Sequential Organ Failure Assessment; CLIF-SOFA: CLIF-Consortium modification of Sequential Organ Failure Assessment; CLIF-C OF: Organ Failure score; MELD: Model of End-Stage Liver Disease.

CONCLUSION

The CLIF-SOFA score surpasses other predictive models in prognosticating short-term mortality in ACLF patients. CLIF-SOFA, CLIF-C ACLF, and CLIF-C AD are accurate in predicting scores for short-term and long-term mortality in patients with ACLF and in predicting adverse outcomes associated with chronic liver disease.

ARTICLE HIGHLIGHTS

Research background

Acute-on-chronic liver failure is a syndrome characterized by decompensation in individuals with chronic liver disease, and is generally secondary to one or more extra-hepatic organ failures, implying an elevated mortality rate. Acute decompensation is the term used for one or more significant consequences of liver disease in a short time and is the most common reason for hospital admission in cirrhotic patients.

Research motivation

The European Association for the Study of Liver-Chronic-Liver Failure (EASL-CLIF) Group modified the intensive care Sequential Organ Failure Assessment score into CLIF-SOFA, which detects the presence of acute-on-chronic liver failure (ACLF) in patients with or without acute decompensation (AD), classifying it into three grades.

Research objectives

To investigate the role of the EASL-CLIF definition for ACLF and the ability of CLIF-SOFA, CLIF-C ACLF, and CLIF-C AD scores for prognosticating ACLF or AD.

Research methods

This study is a literature review using a standardized search method, conducted using the steps following the guidelines for reporting systematic reviews set out by the PRISMA statement. Using specific keywords, relevant articles were found by searching PubMed, ScienceDirect, and BioMed Central-BMC. The databases were searched using the search terms by one reviewer (MSc student), and a list of potentially eligible studies was generated based on the titles and abstracts screened.

Research results

Most of the included studies used the EASL-CLIF definition for ACLF to identify cirrhotic patients with a significant risk of short-term mortality. The primary outcome in all reviewed studies was mortality. Most of the studies' findings were based on an AUROC analysis, which revealed that the CLIF-SOFA, CLIF-C ACLF, and CLIF-C AD scores were preferable to other models in predicting 28-d mortality. They had the greatest AUROC scores predicting overall mortality at 90, 180, and 365 d. A total of 50 articles were included in this study, which found that the CLIF-SOFA, CLIF-C ACLF, and CLIF-C AD scores could predict short-term and long-term mortality in patients with ACLF or AD in more than 50% of the articles found.

Research conclusions

The CLIF-SOFA score surpassed other predictive models in predicting short-term prognosis in ACLF patients. CLIF-SOFA, CLIF-C ACLF, and CLIF-C AD are accurate in predicting scores for short-term and long-term mortality in patients with ACLF and in predicting adverse outcomes associated with chronic liver disease.

Research perspectives

Within the included articles in this study from 2013 to 2022, CLIF-SOFA was superior to other scores for predicting mortality (mainly in the short-term) in ACLF patients in more than 50% of the included articles, followed by CLIF-C ACLF and CLIF-C AD (30% of the articles). CLIF-C OF was accurate at 10%. CTP accurately predicted the score for ACLF patients with HRS and AOVH patients with AD. The MELD score accurately predicted short-term mortality in ACLF patients who underwent LT.

FOOTNOTES

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