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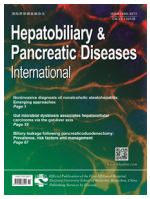

Review Article

The immunological roles in acute-on-chronic liver failure: An update

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ABSTRACT

Background: Acute-on-chronic liver failure (ACLF) refers to the acute deterioration of liver function that occurs in patients with chronic liver disease. ACLF is characterized by acute decompensation, organ failure and high short-term mortality. Numerous studies have been conducted and remarkable progress has been made regarding the pathophysiology and pathogenesis of this disease in the last decade. The present review was to summarize the advances in this field.

Data sources: A comprehensive search in PubMed and EMBASE was conducted using the medical subject words "acute-on-chronic liver failure", "ACLF", "pathogenesis", "predictors", and "immunotherapy" combined with free text terms such as "systemic inflammation" and "immune paralysis". Relevant papers published before October 31, 2018, were included.

Results: ACLF has two marked pathophysiological features, namely, excessive systemic inflammation and susceptibility to infection. The systemic inflammation is mainly manifested by a significant increase in the levels of plasma pro-inflammatory factors, leukocyte count and C-reactive protein. The underlying mechanisms are unclear and may be associated with decreased immune inhibitory cells, abnormal expression of cell surface molecules and intracellular regulatory pathways in immune cells and increased damage-associated molecular patterns in circulation. However, the main cause of susceptibility to infection is immune paralysis. Immunological paralysis is characterized by an attenuated activity of immune cells. The mechanisms are related to elevations of immune inhibitory cells and the concentration of plasma anti-inflammatory molecules. Some immune biological indicators, such as soluble CD163, are used to explore the pathogenesis and prognosis of the disease, and some immunotherapies, such as glucocorticoids and granulocyte colony-stimulating factor, are effective on ACLF.

Conclusions: Overwhelming systemic inflammation and susceptibility to infection are two key features of ACLF. A better understanding of the state of a patient's immune system will help to guide immunotherapy for ACLF.

Introduction

Liver cirrhosis is characterized by the disturbance of blood flow in the liver and portal hypertension, and is the final stage of liver diseases [1]. Cirrhosis is commonly classified into compensated and decompensated cirrhosis. Decompensated cirrhosis is diagnosed with symptoms of ascites, variceal hemorrhage or hepatic encephalopathy [2]. Majority of the patients with decompensated cirrhosis can be reversed to compensated cirrhosis after appropriate treatment. However, some patients will rapidly develop liver failure. The feature of aforementioned liver failure is different from that of acute-on-chronic liver failure (ACLF).

The concept of ACLF was first used in intensive care units to assess the efficacy of artificial liver therapy [3]. In 2009, the Asian Pacific Association for the Study of the Liver (APASL) defined ACLF as "an acute hepatic insult manifesting as jaundice and coagulopathy, complicated within 4 weeks by ascites and/or encephalopathy" [4]. In 2014, APASL revised its definition and added "28-day mortality" [5]. However, the American Association for the Study of Liver Disease (AASLD) and the European Association for the Study of the Liver (EASL) reached a consensus, defining ACLF as "acute deterioration of pre-existing, chronic liver disease, usually related to a precipitating event and associated with increased mortality at 3 months due to multisystem organ failure" [6,7]. Due to the inconsistent definition by researchers, an EASL-Chronic Liver Failure (CLIF) Consortium member initiated a project called the EASL-CLIF Acute-on-Chronic Liver Failure in Cirrhosis (CANONIC) study, a large prospective multicenter study of

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acute decompensated patients [8]. This study aims to distinguish patients with a high-risk of short-term mortality from decompensated cirrhosis and to refine the definition of ACLF. The definition of ACLF in this study is based on three key features: (i) acute decompensated cirrhosis complicated by massive ascites, hepatic encephalopathy, gastrointestinal bleeding, bacterial infection or any combination of these, (ii) organ failure, and (iii) a short-term death rate of at least 15%. In general, ACLF is an acute injury to liver function developed from pre-existing chronic liver disease and is associated with multiple organ failure and high short-term mortality.

CANONIC research suggests that ACLF occurs in a setting of aggravated systemic inflammation. Leukocytes and serum C-reactive protein are good nonspecific indicators of systemic inflammation. The CANONIC study found that these two indicators were significantly elevated in patients with ACLF, and the white blood cell count was positively correlated with disease severity and mortality [8]. The precipitating factors of ACLF include bacterial infections, acute alcoholic hepatitis and hepatitis B virus relapse. These observations led CANONIC researchers to suggest a systemic inflammation hypothesis to explain the pathogenesis of ACLF [9]. This hypothesis has now been widely accepted and has opened a new chapter in the study of the immunological pathogenesis of ACLF. However, little research has been conducted on the pathogenesis of systemic inflammation. Two recent studies [10,11] have described the systemic inflammation in patients with decompensated cirrhosis and ACLF in more detail. This review will focus on the characteristics of systemic inflammation and its associated mechanisms. In addition to systemic inflammation, ACLF patients also exhibit another important feature, namely, susceptibility to infection, the mechanisms of which will be discussed in this review. This review will also introduce the promising immunological predictors and immunotherapy for patients with ACLF. In this paper, for the first time, we summarized the characteristics and underlying mechanisms of both systemic inflammation and immune paralysis in ACLF. The published immunological indicators for the outcome and development of ACLF are collected. We also proposed several immunological indicators to evaluate the immune status of the ACLF patient. The application of prophylactic antibiotics or immune-modulatory treatments is depend on the stage of the disease. We also proposed a proper animal model for the mechanistic study of ACLF and for the medical treatments.

Systemic inflammation

Characteristics of systemic inflammation

ACLF is characterized by an excessive systemic inflammatory response. Two recent studies [10,11] have described the systemic inflammation of ACLF in detail by comparison with patients without ACLF. The former study included 522 patients with decompensated cirrhosis, among which 237 had ACLF, mainly alcohol- or bacterial-induced ACLF [10]. This study focused on the plasma inflammatory cytokines, the redox state of circulating albumin, renin, and copeptin between healthy controls and decompensated cirrhosis with or without ACLF. The study found that systemic inflammation was already present in decompensated cirrhosis, manifested by increased plasma inflammatory factors, the redox state of circulating albumin (a marker of systemic oxidative stress), and renin and copeptin in compensated cirrhosis. These indicators are even higher in ACLF patients. The plasma concentrations of cytokines, such as IL-6, IL-8, TNF-α, IL-10, and IL-1ra, in bacterial-precipitated ACLF are not the same as those in alcohol-precipitated ACLF. The severity of systemic inflammation is closely related to the development and severity of ACLF, and this correlation is much stronger than the correlation of ACLF with systemic circulatory failure.

The latter study included 1108 patients with severe hepatitis B without ACLF and 260 patients with ACLF, all of whom had HBV-precipitated ACLF [11]. The former study by Clària et al. [10] was limited only to the analysis of various factors in plasma, while this study by Wu et al. [11] extended the scope of research to immune cells. This study found that the counts of peripheral blood leukocytes are higher in patients with ACLF than in those without. The neutrophil-to-lymphocyte ratio and monocyte-to-lymphocyte ratio are associated with death in patients with severe hepatitis B, while the neutrophil-to-lymphocyte ratio independently predicts the occurrence of ACLF and short-term mortality. Transcriptome analysis of blood cells from ACLF patients shows higher expression of genes associated with cell migration, movement and bacterial response to wounds and lower expression of genes related to lymphocyte-associated immunity.

Wu et al. [11] also included an analysis of plasma inflammatory factors, but comparing with patients with severe hepatitis B without ACLF, among various plasma inflammatory factors, only IL-10 and granulocyte-macrophage colony stimulating factor (GM-CSF) are increased in ACLF patients, while monocyte chemotactic protein-1 (MCP-1) and chemokine (C-X-C motif) ligand 9 (CXCL9) are significantly reduced. Since the transcriptome analysis found that genes associated with cell migration and motility are highly expressed in ACLF, the decrease in chemokines does not indicate a weaker chemotactic effect in patients with ACLF. A comparison of the studied circulating inflammatory factors is shown in Table 1. The differences may be related to the sensitivity of the test method, the precipitating factors and the stages of included patients.

Precipitating factors

Inducing factors of inflammation are either exogenous or endogenous [12,13]. Exogenous inducers related to ACLF are mainly microorganisms, especially bacteria and the HBV. Bacteria induce inflammatory responses through pathogen-associated molecular patterns (PAMPs) [12,14,15] and virulence factors [12,15]. PAMPs can be recognized by pattern recognition receptors in the host, which stimulate intracellular signaling cascades, thereby activating transcription factors, such as nuclear factor-κB (NF-κB). The activated transcription factors induce a series of inflammation-related gene expression, such as cytokine genes [14,16]. Virulence factors of bacteria are the second type of factors that induce inflammation [12,13,15,17]. These factors are usually not recognized by the receptor but are monitored by so-called functional feature recognition [13,17]. The presence of bacteria is monitored by structural and functional characterization at the site of infection. This monitoring is thought to induce a complement response that eliminates invading bacteria [17].

Endogenous inducers result from necrotic cell release or extracellular matrix degradation [13,18]. These endogenous factors are called damage-associated molecular patterns (DAMPs) [19] because they reflect severe tissue damage to the host's immune system. DAMPs can be recognized by host receptors, and this recognition could induce a sterile inflammatory response.

Exogenous and endogenous factors induced an inflammatory response is usually not very strong under normal conditions. However, when chronic liver inflammation persists, unknown factors might change, aggravating the induced inflammation. When the inflammation is overwhelming, it damages organs and leads to organ failure, which in turn causes death. A series of symptoms caused by this excessive inflammatory response in pre-existing chronic liver diseases are called ACLF.

The most common predisposing factor of ACLF is bacterial infection. In the acute phase of bacterial infection, patients with cirrhosis have higher pro-inflammatory cytokines than those

Table 1
Comparison of circulating inflammatory factors between the two studies.

Cytokines	Clària et al. [10]				Wu et al. [11]			
	HC	No ACLF	ACLF	*P* value*	HC	SE-CHB	HB-ACLF	*P* value*
TNF-α	9	20	29	**<0.05**	0.65	0.88	0.88	>0.05
IL-6	0.3	21	39	**<0.05**	0.88	5.84	7.19	>0.05
IL-8	1.6	37	84	**<0.05**	32.70	98.52	101.82	>0.05
MCP-1	337	318	410	**<0.05**	15.30	8.02	0	**<0.05**
IP-10	328	965	1184	**<0.05**	123.79	208.39	247.78	>0.05
MIP-1β	13	20	28	**<0.05**	44.28	43.88	44.78	>0.05
CXCL9	–	–	–	**–**	79.84	146.77	54.32	**<0.05**
G-CSF	2.1	23	32	**<0.05**	1.32	2.29	2.45	>0.05
GM-CSF	7.5	4.7	7.3	**<0.05**	0.30	0.35	0.55	**<0.05**
IL-10	1.1	3.4	8.1	**<0.05**	0	1.03	2.27	**<0.05**
IL-1ra	7	10	23	**<0.05**	–	–	–	–

Data are expressed as median.
HC: healthy controls; ACLF: acute-on-chronic liver failure; No ACLF: decompensated cirrhosis without ACLF; SE-CHB: severe exacebation of chronic hepatitis B without ACLF; HB-ACLF: hepatitis B-precipitated ACLF.
* *P* value between ACLF and No ACLF or HB-ACLF and SE-CHB.

without cirrhosis [20]. In patients with spontaneous bacterial peritonitis, patients complicated with renal failure show a higher level of pro-inflammatory cytokines compared to those without renal failure [21]. Patients with cirrhosis show higher mortality due to bacterial sepsis than those without cirrhosis [16]. These clinically observed phenomena have been confirmed in rat experiments. Rats with cirrhosis undergo intense inflammation, more severe liver damage and earlier death was observed compared with control rats when stimulated with lipopolysaccharide [LPS, a bacterial (PAMP) recognized by the pattern-recognition receptor toll-like receptor 4 (TLR4)] [16]. Monocytes and peripheral blood mononuclear cells (PBMCs) isolated from patients with cirrhosis also respond stronger to LPS stimulation than those from healthy controls [22–27].

Alcohol-induced ACLF occurs largely because alcohol increases intestinal permeability to bacteria, causing bacterial translocation. Therefore, the most common infection with severe alcoholic hepatitis is spontaneous bacterial peritonitis [28]. However, some patients with severe alcoholic hepatitis have systemic inflammation but no infection [8,29]. Elevated endotoxin was observed in the circulation of these patients [29]. Therefore, these researchers speculated that the bacterial intestinal permeability to PAMPs (such as LPS) is increased in patients with chronic alcoholic liver disease, causing PAMP translocation [29], which induced a strong immune response.

HBV reactivation refers to a re-emergence or elevation of HBV DNA in previously inactive patients. Reactivation can be spontaneous but is most commonly due to immunosuppression (such as cancer chemotherapy, organ transplantation, etc.) or interruption of antiviral therapy [30,31]. HBV reactivation may lead to decompensated liver function or liver failure. In terms of innate immunity, HBV reactivation leads to the upregulation of TLR2/3/4/5/7/9/10 [32–34], and the plasma endotoxin (clinical name of LPS) level is also elevated in HBV-induced decompensated cirrhosis [35]. During LPS stimulation, circulating monocytes secrete higher pro-inflammatory cytokines in hepatitis B precipitated decompensated cirrhosis and ACLF [35,36]. Thus, there is a high possibility that intestinal permeability to LPS is also increased when HBV-related decompensated cirrhosis occurs. However, the cause of this increase in LPS is not conclusive, as it can be caused by decompensated cirrhosis or by reactivation of the HBV. In terms of adaptive immunity, early acute re-flare of HBV results in increased T cell immune response and is associated with liver damage [37]. During spontaneous reactivation, liver damage appears to be mediated by the immune response of expanded T cells to HBV antigen. In an activated immunopathological study, the infiltrating cells were mainly CD8+ T cells (CTL) at the site of hepatic necrosis, and it is generally believed that the HBsAg peptide on the surface of the hepatocytes can activate the infiltration of CTLs [38], which plays an important role in the acute exacerbation of hepatitis.

Characterized by low-grade inflammatory state, obesity has been identified as a risk factor for ACLF [39]. A recent investigation on 95,126 postmenopausal women showed that high body mass index and alcohol consumption were independent risk factors for chronic liver disease [40]. A recent study conducted by Sundaram et al. [41] examined the United Network for Organ Sharing (UNOS) database in which 387,884 with decompensated cirrhosis were enrolled. The study showed that Class III obesity is a risk factor for ACLF patients with decompensated cirrhosis (HR=1.24, 95% CI: 1.09–1.41, $P < 0.001$). The results was confirmed by nationwide inpatient sample (OR=1.30, 95% CI: 1.25–1.35, $P < 0.001$), and the incidence of renal failure was higher in Class III obesity. The increased risk of ACLF in Class III obesity is due to the chronic inflammatory status associated with obesity. Excessive systemic inflammation is the hallmark of ACLF pathophysiology, which is described as acute exacerbation of existing systemic inflammation in decompensated cirrhosis [42], and is characterized by elevation of C-reactive protein and white blood cell count, along with high serum level of IL-6, IL-10 and TNF-α [43]. The adaptive immune response secondary to over nutrition can lead to production of several pro-inflammatory cytokines (such as TNF-α, IL-6), promotion of NF-κB signaling via TLR4 and immune activation, resulting in an elevated inflammatory state in obesity [44]. Free fatty acid is the key molecule in obesity-induced chronic inflammation [45], which activates TLR4 and strengthens inflammatory response. Free fatty acid induced inflammatory pathway also plays an important role in adipose tissue ectopic deposition and insulin resistance [46,47]. Recent studies [43,46–48] have found that over nutrition leads to alteration of intestinal flora, elevates the level of LPS in the circulatory system and activate TLR4 pathway, results in chronic inflammatory response, which suggests that TLR4 is an important link molecule for obesity related inflammatory response. The association between obesity and acute kidney injury in ACLF have been confirmed by multiple studies [41,48–51], and the incidence of acute kidney injury in ACLF patients increases with obesity grading. The association between obesity and acute kidney injury is not unique in cirrhotic patients. Obesity is an independent risk factor for acute kidney injury [48]. The mechanism of obesity-associated acute kidney injury is likely multifactorial in nature, including obesity-related glomerular disease, renal hypoperfusion, and endothelial dysfunction [49–51]. Overall, in

consideration of high disease burden caused by obesity, and its rising prevalence in patients with cirrhosis [52], we emphasize on losing weight among cirrhotic patients with obesity [53]. For patients unable to reach the goal of weight control, bariatric surgery may be an effective measure [54].

In summary, the immune system of some patients with chronic liver disease exhibits an excessive immune response to the stimulation of bacteria or LPS or HBV re-flare. However, the mechanism of this response is still unclear.

Mechanisms of systemic inflammation

There are a few studies on the mechanisms of excessive systemic inflammation, mainly focusing on the inhibitory cell reduction, abnormal expression of surface markers and regulatory pathways in immune cells, and increased pro-inflammatory factors in the plasma [55–57]. A rat cirrhotic model induced by carbon tetrachloride showed a decrease of Tregs in the peripheral blood [55]. T-cell immunoglobulin and mucin domain-containing molecule-3 (Tim-3), an immunoregulatory receptor, is expressed on various immune cells, including monocytes [56,57]. Our previous study found that Tim-3 is downregulated on monocytes in patients with decompensated cirrhosis, and that blocking Tim-3 enhances the secretion of monocyte pro-inflammatory cytokines under LPS stimulation, suggesting that low expression of Tim-3 leads to a monocyte over-response to LPS [35]. In addition, the upregulation of TLR4 and TLR3 may also be responsible for the excessive reaction between immune cells and PAMPs (such as endotoxin) [58,59]. In patients with cirrhosis, except for the upregulation of the TLR4 receptor, there are impaired negative feedback regulatory pathways for TLR4 signaling, including intracellular mechanisms (IRAK-M induction [25] and PI3 kinase induction, AKT-mediated GSK3 inhibition [22]) and IL-10 production in the extracellular environment [22,26]. However, such mechanisms have not yet been studied in decompensated cirrhosis and ACLF. Potassium channel tetramerization domain containing 9 (KCTD9) belongs to the KCTD family. KCTD9 harbors a unique eukaryote-specific DUF3354 domain and penta-peptide repeat that provides bacterial resistance to quinolones by imitating the structure and surface charge of DNA [60]. KCTD9 is highly expressed on circulating and liver NK cells in patients with ACLF. *In vitro*, cell line experiments have shown that KCTD9 enhances the immune activity of NK cells, manifested as higher expression of CD69, enhanced cell killing effect, and cytokine secretions [61]. Furthermore, the DAMP IL-33 levels are elevated in the plasma of patients with HBV-precipitated ACLF. Some *in vitro* experiments have confirmed that IL-33 promotes the immune response of primary monocytes (isolated from ACLF patients) to LPS, possibly pushing patients to enter a vicious cycle [33,36]. In addition, in patients with decompensated cirrhosis and ACLF, peroxidized albumin is increased. Peroxidized albumin promotes PBMC isolated from cirrhosis or healthy controls to secrete more cytokines and to activate cyclooxygenase-2 (COX-2) through the phosphorylation of p38 MAP kinase [62].

Susceptibility to infection

Characteristics of immune paralysis

Bacterial infection is a key predisposing factor of ACLF and a major challenge for its treatment [63]. When a bacterial infection is suspected, timely administration of the correct antibiotics significantly improves survival rate [63]. Our previous study found that patients with ACLF due to extrahepatic factors (50% were bacterial infections) had significantly increased 90-day and 1-year mortality compared to patients with intrahepatic factors, suggesting that bacterial infection plays an important role in disease progression [64]. ACLF patients are prone to fatal secondary nosocomial infections because their immune system are paralyzed. Immunological paralysis is common in ACLF and is significantly associated with the severity of organ failure, the risk of sepsis and high mortality [63]. In recent years, a few studies on the intrinsic immune paralysis mechanism of ACLF showed a decrease in pro-inflammatory immune cells, an increase in inhibitory immune cells, and an increase in anti-inflammatory substances in the microenvironment [65–68].

Earlier studies have found that patients with ACLF exhibited sepsis-like immunoparalysis [63,65]. LPS-stimulated cytokine-producing factors are attenuated like sepsis [65]. Further studies have shown that the ability of monocytes to secrete cytokines is related to the course of the disease [66–68]. The ability of monocytes to secrete IL-1b, TNF-α and IL-12p70 is significantly higher in early ACLF than in healthy individuals, but lower in the late-stage of ACLF compared to healthy individuals. Meanwhile, early ACLF monocytes secrete lower IL-10 than those from both healthy controls and advanced ACLF patients [66]. The expression of monocyte HLA-DR is gradually reduced in chronic hepatitis B, cirrhosis and ACLF, especially in the late stage of ACLF. The expression of HLA-DR on monocytes is significantly lower in dying patients than survivors. The expression of TLR4 on monocytes in patients with liver cirrhosis and all stages of ACLF is higher than that of healthy controls [66]. However, most studies on ACLF do not distinguish between early- and late-stage because there is no clear boundary between the two stages. Thus, studies conducted under the same condition show opposite results [35,36,67,68]. Another innate immune cell, the NK cell, is also lower and impaired in killing functions in ACLF patients than in healthy controls [69].

Mechanisms of immune paralysis

Cells presenting a decreased antibacterial activity may be associated with an increase in immunosuppressive cells in circulation in ACLF. The ratio of regulatory T cells to normal T cells in ACLF patients is higher than that in healthy controls [70]. In addition, the immunosuppressive cells (CD14+ HLA-DR-M-MDSCs) are increased in patients with ACLF. Such cells inhibit T cell expansion and antibacterial immune responses and are associated with secondary infections, disease severity, and prognosis. Activation of TLR3 reverses the expansion of such cells and restores innate immune function [71]. MER receptor tyrosine kinase (MERTK), a receptor expressed on monocytes/macrophages and dendritic cells as well as epithelial cells and reproductive and neuronal tissues, negatively modulates innate immune responses. MERTK+ monocytes/macrophages are elevated in the peripheral blood, liver and lymph nodes of ACLF. In addition to the severity of the disease, MERTK expression is also associated with systemic inflammation. MERTK+ monocytes can pass through endothelial cells and migrate into peripheral lymph nodes and tissues. The expression of METRK also inhibits the response of immune cells to LPS; UNC569, a small MER inhibitor, restores this response [68]. Impaired immune cell function or differentiation in inhibitory cells is often due to the change of the microenvironment. COX-derived lipid mediators, including Prostaglandin E2 (PGE2), have broad immunosuppressive effects [72]. Patients with acute decompensated cirrhosis (including ACLF) show elevated plasma PGE2 levels [67]. Adding plasma from acute decompensated cirrhosis to cultured monocytes isolated from healthy individuals reveals that the plasma enriched PGE2 inhibits the monocyte response to LPS and impairs the bactericidal activity. This increased PGE2 in plasma can be reversed by the supplement of albumin. All these phenomena have been confirmed in mice with decompensated cirrhosis [67]. A summary of the pathogenesis of ACLF is shown in Fig. 1.

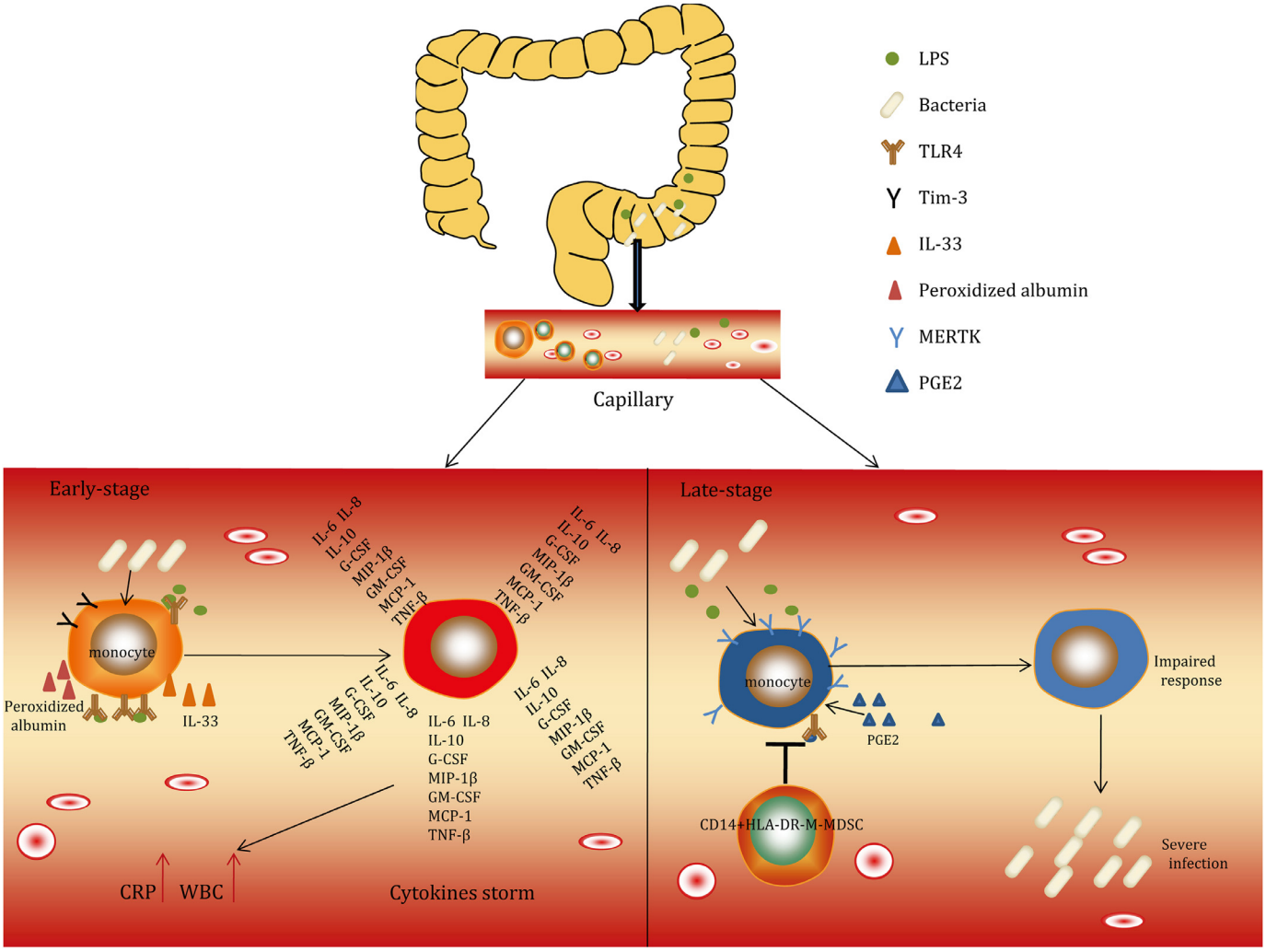

Fig. 1. Summary of the pathogenesis of ACLF. In cirrhosis, decompensated cirrhosis or ACLF, bacteria or LPS in the gut translocate to circulation. In cirrhosis, decompensated cirrhosis or the early stage of ACLF, the response of circulating monocytes to the translocated bacteria or LPS is particularly strong and results in a cytokine storm. These cytokines induce the patients' body to produce more CRP and white blood cells. The underlying mechanisms include downregulated Tim-3, upregulated TLR4, impaired negative feedback of TLR4 signaling, increased plasma IL-33 levels and increased peroxidized albumin. In the late-stage of ACLF, the response of monocytes to the bacteria and LPS is attenuated via the mechanism of upregulated MERTK on monocytes, increased plasma PGE2 levels and increased CD14+ HLA-DR-M-MDSCs. Thus, the late stage of ACLF is susceptible to the development of severe infection.

Immunological indicators for the occurrence and prognosis of ACLF

As the pathogenesis of ACLF is closely related to immunity, the levels of immune markers are predictors of the ACLF. We have summarized the studies that calculate the predictive ability of the immunological biomarkers, shown as area under the receiver operating characteristic curve (AUC) or C-index or odds ratio (OR) [73–80] (Tables 2 and 3).

Immunotherapy

Albumin

Albumin has long been used to treat cirrhosis, and acts by expanding the volume and binding to ligands in plasma. Volume expansion prevents kidney failure. Binding with ligands in plasma increases the solubility of the ligands, making them easier to be transported to the target tissues. In fact, albumin binds to ligands in plasma can prevent them from exerting function. As mentioned above, PGE2 bound to albumin in ACLF patients has no bioavailability [67]. Albumin also binds to LPS to prevent LPS from acting on endothelial cells and causing cell dysfunction [81]. This mechanism explains a previous clinical study that low albumin in serum or ascites was associated with increased risk of infection and spontaneous bacterial peritonitis [82]. Nevertheless, there is still controversy over whether albumin improves the survival of ACLF patients [83,84]. Moreover, normal concentration of albumin does not mean that its function is normal. Studies [67,85] have shown that, in patients with cirrhosis, albumin exhibits a change in post-transcriptional modification that affects its binding function and is associated with disease severity and survival. As mentioned above, plasma peroxidized albumin levels are increased in patients with ACLF and decompensated cirrhosis. This peroxidized albumin also increases the secretion of cytokines in peripheral PBMC, worsening systemic inflammation [62]. Thus, the supplementation with albumin should not only be based on the serum concentrations of albumin but also the concentrations of the dysfunctional albumin that should also be detected in routine laboratory tests.

Glucocorticoids

Glucocorticoids are widely used in clinical inflammatory diseases due to their potent anti-inflammatory effects, although the

Table 2
The immunological biomarkers for short-term mortality of ACLF.

Studies	Year	Study type	Biomarkers	Patient number	Term (d)	Predictive ability
Yu et al. [73]	2016	Retrospective	IL-9	21	90	AUC: 0.84
			IL-10	21	90	AUC: 0.84
Xin et al. [74]	2016	Retrospective	MIP-3α	279	90	AUC: 0.73
Grønbæk et al. [75]	2016	Retrospective	sCD163	137	90	C-index: 0.66 (0.59–0.73)
			sCD163	137	28	C-index: 0.71 (0.63–0.79)
			sMR	137	90	C-index: 0.66 (0.59–0.72)
			sMR	137	28	C-index: 0.67 (0.59–0.75)
Ariza et al. [76]	2016	Retrospective	NGAL+MELD	148	28	AUC: 0.88 (0.83–0.92)
Moreau et al. [77]	2018	Retrospective	NLR	75	90	AUC: 0.71 (0.59–0.82)

AUC: area under the receiver operating characteristic curve; sCD163: soluble CD163; sMR: soluble mannose receptor; NGAL: neutrophil gelatinase-associated lipocalin; MELD: model for end-stage liver disease; NLR: neutrophil-to-lymphocyte ratio.

Table 3
Immunological biomarkers for the development of ACLF.

Studies	Year	Study type	Center	Biomarker	Patient number	Follow-up period	Effect
Sargenti et al. [78]	2016	Prospective	Single	Oxidative burst in monocyte	60	1 yr	AUC: 0.88 (0.74–1.00)
				Oxidative burst in PMN	60	1 yr	AUC: 0.87 (0.72–1.00)
Ariza et al. [76]	2016	Prospective	Multi	Urine NGAL	148	28 d	AUC: 0.74 (0.66–0.81)
Alcaraz-Quiles et al. [79]	2017	Prospective	Multi	IL-1b rs1143623CC	178	–	OR: 0.22 (0.08–0.64)
				IL-1b rs1143623CC +IL-1ra rs4251961TC	178	–	OR: 0.05 (0.01–0.47)
Tan et al. [80]	2018	Prospective	Single	HLA-DR rs3129859	1300	–	OR: 1.83 (1.60–2.09)
				HLA-DR rs3129859	1300	–	OR: 1.86 (1.59–2.18)

AUC: area under the receiver operating characteristic curve; NGAL: neutrophil gelatinase-associated lipocalin; NLR: neutrophil-to-lymphocyte ratio.

specific mechanism of action remains unclear. Whether glucocorticoids can be used in ACLF is still controversial [86]. Some studies have reported that glucocorticoids improve the survival rate of the patients with ACLF [24,87]. However, this effect is not validated by others [88,89]. The main source of this dispute may be that the use of glucocorticoids is highly dependent on the timing of treatment. Theoretically, glucocorticoids should be able to control excessive systemic inflammation and liver inflammation in the early-stage of ACLF, but glucocorticoids aggravate immune paralysis in the late-stage. Therefore, we propose to initiate a clinical study using glucocorticoids to treat the early stages of ACLF and discontinue the drug as early as possible before the onset of advanced immune paralysis. Another noteworthy issue is the appropriate amount of the dose of glucocorticoids. Enzyme clearance in critically ill patients is often reduced [90], which leads to drug accumulation in the body. Therefore, when using glucocorticoids, it is necessary to closely monitor the drug concentration in the blood. This recommendation also needs to be confirmed by further study.

Granulocyte colony-stimulating factor

The physiological role of granulocyte colony-stimulating factor (G-CSF) is to promote the proliferation and differentiation of granulocytes in the bone marrow. In 2012, a pilot study showed that G-CSF is effective on ACLF patients, improving survival rate, decreasing the score of model for end-stage liver disease (MELD), and decreasing the incidences of sepsis, hepatic encephalopathy and renal failure [91]. A later study on the treatment of HBV-related ACLF with G-CSF also achieved consistent results [92]. The specific mechanism remains unclear. However, it is speculated that this treatment mediates the colonization of CD34+ hematopoietic stem cells in the liver [91]. Because of the liver damage, bone marrow-derived pluripotent stem cells provide growth factors and cytokines to promote hepatocyte regeneration [93]. Therefore, we hypothesized that G-CSF mediates the colonization of CD34+ hematopoietic stem cells in the liver and promotes hepatocyte regeneration. After treatment with G-CSF, both circulating bone marrow-like and plasmacytoid dendritic cells and T cells are increased, while CD8+ T cells produced less IFN-γ [93,94]. It seems that CD34+ hematopoietic stem cells proliferate and differentiate into immune cells when the immune system status of the body changes in order to maintain immune homeostasis. Regardless of the mechanisms, the results of these pilot studies show that the application of G-CSF for the treatment of patients with ACLF is promising. Nevertheless, these studies did not exceed 90 days. Therefore, long-term follow-up studies are needed to determine whether G-CSF improves long-term survival and whether there is a risk of cancer.

Artificial liver support

Artificial liver support (ALS) refers to albumin dialysis by the molecular sedimentation recirculating system (MARS) [95] or fractionated plasma separation and absorption (Prometheus) [96]. The basic principle of ALS is to remove poisons from the patient's blood through a semipermeable membrane by means of an albumin-enriched dialysis device [97]. In the treatment of end-stage severe liver disease, ALS is often used as a bridge to liver transplantation [98,99]. However, ALS alone is not efficient. The bilirubin, serum ammonia and creatinine in the blood of ACLF patients can be reduced, but it has no effect on survival. At present, the largest study evaluating the effect of MARS on the survival rate of ACLF shows that MARS does not benefit patients with 28-day transplant-free survival [97]. Moreover, there have been very few studies on the changes of the immune systems in patients with ACLF after treatment. There are only a few studies on whether plasma cytokines can be changed after treatment with artificial liver [74,100–104], and the results are controversial.

Outlook

Different disease stages of ACLF exhibit different immune profiles. The main pathogenesis of ACLF is immunology, and various immunotherapies have different effects in different experimental groups. If immunotherapy is to be used according to the role of immune system in different stage of ACLF, especially using immunosuppressive agents, it is important to evaluate firstly the immune status of the patient. Therefore, one or several immunological indicators are urgently needed to evaluate the immune status of the patient, which would periodize the disease

course so that the prophylactic antibiotics or immune-modulatory treatments fit in the right stage of the disease. With the periodization of the disease determined, studies on the immunological pathogenesis should be re-examined according to the disease stages. In addition, further development and detailed studies are needed for immune-modulatory drugs for the treatment of patients with ACLF. Moreover, a proper animal model is greatly needed to study the pathogenesis and treatment of ACLF.

Contributors

ZHH and CZ proposed the study. CP and WYY performed the research and wrote the first draft. CC and GJ collected and analyzed the related references. CP and WYY contributed equally to this article. All authors contributed to the design and interpretation of the study and to further drafts. ZHH and CZ are the guarantors.

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Ethical approval

Not needed.

Competing interest

No benefits in any form have been received or will be received from a commercial party related directly or indirectly to the subject of this article.

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