Journal of Clinical and Translational Hepatology **2025** vol. 13(5) | 395–408
DOI: 10.14218/JCTH.2024.00452

Original Article

Mitochondrial Membrane Potential of CD8$^+$ T Cells Predicts Bacterial Infection and Rapid Development of Acute-on-chronic Liver Failure in Cirrhotic Patients

Xixuan Wang[1#], Shuling Chen[2#], Jing Fan[3], Yuxiang Gong[2], Hongli Liu[1], Lili Wang[3], Xiaoning Feng[2], Hui Zhou[2], Wenquan Zeng[2], Changhua Yi[4], Caiyun Zhang[3], Qingfang Xiong[1,2], Hao Ren[1,2*] and Yongfeng Yang[1,2*]

[1]Department of Hepatology, The Second Hospital of Nanjing, Medical School, Southeast University, Nanjing, Jiangsu, China; [2]Department of Hepatology, The Second Hospital of Nanjing, Affiliated to Nanjing University of Chinese Medicine, Nanjing, Jiangsu, China; [3]Clinical Research Centre, The Second Hospital of Nanjing, Affiliated to Nanjing University of Chinese Medicine, Nanjing, Jiangsu, China; [4]Department of Biobank, The Second Hospital of Nanjing, Affiliated to Nanjing University of Chinese Medicine, Nanjing, Jiangsu, China

Received: December 02, 2024 | **Revised:** January 22, 2025 | **Accepted:** February 06, 2025 | **Published online:** February 25, 2025

Abstract

Background and Aims: Patients with cirrhosis are at an increased risk of bacterial infection (BI), which is the most common precondition for acute-on-chronic liver failure (ACLF). In this study, we aimed to evaluate the ability of mitochondria-related indicators (mitochondrial mass and mitochondrial membrane potential (MMP)) of T cells in peripheral blood to predict BI and ACLF within 90 days in cirrhotic patients. ***Methods:*** We prospectively studied mitochondria-related indicators in various T cells from 235 cirrhotic patients at the Second Hospital of Nanjing. The outcomes of interest were BI and ACLF. ***Results:*** The restricted cubic spline analysis showed that the MMP of CD8$^+$ T cells had a linear relationship with the risk of BI and ACLF (both $P < 0.001$). Multivariable Cox regression analysis demonstrated that the MMP of CD8$^+$ T cells was an independent risk factor for both BI and ACLF (BI: hazard ratio 0.96, 95% confidence interval 0.94–0.98; $P < 0.001$; ACLF: hazard ratio 0.94, 95% confidence interval 0.90–0.97; $P < 0.001$). The MMP of CD8$^+$ T cells exhibited better diagnostic efficacy than traditional indices in predicting BI (C index: 0.75). The MMP of CD8$^+$ T cells, when combined with traditional models (Child-Turcotte-Pugh and model for end-stage liver disease score), improved their diagnostic efficiency in predicting both BI and ACLF. Additionally, the MMP of CD8$^+$ T cells showed a significant negative correlation with inflammation-related markers ($P < 0.05$). Mitochondrial damage and abnormally activated mitochondrial autophagy were observed in CD8$^+$ T cells from cirrhotic patients with low MMP. ***Conclusions:*** The MMP of CD8$^+$ T cells could serve as a valuable predictor of BI and ACLF within 90 days in cirrhotic patients.

Citation of this article: Wang X, Chen S, Fan J, Gong Y, Liu H, Wang L, *et al*. Mitochondrial Membrane Potential of CD8$^+$ T Cells Predicts Bacterial Infection and Rapid Development of Acute-on-chronic Liver Failure in Cirrhotic Patients. J Clin Transl Hepatol 2025;13(5):395–408. doi: 10.14218/JCTH.2024.00452.

Introduction

Bacterial infection (BI) is common in patients with cirrhosis and is the leading cause of hospital admission.[1] The incidence of BI in cirrhotic patients is as high as 34% per year,[2,3] and about one-third of those admitted to the hospital develop a hospital-acquired infection.[3] BI, which is the most common precondition for acute-on-chronic liver failure (ACLF), can lead to further decompensation, including oesophageal and gastric varices bleeding (EGVB), hepatorenal syndrome, and hepatic encephalopathy.[4] Therefore, the early diagnosis of BI and the identification of associated complications, such as ACLF, are crucial for individualized treatment to improve the poor prognosis of cirrhotic patients with BI.

Early recognition of BI is challenging.[5,6] Cirrhotic patients may not exhibit typical signs of infection, such as fever, in the early stages of infection.[7] Routine serum biomarkers have limited diagnostic performance in cirrhotic patients.[2,8] The white blood cell (WBC) count may be affected by hypersplenism[8] and cirrhosis-associated immune dysfunction (CAID).[9] C-reactive protein (CRP) may serve as an indirect marker of acute inflammatory responses.[10] In cases of renal dysfunction or comorbid conditions, elevated procalcitonin levels may be misinterpreted as a sign of cirrhosis.[11,12] Therefore, there is a need for new diagnostic biomarkers for BI.

The poor prognosis of cirrhotic patients with BI may be linked to immune dysfunction.[13] Adaptive immune deficiency, partly due to suppressed or insufficient T-cell responses,[14] could be a significant risk factor for immune paralysis and

Keywords: Cirrhosis-associated immune dysfunction; T cells; Mitochondria; Mitochondrial autophagy; Bacterial infection; Acute-on-chronic liver failure.
[#]Contributed equally to this work.
[*]**Correspondence to:** Yongfeng Yang and Hao Ren, Department of Hepatology, The Second Hospital of Nanjing, Affiliated to Nanjing University of Chinese Medicine, Zhongfu Road, Nanjing, Jiangsu 210003, China. ORCID: https://orcid.org/0000-0002-3214-0038 (YY). Tel: +86-13951990210 (YY) and +86-19951650426 (HR), E-mail: yyf1997@163.com (YY) and 292951393@qq.com (HR).

bacterial susceptibility in cirrhotic patients.[15] The impaired antimicrobial response in conditions like acute liver failure and alcoholic acute hepatitis has been associated with CD4$^+$ and CD8$^+$ T cells.[16–18]

Mitochondrial metabolic regulation is essential for the transformation of T cells from naïve to active states, and from active to memory cells.[19,20] Chen *et al*.[21] concluded that the mitochondrial function of CD8$^+$ T cells is essential for anti-tumor immunity. Mitochondria regulate T-cell maturation, differentiation, and function in various ways.[22] Mitochondrial function plays an important role in regulating T-cell immune metabolism.[23] The mitochondrial membrane potential (MMP), the potential difference across the mitochondrial inner membrane, reflects the real-time metabolic state of cells.[24] The MMP is involved in the production of ATP and reactive oxygen species (ROS):[25] the higher the MMP, the greater the ATP production rate.[26,27] An abnormal MMP can significantly impair the function and survival of immune cells.[24] Mitochondrial mass (MM) refers to the total amount of protein available in the mitochondrial respiratory chain, representing the upper limit of a cell's metabolic capacity.[28]

We aimed to evaluate the diagnostic efficiency of MM and MMP in T cells from peripheral blood (PB) for predicting BI and ACLF within 90 days after hospitalization in cirrhotic patients.

Methods

Patient selection

A prospective observational cohort study (*TBNK COHORT STUDY*) of cirrhotic inpatients was conducted at the Second Hospital of Nanjing from January to November 2023. This cohort study evaluated changes in the mitochondrial function of T cells, B cells, and NK cells in PB in the progression of cirrhosis and their clinical application. Additionally, we collected PB from patients for cytokine assays to assess whether mitochondria-related indicators reflect systemic inflammatory changes in CAIDs from March 2024 to June 2024. This study was part of the *TBNK Clinical Programme*.

The inclusion criteria were patients aged ≥18 and ≤80 years with cirrhosis, based on histological, biochemical, ultrasonic, or radiological findings. Both patients with compensated and decompensated cirrhosis were included in the study. The exclusion criteria were as follows: (1) ACLF present at admission; (2) acute infection within the previous week or prophylactic antibiotics within one week; (3) human immunodeficiency virus infection; (4) treatment with corticosteroids, immunosuppressants, or cytotoxic drugs; (5) hepatocellular carcinoma and concomitant non-liver-related malignant tumors; (6) previous liver lobectomy or liver transplantation; (7) severe heart failure (New York Heart Association class 4), chronic obstructive pulmonary disease (Global Initiative for Chronic Obstructive Lung Disease grade 3–4), or kidney dysfunction (chronic kidney disease level 5) diagnosed before cirrhosis; (8) pregnancy or lactation; and (9) missing follow-up data.

Study design

Upon admission, patients were evaluated for inclusion and exclusion criteria. Patients meeting the criteria were introduced to the program, and those who agreed to participate signed informed consent. Patients underwent routine physical examinations, laboratory tests, and imaging exams (ultrasound and CT). Three to five milliliters of peripheral blood was collected from patients on the day of admission or the day after admission (depending on the time of admission) and transported to the laboratory for testing within 2 h. Demographic, clinical, laboratory, imaging, and treatment data were collected (antibiotic use was recorded in detail). Baseline indicators in the *Lis-System* were recorded on the day of admission or the day after admission.

The detection time of blood samples was considered day 0. Follow-up was performed at one week, one month, and three months. Phone and WeChat follow-up were conducted as needed. During follow-up, any condition requiring active management was treated accordingly.

The study endpoints were BI and ACLF.

Bacterial infections were diagnosed according to the following criteria: (1) spontaneous bacterial peritonitis: ascites multinucleate cell count ≥ 250/mm^3; (2) urinary tract infection: at least one of the following signs or symptoms (fever ≥ 38°C, urgency to urinate, frequent urination, dysuria, or suprapubic tenderness) and positive urine culture, or at least two signs or symptoms (fever ≥ 38°C, urgency to urinate, frequent urination, dysuria, or suprapubic tenderness) with urine WBC greater than 10/μL; (3) pneumonia: radiographic examination suggesting new or progressive osmotic or cavernous changes, plus at least one of the following: fever ≥ 38°C, WBC ≥ 12,000/mm^3 or ≤4,000/mm^3, and at least one of the following: new purulent sputum or change in sputum character, new cough, dyspnea or shortness of breath (20 breaths per minute), rales or bronchial breathing sounds, or worsening gas exchange, and/or positive culture from blood, pleural effusion, or specimens obtained from the trachea, aspiration, bronchoalveolar lavage, or biopsy; and (4) acute biliary tract infection: radiographic examination (CT or MRCP) showing exudative or necrotic changes in the biliary tract, plus at least one of the following: fever ≥ 38°C, WBC ≥ 12,000/mm^3 or ≤4,000/mm^3, plus local or systemic inflammatory changes (abdominal pain, jaundice, positive Murphy sign, Charcot triad); (5) spontaneous bacteraemia: in the absence of a known infection source, at least one of the following: fever ≥ 38°C, chills, and hypotension, with positive blood cultures (bilateral and 2-culturing-bottle) and (6) the upper respiratory tract infection: acute pharyngitis, tonsillitis, laryngitis, sinusitis, otitis media, with fever and WBC ≥ 12,000/mm^3 or ≤ 4,000/mm^3, with or without clear etiological evidence, and significant improvement after antibiotic treatment (according to 'Guideline for primary care of acute upper respiratory tract infection (2018)', DOI: 10.3760/cma.j.issn.1671-7368.2019.05.005). BI at other sites were diagnosed according to the corresponding diagnostic criteria.

ACLF was diagnosed following the EASL guidelines.[29]

Two physicians regularly reviewed the data to detect errors and assess the accuracy of the collected data. The researchers who tested the cell counts, MM, and MMP were blinded to the clinical status of the patients. After verifying the collected clinical variables, statistical analysis was performed.

Measurement of MM and MMP, mitochondrial ultrastructure observation, autophagy-related protein detection, and cytokine concentration determination

This process is described in detail in the Supplementary File 1.

Ethics

The research adhered to both the Declaration of Helsinki and the Declaration of Istanbul and was approved by the Ethics Committee of the Second Hospital of Nanjing (2023-LY-ky-022). All patients provided written informed consent.

Statistical analysis

Clinical indexes with missing values of less than 10% were

imputed using multiple imputation methods.

The Survival and Survminer packages in R software were used for Kaplan–Meier curves and log-rank statistical calculations. The rms package was used to draw restricted cubic splines, and the ggpubr package was used to draw fitted curves.

Data are presented as frequencies and percentages, means and standard deviations, or medians and interquartile ranges, as appropriate. Baseline characteristics were compared using Fisher's exact test for categorical variables, Student's t-test for continuous variables, and the Wilcoxon rank-sum test for ordinal and continuous variables. Fitted curves were drawn to assess the relationships between cell counts or markers (inflammation, immune exhaustion, and liver disease) and mitochondria-related indicators, and Spearman correlation coefficients were calculated. The receiver operating characteristic (ROC) curves for BI and ACLF at 90 days were plotted, and the areas under the ROC curves (AUROC) were calculated. Considering the possibility of a nonlinear correlation, the optimal cut-off for MM or MMP of T cells was determined by plotting a 5-knot restricted cubic spline (RCS). C-indexes were also calculated to compare the diagnostic efficacy of different indicators. Kaplan–Meier curves based on mitochondria-related indicators are presented. The association between risk factors and the rates of BI and ACLF was assessed using univariable and multivariable Cox proportional hazards regression models, and forward regression (Wald) analysis was employed to select variables for inclusion in the multivariable model. Time-dependent ROC curves were used to evaluate the diagnostic performance of the conventional model combined with T-cell mitochondria-related indicators. The adjusted RCS was plotted for sensitivity analysis. Statistical analyses were performed using GraphPad software (version 9.0), the Statistical Package for Social Sciences (version 22.0), RStudio (version 4.2.1), and Python (version 3.10).

The level of significance was set at the two-sided 5% level.

Results

Cohort baseline characteristics

According to the inclusion and exclusion criteria, 235 cirrhotic patients were analyzed (mean age 52.65 ± 12.93 years, 118 males, 120 in the decompensated stage) (Supplementary Fig. 1), 45 of whom were newly diagnosed with liver disease. The baseline characteristics of the patients are shown in Table 1.

Mitochondria-related indicators to predict infection within 90 days

BI occurred in 97 patients (41.28%) during follow-up, 32 (9.79%) of whom had BI during their hospital stay, 10 (4.25%) of whom were readmitted, and the remaining patients received outpatient or emergency treatment with oral or intravenous antibiotics, nutritional support, etc. The most common sites of infection were the upper respiratory tract (52, 53.61%) and urinary tract (20, 20.62%). The distribution of infection sites is shown in Supplementary Figure 2. Notably, the 90-day incidence of BI was significantly different between decompensated and compensated patients (hazard ratio (HR) 5.41, 95% confidence interval (CI): 3.62–8.08, $P < 0.001$, Supplementary Fig. 3), as was the distribution of causes ($P = 0.009$, Supplementary Fig. 2).

Compared with patients who did not develop BI within 90 days, those who did had fewer CD3$^+$ T cells, CD4$^+$ T cells, and CD8$^+$ T cells (all $P < 0.001$, Supplementary Table 1A). These results suggested that the immune function of patients with cirrhosis was significantly impaired and that those at high risk for infection had more severe immune deficiencies. There were significant differences in mitochondria-related indicators of T cells between patients who developed and did not develop BI within 90 days (Supplementary Table 1A).

Previous studies have suggested that T-cell mitochondrial function is closely related to T-cell fate.[19] We generated a fitting curve to assess the correlation between T-cell count and MM or MMP. MM did not correlate with cell count for T cells, CD4$^+$ T cells, or CD8$^+$ T cells ($P > 0.05$), while MMP was weakly correlated with cell count (Supplementary Fig. 4).

The AUROCs of MM and MMP for predicting BI within 90 days were calculated. The MMP of CD8$^+$ T cells demonstrated the best predictive performance (AUROC = 0.79, 95% CI 0.73–0.85, $P < 0.001$) (Table 2A).

As shown in Supplementary Figure 5, we used RCS modeling and visualization to predict the relationship between MM or MMP of T cells and the risk of infection. There was a nonlinear relationship between MM and infection risk (nonlinear $P < 0.05$) (Supplementary Fig. 5). When the MM of CD3$^+$ cells was less than 2.07, the risk of infection remained relatively stable, but above that threshold, it began to increase rapidly (nonlinear $P < 0.001$) (Supplementary Fig. 5A). When the number of CD8$^+$ T cells was less than 0.86, the risk of infection remained stable, and above that value, it increased rapidly (nonlinear $P < 0.05$) (Supplementary Fig. 5E). In contrast, MMP had a linear relationship with the risk of infection: as MMP decreased, the risk of infection gradually increased. The cut-off values were 34.87 (the MMP of T cells), 19.66 (the MMP of CD4$^+$ T cells), and 49.48 (the MMP of CD8$^+$ T cells) (Supplementary Fig. 5B, D, F).

Mitochondria-related indicators to predict ACLF within 90 days

Twenty-nine (12.34%) patients developed ACLF. Consistent with the literature, BI (19, 68.97%) was the most common risk factor. Pulmonary infection (13, 44.83%) accounted for the majority, with iatrogenic and healthcare-associated infections being the most common pulmonary infections. There were significant differences in mitochondria-related indicators of T cells between patients who developed and did not develop ACLF within 90 days (Supplementary Table 1B).

The AUROCs of MM and MMP for predicting ACLF within 90 days were calculated, with the MMP of CD3$^+$CD8$^+$ cells showing superior performance (AUROC = 0.75, 95% CI 0.66–0.83, $P < 0.001$) (Table 3). Unexpectedly, the mitochondria-related indicators of CD4$^-$CD8$^-$ T cells also predicted ACLF well (MM: AUROC = 0.74, 95% CI 0.64–0.83, $P < 0.001$; MMP: AUROC = 0.72, 95% CI 0.63–0.81, $P < 0.001$) (Table 3), even though they were not good predictors of BI.

As shown in Supplementary Figure 6, RCS modeling and visualization were used to predict the relationship between MM or MMP of T cells and the risk of ACLF. The MMP of CD8$^+$ T cells had the strongest correlation with the risk of ACLF, and the threshold value was similar to that for BI prediction (49.48 to 49.45) (Supplementary Fig. 6).

Patients with low MMP in CD8$^+$ T cells had a higher risk of BI and ACLF

We then performed a Cox analysis to predict BI. In the univariable analysis, 41 factors were included as candidate variables in the multivariable model (Table 4). The multivariable Cox regression model showed that the MMP of CD8$^+$ T cells

Table 1. Baseline characteristics of the full cohort

	Patients with compensated cirrhosis (N = 115)	Patients with decompensated cirrhosis (N = 120)	*P*-value
Basic characteristics			
Age (years)	48.50 ± 12.67	56.63 ± 11.94	<0.001
Sex (males/females)	49/66	69/51	0.02
Aetiology (AIH/PBC-AIH overlapping syndrome/PBC/Alcoholic /HCV/HBV/MASH)	19/15/18/4/10/35/14	19/8/11/10/10/61/1	<0.001
Complications			
Ascites (yes/no)	0/115	105/15	<0.001
EGV (yes/no)	1/114	56/64	<0.001
HE (yes/no)	0/115	26/94	<0.001
Splenectomy (yes/no)	1/114	20/100	<0.001
TIPS (yes/no)	0/115	4/116	0.048
Diabetes (yes/no)	18/97	27/93	0.18
Cardiovascular disease (yes/no)	36/79	33/87	0.52
chronic kidney diseases (yes/no)	1/114	6/114	0.06
Chronic lung disease (yes/no)	3/112	1/119	0.29
Aetiological treatment			
Receiving the aetiological treatment (yes/no)	92/23	87/33	0.17
Aetiological control or Biochemical response* (yes/no)	26/89	39/81	0.09
Clinical characteristics			
Higher than ULN of high sensitivity CRP (yes/no)	10/105	41/79	<0.001
Higher than ULN of CRP (yes/no)	6/109	15/105	0.05
White blood cells ($*10^9$/L)	5.48 ± 2.03	4.50 ± 1.98	<0.001
Neutrophils percentage (%)	55.18 ± 12.37	57.29 ± 14.86	0.24
Haemoglobin (g/L)	130.19 ± 25.11	116.15 ± 25.47	<0.001
Platelet ($*10^{12}$/L)	172.82 ± 75.21	93.56 ± 60.78	<0.001
Prothrombin time (s)	11.51 ± 1.41	15.22 ± 5.25	<0.001
International normalized ratio	1.04 ± 0.13	1.42 ± 0.52	<0.001
Total bilirubin (μmol/L)	13.00 (8.80, 29.30)	27.60(17.40, 59.80)	<0.001
Albumin (g/L)	41.08 ± 6.85	36.84 ± 15.32	0.007
Globulin (g/L)	27.24 ± 5.45	30.44 ± 7.45	<0.001
Alanine transaminase (U/L)	79.60 (35.40, 182.60)	28.10 (19.20, 50.30)	<0.001
Aspartate aminotransferase (U/L)	44.30 (29.30, 117.10)	36.70 (24.00, 75.60)	0.08
Gamma-glutamyl transpeptidase (U/L)	79.70 (36.60, 171.20)	50.00 (21.40, 92.40)	<0.001
Alkaline phosphatase (U/L)	93.00 (57.00,155.00)	123.00 (90.00, 164.00)	0.18
Creatinine (μmol/L)	63.55 ± 15.27	72.6 ± 37.00	0.02
Blood urea nitrogen (mmol/L)	4.53 ± 1.24	5.59 ± 4.95	0.02
Serum sodium (mmol/L)	138.78 ± 10.08	139.58 ± 3.91	0.42
C3 (g/L)	1.13 ± 0.67	0.79 ± 0.32	<0.001

(continued)

Table 1. *(continued)*

	Patients with compensated cirrhosis (N = 115)	Patients with decompensated cirrhosis (N = 120)	*P*-value
C4 (g/L)	0.54 ± 0.43	0.15 ± 0.10	0.21
IgA (g/L)	2.50 ± 1.17	4.19 ± 2.71	<0.001
IgG (g/L)	14.95 ± 4.80	18.41 ± 6.75	<0.001
IgM (g/L)	2.01 ± 1.93	2.17 ± 1.76	0.53
Liver stiffness (kPa)	16.34 ± 5.71	31.82 ± 22.59	<0.001
Immune-related index			
Absolute value of CD45$^+$ cells (/μL)	576.18 ± 232.68	427.00 ± 228.96	<0.001
Percentage of CD45$^+$ cells (%)	39.04 ± 11.92	32.68 ± 13.61	<0.001
Absolute value of CD3$^+$ cells (/μL)	1,538.26 ± 640.40	947.10 ± 585.53	<0.001
Percentage of CD3$^+$ cells (%)	75.00 ± 8.54	67.39 ± 12.89	<0.001
Absolute value of CD3$^+$CD4$^+$ cells (/μL)	895.74 ± 426.47	556.13 ± 365.36	<0.001
Percentage of CD3$^+$CD4$^+$ cells (%)	43.42 ± 8.87	39.83 ± 12.08	0.01
Absolute value of CD3$^+$CD8$^+$ cells (/μL)	5,167.86 ± 260.02	311.26 ± 232.13	<0.001
Percentage of CD3$^+$CD8$^+$ cells (%)	25.42 ± 8.99	21.69 ± 9.28	0.002
Absolute value of CD3-CD19$^+$ cells (/μL)	227.14 ± 177.02	202.14 ± 198.22	0.31
Percentage of CD3-CD19$^+$ cells (%)	10.87 ± 6.09	13.88 ± 9.51	0.004
Absolute value of CD3-CD56$^+$ cells (/μL)	244.80 ± 206.98	245.93 ± 243.40	0.97
Percentage of CD3-CD56$^+$ cells (%)	11.90 ± 6.63	15.92 ± 10.22	<0.001
Ratio of CD4 positive cells to CD8 positive cells	2.03 ± 1.12	2.25 ± 1.51	0.21
MM of CD3$^+$ cells	2.04 ± 1.38	2.58 ± 1.55	0.005
MM of CD3$^+$CD4$^+$ cells	2.74 ± 1.54	3.30 ± 1.90	0.01
MM of CD3$^+$CD8$^+$ cells	1.02 ± 0.81	1.44 ± 1.23	0.002
MM of CD3$^+$CD4$^+$CD8$^+$ cells	2.52 ± 1.84	3.17 ± 2.18	0.02
MM of CD3$^+$CD4$^-$CD8$^-$ cells	0.56 ± 0.43	0.75 ± 0.90	0.04
MM of CD3-CD19$^+$ cells	1.01 ± 0.75	1.15 ± 1.07	0.23
MM of CD3-CD56$^+$ cells	0.80 ± 0.79	1.03 ± 1.38	0.12
MMP of CD3$^+$ cells (%)	37.64 ± 11.70	32.55 ± 11.73	0.001
MMP of CD3$^+$CD4$^+$ cells (%)	24.75 ± 13.13	20.97 ± 13.54	0.03
MMP of CD3$^+$CD8$^+$ cells (%)	54.30 ± 11.91	46.15 ± 12.82	<0.001
MMP of CD3$^+$CD4$^+$CD8$^+$ cells (%)	33.96 ± 17.37	29.17 ± 17.37	0.03
MMP of CD3$^+$CD4-CD8- cells (%)	62.74 ± 15.49	57.52 ± 19.27	0.02
MMP of CD3-CD19$^+$ cells (%)	50.47 ± 16.51	48.94 ± 17.01	0.48
MMP of CD3-CD56$^+$ cells (%)	60.03 ± 16.63	56.16 ± 19.98	0.11

*Aetiological control or biochemical response: for AIH: Biochemical remission (normalization of serum aminotransferase and IgG levels) and liver histological remission (Ishak system HAI score <4 or Scheuer grading system G ≤ 1); for PBC: After one year of UDCA treatment, ALP ≤ 3*ULN, AST ≤ 2*ULN, and TB ≤ 1 mg/dL; for AIH-PBC: the above conditions for both AIH and PBC were satisfied at the same time; for alcoholic liver cirrhosis: abstinence for more than six months; for liver cirrhosis associated with HCV or HBV: the viral load was less than the minimum detectable amount for more than one year; for MASH: weight loss and recovery from metabolic syndrome were noted. AIH, autoimmune hepatitis; PBC, primary biliary cholangitis; HCV, hepatitis C virus; HBV, hepatitis B virus; MASH, metabolic-associated steatohepatitis; EGV, esophageal and gastric varices; HE, hepatic encephalopathy; TIPS, transjugular intrahepatic portosystemic shunt; ULN, upper limit of normal; CRP, C-reactive protein; MM, mitochondrial mass; MMP, mitochondrial membrane potential.

Table 2. Performance of the mitochondria-related indicators in predicting BI within 90 days

Mitochondria-related indicators	AUROC (95% CI)	*P*-value
(A) *Performance of the mitochondria-related indicators in predicting BI within 90 days in the total cohort*		
MM of CD3$^+$ cells	0.65 (0.58, 0.72)	<0.001
MM of CD3$^+$CD4$^+$ cells	0.63 (0.56, 0.71)	<0.001
MM of CD3$^+$CD8$^+$ cells	0.72 (0.65,0.78)	<0.001
MM of CD3$^+$CD4$^+$CD8$^+$ cells	0.59 (0.52, 0.67)	0.01
MM of CD3$^+$CD4$^-$CD8$^-$ cells	0.64 (0.56, 0.71)	<0.001
MMP of CD3$^+$ cells	0.65 (0.58, 0.72)	<0.001
MMP of CD3$^+$CD4$^+$ cells	0.62 (0.54, 0.69)	0.002
MMP of CD3$^+$CD8$^+$ cells	0.79 (0.73, 0.85)	<0.001
MMP of CD3$^+$CD4$^+$CD8$^+$ cells	0.58 (0.50, 0.65)	0.049
MMP of CD3$^+$CD4$^-$CD8$^-$ cells	0.64 (0.57, 0.71)	<0.001
(B) *Performance of the mitochondria-related indicators in predicting BI within 90 days in compensated patients*		
MM of CD3$^+$ cells	0.56 (0.42, 0.70)	0.43
MM of CD3$^+$CD4$^+$ cells	0.53 (0.40, 0.67)	0.66
MM of CD3$^+$CD8$^+$ cells	0.62 (0.48,0.78)	0.09
MM of CD3$^+$CD4$^+$CD8$^+$ cells	0.63 (0.50, 0.76)	0.08
MM of CD3$^+$CD4$^-$CD8$^-$ cells	0.60 (0.46, 0.74)	0.18
MMP of CD3$^+$ cells	0.65 (0.51, 0.78)	0.04
MMP of CD3$^+$CD4$^+$ cells	0.56 (0.41, 0.71)	0.40
MMP of CD3$^+$CD8$^+$ cells	0.69 (0.56, 0.81)	0.01
MMP of CD3$^+$CD4$^+$CD8$^+$ cells	0.66 (0.54, 0.79)	0.02
MMP of CD3$^+$CD4$^-$CD8$^-$ cells	0.72 (0.60, 0.83)	0.003
(C) *Performance of the mitochondria-related indicators in predicting BI within 90 days in decompensated patients*		
MM of CD3$^+$ cells	0.66 (0.56, 0.76)	0.005
MM of CD3$^+$CD4$^+$ cells	0.66 (0.55, 0.76)	0.005
MM of CD3$^+$CD8$^+$ cells	0.75 (0.66,0.85)	<0.001
MM of CD3$^+$CD4$^+$CD8$^+$ cells	0.53 (0.43, 0.64)	0.54
MM of CD3$^+$CD4$^-$CD8$^-$ cells	0.68 (0.58, 0.78)	0.001
MMP of CD3$^+$ cells	0.60 (0.49, 0.71)	0.06
MMP of CD3$^+$CD4$^+$ cells	0.59 (0.49, 0.70)	0.09
MMP of CD3$^+$CD8$^+$ cells	0.81 (0.73, 0.85)	<0.001
MMP of CD3$^+$CD4$^+$CD8$^+$ cells	0.52 (0.41, 0.62)	0.76
MMP of CD3$^+$CD4$^-$CD8$^-$ cells	0.60 (0.49, 0.70)	0.08

BI, bacterial infection; AUROC, area under the receiver operating characteristic curve; MM, mitochondrial mass; MMP, mitochondrial membrane potential.

was an independent risk factor for BI (HR 0.96, 95% CI 0.94–0.98; $P < 0.001$; Table 4). We also performed a Cox analysis for predicting ACLF, which concluded that CRP, total bilirubin, international normalized ratio, creatine, CD8$^+$ T cell counts, and the MMP of CD8$^+$ T cells were independent risk factors (Table 5).

Patients in the cohort were divided into two groups according to the cut-off value for the MMP of CD8$^+$ T cells. The BI-free survival rate differed between patients with high and low MMP of CD8$^+$ T cells (HR 4.09, 95% CI 2.73–6.14; $P < 0.001$; C-index: 0.68 ± 0.02; Fig. 1A). The ACLF-free survival rate also differed between patients with high and low MMP of CD8$^+$ T cells (HR 4.47, 95% CI 2.15–9.27; $P < 0.001$; C-index: 0.67 ± 0.04; Fig. 1D).

The diagnostic efficiency of MMP of CD8$^+$ T cells was superior to traditional indices

We also calculated C-indices to compare the diagnostic efficacy of different indicators. In predicting the occurrence of BI within 90 days, the MMP of CD8$^+$ T cells, as a single index, demonstrated better diagnostic efficacy than traditional indices, including absolute T cell count, high sensitivity CRP, CRP, WBC, and the percentage of neutrophils (C-index: 0.75 vs 0.70, 0.69, 0.58, 0.55, and 0.63, Supplementary Table

Table 3. Performance of mitochondria-related indicators for predicting ACLF

Mitochondria-related indicators	AUROC (95% CI)	*P*-value
(A) *Performance of the mitochondria-related indicators in predicting ACLF within 90 days in the total cohort*		
MM of $CD3^+$ cells	0.71(0.62, 0.80)	<0.001
MM of $CD3^+CD4^+$ cells	0.71 (0.61, 0.80)	<0.001
MM of $CD3^+CD8^+$ cells	0.69 (0.60,0.78)	<0.001
MM of $CD3^+CD4^+CD8^+$ cells	0.54 (0.42, 0.66)	0.49
MM of $CD3^+CD4^-CD8^-$ cells	0.74 (0.64, 0.83)	<0.001
MMP of $CD3^+$ cells	0.66 (0.57, 0.75)	0.005
MMP of $CD3^+CD4^+$ cells	0.60 (0.48, 0.71)	0.10
MMP of $CD3^+CD8^+$ cells	0.75 (0.66, 0.83)	<0.001
MMP of $CD3^+CD4^+CD8^+$ cells	0.51 (0.40, 0.63)	0.80
MMP of $CD3^+CD4^-CD8^-$ cells	0.72 (0.63, 0.81)	<0.001
(B) *Performance of the mitochondria-related indicators in predicting ACLF within 90 days in decompensated patients*		
MM of $CD3^+$ cells	0.67 (0.56, 0.77)	0.007
MM of $CD3^+CD4^+$ cells	0.67 (0.56, 0.78)	0.007
MM of $CD3^+CD8^+$ cells	0.64 (0.54,0.75)	0.02
MM of $CD3^+CD4^+CD8^+$ cells	0.51 (0.38, 0.64)	0.84
MM of $CD3^+CD4^-CD8^-$ cells	0.74 (0.63, 0.84)	<0.001
MMP of $CD3^+$ cells	0.60 (0.49, 0.71)	0.11
MMP of $CD3^+CD4^+$ cells	0.54 (0.42, 0.66)	0.54
MMP of $CD3^+CD8^+$ cells	0.68 (0.57, 0.79)	0.004
MMP of $CD3^+CD4^+CD8^+$ cells	0.57 (0.45, 0.69)	0.26
MMP of $CD3^+CD4^-CD8^-$ cells	0.70 (0.60, 0.80)	0.001

ACLF, acute-on-chronic liver failure; AUROC, area under the receiver operating characteristic curve; MM, mitochondrial mass; MMP, mitochondrial membrane potential.

2A). In predicting ACLF, however, the diagnostic efficacy of the single index, the MMP of $CD8^+$ T cells, was inferior to high-sensitivity CRP, total T cell counts, and the percentage of neutrophils (Supplementary Table 2A).

Time-dependent ROC curves were then drawn to evaluate the diagnostic efficacy of combining MMP with traditional models. The MMP of $CD8^+$ T cells improved the ability of Child-Turcotte-Pugh (CTP) score and model for end-stage liver disease (MELD) score to predict BI (AUROC at 90 days: CTP: 0.81 to 0.89; MELD: 0.78 to 0.89; Supplementary Fig. 7). The predictive abilities of the CTP score and MELD score for ACLF could also be strengthened by combining them with the MMP of $CD8^+$ T cells (AUROC at 90 days: CTP: 0.94 to 0.96; MELD: 0.95 to 0.96) (Supplementary Fig. 8).

Sensitivity analysis

The mitochondria-related indicators differed between compensated and decompensated cirrhotic patients. Therefore, stratified analyses were performed. The advantage of the ability to predict BI was observed in patients with decompensated cirrhosis (Table 2C), while for compensated patients, the MMP of $CD4^-CD8^-$ T cells showed better diagnostic efficiency (Table 2B).

The MMP of $CD8^+$ T cells demonstrated the best performance in predicting BI and ACLF in the total cohort. To better understand which aspects of CAIDs were reflected by the MMP of $CD8^+$ T cells, we performed correlation analyzes with markers of inflammation, immune exhaustion, liver disease, and complications (Supplementary Fig. 9). These results suggested that the MMP of $CD8^+$ T cells was associated with age and the stage of liver disease and that the MMP of $CD8^+$ T cells was significantly correlated with inflammation-related markers.

We then performed subgroup analysis based on the stage of cirrhosis. Although the HR varied among subgroups, the MMP of $CD8^+$ T cells demonstrated strong predictive ability for BI in subgroups. The BI-free rate differed between compensated patients with high and low MMP of $CD8^+$ T cells (hazard ratio 2.95, 95% CI 1.12–7.74; $P = 0.01$; Fig. 1B). In the absence of positive events, the hazard ratio could not be calculated (Supplementary Fig. 6B). BI was more common in decompensated cirrhotic patients with high MMP of $CD8^+$ T cells (hazard ratio 3.35, 95% CI 2.15–4.97; $P < 0.001$; Fig. 1C). Decompensated patients with high MMP were more likely to develop ACLF(hazard ratio 2.73, 95% CI 1.30–5.71; $P < 0.001$; Fig. 2C). Compared with the traditional index, the results of the subgroup analysis suggested that the advantage of MMP of $CD8^+$ T cells was mainly in decompensated patients (Supplementary Table 2C).

The results of stratification analysis according to ages were similar to the main findings (Supplementary Table 3).

Figure 2 showed the persistent association of the MMP of $CD8^+$ T cells with the occurrence of BI and ACLF after adjustment for age and cirrhosis stage. We observed that the lower limit of the hazard ratio with the 95% CI was greater than 1 at the MMP of less than 37.3 in Figure 2A. There was a nonlinear relationship between MMP and ACLF risk (nonlinear $P < 0.05$, Fig. 2B).

Table 4. Cox regression evaluation of factors associated with BI

Variable	Univariable analysis		Multivariable analysis	
	Hazard ratio	***P*-value**	**Hazard ratio**	***P*-value**
Age (y)	1.03 (1.01, 1.04)	0.002	…	…
Stage (compensated/decompensated)	5.29 (3.19, 8.75)	<0.001	1.92 (1.02. 3.60)	0.04
Splenectomy (no/yes)	0.37 (0.22, 0.62)	<0.001	…	…
Receiving the aetiological treatment (no/yes)	0.56 (0.37, 0.86)	0.008	…	…
Ascites (no/yes)	3.61 (2.32, 5.62)	<0.001	…	…
EGV (no/yes)	3.11 (2.08, 4.67)	<0.001	1.86 (1.15, 3.01)	0.01
HE (no/yes)	5.01 (3.10, 8.09)	<0.001	…	…
Higher than ULN of high sensitivity CRP (yes/no)	7.33 (4.79, 11.22)	<0.001	3.01 (1.84, 4.93)	<0.001
Higher than ULN of CRP (yes/no)	5.60 (3.29, 9.52)	<0.001	2.25 (1.20, 4.20)	0.01
Neutrophils percentage (%)	1.03 (1.01, 1.05)	0.001	…	…
Haemoglobin (g/L)	0.98 (0.98, 0.99)	<0.001	0.99 (0.98, 1.00)	0.44
Platelet ($*10^{12}$/L)	0.99 (0.99, 0.99)	<0.001	…	…
Prothrombin time (s)	1.14 (1.11, 1.17)	<0.001	0.83 (0.73, 0.96)	0.01
International normalized ratio	3.49 (2.67, 4.55)	<0.001	12.80 (3.188, 51.40)	<0.001
Total bilirubin (μmol/L)	1.01 (1.00, 1.01)	<0.001	…	…
Albumin (g/L)	0.92 (0.89, 0.95)	<0.001	…	…
Globulin (g/L)	1.05 (1.02, 1.08)	0.002	…	…
Aspartate aminotransferase (U/L)	1.00 (1.00, 1.00)	0.01	…	…
Alkaline phosphatase (U/L)	1.00 (1.00, 1.00)	0.004	1.00 (1.00, 1.00)	0.01
C3 (g/L)	0.13 (0.06, 0.27)	<0.001	…	…
C4 (g/L)	0.00 (0.00, 0.02)	<0.001	…	…
IgA (g/L)	1.20 (1.13, 1.27)	<0.001	…	…
IgG (g/L)	1.06 (1.03, 1.09)	<0.001	…	…
IgM (g/L)	1.10 (1.02, 1.12)	0.02	…	…
Absolute value of $CD45^+$ cells (/μL)	1.00 (1.00, 1.00)	<0.001	…	…
Percentage of $CD45^+$ cells (%)	0.96 (0.94, 0.98)	<0.001	…	…
Absolute value of $CD3^+$ cells (/μL)	1.00 (1.00, 1.00)	<0.001	…	…
Percentage of $CD3^+$ cells (%)	0.97 (0.95, 0.98)	<0.001	…	…
Absolute value of $CD3^+CD4^+$ cells (/μL)	1.00 (1.00, 1.00)	<0.001	…	…
Percentage of $CD3^+CD4^+$ cells (%)	0.97 (0.95, 0.99)	0.001	…	…
Absolute value of $CD3^+CD8^+$ cells (/μL)	1.00 (1.00, 1.00)	<0.001	…	…
MM of $CD3^+$ cells	1.21 (1.08, 1.34)	<0.001	…	…
MM of $CD3^+CD4^+$ cells	1.16 (1.05, 1.28)	0.003	…	…
MM of $CD3^+CD8^+$ cells	1.38 (1.21, 1.57)	<0.001	…	…
MM of $CD3^+CD4^+CD8^+$ cells	1.11 (1.02, 1.21)	0.02	…	…
MM of $CD3^+CD4^-CD8^-$ cells	1.31 (1.10, 1.56)	0.002	…	…
MMP of $CD3^+$ cells (%)	0.97 (0.95, 0.99)	<0.001	…	…
MMP of $CD3^+CD4^+$ cells (%)	0.98 (0.96, 1.00)	0.02	…	…
MMP of $CD3^+CD8^+$ cells (%)	0.93 (0.92, 0.95)	<0.001	0.96 (0.94, 0.98)	<0.001
MMP of $CD3^+CD4^+CD8^+$ cells (%)	0.99 (0.98, 1.00)	0.11	…	…
MMP of $CD3^+CD4^-CD8^-$ cells (%)	0.98 (0.97, 0.99)	<0.001	…	…

BI, bacterial infection; EGV, esophageal and gastric varices; HE, hepatic encephalopathy; ULN, upper limit of normal; CRP, C-reactive protein; MM, mitochondrial mass; MMP, mitochondrial membrane potential.

Table 5. Cox regression evaluation of factors associated with ACLF

Variable	Univariable analysis		Multivariable analysis	
	Hazard ratio	***P*-value**	**Hazard eatio**	***P*-value**
Stage (compensated/decompensated)	69.18 (3.48, 1,375.44)	0.005	…	…
Ascites (no/yes)	16.86 (4.01, 70.93)	<0.001	…	…
EGV (no/yes)	3.07 (1.48, 6.35)	0.003	…	…
HE (no/yes)	10.99 (5.27, 22.91)	<0.001	…	…
Higher than ULN of high sensitivity CRP (yes/no)	21.24 (8.08, 55.84)	<0.001	5.94 (2.14, 16.51)	<0.001
Higher than ULN of CRP (yes/no)	8.91 (4.23, 18.74)	<0.001	…	…
Neutrophils percentage (%)	1.07 (1.03, 1.10)	<0.001	…	…
Haemoglobin (g/L)	0.98 (0.97, 0.99)	<0.001	…	…
Platelet (*10^{12}/L)	0.99 (0.98, 0.99)	<0.001	…	…
Prothrombin time (s)	1.23 (1.17, 1.28)	<0.001		
International normalized ratio	6.03 (4.07, 8.93)	<0.001	4.36 (2.31, 8.22)	<0.001
Total bilirubin (μmol/L)	1.01 (1.01, 1.01)	<0.001	1.01 (1.00, 1.01)	<0.001
Globulin (g/L)	1.06 (1.01, 1.11)	0.02	…	…
Aspartate aminotransferase (U/L)	1.00 (1.00, 1.00)	0.03	…	…
Creatinine (μmol/L)	1.01 (1.00, 1.02)	0.003	1.01 (1.01, 1.02)	0.001
C3 (g/L)	0.02 (0.00, 0.06)	<0.001		
C4 (g/L)	0.00 (0.00, 0.01)	<0.001	…	…
IgA (g/L)	1.24 (1.14, 1.35)	<0.001	…	…
IgG (g/L)	1.10 (1.06, 1.15)	<0.001	…	…
Absolute value of CD45$^+$ cells (/μL)	1.00 (0.99, 1.00)	<0.001	…	…
Percentage of CD45$^+$ cells (%)	0.94 (0.91, 0.97)	<0.001	…	…
Absolute value of CD3$^+$ cells (/μL)	1.00 (1.00, 1.00)	<0.001	…	…
Percentage of CD3$^+$ cells (%)	0.97 (0.94, 0.99)	0.01	…	…
Absolute value of CD3$^+$CD4$^+$ cells (/μL)	1.00 (1.00, 1.00)	<0.001	…	…
Absolute value of CD3$^+$CD8$^+$ cells (/μL)	0.99 (0.99, 1.00)	<0.001	1.00 (0.99, 1.00)	0.007
MM of CD3$^+$ cells	1.28 (1.06, 1.54)	0.009	…	…
MM of CD3$^+$CD4$^+$ cells	1.31 (1.12, 1.53)	0.001	…	…
MM of CD3$^+$CD8$^+$ cells	1.30 (1.02, 1.66)	0.04	…	…
MM of CD3$^+$CD4$^-$CD8$^-$ cells	1.51 (1.180, 1.93)	0.001	…	…
MMP of CD3$^+$ cells (%)	0.96 (0.92, 0.99)	0.007	…	…
MMP of CD3$^+$CD8$^+$ cells (%)	0.93 (0.91, 0.96)	<0.001	0.94 (0.90, 0.97)	<0.001
MMP of CD3$^+$CD4$^-$CD8$^-$ cells (%)	0.96 (0.94, 0.98)	<0.001	…	…

ACLF, acute-on-chronic liver failure; EGV, esophageal and gastric varices; HE, hepatic encephalopathy; ULN, upper limit of normal; CRP, C-reactive protein; MM, mitochondrial mass; MMP, mitochondrial membrane potential.

Mitochondria damage and abnormally activated mitochondrial autophagy in CD8$^+$ T cells

The majority of mitochondrial structures in CD8$^+$ T cells from patients with high MMP were roughly normal (Fig. 3A). In patients with CD8$^+$ T cells with low MMP, abnormal mitochondria were observed (mitochondrial cristae contracted along the outer membrane, and some mitochondrial cristae were unclear), and damaged mitochondria accumulated. The mitochondrial phagosomes stuck to lysosomes, with damaged mitochondria being engulfed by lysosomes, and the mitochondrial membrane protruded into vesicle-like structures (Fig. 3B). Many typical autophagy structures were observed under transmission electron microscopy (Fig. 3B).

Then we collected CD8$^+$ T cells for mitochondrial autophagy-related protein detection, which revealed that the levels of autophagy-related protein 8a (LC3) and classical mitochondrial autophagy pathway proteins PINK1 and Parkin accumulated in patients with low-MMP T cells, while the ubiquitin-binding scaffold protein (p62) was significantly downregulated (Supplementary Fig. 10A). When we measured the intracellular ROS content in the CD3$^+$ T cells, the results indicated that the intracellular ROS content in the T cells of patients with low MMP was significantly decreased (Supple-

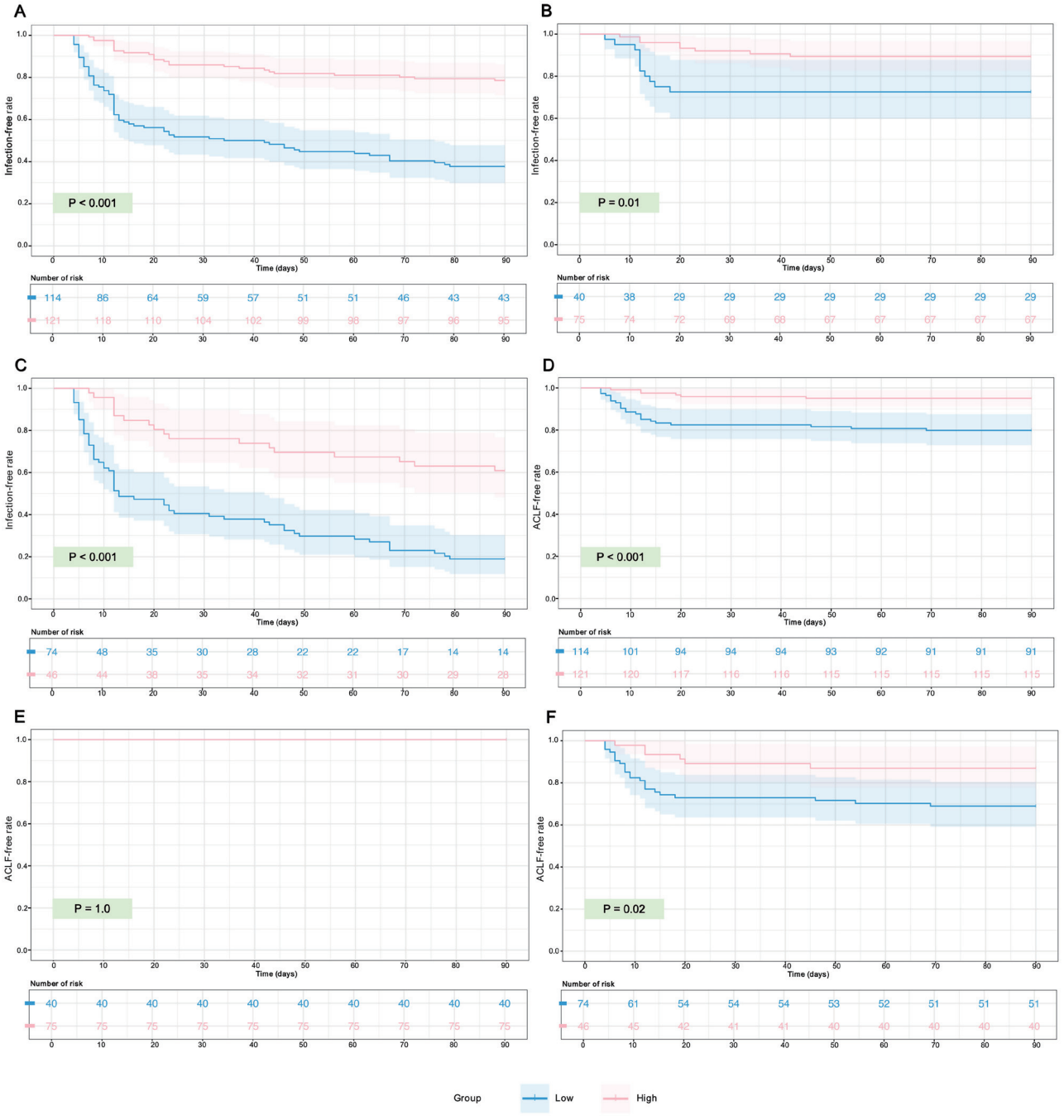

Fig. 1. Comparison of the rates of BI-free survival and ACLF-free survival between patients with high and low MMP in CD8$^+$ T cells. (A) Comparison of BI-free survival rate between patients with high and low MMP of CD8$^+$ T cells; (B) Comparison of BI-free survival rate between patients with high and low MMP of CD8$^+$ T cells for compensated cirrhosis; (C) Comparison of BI-free survival rate between patients with high and low MMP of CD8$^+$ T cells for decompensated cirrhosis. (D) Comparison of the rate of ACLF-free status between patients with high- and low-MMP CD8$^+$ T cells. (E) Comparison of the rate of ACLF-free status between compensated patients with high- and low-MMP CD8$^+$ T cells. (F) Comparison of the rate of ACLF-free status between decompensated patients with high- and low-MMP CD8$^+$ T cells. BI, bacterial infections; ACLF, acute-on-chronic liver failure; MMP, mitochondrial membrane potential.

mentary Fig. 10B). These findings suggest that T cells with low MMP have abnormal mitochondrial autophagy activation.

Patients with low CD8$^+$ T-cells MMP had high systemic inflammatory response

Supplementary Figure 9 showed that the MMP of CD8$^+$ T cells was significantly correlated with inflammation-related markers. We further collected peripheral blood samples for cytokine assays to assess whether these markers reflected systemic inflammatory changes in CAIDs. TNF-α and IL-6 levels were also found to be moderately correlated with the MMP of CD8$^+$ T cells ($0.3<r<0.5$, $P < 0.05$, Supplementary Fig. 11).

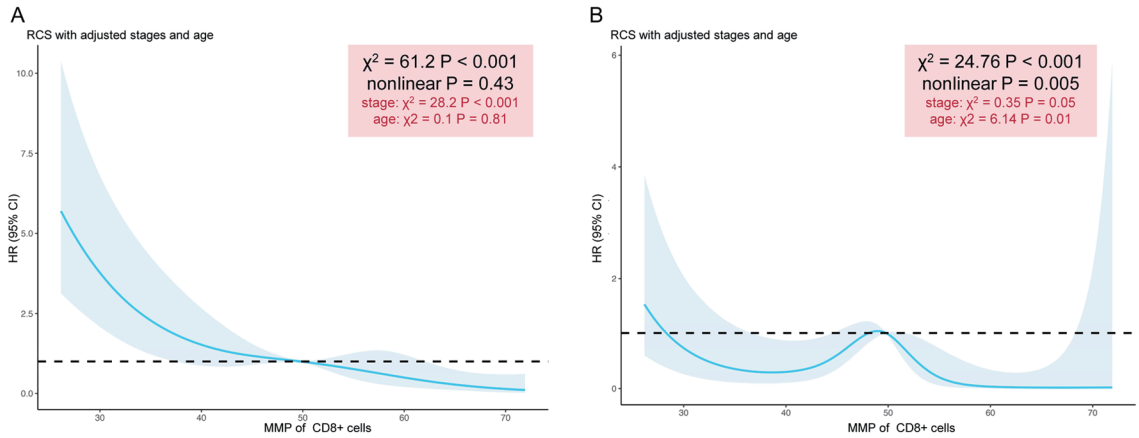

Fig. 2. Continuous association between the MMP of CD8+ T cells and incident risk of BI and ACLF adjusted with ages and stages of cirrhosis. (A) Continuous association between the MMP of CD8+ T cells and incident risk of BI adjusted with ages and stages of cirrhosis. (B) Continuous association between the MMP of CD8+ T cells and incident risk of ACLF adjusted with ages and stages of cirrhosis. ACLF, acute-on-chronic liver failure; BI, bacterial infections; RCS, restricted cubic spline; HR, hazard ratio; MMP, mitochondrial membrane potential.

Discussion

Patients with cirrhosis have a unique spectrum of immune changes that characterize the course of end-stage liver disease.[9] Immune deficiency is one of the key manifestations of CAID.[8] The severity of CAID is related to the stage of cirrhosis.[9] The progression of cirrhosis has a significant impact on the intensity of CAID,[30,31] which is associated with the severity of liver insufficiency, bacterial translocation, and organ failure.[32] Patients with compensated cirrhosis and stable decompensated cirrhosis exhibit an overactive immune system, but immune function, including the ability to fight infection, is not significantly impaired. However, in patients with ACLF, there is significant immune dysfunction, which increases the risk of infection and worsens prognosis.[9,31] It was previously thought that the overall effector response of cirrhotic patients in the compensated and stable-decompensated stages was not significantly impaired. However, there is a marked difference in the incidence of BI between the two stages. While both compensated and stable decompensated patients exhibit similar low-grade systemic inflammatory phenotypes,[9] they differ in T-cell mitochondrial function. In our study, we found that the MM of total T cells, CD4+ T cells, and CD8+ T cells increased, while MMP decreased significantly during decompensation.

T-cell depletion occurs in patients with ACLF.[33,34] Throughout the life cycle of immune cells, energy and substrate requirements change dramatically, and metabolic pathways are activated or inhibited to adapt to cell development and activation.[35] Mitochondrial changes occur in T cells with different phenotypes and at various stages of the T-cell life cycle.[36,37] Therefore, we focused on T-cell mitochondrial function to clarify the relationship between T-cell mitochondrial function indicators and BI in patients with cirrhosis. We recorded the MM and MMP of patients' total T cells, CD4+ T cells, CD8+ T cells, CD4+CD8+ T cells, and CD4+CD8+ T cells. Among many T-cell mitochondrial indicators, the MMP of CD8+ T cells showed the best performance in predicting BI and ACLF and surprisingly performed better than traditional indicators. The multivariable Cox regression model showed that the MMP of CD8+ T cells was an independent risk factor for BI.

Our study found that the MMP of CD8+ T cells was significantly and weakly correlated with age and liver disease stages. This is in line with previous literature, which suggests that CAIDs are related to liver disease stages and age, indicating that the MMP of CD8+ T cells might reflect the severity of CAIDs. Sensitivity analyses were performed to account for the possible confounding role of age and liver disease stage. The RCS curves, adjusted for age and liver disease stage, still showed that a decreased MMP of CD8+ T cells was associated with an increased risk of BI. Subgroup analysis revealed that the advantage of the MMP of CD8+ T cells, compared with traditional inflammation indices, was mainly observed in patients with decompensated cirrhosis and was not as pronounced in compensated cirrhotic patients.

CAIDs mainly include immune exhaustion and abnormal systemic inflammation.[9,31] Alterations in T cells have been shown to be associated with the development of secondary infections in patients with immune paralysis and prolonged sepsis.[38] Our study suggests that the MMP of CD8+ T cells is related to markers of inflammation, but the role of MMPs in CAIDs still requires further study.

Mitochondria are involved in the mechanism of autophagy, and damaged mitochondria caused by minor stress can be cleared through mitochondrial autophagy.[39] Intense stress can lead to an increase in the number of osmotic transition pores in the mitochondrial membrane, which affects mitochondrial autophagy.[40] Abnormally activated mitochondrial autophagy in cells is characterized by decreased membrane potential.[41] Abnormal mitochondria accumulated and mitochondrial autophagy increased in peripheral blood T cells of patients with low MMP in CD8+ T cells, as observed under electron microscopy. Protein detection indicated that, compared with patients with high MMP in CD8+ T cells, peripheral circulating total T cells and CD8+ T cells in patients with low MMP CD8+ T cells showed activation of abnormal mitochondrial autophagy. The low MMP of T cells in cirrhotic patients could be partially explained by mitochondrial morphological abnormalities and abnormal autophagy activation. The underlying mechanism still needs to be further explored.

We identified a novel biomarker, the MMP of CD8+ T cells, for the early identification of BI and ACLF in cirrhotic patients within 90 days of hospitalization. The results of the subgroup analysis suggested that the advantage of the MMP of CD8+ T cells in predicting BI and ACLF was primarily observed in decompensated patients. The MMP of CD8+ T cells was significantly and weakly correlated with inflammation-related markers. Our study suggests that mitochondrial abnormalities in T cells in peripheral blood might be actively involved

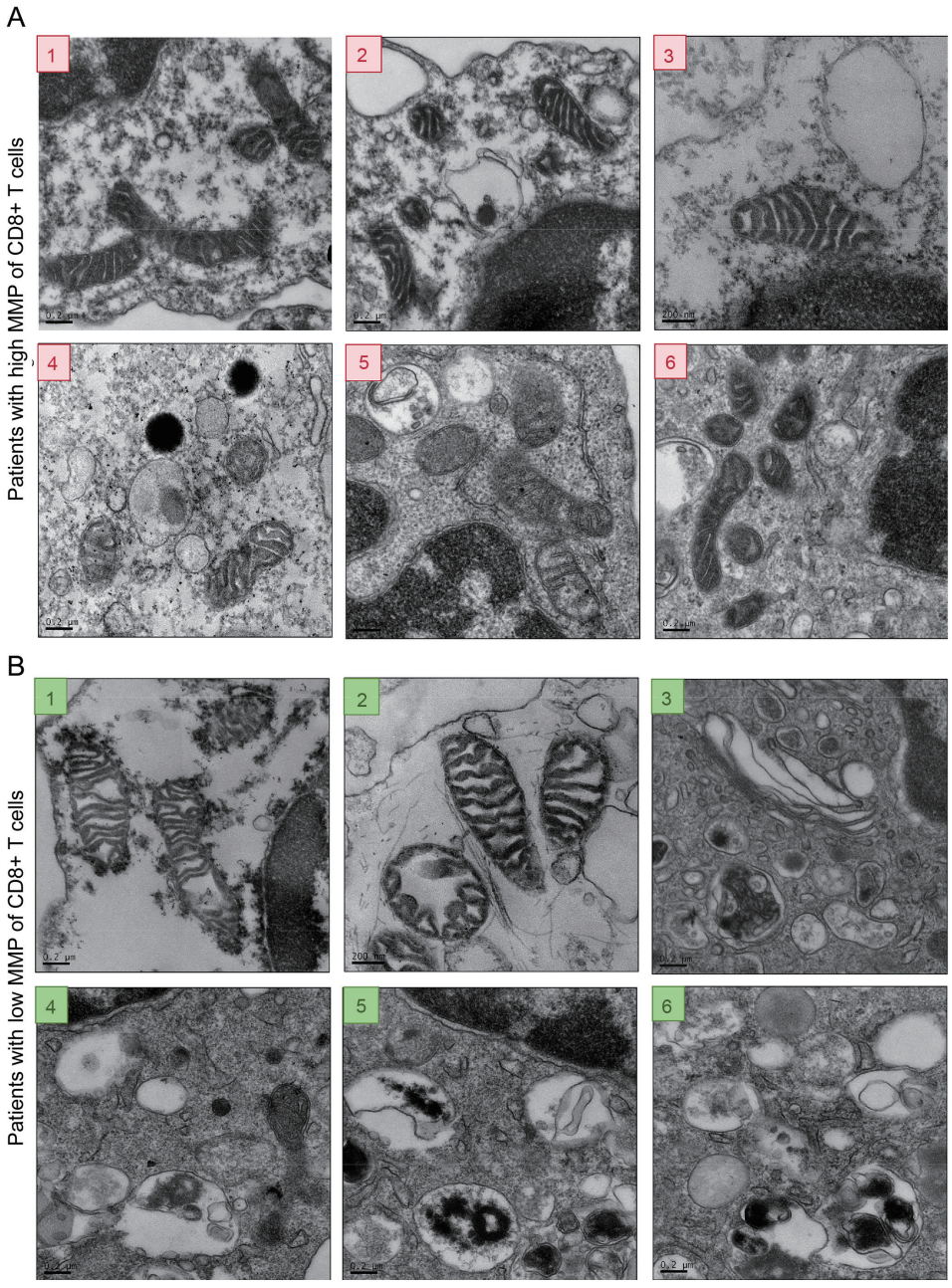

Fig. 3. CD8$^+$ T-cell mitochondria observed by transmission electron microscopy. (A) CD8$^+$ T-cell mitochondria from high-MMP patients observed by transmission electron microscopy: The morphology of mitochondria was generally normal, with some mitochondria showing slight structural damage near lysosomes. 1 & 2: A PBC patient with cirrhosis, hospitalized for review of the stomach to assess esophageal gastric varices, with long-term oral use of ursodiol and carvedilol. Total bilirubin and liver elasticity were controlled (13.6 kPA. No BI occurred during the 90-day follow-up period. 3 & 4: A patient with liver cirrhosis and a small amount of perihepatic ascites, admitted to the hospital for clarification of the cause of liver disease. The patient had not received prior liver disease treatment and was found to have hepatitis C cirrhosis complicated by fatty liver. After discharge, regular antiviral therapy was started, and no BI occurred during the 90-day follow-up period. 5 & 6: A patient with hepatitis B cirrhosis, admitted to the hospital for marked thrombocytopenia, received intermittent antiviral therapy. Regular antiviral therapy was given after discharge. Sixty-seven days later, a respiratory tract infection developed, and oral antibiotics combined with symptomatic and supportive treatment were administered. (B) CD8$^+$ T-cell mitochondria from low-MMP patients observed by transmission electron microscopy: Abnormal mitochondria were observed in T cells (mitochondrial cristae contracted along the outer membrane, and some mitochondrial cristae were unclear), with damaged mitochondria accumulating, mitochondrial phagosomes adhering to lysosomes, and the damaged mitochondria being engulfed by lysosomes. Mitochondrial membranes protruded into vesicle-like structures. Many typical autophagy structures were observed under transmission electron microscopy. 1 & 2: A patient with hepatitis B cirrhosis, admitted to the hospital due to massive ascites and planned to receive ascites treatment, was treated with regular antiviral therapy. After 12 days of high fever and ascites culture positive for gram-positive bacteria, satisfactory results were achieved with anti-infection treatment. 3 & 4: A patient with hepatitis B cirrhosis, admitted with stage 0–1 hepatic encephalopathy, was treated with regular antiviral therapy. On day 8, pneumonia developed, and anti-infective treatment was ineffective. Respiratory failure and progressive hepatic encephalopathy occurred on day 12, leading to a diagnosis of ACLF. The patient died on day 47 after active treatment. 5 & 6: A patient with alcoholic cirrhosis, admitted with upper gastrointestinal bleeding, had not abstained from alcohol. Upon admission, preventive anti-infection treatment was administered. Pneumonia developed on day 5, and infection control was achieved after upgrading antibiotics. ACLF, acute-on-chronic liver failure; BI, bacterial infections; MMP, mitochondrial membrane potential; PBC, primary biliary cholangitis.

in the pathogenesis of CAID, which will be the focus of our upcoming research. Further studies are also needed to determine whether prophylactic use of antibiotics or exogenous administration of immune molecules can improve prognosis in cirrhotic patients with low-MMP CD8$^+$ T cells.

The limitations of this study are as follows: (1) Due to the nature of this single-center observational study, selection bias was inevitable; (2) Most patients in the compensated stage were hospitalized for pathological examinations or to assess complications, while most patients in the decompensated stage were hospitalized for ascites, occult or microhepatic hepatic encephalopathy, and jaundice. This prevents us from extending the conclusions of the study to post-EGVB patients (only two were hospitalized for EGVB); (3) Our study does not determine whether the MMP of CD8$^+$ T cells can predict ACLF in cirrhotic patients with BI, as patients who suffered BI within one week and received antibiotics were excluded at the beginning of the study; (4) Although the MMP of CD8$^+$ T cells demonstrated a reasonable ability to predict transplant-free survival, continued follow-up is needed because there were too few outcome events to draw definitive conclusions; (5) Due to ethical requirements (blood samples collected for TME exceeding 40 mL), our results in the TME were not detailed enough to extend to clinically unstable patients; (6) The cohort was not established with the concept of recompensation in mind; and (7) Our findings cannot be extended to patients with liver cancer or other organ failures.

Conclusions

This study demonstrates the potential of the MMP of CD8$^+$ T cells to predict BI and ACLF within 90 days of hospitalization, which could be partially explained by mitochondrial damage in peripheral circulating CD8$^+$ T cells and abnormally activated mitochondrial autophagy.

Acknowledgments

The authors would like to deeply thank all the patients who donated their blood, without whose help this study would not have been possible. We also extend our thanks to the clinical doctors and nurses from Ward 113 and Ward 111 in the Department of Hepatology at the Second Hospital of Nanjing for their assistance with patient screening and blood sample collection for the *TBNK COHORT STUDY*. We are grateful to our colleagues in the Clinical Laboratory for their guidance and technical support, Dr. Hao Zhang at Nanjing Drum Tower Hospital for his guidance on visualization, Dr. Weigang Ren at Nanjing Drum Tower Hospital for his guidance on transmission electron microscopy image recognition, and Dr. Wei Zhang at Nanjing Drum Tower Hospital for his valuable suggestions. The authors would also like to thank Nanjing JINGXIAN Biotechnology Co., LTD for providing electron microscopy services.

Funding

This work was supported by the Innovation Center for Infectious Disease of Jiangsu Province (No. CXZX202232), Key Projects of Jiangsu Provincial Health Commission (ZD2021061), and the SEU Innovation Capability Enhancement Plan for Doctoral Students (CXJH_SEU 24230).

Conflict of interest

The authors have no conflict of interests related to this publication.

Author contributions

Conceptualization (XW, SC, YY, HR), data curation, biological sample collection (HR, YG, HL, LW, JF, XF, HZ, WZ, CY), formal analysis (XW, SC, JF), funding acquisition (YY, XW), investigation (XW, HR, HL, LW, JF, XF, HZ, WZ, SC, project administration (QX, CY, CZ, HR, YY), resources (QX, CY, CZ, YY), validation (XW, SC, HR), visualization (XW), writing-original draft (XW, SC), and writing-review & editing (HR, YY). All authors have approved the final version and publication of the manuscript.

Ethical statement

The research adhered to both the Declaration of Helsinki and the Declaration of Istanbul and was approved by the Ethics Committee of the Second Hospital of Nanjing (2023-LY-ky-022). All patients included gave written informed consent.

Data sharing statement

Participant data from the *TBNK-Cirrhosis* cohort in this article can be requested by contacting YY (E-mail: yyf1997@163.com) and HR (E-mail: 292951393@qq.com). Data anonymized to protect patient characteristics will be provided for studies whose aims and objectives align with the study protocols. Only proposals where data will be used for statistical and scientific studies will be considered. Data will be shared through Excel electronic forms after the signing of a data access agreement.

References

[1] Piano S, Singh V, Caraceni P, Maiwall R, Alessandria C, Fernandez J, *et al*. Epidemiology and Effects of Bacterial Infections in Patients With Cirrhosis Worldwide. Gastroenterology 2019;156(5):1368–1380.e10. doi:10.1053/j.gastro.2018.12.005, PMID:30552895.
[2] Xu Z, Zhang X, Chen J, Shi Y, Ji S. Bacterial Infections in Acute-on-chronic Liver Failure: Epidemiology, Diagnosis, Pathogenesis, and Management. J Clin Transl Hepatol 2024;12(7):667–676. doi:10.14218/JCTH.2024.00137. PMID:38993512.
[3] Fernández J, Prado V, Trebicka J, Amoros A, Gustot T, Wiest R, *et al*. Multidrug-resistant bacterial infections in patients with decompensated cirrhosis and with acute-on-chronic liver failure in Europe. J Hepatol 2019;70(3):398–411. doi:10.1016/j.jhep.2018.10.027, PMID:30391380.
[4] European Association for the Study of the Liver. EASL Clinical Practice Guidelines on acute-on-chronic liver failure. J Hepatol 2023;79(2):461–491. doi:10.1016/j.jhep.2023.04.021, PMID:37364789.
[5] Bajaj JS, Kamath PS, Reddy KR. The Evolving Challenge of Infections in Cirrhosis. N Engl J Med 2021;384(24):2317–2330. doi:10.1056/NEJMra2021808, PMID:34133861.
[6] Kulkarni AV, Premkumar M, Arab JP, Kumar K, Sharma M, Reddy ND, *et al*. Early Diagnosis and Prevention of Infections in Cirrhosis. Semin Liver Dis 2022;42(3):293–312. doi:10.1055/a-1869-7607, PMID:35672014.
[7] Piano S, Brocca A, Mareso S, Angeli P. Infections complicating cirrhosis. Liver Int 2018;38(Suppl 1):126–133. doi:10.1111/liv.13645, PMID:29427501.
[8] Albillos A, Lario M, Álvarez-Mon M. Cirrhosis-associated immune dysfunction: distinctive features and clinical relevance. J Hepatol 2014;61(6):1385–1396. doi:10.1016/J.JHEP.2014.08.010, PMID:25135860.
[9] Albillos A, Martin-Mateos R, Van der Merwe S, Wiest R, Jalan R, Álvarez-Mon M. Cirrhosis-associated immune dysfunction. Nat Rev Gastroenterol Hepatol 2022;19(2):112–134. doi:10.1038/S41575-021-00520-7, PMID:34703031.
[10] van Leeuwen MA, van Rijswijk MH. Acute phase proteins in the monitoring of inflammatory disorders. Baillieres Clin Rheumatol 1994;8(3):531–552. doi:10.1016/s0950-3579(05)80114-1, PMID:7525084.
[11] Dong R, Wan B, Lin S, Wang M, Huang J, Wu Y, *et al*. Procalcitonin and Liver Disease: A Literature Review. J Clin Transl Hepatol 2019;7(1):51–55. doi:10.14218/JCTH.2018.00012, PMID:30944820.
[12] Bunchorntavakul C, Chamroonkul N, Chavalitdhamrong D. Bacterial infections in cirrhosis: A critical review and practical guidance. World J Hepatol 2016;8(6):307–321. doi:10.4254/wjh.v8.i6.307, PMID:26962397.
[13] Lin CY, Tsai IF, Ho YP, Huang CT, Lin YC, Lin CJ, *et al*. Endotoxemia contributes to the immune paralysis in patients with cirrhosis. J Hepatol 2007;46(5):816–826. doi:10.1016/j.jhep.2006.12.018, PMID:17328986.
[14] Schietinger A, Greenberg PD. Tolerance and exhaustion: defining mechanisms of T cell dysfunction. Trends Immunol 2014;35(2):51–60. doi:10.1016/j.it.2013.10.001, PMID:24210163.
[15] Dirchwolf M, Podhorzer A, Marino M, Shulman C, Cartier M, Zunino M,

et al. Immune dysfunction in cirrhosis: Distinct cytokines phenotypes according to cirrhosis severity. Cytokine 2016;77:14–25. doi:10.1016/j.cyto.2015.10.006, PMID:26517154.
[16] Lebossé F, Gudd C, Tunc E, Singanayagam A, Nathwani R, Triantafyllou E, *et al*. CD8(+)T cells from patients with cirrhosis display a phenotype that may contribute to cirrhosis-associated immune dysfunction. EBioMedicine 2019;49:258–268. doi:10.1016/j.ebiom.2019.10.011, PMID:31678004.
[17] Arruvito L, Payaslián F, Baz P, Podhorzer A, Billordo A, Pandolfi J, *et al*. Identification and clinical relevance of naturally occurring human CD8+HLA-DR+ regulatory T cells. J Immunol 2014;193(9):4469–4476. doi:10.4049/jimmunol.1401490, PMID:25261474.
[18] Machicote A, Belén S, Baz P, Billordo LA, Fainboim L. Human CD8(+) HLA-DR(+) Regulatory T Cells, Similarly to Classical CD4(+)Foxp3(+) Cells, Suppress Immune Responses via PD-1/PD-L1 Axis. Front Immunol 2018;9:2788. doi:10.3389/fimmu.2018.02788, PMID:30555473.
[19] Buck MD, O'Sullivan D, Klein Geltink RI, Curtis JD, Chang CH, Sanin DE, *et al*. Mitochondrial Dynamics Controls T Cell Fate through Metabolic Programming. Cell 2016;166(1):63–76. doi:10.1016/j.cell.2016.05.035, PMID:27293185.
[20] Chang CH, Curtis JD, Maggi LB Jr, Faubert B, Villarino AV, O'Sullivan D, *et al*. Posttranscriptional control of T cell effector function by aerobic glycolysis. Cell 2013;153(6):1239–1251. doi:10.1016/j.cell.2013.05.016, PMID:23746840.
[21] Chen C, Zheng H, Horwitz EM, Ando S, Araki K, Zhao P, *et al*. Mitochondrial metabolic flexibility is critical for CD8(+) T cell antitumor immunity. Sci Adv 2023;9(49):eadf9522. doi:10.1126/sciadv.adf9522, PMID:38055827.
[22] Bailis W, Shyer JA, Zhao J, Canaveras JCG, Al Khazal FJ, Qu R, *et al*. Distinct modes of mitochondrial metabolism uncouple T cell differentiation and function. Nature 2019;571(7765):403–407. doi:10.1038/s41586-019-1311-3, PMID:31217581.
[23] Bantug GR, Fischer M, Grählert J, Balmer ML, Unterstab G, Develioglu L, *et al*. Mitochondria-Endoplasmic Reticulum Contact Sites Function as Immunometabolic Hubs that Orchestrate the Rapid Recall Response of Memory CD8(+) T Cells. Immunity 2018;48(3):542–555.e6. doi:10.1016/j.immuni.2018.02.012, PMID:29523440.
[24] Begum HM, Shen K. Intracellular and microenvironmental regulation of mitochondrial membrane potential in cancer cells. WIREs Mech Dis 2023;15(3):e1595. doi:10.1002/wsbm.1595, PMID:36597256.
[25] Suzuki R, Hotta K, Oka K. Transitional correlation between inner-membrane potential and ATP levels of neuronal mitochondria. Sci Rep 2018;8(1):2993. doi:10.1038/s41598-018-21109-2, PMID:29445117.
[26] Wu KC, Cheng KS, Wang YW, Chen YF, Wong KL, Su TH, *et al*. Perturbation of Akt Signaling, Mitochondrial Potential, and ADP/ATP Ratio in Acidosis-Challenged Rat Cortical Astrocytes. J Cell Biochem 2017;118(5):1108–1117. doi:10.1002/jcb.25725, PMID:27608291.
[27] Ouyang Y, Jeong MY, Cunningham CN, Berg JA, Toshniwal AG, Hughes CE, *et al*. Phosphate starvation signaling increases mitochondrial membrane potential through respiration-independent mechanisms. Elife 2024;13:e84282. doi:10.7554/eLife.84282, PMID:38251707.
[28] Zhang L, Zhang W, Li Z, Lin S, Zheng T, Hao B, *et al*. Mitochondria dysfunction in CD8+ T cells as an important contributing factor for cancer development and a potential target for cancer treatment: a review. J Exp Clin Cancer Res 2022;41(1):227. doi:10.1186/s13046-022-02439-6, PMID:35864520.
[29] European Association for the Study of the Liver. EASL Clinical Practice Guidelines for the management of patients with decompensated cirrhosis. J Hepatol 2018;69(2):406–460. doi:10.1016/j.jhep.2018.03.024, PMID:29653741.
[30] Arroyo V, Angeli P, Moreau R, Jalan R, Clària J, Trebicka J, *et al*. The systemic inflammation hypothesis: Towards a new paradigm of acute decompensation and multiorgan failure in cirrhosis. J Hepatol 2021;74(3):670–685. doi:10.1016/j.jhep.2020.11.048, PMID:33301825.
[31] Martin-Mateos R, Alvarez-Mon M, Albillos A. Dysfunctional Immune Response in Acute-on-Chronic Liver Failure: It Takes Two to Tango. Front Immunol 2019;10:973. doi:10.3389/fimmu.2019.00973, PMID:31118937.
[32] Kubes P, Jenne C. Immune Responses in the Liver. Annu Rev Immunol 2018;36:247–277. doi:10.1146/annurev-immunol-051116-052415, PMID:29328785.
[33] Albillos A, Hera Ad Ade L, Reyes E, Monserrat J, Muñoz L, Nieto M, *et al*. Tumour necrosis factor-alpha expression by activated monocytes and altered T-cell homeostasis in ascitic alcoholic cirrhosis: amelioration with norfloxacin. J Hepatol 2004;40(4):624–631. doi:10.1016/j.jhep.2003.12.010, PMID:15030978.
[34] Hossain M, Kubes P. Innate immune cells orchestrate the repair of sterile injury in the liver and beyond. Eur J Immunol 2019;49(6):831–841. doi:10.1002/eji.201847485, PMID:31001813.
[35] Faas MM, de Vos P. Mitochondrial function in immune cells in health and disease. Biochim Biophys Acta Mol Basis Dis 2020;1866(10):165845. doi:10.1016/j.bbadis.2020.165845, PMID:32473386.
[36] Geltink RIK, Kyle RL, Pearce EL. Unraveling the Complex Interplay Between T Cell Metabolism and Function. Annu Rev Immunol 2018;36:461–488. doi:10.1146/annurev-immunol-042617-053019, PMID:29677474.
[37] Chávez MD, Tse HM. Targeting Mitochondrial-Derived Reactive Oxygen Species in T Cell-Mediated Autoimmune Diseases. Front Immunol 2021;12:703972. doi:10.3389/fimmu.2021.703972, PMID:34276700.
[38] Hotchkiss RS, Moldawer LL, Opal SM, Reinhart K, Turnbull IR, Vincent JL. Sepsis and septic shock. Nat Rev Dis Primers 2016;2:16045. doi:10.1038/nrdp.2016.45, PMID:28117397.
[39] Ashrafi G, Schwarz TL. The pathways of mitophagy for quality control and clearance of mitochondria. Cell Death Differ 2013;20(1):31–42. doi:10.1038/cdd.2012.81, PMID:22743996.
[40] Kominsky DJ, Campbell EL, Colgan SP. Metabolic shifts in immunity and inflammation. J Immunol 2010;184(8):4062–4068. doi:10.4049/jimmunol.0903002, PMID:20368286.
[41] Picca A, Lezza AMS, Leeuwenburgh C, Pesce V, Calvani R, Landi F, *et al*. Fueling Inflamm-Aging through Mitochondrial Dysfunction: Mechanisms and Molecular Targets. Int J Mol Sci 2017;18(5):933. doi:10.3390/ijms18050933, PMID:28452964.