

Original Article

Systemic immune-inflammatory disturbance and multiorgan dysfunction in patients with sepsis-associated acute-on-chronic liver failure

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Abstract: Objective: This study aimed to investigate the clinical features of immune-inflammatory disturbance and clarify their correlations with multiorgan dysfunction and 28-day mortality in patients with sepsis-associated acute-on-chronic liver failure (ACLF). Methods: We retrospectively enrolled 116 patients with sepsis-associated ACLF (January 2021-December 2025), divided by 28-day prognosis into survivor (n=48) and non-survivor (n=68) groups. Baseline data within 24 hours of admission, including severity scores [sequential organ failure assessment (SOFA), chronic liver failure consortium ACLF score (CLIF-C ACLF), model for end-stage liver disease (MELD), albumin-bilirubin score (ALBI)], serum inflammatory cytokines [interleukin-6 (IL-6), interleukin-18 (IL-18), interleukin-10 (IL-10), procalcitonin (PCT), etc.], cellular immune markers [CD4⁺/CD8⁺ ratio, human leukocyte antigen-DR (HLA-DR), neutrophil-to-lymphocyte ratio (NLR), systemic immune-inflammation index (SIRI), platelet-to-lymphocyte ratio (PLR), lymphocyte-to-monocyte ratio (LMR)], and organ function indices, were collected and compared between groups. Correlations between immune-inflammatory markers and multiorgan dysfunction were analyzed using Spearman correlation. Results: The 28-day mortality was 58.62% (68/116). Non-survivors had significantly higher severity scores (all $P < 0.001$), elevated pro-inflammatory cytokines IL-6, IL-18, and PCT ($P < 0.05$), lower IL-10 ($P = 0.004$), and reduced CD4⁺ T cells, CD4⁺/CD8⁺ ratio, and HLA-DR expression (all $P < 0.001$). Composite inflammatory indices (NLR, SIRI, PLR) were significantly elevated and LMR decreased in non-survivors (all $P < 0.001$). Non-survivors also exhibited worse organ function and significantly higher incidences of acute kidney injury, acute respiratory distress syndrome, hepatic encephalopathy, and circulatory failure (all $P < 0.05$). Spearman analysis showed that IL-6, NLR, SIRI, and PLR were positively correlated with the number of organ dysfunctions, while CD4⁺/CD8⁺ ratio and LMR were negatively correlated (all $P < 0.05$). Conclusions: Sepsis-associated ACLF presents with systemic immune-inflammatory disturbance characterized by concurrent pro-inflammatory cytokine storm and immunoparalysis. Dynamic monitoring of immune-inflammatory biomarkers may facilitate early risk stratification and provide a basis for immune-guided stratified therapy.

Keywords: Sepsis, acute-on-chronic liver failure, immune-inflammatory disturbance, multiorgan dysfunction, retrospective study

Introduction

Acute-on-chronic liver failure (ACLF) is not merely an acute exacerbation of chronic liver disease but rather an independent disease entity with distinct pathophysiologic mechanisms and a poor prognosis [1, 2]. According to the CANONIC study, the 28-day mortality rate for ACLF patients is 30-50%, rising to over 60-90% at 90 days, posing a serious survival threat [3]. Although liver transplantation is an effective curative treatment, its application is limited by donor shortages, timing of surgery,

and the patient's general condition, leaving most patients dependent on comprehensive medical therapy [4-6]. Therefore, elucidating the pathogenesis of ACLF and identifying early warning biomarkers and therapeutic targets is of great significance for improving outcomes.

There are different triggers of ACLF, among which sepsis and bacterial infection have a major effect and they are the main cause of illness in the patients of ACLF due to the different etiological backgrounds of the patients [7, 8]. In Europe and North America, alcoholic cirrho-

sis mainly leads to liver issues, making bacterial infections and sepsis the typical culprits. In the Asia-Pacific region, though, Hepatitis B reactivation is the top reason for ACLF. Still, secondary sepsis is a key factor there too, causing multiorgan dysfunction syndrome (MODS) and rapidly worsening patients' conditions [9]. A study conducted by Kumar et al. [10] showed that sepsis as a standalone risk factor dramatically exacerbated the clinical outcomes of metabolic dysfunction-associated steatotic liver disease-related ACLF. A vicious cycle exists between sepsis and ACLF. For one, patients with cirrhosis and ACLF have a disrupted intestinal barrier and gut microbiota can leak, causing pathogen-associated molecular patterns (PAMPs) to enter the system constantly, raising inflammation. At the same time, immunoparalysis in ACLF makes patients prone to infections, sparking powerful inflammatory cascades that speed up organ failure. So, sepsis-triggered ACLF is a frequent problem and its underlying mechanisms need more research.

Systemic immune-inflammatory disturbance is thought to be the main driving force that causes sepsis-associated ACLF at the cellular level and probably sustains the entire course of disease from initiation and progression to the final outcome. Traditionally, the pathophysiologic cascade of ACLF is thought to start with a local inflammation of the liver. This is later followed by a systemic inflammatory response where both immune activation and immunoparalysis coexist [11]. Intense systemic inflammatory response and excessive immune response result in immune-metabolic dysregulation, which leads to multiorgan failure and progression to death in ACLF [12]. Recently, using multi-omics techniques such as single-cell sequencing, transcriptomics, metabolomics, and proteomics, the detailed mechanisms of immune-inflammatory disturbance in ACLF have been better revealed. Sepsis and ACLF are very specific pathologic situations where the "over-activation" of the innate immune system arises in conjunction with the "functional suppression" of the adaptive immunity, which causes a paradoxical situation of concurrent immunoparalysis and cytokine storm. Monocytes and macrophages respond to pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs) by stimulation of pattern recognition receptors such as Toll-like receptors (TLRs) and NOD-like recep-

tors (NLRs), which activate downstream signaling pathways and produce a cascade release of large amounts of pro-inflammatory cytokines [13, 14]. Besides causing direct damage to hepatocytes, these pro-inflammatory cytokines also bring about endothelial dysfunction, microcirculatory disturbances, and mitochondrial oxidative stress injury, finally leading to secondary damage of extrahepatic organs such as the kidneys, lungs, central nervous system, and the coagulation system [15, 16].

We have made great strides in unraveling the immune-inflammatory mechanisms of ACLF. However, there are still a few crucial knowledge gaps about sepsis-associated ACLF, which is a common clinical subgroup with a very poor prognosis. The majority of prior research to date has mainly concentrated on either the global ACLF patient population or on cohorts with a single etiology, thereby missing out on revealing immune-inflammatory features that are not only characteristic but also exclusive to ACLF patients where sepsis was a trigger. Most prior research has focused on a single time point or specific category of inflammatory markers. There has been a shortage of thorough integrative analysis that includes pro-inflammatory cytokines, anti-inflammatory cytokines, cellular immune markers, and composite inflammatory indices altogether. A comprehensive description of the entire spectrum of immune-inflammatory disruption in sepsis-associated ACLF is quite challenging. Furthermore, the association between immune-inflammatory markers and the progression of MODS remains unclear; in particular, the specific mechanisms and key nodes by which immune-inflammatory disturbance drives extrahepatic organ failure require further exploration. Therefore, systematically characterizing systemic immune-inflammatory disturbance in patients with sepsis-associated ACLF and clarifying the correlations between key immune-inflammatory markers, MODS, and short-term prognosis is of great theoretical significance and clinical value for achieving early risk stratification, guiding immunomodulatory therapy, and improving outcome in this patient population.

Based on the above background, this study retrospectively analyzed the clinical data of patients with sepsis-associated ACLF to describe the clinical characteristics, distribution of underlying liver disease etiologies, features

Immune-inflammatory disturbance in sepsis-associated ACLF

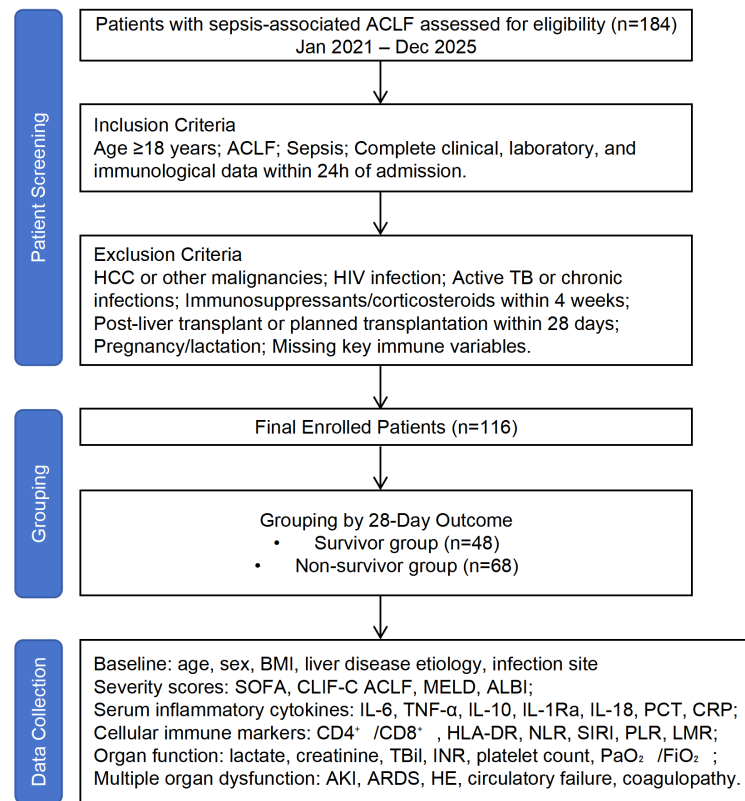


Figure 1. Research workflow. Abbreviations: ACLF, acute-on-chronic liver failure; 24 h, 24 hours; HCC, hepatocellular carcinoma; HIV, human immunodeficiency virus; TB, tuberculosis; BMI, body mass index; SOFA, Sequential Organ Failure Assessment; CLIF-C ACLF, Chronic Liver Failure Consortium ACLF score; MELD, Model for End-Stage Liver Disease; ALBI, Albumin-Bilirubin score; IL-6, interleukin-6; TNF- α , tumor necrosis factor- α ; IL-10, interleukin-10; IL-1Ra, interleukin-1 receptor antagonist; IL-18, interleukin-18; PCT, procalcitonin; CRP, C-reactive protein; CD4⁺/CD8⁺, CD4-positive/CD8-positive T lymphocyte ratio; HLA-DR, human leukocyte antigen-DR; NLR, neutrophil-to-lymphocyte ratio; SIRI, systemic immune-inflammation index; PLR, platelet-to-lymphocyte ratio; LMR, lymphocyte-to-monocyte ratio; TBil, total bilirubin; INR, international normalized ratio; PaO₂/FiO₂, oxygenation index; AKI, acute kidney injury; ARDS, acute respiratory distress syndrome; HE, hepatic encephalopathy.

of infection sites, and occurrence of MODS in this patient population. This can clarify the epidemiologic and clinical phenotypic features of this group. This study compared immune-inflammatory markers between 28-day survivors and non-survivors to reveal systemic immune-inflammatory disturbance in sepsis-associated ACLF. Through the above analyses, this study sought to provide clinical evidence-based support for understanding the immunopathologic mechanisms of sepsis-associated ACLF and to offer biomarker support for immune-guided stratified therapy and precision management of these patients.

Patients and methods

Study population

This single-center retrospective cohort study enrolled patients with sepsis-associated ACLF (January 2021-December 2025), dividing them into survivor and non-survivor groups based on 28-day prognosis. It was approved by Ethics Committee of Beijing Ditan Hospital, Capital Medical University (No.: DTEC-KY2021-045-02), which waived informed consent. All patient data were anonymized, adhering strictly to the Declaration of Helsinki.

Inclusion criteria: Age ≥ 18 years; ACLF per criteria [17]: acute hepatic decompensation within 4 weeks [total bilirubin (TBil) ≥ 5 mg/dL, international normalized ratio (INR) ≥ 1.5 or prothrombin activity $< 40\%$, plus ascites and/or encephalopathy] in known or unknown chronic liver disease; sepsis per criteria [18]: confirmed/suspected infection with sequential organ failure assessment (SOFA) increase ≥ 2 from baseline; and comprehensive clinical, lab, and immunological testing completed within 24 hours of admission.

Exclusion criteria: Presence of primary hepatocellular carcinoma or other malignant tumors; human immunodeficiency virus (HIV) infection; active tuberculosis or other uncontrolled chronic infections; use of glucocorticoids, immunosuppressants, or biological agents within 4 weeks before admission; history of liver transplantation or planned liver transplantation within 28 days; pregnancy or lactation; incomplete clinical data with missing key immunological indices.

Baseline clinical, immune-inflammatory, and organ function indicators were compared to assess associations between immune-inflammatory disturbance, MODS progression, and short-term prognosis (see **Figure 1**).

Treatment regimen

All patients received standardized comprehensive treatment for sepsis-associated ACLF, including the following: (1) Anti-infective therapy: Pathogen specimens (blood culture, ascitic fluid culture, sputum culture, etc.) were collected within 2 hours of admission, and empiric broad-spectrum antibiotics were administered, followed by de-escalation therapy based on antimicrobial susceptibility testing results. (2) Organ function support: Circulatory: fluid resuscitation plus vasoactive agents if needed to maintain mean arterial pressure (MAP) ≥ 65 mmHg. Respiratory: nasal cannula/face mask oxygen; non-invasive or invasive ventilation as required. Renal: continuous renal replacement therapy (CRRT) for acute kidney injury with poor diuretic response. Liver: plasma exchange, molecular adsorbent recirculating system (MARS), or dual plasma molecular adsorption system (DPMAS) depending on patient condition and resource availability. (3) Other treatments: These include correcting coagulation disorders, preventing gastrointestinal bleeding, managing hepatic encephalopathy, and providing nutritional support.

Collection of baseline data

Within 24 hours of admission, we collected baseline data from the hospital's electronic medical records, including demographics [age, sex, body mass index (BMI)], underlying liver disease information (etiology, infection site), and disease severity scores [SOFA, chronic liver failure consortium ACLF score (CLIF-C ACLF), model for end-stage liver disease (MELD), albumin-bilirubin score (ALBI)]. Two researchers independently extracted and verified all data for accuracy.

Laboratory indicator measurement

All blood samples were collected within 24 hours after admission and were tested according to standardized laboratory procedures. Serum inflammatory cytokines [interleukin-6 (IL-6), tumor necrosis factor- α (TNF- α), interleukin-10 (IL-10), interleukin-1 receptor antagonist (IL-1Ra), interleukin-18 (IL-18), procalcitonin (PCT), C-reactive protein (CRP)] were measured by enzyme-linked immunosorbent assay (ELISA) or chemiluminescence immunoassay per manufacturer's instructions, with

intra- and inter-assay coefficients of variation (CVs) $<10\%$ and $<15\%$, respectively. Cellular immune markers [CD4⁺/CD8⁺ ratio, monocyte human leukocyte antigen-DR (HLA-DR) expression rate, neutrophil-to-lymphocyte ratio (NLR), systemic immune-inflammation index (SIRI), platelet-to-lymphocyte ratio (PLR), lymphocyte-to-monocyte ratio (LMR)] were measured by flow cytometry using fluorescent monoclonal antibodies [CD3-fluorescein isothiocyanate (FITC), CD4-phycoerythrin (PE), CD8-peridinin chlorophyll protein-cyanine 5.5 (PerCP-Cy5.5), HLA-DR-allophycocyanin (APC)] on a FACS Calibur cytometer. Organ function indices: Arterial blood lactate (mmol/L), serum creatinine ($\mu\text{mol/L}$), total bilirubin ($\mu\text{mol/L}$), INR, platelet count ($\times 10^9/\text{L}$), oxygenation index ($\text{PaO}_2/\text{FiO}_2$, mmHg).

Clinical outcome measures

Primary outcome: 28-day all-cause mortality from admission; Secondary outcomes: Multiorgan dysfunction, including acute kidney injury (KDIGO 2012), acute respiratory distress syndrome (Berlin Definition 2012), hepatic encephalopathy (West-Haven criteria), circulatory failure (requiring vasoactive agents), and coagulation dysfunction (INR ≥ 1.5 or platelets $< 50 \times 10^9/\text{L}$).

Statistical analysis

Data were analyzed using SPSS 26.0 (IBM Corp., Armonk, NY, USA). The normality of continuous variables was assessed using the Shapiro-Wilk test. Continuous variables with a normal distribution were presented as mean $\pm$ standard deviation (SD) and were compared between the survivor and non-survivor groups using the independent samples t-test. Continuous variables with a non-normal distribution were presented as median with interquartile range (IQR) and were compared using the Mann-Whitney U test. Categorical variables were expressed as numbers (percentages) and were compared using the Chi-square (χ^2) test, or Fisher's exact test. Spearman's rank correlation coefficient was used to evaluate the correlations between immune-inflammatory markers and the number of dysfunctional organs, as these variables were not normally distributed. All statistical tests were two-sided, and a *P*-value of <0.05 was considered significant.

Table 1. Comparison of baseline characteristics

Variable	All patients (n=116)	Survivor group (n=48)	Non-survivor group (n=68)	t/ χ^2 /Z	P-value
Age (years)	52.45±6.14	53.38±6.35	51.79±5.94	1.372	0.173
Sex					
Male	82 (70.69)	32 (66.67)	50 (73.53)	0.640	0.424
Female	34 (29.31)	16 (33.33)	18 (26.47)		
BMI (kg/m ²)	22.85±1.77	22.57±1.67	23.04±1.82	-1.392	0.167
Etiology of liver disease					
Hepatitis B virus infection	74 (63.79)	32 (66.67)	42 (61.76)	0.839	0.875
Alcoholic liver disease	28 (24.14)	10 (20.83)	18 (26.47)		
Autoimmune liver disease	8 (6.90)	3 (6.25)	5 (7.35)		
Others	6 (5.17)	3 (6.25)	3 (4.41)		
Infection site					
Spontaneous bacterial peritonitis	45 (38.79)	20 (41.67)	25 (36.76)	1.485	0.943
Pulmonary infection	34 (29.31)	13 (27.08)	21 (30.88)		
Urinary tract infection	18 (15.52)	7 (14.58)	11 (16.18)		
Biliary tract infection	10 (8.62)	4 (8.33)	6 (8.82)		
Bloodstream infection	6 (5.17)	2 (4.17)	4 (5.88)		
Skin and soft tissue infection	3 (2.59)	2 (4.17)	1 (1.47)		

Abbreviation: BMI, body mass index.

Table 2. Comparison of disease severity scores

Variable	All patients (n=116)	Survivor group (n=48)	Non-survivor group (n=68)	t/Z	P-value
SOFA score	11.50 (7.00, 14.00)	7.50 (5.00, 12.00)	13.00 (9.00, 15.25)	-5.314	<0.001
CLIF-C ACLF score	51.54±7.85	44.33±4.65	56.63±5.19	-13.112	<0.001
MELD score	24.43±4.49	21.44±3.11	26.54±4.10	-7.622	<0.001
ALBI score	-0.97 (-1.27, -0.80)	-1.25 (-1.51, -0.99)	-0.86 (-1.02, -0.72)	-5.963	<0.001

Abbreviations: SOFA, Sequential Organ Failure Assessment; CLIF-C ACLF, Chronic Liver Failure Consortium ACLF score; MELD, Model for End-Stage Liver Disease; ALBI, Albumin-Bilirubin score.

Results

Characteristics of study population

Table 1 summarizes the 116 sepsis-associated ACLF patients (70.69% male; 28-day mortality 58.62%, n=68). Survivor and non-survivor groups were comparable in age, sex, and BMI ($P>0.05$). Most common underlying liver diseases: HBV-related cirrhosis (63.79%) and alcoholic liver disease (24.14%; $P=0.679$). Main infection sites: spontaneous bacterial peritonitis (38.79%), pulmonary (29.31%), and urinary tract (15.52%; $P=0.747$). These results indicate a good balance between the non-survivor and survivor groups in terms of demographic characteristics, composition of underlying liver diseases, and types of infection. Therefore,

subsequent intergroup differences can be primarily attributed to disease-related immune-inflammatory and organ function status.

Disease severity scores

Table 2 shows that non-survivors had significantly higher baseline scores than survivors for SOFA ($Z=-5.314$, $P<0.001$), CLIF-C ACLF ($t=-13.112$, $P<0.001$), MELD ($t=-7.622$, $P<0.001$), and ALBI ($Z=-5.963$, $P<0.001$).

Serum inflammatory cytokine levels

Within 24 hours of admission, non-survivors had significantly higher pro-inflammatory cytokines than survivors: IL-6 ($t=-4.284$, $P<0.001$), IL-18 ($t=-2.361$, $P=0.020$), and PCT ($Z=-3.457$,

Table 3. Comparison of serum inflammatory cytokine levels

Variable	All patients (n=116)	Survivor group (n=48)	Non-survivor group (n=68)	t/Z	P-value
Pro-inflammatory cytokines					
IL-6 (pg/mL)	81.15±24.68	70.26±17.19	88.84±26.33	-4.284	<0.001
TNF-α (pg/mL)	29.45 (23.55, 35.92)	28.20 (22.75, 35.28)	30.35 (24.58, 37.10)	-1.376	0.169
IL-18 (pg/mL)	61.92±19.01	57.05±17.79	65.35±19.21	-2.361	0.020
PCT (ng/mL)	4.35 (3.58, 5.32)	3.80 (2.65, 5.00)	4.85 (4.07, 5.80)	-3.457	0.001
CRP (mg/L)	69.80±20.42	65.98±17.09	72.49±22.21	-1.704	0.091
Anti-inflammatory cytokines					
IL-10 (pg/mL)	32.38±9.05	35.22±8.80	30.38±8.75	2.925	0.004
IL-1Ra (pg/mL)	479.50±83.55	476.32±71.83	481.74±91.38	-0.343	0.732

Abbreviations: IL-6, interleukin-6; TNF-α, tumor necrosis factor-alpha; IL-18, interleukin-18; PCT, procalcitonin; CRP, C-reactive protein; IL-10, interleukin-10; IL-1Ra, interleukin-1 receptor antagonist.

Table 4. Comparison of cellular immune markers

Variable	All patients (n=116)	Survivor group (n=48)	Non-survivor group (n=68)	t/Z	P-value
CD4 ⁺ T lymphocytes (cells/μL)	438.00 (301.50, 690.50)	474.00 (393.25, 708.00)	365.00 (253.75, 620.00)	2.831	0.005
CD8 ⁺ T lymphocytes (cells/μL)	440.00 (288.75, 731.75)	404.00 (286.25, 624.25)	467.50 (289.75, 924.00)	-1.519	0.129
CD4 ⁺ /CD8 ⁺ ratio	0.99±0.36	1.25±0.31	0.82±0.27	7.932	<0.001
Monocyte HLA-DR expression rate (%)	35.08±8.84	38.66±8.15	32.55±8.47	3.880	<0.001

Abbreviation: HLA-DR, human leukocyte antigen-DR.

Table 5. Comparison of integrated inflammatory indices

Variable	All patients (n=116)	Survivor group (n=48)	Non-survivor group (n=68)	t	P-value
NLR	14.19±5.15	9.28±2.45	17.65±3.44	-15.300	<0.001
SIRI	8.02±2.95	5.45±1.73	9.84±2.17	-11.595	<0.001
PLR	174.43±43.73	154.22±32.45	188.69±45.21	-4.520	<0.001
LMR	1.53±0.46	1.82±0.37	1.32±0.42	6.615	<0.001

Abbreviations: NLR, neutrophil-to-lymphocyte ratio; SIRI, systemic immune-inflammation index; PLR, platelet-to-lymphocyte ratio; LMR, lymphocyte-to-monocyte ratio.

P=0.001); TNF-α and CRP showed no differences (P=0.169, P=0.091). For anti-inflammatory cytokines, non-survivors had lower IL-10 (t=2.925, P=0.004), with no difference in IL-1Ra (P=0.732). See **Table 3**.

Cellular immune markers

Non-survivors had significantly lower CD4⁺ T cells (Z=2.831, P=0.005), CD4⁺/CD8⁺ ratio (t=7.932, P<0.001), and monocyte HLA-DR expression (t=3.880, P<0.001), but no difference in CD8⁺ counts (P=0.129). These findings indicate that patients in the non-survivor group had more severe adaptive immune suppression, manifested by reduced CD4⁺ T cells, an inverted CD4⁺/CD8⁺ ratio, and impaired monocyte antigen-presenting function (downregulat-

ed HLA-DR expression), representing a classic state of “immunoparalysis”. See **Table 4**.

Composite inflammatory indices

Non-survivors had significantly different composite inflammatory indices than survivors: higher NLR (t=-15.300), SIRI (t=-11.595), and PLR (t=-4.520), but lower LMR (t=6.615); all P<0.001. These findings indicate a more severe systemic inflammatory response and greater immune dysregulation in non-survivors at the peripheral blood cell level. See **Table 5**.

Organ function indices

Table 6 lists 24-hour admission lab and physiologic indices. Non-survivors had significantly higher lactate (t=-13.863, P<0.001), creatinine

Table 6. Comparison of organ function indices

Variable	All patients (n=116)	Survivor group (n=48)	Non-survivor group (n=68)	t/Z	P-value
Arterial lactate (mmol/L)	3.93±1.46	2.62±0.45	4.85±1.21	-13.863	<0.001
Serum creatinine (μmol/L)	184.97±48.91	168.55±40.36	196.55±51.32	-3.153	0.002
Total bilirubin (μmol/L)	225.33±53.63	196.54±45.63	245.66±49.64	-5.426	<0.001
INR	2.00 (1.50, 2.60)	1.80 (1.58, 2.10)	2.30 (1.50, 3.23)	-2.713	0.007
Platelet count (×10 ⁹ /L)	92.32±30.83	102.33±33.63	85.25±26.74	2.926	0.004
PaO ₂ /FiO ₂ (mmHg)	267.43±73.33	312.44±63.78	235.66±62.45	6.465	<0.001

Abbreviations: INR, international normalized ratio; PaO₂/FiO₂, oxygen index.

Table 7. Comparison of multiple organ dysfunction incidence

Variable	All patients (n=116)	Survivor group (n=48)	Non-survivor group (n=68)	χ ²	P-value
AKI	59 (50.86)	19 (39.58)	40 (58.82)	4.168	0.041
ARDS	42 (36.21)	12 (25.00)	30 (44.12)	4.452	0.035
HE	52 (44.83)	14 (29.17)	38 (55.88)	8.120	0.004
Circulatory failure	51 (43.97)	15 (31.25)	36 (52.94)	5.374	0.020
Coagulopathy	83 (71.55)	31 (64.58)	52 (76.47)	1.953	0.162

Abbreviations: AKI, acute kidney injury; ARDS, acute respiratory distress syndrome; HE, hepatic encephalopathy.

Table 8. Spearman correlation analysis between immune-inflammatory markers and the number of dysfunctional organs

Immune-inflammatory marker	ρ value	P-value
IL-6 (pg/mL)	0.265	0.004
TNF-α (pg/mL)	0.064	0.497
IL-18 (pg/mL)	0.114	0.224
PCT (ng/mL)	0.164	0.078
CRP (mg/L)	0.123	0.190
IL-10 (pg/mL)	-0.114	0.222
IL-1Ra (pg/mL)	0.067	0.478
CD4 ⁺ /CD8 ⁺ ratio	-0.320	<0.001
Monocyte HLA-DR expression rate (%)	-0.138	0.140
NLR	0.483	<0.001
SIRI	0.366	<0.001
PLR	0.275	0.003
LMR	-0.308	0.001

Abbreviations: IL-6, interleukin-6; TNF-α, tumor necrosis factor-alpha; IL-18, interleukin-18; PCT, procalcitonin; CRP, C-reactive protein; IL-10, interleukin-10; IL-1Ra, interleukin-1 receptor antagonist; HLA-DR, human leukocyte antigen-DR; NLR, neutrophil-to-lymphocyte ratio; SIRI, systemic immune-inflammation index; PLR, platelet-to-lymphocyte ratio; LMR, lymphocyte-to-monocyte ratio.

(t=-3.153, P=0.002), total bilirubin (t=-5.426, P<0.001), and median INR (Z=-2.713, P=0.007), but lower platelet count (t=2.926,

P=0.004) and oxygenation index (t=6.465, P<0.001), indicating more severe tissue hypoperfusion, renal, hepatic, coagulation, and respiratory dysfunction. Overall, non-survivors had more extensive multiorgan dysfunction.

Multiorgan dysfunction

As shown in **Table 7**, compared to the survivor group, the non-survivor group had significantly higher incidences of acute kidney injury (χ²=4.168, P=0.041), acute respiratory distress syndrome (χ²=4.452, P=0.035), hepatic encephalopathy (HE) (χ²=8.120, P=0.004), and circulatory failure (χ²=5.374, P=0.020). No significant difference was observed for coagulation dysfunction (P=0.162). These findings suggest that patients in the non-survivor group were more prone to develop AKI, ARDS, HE, and circulatory failure, indicating that poor prognosis in sepsis-associated ACLF was closely associated with the failure of extrahepatic organs.

Spearman correlation analysis

Table 8 presents the results of the correlation analysis between key immune-inflammatory markers and the number of organ dysfunctions in patients. The results showed that the pro-inflammatory cytokine IL-6 was significantly positively correlated with the number of

organ dysfunctions ($p=0.265$, $P=0.004$). The composite inflammatory indices NLR ($p=0.483$, $P<0.001$), SIRI ($p=0.366$, $P<0.001$), and PLR ($p=0.275$, $P=0.003$) were also significantly positively correlated with the number of organ dysfunctions. In contrast, the $CD4^+/CD8^+$ ratio ($p=-0.320$, $P<0.001$) and LMR ($p=-0.308$, $P=0.001$), which reflect immune function, were significantly negatively correlated with the number of organ dysfunctions. No significant correlations were found between $TNF-\alpha$, IL-18, PCT, CRP, IL-10, IL-1Ra, or monocyte HLA-DR expression rate and the number of organ dysfunctions (all $P>0.05$). These findings suggest that immune-inflammatory disturbances characterized by elevated IL-6, elevated NLR/SIRI, and a decreased $CD4^+/CD8^+$ ratio are closely associated with the severity of multiorgan dysfunction in patients with sepsis-associated ACLF. Dynamic monitoring of these indicators may facilitate early identification of high-risk patients.

Discussion

Sepsis-associated acute on chronic liver failure (ACLF) is one of the most severe clinical ACLF subtypes. Its pathogenesis is dominated by systemic immune-inflammatory network disorders. These disorders not only aggravate local liver injury but also trigger systemic release of inflammatory mediators, further inducing MODS. However, clinical studies focusing on the immune-inflammatory characteristics of patients with sepsis-associated ACLF and their association with MODS progression remain scarce. This retrospective study enrolled 116 patients with sepsis-associated ACLF to systematically characterize systemic immune-inflammatory abnormalities in this patient cohort. We confirmed the coexistence of pro-inflammatory cytokine storm and immunoparalysis in such patients. We also clarified the close correlations between core immune-inflammatory markers, multiorgan dysfunction, and 28-day mortality.

Our clinical data revealed consistent phenotypic differences between non-survivors and survivors. Compared to survivors, non-survivors had significantly elevated levels of the pro-inflammatory cytokines (IL-6, IL-18) and PCT. In contrast, non-survivors showed decreased IL-10 levels, reduced $CD4^+$ T cell

counts, reversed $CD4^+/CD8^+$ T cell ratio, and downregulated HLA-DR expression. These alterations are typical manifestations of immunosuppression. Such immune imbalance is closely associated with a higher incidence of extrahepatic organ failure and a poor short-term prognosis. These findings provide credible clinical evidence elucidating the immunopathologic mechanisms of sepsis-associated ACLF.

Systemic immune-inflammatory disturbance serves as the initiating trigger and core driving factor for sepsis-associated ACLF progression. The pathologic cascade is activated when pathogen-associated molecular patterns (PAMPs) and DAMPs excessively activate the innate immune system. ACLF is defined by severe immune system imbalance, which causes concurrent systemic inflammation and immunoparalysis, a paradoxical immune state reported by Drago et al. [11]. Our study verified that serum IL-6 levels were markedly higher in non-survivors than in survivors, which was consistent with the research from Elias et al. [19].

Sepsis-induced endothelial dysfunction exacerbates organ damage in ACLF through angiotensin-2 (Ang-2) and hepatocyte growth factor-related signaling pathways [19]. As a critical pro-inflammatory mediator, IL-6 is strongly correlated with Ang-2, acting as a key bridge linking inflammation and organ injury [20]. Transcriptomic studies have confirmed that acute phase response-related transcription factors are significantly upregulated in peripheral blood mononuclear cells of ACLF patients. Excessive pro-inflammatory signal activation is closely related to disease severity and short-term prognosis [21].

IL-18, a downstream product of the NOD-like receptor family pyrin domain containing 3 (NLRP3) inflammasome, was elevated in the non-survivor group. Increased IL-18 amplifies inflammatory cascades and promotes hepatocyte apoptosis and necrosis [22]. In addition, elevated PCT in non-survivors not only reflects the severity of bacterial infection but also indicates persistent innate immune overactivation [23].

Pro-inflammatory cytokine storm is accompanied by compensatory anti-inflammatory response syndrome and prominent adaptive

immune suppression, namely immunoparalysis. In this study, non-survivors had significantly lower CD4⁺ T cell counts, lower CD4⁺/CD8⁺ ratios, and reduced monocyte HLA-DR expression than survivors, indicating severe adaptive immune impairment in sepsis-associated ACLF.

Multiple studies have suggested the mechanisms of immune dysfunction in ACLF. Single-cell sequencing analysis has demonstrated high heterogeneity of peripheral blood T cells in ACLF patients. Exhausted T cells accumulate with disease progression, which is a core cause of immune dysfunction [24]. In patients with hepatitis B virus-related ACLF, circulating and intrahepatic CD4⁺ T cells show significant upregulation of B and T lymphocyte attenuator, of which the expression level is positively correlated with disease severity, poor prognosis, and infectious complications [25]. Targeted inhibition or antibody blockade of this molecule can restore cytokine secretion, reduce mortality, and alleviate bacterial infection [26].

Moreover, ACLF patients have increased numbers of MER tyrosine kinase (MERTK)-expressing regulatory immune cells in peripheral blood and liver tissues. Upregulated MERTK induces monocyte-macrophage dysfunction and inhibits innate immunity, increasing the risk of secondary bacterial infection. CD14⁺ CD15⁻ HLA-DR⁻ myeloid-derived suppressor cells (M-MDSCs) are also significantly expanded in ACLF patients. These cells suppress T cell function and impair antibacterial innate immunity, and their abundance is positively correlated with secondary infection, disease severity, and poor prognosis [27]. The decreased monocyte HLA-DR expression observed in our study directly reflects immunoparalysis in sepsis-associated ACLF. This defect impairs antigen presentation, weakens pathogen clearance, increases superinfection risk, and accelerates multi-organ failure progression.

Routine peripheral blood inflammatory indices combined with cytokines can effectively stratify the prognosis of sepsis-associated ACLF. Our results showed that compared to survivors, non-survivors had significantly higher levels of NLR, SIRI and PLR, but a lower LMR. All these markers were significantly correlated with the number of failed organs.

Previous studies have validated the prognostic value of these routine inflammatory markers. A

NLR value above 5 predicts higher in-hospital mortality in acute coronary syndrome patients [28]. Elevated baseline NLR is an independent risk factor for 90-day non-transplant mortality in cirrhotic patients, reflecting aggravated systemic inflammation and poor prognosis [29]. Dynamic changes in SIRI are superior to single inflammatory markers for evaluating immune-inflammatory imbalance in hepatitis B virus-related ACLF [30]. LASSO regression-based nomogram studies have confirmed the prognostic value of SIRI in ACLF, while also indicating that combined indicators are required to improve predictive efficacy [31].

Dynamic PLR changes are closely related to treatment response and prognosis in ACLF. PLR levels decrease after artificial liver support therapy, and the reduction is more significant in improved patients than in non-improved patients [32]. PLR is also an independent prognostic factor for ACLF [33]. LMR exhibits excellent predictive performance for 28-day mortality in ACLF, with prognostic accuracy superior to the SOFA score [34].

These blood routine-derived composite indices are low-cost, highly repeatable, and easy to operate. They are suitable for dynamic monitoring and early risk warning in sepsis-associated ACLF patients, especially in resource-limited clinical settings.

Immune-inflammatory disturbance is not only a core pathologic feature of sepsis-associated ACLF but also a direct driver of progressive multi-organ dysfunction. In our cohort, non-survivors had significantly higher incidences of acute kidney injury, acute respiratory distress syndrome, hepatic encephalopathy, and circulatory failure than survivors. Correlation analysis showed that IL-6, NLR, SIRI, and PLR were positively correlated with the number of dysfunctional organs, while CD4⁺/CD8⁺ ratio and LMR were negatively correlated with organ failure burden.

Systemic inflammation-induced endothelial dysfunction, microcirculatory disturbance, mitochondrial oxidative stress, and energy metabolism disorder constitute the common pathologic basis of extrahepatic organ injury in ACLF [19]. Animal experiments have further verified that systemic inflammation directly causes renal, pulmonary, and central nervous system

dysfunction in ACLF [35, 36]. Persistent high IL-6 levels activate vascular endothelial cells, increase vascular permeability, and promote leukocyte infiltration into tissues, ultimately leading to renal and respiratory failure [37]. Elevated NLR and SIRI reflect an imbalance between excessive neutrophil activation and lymphocyte depletion. This immune imbalance aggravates immune-mediated tissue damage and amplifies the organ failure cascade [38]. Hepatic encephalopathy is mainly caused by systemic inflammation-induced blood-brain barrier disruption and neuroinflammation [39]. Circulatory failure results from inflammation-mediated vasodilation and insufficient effective circulating blood volume, as well as mitochondrial dysfunction-induced energy metabolism collapse [40].

In recent years, biomarker-targeted immunomodulatory strategies have become a new research direction for ACLF treatment. Circulating vascular endothelial growth factor A (VEGF-A) levels and immune cell population clustering can predict transplant-free survival and granulocyte colony-stimulating factor treatment response, providing a basis for precise patient screening before immunomodulatory therapy [41]. Programmed cell death protein 1 (PD-1) blockade can restore the phagocytic function of Kupffer cells and monocytes, enhance bacterial clearance capacity, and is a promising immune checkpoint therapy for acute liver failure [42]. In addition, targeted inhibition of IL-6, intravenous immunoglobulin therapy, and stem cell therapy have shown potential therapeutic effects on ACLF by regulating immune-inflammatory pathways, though high-level clinical evidence is still lacking [43, 44]. Further disease-specific clinical trials are urgently needed to validate the efficacy of targeted immunomodulatory interventions in sepsis-associated ACLF patients.

This study had several limitations that need to be acknowledged. First, this was a single-center retrospective study. All clinical and laboratory data were extracted from routine electronic medical records rather than standardized research databases, which may have led to information bias and selection bias, as well as partial data inconsistency and missing values. Second, the limited sample size and single-center enrollment characteristics may have

restricted the generalizability of our conclusions to sepsis-associated ACLF patients in different medical centers and regions.

Third, we detected baseline immune-inflammatory markers only within 24 hours of admission. No dynamic longitudinal monitoring was performed throughout the disease course. Sepsis-associated ACLF is a rapidly progressive disease with highly dynamic immune-inflammatory changes. Single time-point cross-sectional detection cannot reflect the dynamic evolution of cytokine storm and immunoparalysis. It also fails to clarify the temporal causal relationship between immune-inflammatory fluctuations and progressive organ dysfunction or poor prognosis. Therefore, this study cannot determine whether immune imbalance is the primary cause or secondary manifestation of organ failure.

Fourth, this observational study grouped patients solely based on 28-day survival outcomes. We did not perform multivariate adjustment for confounding factors, including baseline treatment regimens, empirical antibiotic selection, and the timing and application of artificial liver support. These unadjusted confounding factors may have affected the reliability of the correlation results.

Fifth, this study only analyzed the correlation between clinical markers and prognosis. No multi-omics detection (single-cell sequencing, transcriptomics, metabolomics) or *in vitro* and *in vivo* functional verification was performed. The molecular regulatory mechanisms of immune-inflammatory disturbance in sepsis-associated ACLF remain speculative based on previous literature, lacking direct experimental evidence. The multidimensional regulatory relationships among cytokines, immune cell subsets, and organ injury signaling pathways could not be fully elucidated in this study.

Finally, we did not construct multivariate prognostic models or nomograms to verify the independent predictive value of included markers. Collinearity diagnosis of multiple immune-inflammatory indicators was not conducted, which may have interfered with the interpretation of individual marker efficacy. The limited sample size also prevented stratified subgroup analysis based on underlying liver disease etiology and infection sites. In addition, we used

only a 28-day follow-up and did not evaluate the long-term prognostic value of these markers.

Future high-quality studies are required to address these deficiencies. We will design prospective multicenter studies with larger sample sizes, conduct dynamic longitudinal monitoring of biomarkers, perform collinearity analysis and stratified subgroup analysis, and construct multivariate prognostic models. Ultimately, biomarker-guided randomized controlled trials are needed to optimize targeted immunomodulatory strategies and improve the clinical prognosis of sepsis-associated ACLF patients.

In conclusion, this study systematically analyzed 116 sepsis-associated ACLF patients and defined the core immune-inflammatory characteristics of this population, namely the coexistence of pro-inflammatory cytokine storm and immunoparalysis. We validated that key immune-inflammatory markers (IL-6, NLR, SIRI, CD4⁺/CD8⁺ ratio, and LMR) were closely correlated with multi-organ dysfunction and 28-day short-term prognosis. These findings deepen the understanding of the immunopathologic mechanisms of sepsis-associated ACLF, and provide biomarker evidence for early risk stratification and immune-targeted stratified clinical therapy for sepsis-associated ACLF.

Disclosure of conflict of interest

None.

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