Journal of Clinical and Translational Hepatology **2023** vol. 11(4) | 908–917
DOI: 10.14218/JCTH.2022.00254

Original Article

Double Plasma Molecular Adsorption System with Sequential Low-dose Plasma Exchange in Patients with Hepatitis B Virus-related Acute-on-chronic Liver Failure: A Prospective Study

Lu Wang[1,2#], Wenxiong Xu[2,3#], Shu Zhu[2], Guoli Lin[2,3], Jing Lai[2,3], Yufeng Zhang[2,3], Ying Liu[2,3], Lihua Zheng[2], Qiumin Luo[2,3], Zhiliang Gao[2,3], Chan Xie[2,3*] and Liang Peng[2,3*]

[1]Second School of Clinical Medicine, Binzhou Medical University, Yantai, Shandong, China; [2]Department of Infectious Diseases, Third Affiliated Hospital of Sun Yat-Sen University, Guangzhou, Guangdong, China; [3]Key Laboratory of Tropical Disease Control (Sun Yat-sen University), Ministry of Education, Guangzhou, Guangdong, China

Received: 25 May 2022 | **Revised:** 20 July 2022 | **Accepted:** 17 October 2022 | **Published online:** 9 January 2023

Abstract

Background and Aims: To investigate the safety and efficacy of double plasma molecular adsorption system (DPMAS) with sequential low-dose plasma exchange (LPE) in treating early hepatitis B virus-related acute-on-chronic liver failure (HBV-ACLF). ***Methods:*** Clinical data of patients with HBV-ACLF were prospectively collected, including patients in a DPMAS with sequential LPE (DPMAS+LPE) group and those in a standard medical treatment (SMT) group. The primary endpoint was death or liver transplantation (LT) at 12 weeks of follow-up. Propensity-score matching was performed to control the effects of confounding factors on prognosis between the two groups. ***Results:*** After 2 weeks, total bilirubin, alanine aminotransferase, blood urea nitrogen levels, and Chinese Group on the Study of Severe Hepatitis B score, were significantly lower in the DPMAS+LPE group than those in the SMT group (p<0.05). After 4 weeks, laboratory parameters of the two groups were similar. The cumulative survival rate of the DPMAS+LPE group was significantly higher than that of the SMT group at 4 weeks (97.9% vs. 85.4%, p=0.027), but not at 12 weeks (85.4% vs. 83.3%, p=0.687). Cytokine levels were significantly lower in 12-week survival group than in the death-or-LT group (p<0.05). Functional enrichment analysis showed that downregulated cytokines were mainly involved in positive regulation of proliferation and activation of lymphocytes and monocytes, regulation of immune effect response, regulation of endotoxin response, and glial cell proliferation. ***Conclusion:*** DPMAS+LPE significantly improved the 4-week cumulative survival rate, and ameliorated the inflammatory response in patients. DPMAS+LPE may be a promising modality for patients with early HBV-ACLF.

Citation of this article: Wang L, Xu W, Zhu S, Lin G, Lai J, Zhang Y, *et al*. Double Plasma Molecular Adsorption System with Sequential Low-dose Plasma Exchange in Patients with Hepatitis B Virus-related Acute-on-chronic Liver Failure: A Prospective Study. J Clin Transl Hepatol 2023;11(4):908–917. doi: 10.14218/JCTH.2022.00254.

Introduction

Hepatitis B virus-related acute-on-chronic liver failure (HBV-ACLF) is a common clinical critical illness with high short-term mortality.[1–3] In the early stage of liver failure, many inflammatory mediators accumulate, resulting in secondary immunodeficiency due to liver disease.[4] Artificial liver support system (ALSS), which is a common treatment regimen for HBV-ACLF,[5] removes inflammatory cytokines, blocks the cytokine storm, alleviates liver damage, and improves the clinical outcomes of these patients.[6] In recent years, ALSS has been extensively used in clinical practice to treat patients with HBV-ACLF. Double plasma molecular adsorption system (DPMAS) with sequential plasma exchange (PE) is commonly used to treat HBV-ACLF.[7] This combined system complements the beneficial effects of individual approaches to effectively remove bilirubin and inflammatory mediators, and to supplement coagulation factors and albumin.

An earlier study[8] showed that patients with early HBV-ACLF treated with DPMAS with sequential low-dose plasma exchange (DPMAS+LPE, 1,000 mL fresh frozen plasma) had a 4-week survival rate similar to that of patients treated with DPMAS with sequential full-dose PE (DPMAS+PE, 2,000 mL fresh frozen plasma). In addition, DPMAS+LPE reduces plasma consumption and incidence of adverse reactions related to blood products, and helps to avoid a delay in

Keywords: Plasma exchange; Double plasma molecular adsorption system; Acute-on-chronic liver failure; Prognosis.

Abbreviations: ALSS, artificial liver support system; COSSH, Chinese Group on the Study of Severe Hepatitis B; COSSH-ACLF, Chinese Group on the Study of Severe Hepatitis B-Acute-on-Chronic Liver Failure; DPMAS, double plasma molecular adsorption system; DPMAS+LPE, DPMAS with sequential LPE; FLT3L, fms-like tyrosine kinase 3 ligand; HBV-ACLF, hepatitis B virus-related acute-on-chronic liver failure; HR, hazard ratio; IL, interleukin; LPE, low-dose plasma exchange; LT, liver transplantation; MCP, monocyte chemoattractant protein; M-CSF, macrophage colony stimulating factor; MELD, model for end-stage liver disease; NEU, neutrophil; PLT, platelet; PSM, propensity-score matching; SMT, standard medical treatment; TBIL, total bilirubin; TGF-α, transforming growth factor alpha; TNF-α, tumor necrosis factor alpha.

[#]Contributed equally to this work.

[*]**Correspondence to:** Liang Peng and Chan Xie Department of Infectious Diseases, Third Affiliated Hospital of Sun Yat-Sen University, Key Laboratory of Tropical Disease Control (Sun Yat-sen University), Ministry of Education, #600 Tianhe Road, Guangzhou, Guangdong, China. ORCID: https://orcid.org/0000-0001-6184-5750 (LP) and https://orcid.org/0000-0001-6344-9380 (CX). Tel: +86-13533978874 (LP) and +86-13434383329 (CX); Fax: +86-20-85252250, E-mail: pliang@mail.sysu.edu.cn (LP) and xchan@mail.sysu.edu.cn (CX)

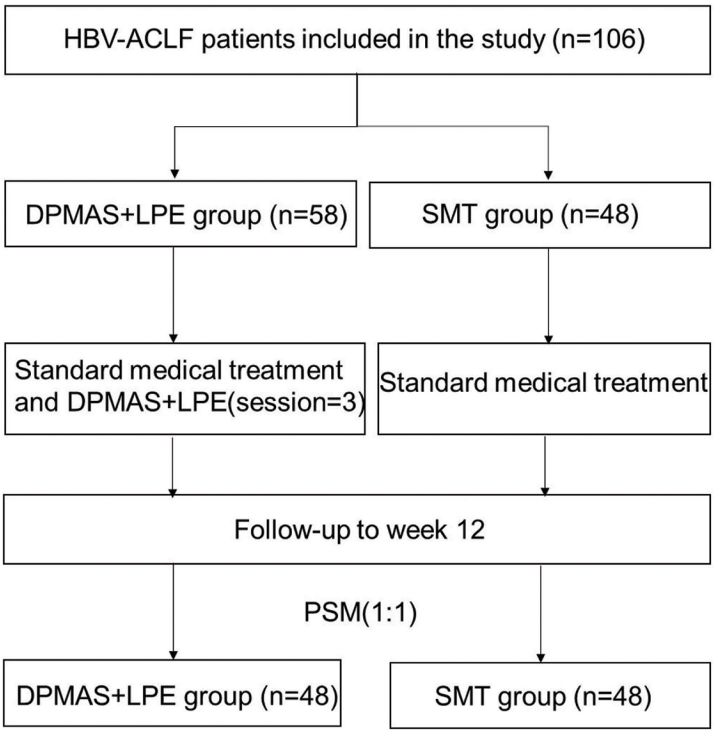

Fig. 1. Flowchart of patient selection. HBV-ACLF, hepatitis B-related acute-on-chronic liver failure; DPMAS+LPE, double plasma molecular adsorption system with sequential low-dose plasma exchange; SMT, standard medical treatment; PSM, propensity-score matching.

treatment due to the limited supply of plasma. However, DPMAS+LPE has mostly been evaluated in single-center retrospective studies having small patient cohorts and low levels of evidence. Prospective cohort studies on DPMAS+LPE are lacking. The treatment regimen of DPMAS+LPE varied in different studies, with differences in treatment protocols, frequency and course of treatment, and intervention timing. In this study, we evaluated the safety and efficacy of DPMAS+LPE in the treatment of patients with early HBV-ACLF and examined the effects of this modality on cytokine levels. We hope to develop a set of standardized ALSS treatment regimen for patients with HBV-ACLF.

Methods

Study participants

This prospective cohort study included patients diagnosed with HBV-ACLF at the Department of Infectious Diseases, the Third Affiliated Hospital of Sun Yat-sen University (Guangdong Province, China) from December 1, 2020 to December 1, 2021. The patients were divided into a DPMAS+LPE group (n=58) and a standard medical treatment (SMT) (n=48) group based on their treatment preference. All patients were followed up for 12 weeks. Propensity-score matching (1:1) was performed to control the effects of confounding factors on prognosis between the two groups (Fig. 1). Inclusion criteria were: (1) between 18 and 65 years of age; (2) diagnosed with chronic hepatitis B based on the Chinese Guidelines for the Prevention and Treatment of Chronic Hepatitis B (2019 edition);[9] (3) diagnosis of ACLF in agreement with Chinese Group on the Study of Severe Hepatitis B (COSSH)-ACLF I criteria;[10] (4) ACLF in early stage with 30% < prothrombin activity (PTA) ≤ 40, or 1.5 < international normalized ratio (INR) ≤ 2.6; (5) platelet count (PLT) >50×10^9/L; (6) with > 1-day hospitalization; and (7) having complete clinical data including demographics, laboratory parameters, adverse events, and survival. Exclusion criteria were: (1) other causes of liver disease; (2) liver cancer and/or other types of tumors; (3) pregnant or breastfeeding; (4) infection with human immunodeficiency virus or other immunodeficiency-related diseases; (5) autoimmune diseases, unstable infarction caused by cardiovascular and/or cerebrovascular events, and a history of organ transplantation and/or other organ dysfunction or failure; (6) other serious complications such as severe infection, active bleeding, disseminated intravascular coagulation, Grade III-IV hepatic encephalopathy, or hepatorenal syndrome; (7) received treatment using ALSS a week before admission.

Treatment methods

Standard medical treatment (SMT): Both groups of patients with HBV-ACLF were administered a high-carbohydrate, low-fat, moderate-protein diet to maintain water and electrolyte balance at the Department of Internal Medicine. Patients that tested positive for HBV-DNA were immediately administered first-line nucleoside (acid)-analog antiviral therapy. All patients were infused with fresh plasma (average 200 mL per day) and human albumin to improve hypoalbuminemia and coagulation. Patients also received regimens for active prevention and treatment of complications such as infection, hepatic encephalopathy, and upper gastrointestinal bleeding.

ALSS protocol: In addition to SMT, patients in the DPMAS+LPE group received three sessions of ALSS treatment (DPMAS was performed before LPE). All patients were treated in a special ALSS unit. DPMAS+LPE was administered once every 2–3 days according to the condition of each patient. During the course of treatment, unfractionated heparin sodium was used to achieve anticoagulation; specific dosage

of unfractionated heparin sodium was determined based on the coagulation function of each individual patient. During DPMAS treatment, blood flow rate ranged from 80 to 120 mL/m, and plasma separation rate ranged from 25 to 30 mL/m; the total adsorption plasma during each treatment was about 5,000 mL. PE volume was 1,000 mL during each treatment, and the rates of blood flow and plasma separation were the same as those described for DPMAS. When the plasma volume of DPMAS treatment reached 5,000 mL, DPMAS was stopped, and LPE treatment immediately followed using the same blood vessels and equipment.

Follow-up and clinical data collection

All patients were followed up at baseline, before and after three sessions of ALSS treatment, and at 2-, 4-, and 12-weeks post-treatment. Demographic data, treatment-related adverse events, changes in laboratory parameters, and 4- and 12-week survival of patients in the two groups were evaluated. Laboratory parameters included: (1) routine blood tests including white blood cell (WBC) count, red blood cell count, hemoglobin (HGB) level, PLT, and neutrophil (NEU) percentage; (2) alanine aminotransferase (ALT), aspartate aminotransferase (AST), albumin (ALB), gamma-glutamyl transpeptidase (GGT), total bilirubin (TBIL), indirect bilirubin (DBIL), and total bile acid (TBA) levels; (3) prothrombin time (PT) and PT-INR; (4) blood urea nitrogen (BUN) and creatinine (Cr) levels; (5) procalcitonin (PCT) assessment; and (6) model for end-stage liver disease (MELD) and COSSH-ACLF II scores.

Study endpoints

The primary endpoint was the clinical adverse events at 12-week follow-up, including death or liver transplantation (LT). Secondary endpoints were changes in TBIL, PT, ALB, and MELD and COSSH-ACLF II scores of the patients from baseline to post-treatment using ALSS, and at 2- and 4-weeks.

Detection of cytokine levels

Study participants and specimen collection: Ten patients from the DPMAS+LPE group were subdivided into a survival group (n=5) and a death or LT group (n=5) based on their survival at the 12-week follow-up. None of the patients had used any immune suppressants. Whole blood samples obtained from patients in the DPMAS+LPE group were collected 2 hours before and 2 hours after the three treatments using DPMAS+LPE.

Measurement of serum cytokine levels: Previous studies focused primarily on common pro-inflammatory factors and anti-inflammatory factors such as interleukin (IL)-6, IL10, and tumor necrosis factor alpha (TNF-α). However, we wanted to evaluate cytokines (e.g., growth factors, chemokines) that have not been reported in earlier studies on DPMAS+LPE therapy. Measurement of the levels of 44 cytokines in serum samples obtained from study participants was performed using the HCYTA-60K MILLIPLEX Human Cytokine/Chemokine/Growth Factor Panel A (Merck Serono, Germany). The five types of cytokines include chemokines, interferons (IFNs), ILs, TNFs, and growth factors (Supplementary Table 1). The obtained data were analyzed using the supporting MILLIPLEX Analyst 5.1 software (EMD Millipore, Billerica, MA). Gene ontology (GO) functional enrichment analysis was performed using Cluster Profiler package in R (v 4.0.1) to explore the biological functions of cytokines present at significantly different levels in the two groups. A significant biological process was selected according to the adjusted p<0.05.

Statistical analysis

Matchit package in R (v4.0.1) was used to perform propensity-score matching (PSM), and nearest-neighbor matching was used to match the two groups of patients with a 1:1 ratio. This matching method takes the SMT group as the standard and finds individuals with the slightest difference in propensity-score from the DPMAS+LPE group. Patients who did not meet the matching criteria were removed from the DPMAS+LPE group, and the SMT group kept the original number of cases. The matching factors included: age, cirrhosis ratio, PT, and MELD and COSSH-ACLF II scores. The standardized difference between the two groups was 10%. SPSS 25.0 (IBM, Armonk, NY, USA) was used for data analysis. Continuous variables were reported as mean ± standard deviation and medians (upper and lower quartile). Differences between groups were compared using independent sample t-tests, paired sample t-tests, or Mann-Whitney U tests. Categorical variables were reported as frequency and percentage, and differences between groups were compared using chi-square or Fisher's tests. Risk factors for adverse outcomes at the 4- and 12-week follow-up were screened using multivariate logistic regression analysis. All clinical variables were logarithmically transformed before logistic regression analysis. Forward stepwise selection was used to construct the final models with the same significance level (p<0.05) for including or excluding variables. The cumulative survival rate was evaluated by the Kaplan-Meier method and log-rank test. P-values <0.05 were considered statistically significant.

Results

Analysis of clinical baseline data obtained in the cohort before and after PSM

Before PSM, 106 patients met the inclusion criteria, including the DPMAS+LPE group (n=58) and SMT group (n=48, Supplementary Table 2). Patients in the SMT group were significantly older than the patients in DPMAS+LPE group (p<0.05), and the levels of ALT and HBV-DNA were significantly higher in the DPMAS+LPE group than in the SMT group (p<0.05). To reduce the impact of confounding factors (e.g., age) on prognosis of the two groups, PSM and nearest-neighbor matching were used to control for bias. After PSM, the cohort included the DPMAS+LPE group (n=48) and SMT group (n=48, Table 1). No significant differences in age, sex, presence of cirrhosis, infection rate, laboratory parameters, or the scores of liver disease severity were found between the two groups (p>0.05).

Comparison of changes in laboratory parameters from baseline at 2 weeks of follow-up

At 2 weeks of follow-up, decreases in the levels of TBIL, ALT, and BUN, and the COSSH-ACLF II scores from baseline were significantly higher in the DPMAS+LPE group than in the SMT group (p<0.05). There were no significant differences in other biochemical measurements of decline from baseline between the two groups (p>0.05, Table 2).

Comparison of changes in laboratory parameters from baseline at 4 weeks of follow-up

At 4 weeks of follow-up, decreases in ALT, Cr, and BUN levels from baseline were significantly higher in the DPMAS+LPE group than in the SMT group (p<0.05, Supplementary Table 3). However, decreases in the MELD and COSSH-ACLF II scores from baseline were not significantly different between the two groups (p>0.05).

Table 1. Clinical baseline characteristics in our cohort after PSM

Parameter	DPMAS+LPE, *n*=48	SMT, *n*=48	*p*-value
Age (years)	46±10	49±10	0.082
Sex, male (%)	44 (91.7)	46 (95.8)	0.399
Cirrhosis (%)	29 (60.4)	31 (64.6)	0.673
Etiology			0.651
Without antiviral therapy (%)	39 (81.3)	36 (76.6)	
Withdrawal of NA (%)	7 (14.6)	10 (21.3)	
Other (%)	2 (4.1)	1 (2.1)	
Infection (%)	15 (31.3)	22 (45.8)	0.142
TBIL (μmol/L)	372±116	375±139	0.908
DBIL (μmol/L)	199±66	212±80	0.394
AST (U/L)	212 (94, 530)	157 (103, 301)	0.328
ALT (U/L)	253 (167, 692)	169 (80, 393)	0.051
ALB (g/L)	34±4	34±5	0.968
GGT (U/L)	101 (71, 153)	74 (62, 137)	0.939
TBA (μmol/L)	275±100	255±96	0.301
PT (sec)	21.6±3.2	22.2±2.9	0.331
PT-INR	1.9±0.3	2.0±0.3	0.310
BUN (mmol/L)	3.9±1.4	4.2±1.6	0.316
Cr (μmol/L)	71±16	70±14	0.623
eGFR	107±16	107±24	0.847
K (mmol/L)	3.7±0.5	3.8±0.5	0.642
Na (mmol/L)	136±15	137±4.0	0.697
WBC (10^9/L)	7.6±2.5	7.3±3.3	0.651
NEU (%)	0.69±0.08	0.67±0.11	0.477
LY (%)	0.18±0.07	0.19±0.08	0.593
MO (%)	0.11±0.03	0.11±0.04	0.796
HGB (g/L)	123±16	118±21	0.156
PLT (10^9/L)	133±58	120±47	0.226
PCT	0.73±0.34	0.85±0.57	0.274
lgHBV-DNA	5.1±1.9	4.6±1.7	0.210
MELD score	22.6±3.2	22.7±3.8	0.828
COSSH-ACLF II score	6.7±0.5	6.9±0.6	0.095

Independent sample *t*-test and Mann-Whitney *U* test were used to compare between-group differences in continuous variables. Data are mean ± standard deviation, medians (interquartile range); categorical variables are frequency (percentage) and were compared by the chi-square tests. NA, nucleotide analog; TBIL, total bilirubin; DBIL, indirect bilirubin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALB, albumin; GGT, gamma-glutamyl transpeptidase; TBA, total bile acid; PT, prothrombin time; INR, international normalized ratio; BUN, blood urea nitrogen; Cr, creatinine; eGFR, estimated glomerular filtration rate; K, serum kalium; Na, serum sodium; WBC, white blood cell; NEU, neutrophil; HGB, hemoglobin; platelet, PLT; PCT, procalcitonin; MELD, model for end-stage liver disease; COSSH-ACLF, Chinese Group on the Study of Severe Hepatitis B-Acute-on-Chronic Liver Failure.

Comparison of cumulative survival rates at 4 and 12 weeks in the entire cohort

In the entire cohort, at 4 weeks of follow-up, one patient in the DPMAS+LPE group and seven in the SMT group had died. By the 12-week follow-up, three patients died and three patients received LTs in the DPMAS+LPE group, and eight patients in the SMT group died (Supplementary Table 4). The 4-week cumulative survival rate was 98.3% in those treated with DPMAS+LPE vs. 85.4% for the SMT group [hazard ratio (HR) 0.112, 95% CI: (0.042 to 0.682), p=0.013)]. There was no significant difference in the 12-week cumulative survival rate between the PE group and DPMAS+PE group (87.9% vs. 83.3%, p=0.453, Supplementary Fig. 1).

Comparison of cumulative survival rates at 4 and 12 weeks in the PSM cohort

In the PSM cohort, one patient in the DPMAS+LPE group and seven in the SMT group had died at 4 weeks. Two pa-

Table 2. Comparison of changes in laboratory parameters from baseline to the week-2 follow-up

Parameter	DPMAS+LPE, *n*=48	SMT, *n*=48	*p*-value
ΔTBIL (2W)	130 (24, 199)	61 (17, 93)	0.002
ΔALT (2W)	208 (73, 616)	99 (11, 291)	0.035
ΔALB (2W)	−2 (6, 0)	−2 (−6, 3)	0.474
ΔTBA (2W)	67 (−22, 150)	23 (−35, 105)	0.204
ΔGGT (2W)	35 (8, 82)	23 (3, 53)	0.154
ΔPT (2W)	−0.4 (−3.7, 30)	−0.8 (−2.2, 1.9)	0.772
ΔPT-INR	0.05 (−0.42, 0.37)	−0.07 (−0.26, 0.20)	0.652
ΔBUN (2W)	0.7 (0.1, 2.1)	0.4 (−0.9, 1.1)	0.080
ΔCr (2W)	2 (−6, 7)	−0.5 (−5, 8)	0.752
ΔWBC (2W)	1.4 (0.1, 3.5)	1.2 (−0.4, 2.4)	0.375
ΔNEU% (2W)	0.09 (0.03,0.14)	0.06 (0.02, 0.12)	0.065
ΔHGB (2W)	16 (10, 23)	15 (3, 25)	0.375
ΔPLT (2W)	37 (7, 56)	23 (1, 43)	0.145
ΔMELD score (2W)	2.6 (−1.1, 5.5)	0.7 (−1.1, 3.0)	0.056
ΔCOSSH-ACLF II score (2W)	0.5 (−0.1, 1.2)	0.2 (−0.1, 0.5)	0.034

Continuous variables are medians (interquartile range) and were compared with Mann-Whitney *U* tests. TBIL, total bilirubin; ALT, alanine aminotransferase; ALB, albumin; GGT, gamma-glutamyl transpeptidase; TBA, total bile acid; PT, prothrombin time; INR, international normalized ratio; BUN, blood urea nitrogen; Cr, creatinine; WBC, white blood cell; NEU, neutrophil; HGB, hemoglobin; platelet, PLT; PCT, procalcitonin; MELD, model for end-stage liver disease; COSSH-ACLF, Chinese Group on the Study of Severe Hepatitis B-Acute-on-Chronic Liver Failure.

tients in the DPMAS+LPE group died and three received LTs. Eight patients in the SMT group had died at 12 weeks (Supplementary Table 5). Organ failures of patients who died or underwent LT in both groups are shown in Supplementary Table 6. Patients in the DPMAS+LPE group had a significantly higher 4-week cumulative survival rate than patients in the SMT group (97.9% vs. 85.4%, HR: 0.135, 95% CI: (0.052 to 0.838), p=0.027). The 12-week cumulative survival rate of DPMAS+LPE group also was higher than that in the SMT group (85.4% vs. 83.3%, p=0.687, Fig. 2).

Analysis of risk factors for 4- and 12-week prognosis

Results of multivariate logistic regression analysis showed that ΔNEU% (2W) was independent risk factors affecting patient prognosis by 4-week follow-up (P<0.05, Supplementary Table 7). We found co-infection and ΔMELD score (2W) to be significantly associated with patient prognosis as of 12-week follow-up (P<0.05, Supplementary Table 8).

DPMAS+LPE-related adverse events

Seven treatment-related adverse events were recorded in the DLMAS+LPE group (Table 3) and included decreased blood pressure (n=3), decreased heart rate (n=1), internal jugular vein thrombosis (n=2), and bleeding at the internal jugular vein puncture site (n=1). The HGB and PLT levels in patients were significantly reduced after DLMAS+LPE treatment (p<0.05). A week after completing ALSS treatment, the HGB level decreased significantly compared with baseline (p<0.05), and the PLT level increased compared with that at baseline (p>0.05, Supplementary Fig. 2).

Detection of cytokine levels in study participants

Analysis of baseline clinical data of patients in the survival and death-or-LT groups: Baseline clinical data,

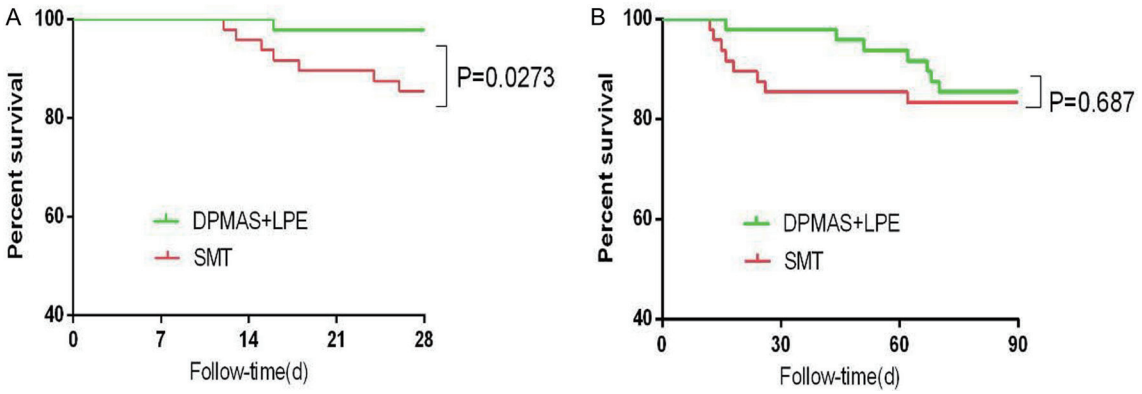

Fig. 2. Kaplan-Meier survival curves of SMT and DPMAS+LPE patients in the propensity-score-matched cohort (A) at 4 weeks and (B) at 12 weeks. DPMAS+LPE, double plasma molecular adsorption system with sequential low-dose plasma exchange; SMT, standard medical treatment.

Table 3. DPMAS+LPE-related adverse events

Adverse event	Entire cohort, case (%)	Propensity-score-matched cohort, case (%)
Decreased blood pressure	1 (1.7)	1 (2.1)
Decreased heart rate	2 (3.4)	2 (4.2)
Internal jugular vein thrombosis	1 (1.7)	1 (2.1)
Bleeding of the internal jugular vein puncture site	3 (5.2)	3 (5.2)

DPMAS, double plasma molecular adsorption system; DPMAS+LPE, DPMAS with sequential LPE.

especially indicators of infection (e.g., PCT, and IL6), were assayed in the survival and death-or-LT groups at 12 weeks. Through case-control matching, five pairs of patients in the DPMAS+LPE group and five pairs of patients in the SMT group were identified. No significant differences in age, sex, proportions of cirrhosis and infections, or cause of disease were found between the survival and death-or-LT groups. Baseline laboratory parameters and scores of liver disease severity were comparable for the two groups of patients ($p>0.05$, Supplementary Table 9).

Comparison of cytokine levels before and after first, second, and third treatment using ALSS: After the first ALSS treatment (Supplementary Table 10), the levels of the majority of pro-inflammatory cytokines and anti-inflammatory cytokines such as IL8, monocyte chemoattractant protein (MCP)-1, IL6, TNFα, and IL9) in the survival group were lower than at baseline ($p>0.05$). After the second ALSS treatment (Supplementary Table 11), most cytokines in both groups decreased from baseline levels ($p>0.05$). After the third treatment using ALSS (Table 4), the levels of several pro-inflammatory cytokines in the survival group were significantly decreased compared with their baseline levels ($p<0.05$); however, only a few pro-inflammatory cytokines in the death-or-LT group had significantly decreased levels ($p<0.05$).

Comparison of cytokine levels after the third treatment using ALSS in the two groups of patients: After the third ALSS treatment (Fig. 3), the levels of MCP1, MCP3, IL1β, IL5, IL6, IL27, TNF-α, IL13, fms-like tyrosine kinase 3 ligand (FLT3L), macrophage colony stimulating factor (M-CSF), and transforming growth factor alpha (TGFα) were significantly lower than their baseline levels ($p<0.05$). However, there was no significant decrease in cytokines in the death-or-LT group. Of note, the level of IL12 (p40) in the death-or-LT group was significantly decreased compared with its baseline level ($p<0.05$).

GO functional enrichment analysis of cytokines present at significantly different levels in the two groups: By GO enrichment analysis, 12 cytokines present at significantly different levels between the two groups were involved in 70 biological pathways. We focused on the top 10 pathways with the lowest adjusted *p*-values (Supplementary Fig. 3). Downregulated cytokines were mainly involved in these biological processes: cytokine-mediated signaling pathway, positive regulation of monocyte proliferation, receptor signaling pathway via JAK-STAT, positive regulation of peptide-tyrosine phosphorylation, positive regulation of lymphocyte proliferation and activation, positive regulation of immune effector response, response to lipopolysaccharides, and positive regulation of glial cell activation.

Discussion

The results of this prospective cohort study indicate that DPMAS+LPE was a safe treatment modality. Decreased blood pressure was the most common adverse event after therapy, and it resolved after administering symptomatic treatment with vasopressors. There were no serious adverse events during DPMAS+LPE therapy. The PLT levels of participants were significantly decreased after DPMAS+LPE treatment, which may have been related to mechanical destruction of the plasma filter and blood vessels and the administration of heparin.[11] Still, the PLT levels returned to baseline a week after ALSS treatment, indicating that the PLT destruction by ALSS was temporary.[12] In addition, we found that the level of HGB decreased after DPMAS+LPE treatment. The possible reasons are that ALSS therapy inevitably involves the mechanical destruction of red blood cells by the plasma separator, resulting in decreased hemoglobin. The poor nutritional status of patients with ACLF also leads to insufficient raw materials for hemoglobin synthesis, and using such drugs as proton pump inhibitors, and antibiotics can aggravate anemia. These results suggest that clinicians should pay increased attention to the changes in blood cells before and after ALSS therapy.

DPMAS+LPE therapy significantly reduced the level of TBIL, while the effect of DPMAS+LPE on TBA clearance was unclear, which differs from a previous study.[8] DPMAS+LPE did not significantly improve coagulation in patients with HBV-ACLF. Compared with SMT alone, DPMAS+LPE significantly increased the cumulative survival rate at 4 weeks ($p=0.027$), but not at 12 weeks, although a trend toward an improved cumulative survival rate was seen. The best timing of intervention using DPMAS+LP remains debatable. Zhong *et al*.[8] proposed that the efficacy of DPMAS+LPE was significantly higher than that of PE alone in patients with early HBV-ACLF (83.7% vs. 55.6%, $p<0.05$). In contrast to the results obtained in this study, Yao *et al*.[13] showed that DPMAS+LPE effectively improved the 4-week survival rate of patients with moderate to advanced HBV-ACLF. This study differs from earlier studies in several ways. First, in our prospective cohort study, we used a unified ALSS treatment regimen (i.e. combination mode of ALSS, frequency of administration, and timing of intervention). Second, most previous studies used PE therapy as controls. However, the lack of randomized controlled studies on PE in the treatment of HBV-ACLF, and the controversial efficacy of PE, should be considered. Conversely, this study used SMT as a control. Third, in this study, patients in the DPMAS+LPE group had a higher 4-week cumulative survival rate compared with that reported in earlier studies.[8,13] This discrepancy may be related to our enrollment of patients with early HBV-ACLF. consequently, our patients had relatively mild disease and fewer complications compared with those described in other studies.

Our results indicate that co-infection at disease onset was an independent risk factor affecting the 12-week prognosis of HBV-ACLF patients, which agrees with findings obtained in earlier studies.[14,15] Second, in addition to the baseline indicators of patients, the dynamic changes in these indicators, such as ΔMELD score (2W) and ΔNEU% (2W), should also

Table 4. Comparison of cytokine levels before the first, and after the third, treatment with ALSS

Cytokines (pg/mL)	Survival group, *n*=5		Death-or-LT group, *n*=5	
	Before first ALSS	**After third ALSS**	**Before first ALSS**	**After third ALSS**
Pro-inflammatory				
Eotaxin	121.59 (93, 165)	89.83 (74, 135)	143 (84, 166)	87 (50, 128)
Fractalkine	156 (156, 455)	114 (88, 180)	171 (116, 347)	118 (78, 161)
GROa	35 (16, 84)	21 (4, 28)	31 (13, 89)	14 (8, 40)
IL-8	179 (86, 346)	54 (21, 76)	49 (38, 109)	58 (31, 332)
CXCL9	9,577 (5,184, 13,275)	7,004 (4,554, 23,605)	8,212 (4,372, 67,171)	12,530 (6,918, 23,710)
IP-10	2,497 (1,087, 4,762)	1,699 (908, 4,763)	3,201 (1,261.39, 16,939)	2,778 (2,041, 7,868)
MCP-1	811 (554, 973)	384 (337, 425)*	374.42 (309, 680)	386 (302, 506)
MCP-3	66 (30, 109)	22 (18, 54)*	41 (26, 91)	39 (14, 48)
MDC	584 (300, 83)	343 (215, 52)	449 (159, 587)	227 (133, 321)
MIP-1A	31 (30, 58)	19 (16, 32)	34 (25, 80)	33 (19, 60)
MIP-1B	86 (47, 112)	74 (35, 113)	71 (55, 95)	63 (36, 106)
RANTES	2,980 (2,659, 3,957)	2,538 (1,880, 3,604)	2,013 (13,334, 3,934)	2,050 (1,747, 2,645)
IFNa2	64 (62, 146)	58 (45, 83)	91 (70, 248)	62 (44, 123)
IFNy	12 (5, 23)	0.9 (0.6, 2.6)	6 (3, 41.0)	2.3 (1.3, 8.5)
IL-1α	19 (12, 69)	8 (4, 13)	19 (14, 116)	10 (8, 24)
IL-1β	26 (14, 112)	7 (3, 28)*	15 (12, 114)	15 (4, 41)
IL-5	11 (6, 19)	6 (3, 7)*	6 (4, 12)	4 (3, 37)
IL-6	21 (9, 31)	8 (3, 12)*	13 (12, 26)	17 (10, 38)
IL-12 (p40)	49 (28, 127)	32 (15, 34)	88 (66, 133)	50 (35, 70)#
IL-12 (p70)	5 (3, 11)	1.9 (1.8, 4.3)	7 (4, 22)	4 (2, 7)
IL-15	31 (20, 42)	16 (12, 23)	27 (15, 39)	18 (15, 20)
IL-17A	5 (4, 35)	2 (1, 7)	6 (4, 61)	3 (2, 16)
IL-17E	423 (296, 1,245)	316 (194, 589)	586 (423, 2,790)	407 (242, 1,114)
IL-18	178 (82, 205)	43 (32, 51)*	124 (91, 150)	65 (39, 87)#
IL-27	14,064 (5,889, 15,535)	6,033 (3,728, 6,962)*	9,426 (7,053, 10,560)	5,715 (5,159, 7,222)
TNF-α	70.1 (49, 87)	32 (24, 44)*	56 (38, 76)	42 (26, 53)
TNF-β	20 (15, 47)	8 (3, 14)*	14 (9, 26)	5. (3, 7)#
sCD40L	10,578 (8,854, 26,683)	5,186 (1,986, 28,247)	3,050 (1,457, 17,565)	3,013 (1,555, 6,179)
Anti-inflammatory				
IL-1RA	8 (4, 39)	3 (3, 9)	9 (7, 50)	8 (3, 15)
IL-2	2 (1, 12)	0.8 (0.3, 1.1)	2.4 (0.6, 18.8)	0.6 (0.4, 3.6)
IL-4	2.5 (1.2, 8.2)	1.1 (0.5, 1.6)	2.9 (2.1, 13.5)	3.0 (0.8, 3.4)
IL-7	8 (3, 60)	2 (1, 22)	6 (3, 21)	2 (1, 6)
IL-9	24 (8, 8)	3 (1, 10)	7 (4, 45)	9 (1, 19)

(continued)

Table 4. *(continued)*

Cytokines (pg/mL)	Survival group, *n*=5		Death-or-LT group, *n*=5	
	Before first ALSS	After third ALSS	Before first ALSS	After third ALSS
IL-10	21 (8, 2,361)	5 (2, 432)	29 (5, 54)	3. (1, 11)
IL-13	193 (117, 690)	42 (19, 147)*	169 (78, 207)	64 (32, 93)
Growth factors				
EGF	134 (87, 456)	74 (40, 336)	45 (35, 85)	35 (17, 99)
FGF2	58 (43, 236)	37 (29, 68)	100 (72, 364)	44 (33, 137)
FLT3L	11 (5, 25)	3 (1, 4)*	14 (6, 19)	6 (4, 11)
GCSF	38 (14, 87)	9 (4, 14)	24 (8, 81)	16 (6, 20)
MCSF	475 (258, 682)	104 (39, 176)*	257 (120, 390)	65 (34, 227)
PDGF-AA	4,087 (3,304, 8,370)	2,337 (1,596.5, 6,143)	3,853 (1,971, 5,785)	2,313 (2,012, 3,454)
PDGF-ABBB	34,730 (22,888, 43,003.5)	18,185 (13,942.5, 35,291)	20,224 (12,286, 30,659)	17,694 (15,440, 20,015)
TGF-α	58 (20, 188)	4 (3, 28)*	12 (7, 32)	6 (3, 14)
VEGF-A	285 (40, 685)	9 (7, 38)*	125 (31, 383)	15 (11, 29)#

Non-normally distributed continuous variables are medians (interquartile range) and between-group differences were compared with Mann-Whitney *U* tests. *$p<0.05$, significant difference relative to the survival group before the first treatment using ALSS; #$p<0.05$, significant difference relative to the death-or-LT group before the first treatment using ALSS. LT, liver transplantation; ALSS, artificial liver support system; IL, interleukin; MCP, monocyte chemoattractant protein; FLT3L, fms-like tyrosine kinase 3 ligand; M-CSF, macrophage colony stimulating factor; TNF-α, tumor necrosis factor alpha; CXCL9, chemokine (C-X-C motif) ligand 9; IP, interferon gamma-induced protein; MDC, macrophage-derived chemokine; MIP, macrophage inflammatory protein; RANTES, regulated upon activation, normal T cell expressed and presumably secreted; EGF, epidermal growth factor; FGF, fibroblast growth factor; GCSF, colony-stimulating factor; PDGF, platelet-derived growth factor; TGF-α, transforming growth factor alpha; VEGF, vascular endothelial growth factor.

be closely monitored as the disease progresses to adopt appropriate treatment regimens. In the early stages of ACLF, cytokine levels reflect the inflammatory state of the body and are closely related to the short-term prognosis of the patients.[16–18] The results of this study show that DPMAS+LPE decreased several pro-inflammatory cytokines and growth factors (e.g., MCP1, MCP3, and MCSF) in patients, which has not been reported in earlier studies. In addition, the ALSS treatment–mediated cytokine clearance rate was closely related to the sessions of DPMAS+LPE therapy. Decreases in cytokine levels were more significant after the third ALSS treatment than those observed after the first and second treatments. Notably, the levels of pro-inflammatory cytokines and growth factors decreased more significantly in the survival group than in the death or LT group. Through the GO enrichment analysis of differential cytokines, we found that DPMAS+LPE therapy inhibited the activation and proliferation of lymphocytes and monocytes and reduced the inflammatory response, which is consistent with the findings of Rakhi *et al*.[19] Previous studies[20] have shown endotoxemia was closely related to the occurrence and development of liver failure. Our results showed that DPMAS+LPE treatment downregulated endotoxin-mediated immune responses, improving endotoxemia and reducing disease progression in patients. In general, DPMAS+LPE therapy effectively ameliorated the inflammatory response and improved clinical outcomes by decreasing cytokine production.

The study had several limitations. First, it was a single-center study with a small sample size, and the findings should be verified by a large multicenter cohort study. Also, more rigorous statistical methods should obtain desirable results. Second, the SMT group served as a control group in the present study. DPMAS+LPE treatment should be compared with other ALSS models to explore the effectiveness of DPMAS+LPE in patients with HBV-ACLF. Third, the relationship between cytokine levels and the prognosis of patients with ACLF needs further investigation further in future studies.

This study found DPMAS+LPE to be a safe and effective modality in the treatment of patients with HBV-ACLF. In this group of patients, DPMAS+LPE treatment improved the short-term survival rate. DPMAS+LPE also effectively promoted cytokine clearance and reduced the inflammatory response. The study provides preliminary evidence that DPMAS+LPE (1,000 mL plasma, administered once every 2–3 days, for three times) was a beneficial ALSS type in treating patients with early HBV-ACLF.

Funding

This study was supported by grants from the National major science and technology project for the prevention and treatment of AIDS and viral hepatitis (2018ZX10302204-002-002 to LP, 2018ZX10302205-002 to CX), Natural Science Foundation of China (No. 81873572 to LP, 82070611 to LP), Guangzhou Science and Technology Plan Projects (201904010442 to CX, 202102010204 to LP), Guangdong Science and Technology Plan Projects (2020A1515010317 to CX), Sun Yat-Sen University Clinical Research 5010 Program (2018009 to CX, 2020007 to LP), and the Five-Year Plan of Third Affiliated Hospital of Sun Yat-Sen University (K00006 to LP).

Conflict of interest

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

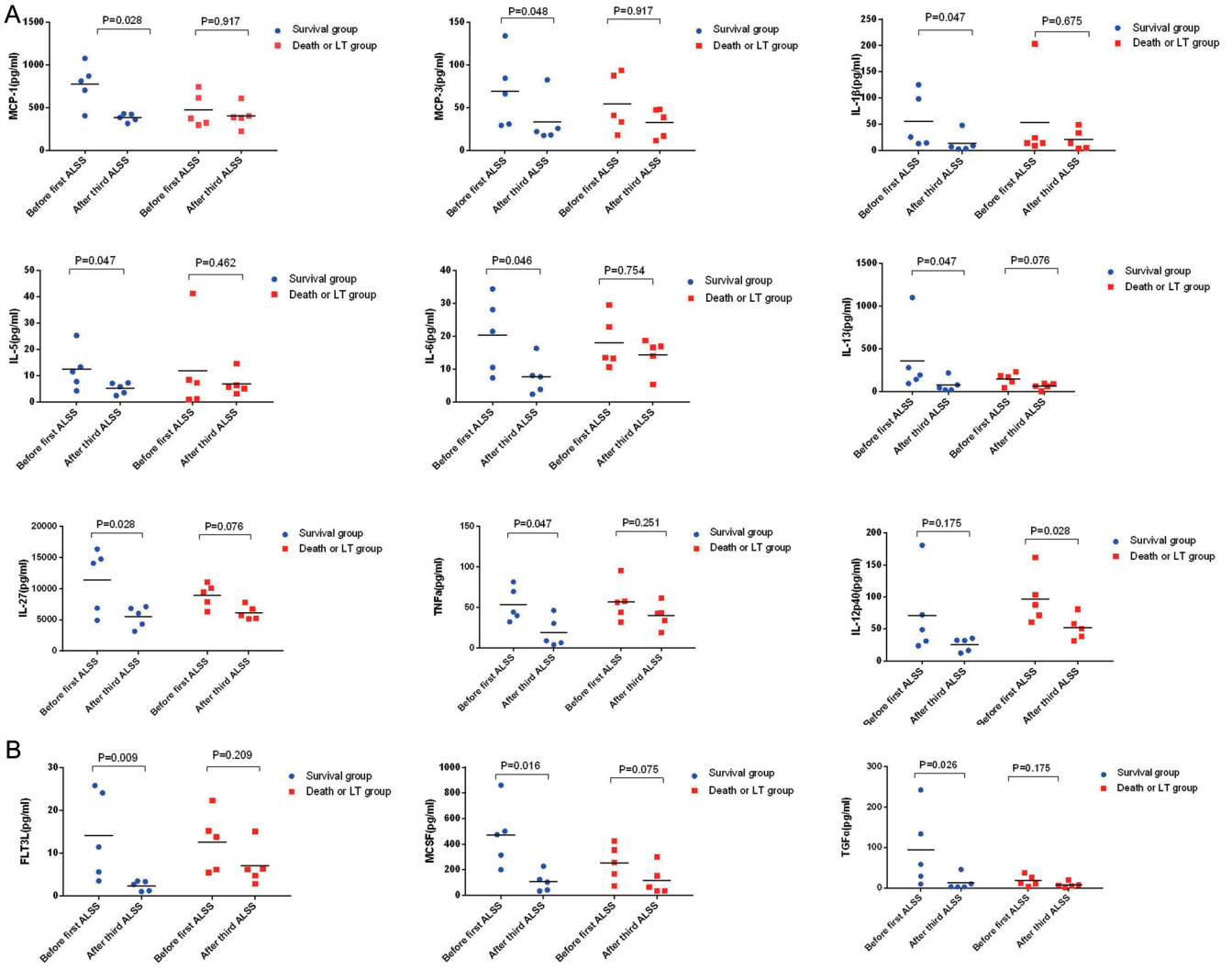

Fig. 3. Comparison of cytokine levels in the survival and the death or liver transplantation groups. (A) Pro-inflammatory and anti-inflammatory cytokine levels; (B) Growth factor levels. IL, interleukin; MCP, monocyte chemoattractant protein; FLT3L, fms-like tyrosine kinase 3 ligand; M-CSF, macrophage colony stimulating factor; TGFα, transforming growth factor alpha; TNF-α, tumor necrosis factor alpha; LT, liver transplantation; ALSS, artificial liver support system.

Author contributions

All authors contributed to this study's concept and design, patient recruitment, and follow-up. had full access to the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis (LW, WX), were responsible for the acquisition, analysis, and interpretation of data (SZ, GL, JL, YZ, YL, LZ, QL, ZG), drafted the manuscript (LW), obtained the funding and took responsibility for the supervision (LP, CX), had access to the study data and reviewed and approved the final manuscript (All authors).

Ethical statement

This prospective cohort study was conducted under the Declaration of Helsinki, and the protocol was approved by the Ethics Committee of the Third Affiliated Hospital of Sun Yat-sen University (Approval number: [2020]-02-173-01; Trial registration number: NCT04597164). All patients signed the written informed consent before participating in the study.

Data sharing statement

The data used to support the findings of this study are available from the corresponding author upon request.

References

[1] Sarin SK, Choudhury A, Sharma MK, Maiwall R, Al Mahtab M, Rahman S, *et al*. Acute-on-chronic liver failure: consensus recommendations of the Asian Pacific association for the study of the liver (APASL): an update. Hepatol Int 2019;13(4):353–390. doi:10.1007/s12072-019-09946-3, PMID:31172417.

[2] Bernal W, Jalan R, Quaglia A, Simpson K, Wendon J, Burroughs A. Acute-on-chronic liver failure. Lancet 2015;386(10003):1576–1587. doi:10.1016/S0140-6736(15)00309-8, PMID:26423181.

[3] Chen JF, Weng WZ, Huang M, Peng XH, He JR, Zhang J, *et al*. Derivation and Validation of a Nomogram for Predicting 90-Day Survival in Patients With HBV-Related Acute-on-Chronic Liver Failure. Front Med (Lausanne) 2021;8:692669. doi:10.3389/fmed.2021.692669, PMID:34222294.

[4] Wu W, Yan H, Zhao H, Sun W, Yang Q, Sheng J, *et al*. Characteristics of systemic inflammation in hepatitis B-precipitated ACLF: Differentiate it from No-ACLF. Liver Int 2018;38(2):248–257. doi:10.1111/liv.13504, PMID:28646630.

[5] Chinese Society of Infectious Diseases, Chinese Medical Association Chinese Society of Hepatology. Guideline for non-bioartificial liver support systems in treatment of liver failure:2016 update. Chin J of Infect Dis

2016;9(2):97–103. doi:10.3760/cma.j.issn.1674-2397.2016.02.001.
[6] Maiwall R, Bajpai M, Choudhury AK, Kumar A, Sharma MK, Duan Z, *et al*. Therapeutic plasma-exchange improves systemic inflammation and survival in acute-on-chronic liver failure: A propensity-score matched study from AARC. Liver Int 2021;41(5):1083–1096. doi:10.1111/liv.14806, PMID:33529450.
[7] Yang Q, Geng Q, Sun CF, Liu XL, Sun SY, Sheng YJ, *et al*. Efficacy of plasma exchange combined with dual plasma molecular adsorption system in the treatment of patients with hepatitis B virus related acute-on-chronic liver failure. Chin J of Infect Dis 2021;07(07):430–435. doi:10.3760/cma.j.cn311365-20201021-00820.
[8] Yao J, Li S, Zhou L, Luo L, Yuan L, Duan Z, *et al*. Therapeutic effect of double plasma molecular adsorption system and sequential half-dose plasma exchange in patients with HBV-related acute-on-chronic liver failure. J Clin Apher 2019;34(4):392–398. doi:10.1002/jca.21690, PMID:30758886.
[9] Chinese Society of Infectious Diseases, Chinese Medical Association Chinese Society of Hepatology, Chinese Medical Association. The guidelines of prevention and treatment for chronic hepatitis B (2019 version). J of Prac Hepatol 2020;23(1):9–32. doi:10.3760/cma.j.issn.1007-3418.2019.12.007.
[10] Wu T, Li J, Shao L, Xin J, Jiang L, Zhou Q, *et al*. Development of diagnostic criteria and a prognostic score for hepatitis B virus-related acute-on-chronic liver failure. Gut 2018;67(12):2181–2191. doi:10.1136/gutjnl-2017-314641, PMID:28928275.
[11] Ocskay K, Kanjo A, Gede N, Szakács Z, Pár G, Erőss B, *et al*. Uncertainty in the impact of liver support systems in acute-on-chronic liver failure: a systematic review and network meta-analysis. Ann Intensive Care 2021;11(1):10. doi:10.1186/s13613-020-00795-0, PMID:33462764.
[12] Kurokawa T, Ohkohchi N. Platelets in liver disease, cancer and regeneration. World J Gastroenterol 2017;23(18):3228–3239. doi:10.3748/wjg.v23.i18.3228, PMID:28566882.
[13] Guo X, Wu F, Guo W, Zhang J, Yang Y, Lu Y, *et al*. Comparison of plasma exchange, double plasma molecular adsorption system, and their combination in treating acute-on-chronic liver failure. J Int Med Res 2020;48(6):300060520932053. doi:10.1177/0300060520932053, PMID:32552092.
[14] Li J, Liang X, You S, Feng T, Zhou X, Zhu B, *et al*. Development and validation of a new prognostic score for hepatitis B virus-related acute-on-chronic liver failure. J Hepatol 2021;75(5):1104–1115. doi:10.1016/j.jhep.2021.05.026, PMID:34090929.
[15] Wu W, Sun S, Wang Y, Zhao R, Ren H, Li Z, *et al*. Circulating Neutrophil Dysfunction in HBV-Related Acute-on-Chronic Liver Failure. Front Immunol 2021;12:620365. doi:10.3389/fimmu.2021.620365, PMID:33717119.
[16] Li L, Chen L, Lin F, Mu J, Wang D, Zhang W, *et al*. Study of the Expression of Inflammatory Factors IL-4, IL-6, IL-10, and IL-17 in Liver Failure Complicated by Coagulation Dysfunction and Sepsis. J Inflamm Res 2021;14:1447–1453. doi:10.2147/JIR.S302975, PMID:33883921.
[17] Cao S, Liu M, Sehrawat TS, Shah VH. Regulation and functional roles of chemokines in liver diseases. Nat Rev Gastroenterol Hepatol 2021;18(9):630–647. doi:10.1038/s41575-021-00444-2, PMID:33976393.
[18] Queck A, Bode H, Uschner FE, Brol MJ, Graf C, Schulz M, *et al*. Systemic MCP-1 Levels Derive Mainly From Injured Liver and Are Associated With Complications in Cirrhosis. Front Immunol 2020;11:354. doi:10.3389/fimmu.2020.00354, PMID:32218781.
[19] Sepehrinezhad A, Zarifkar A, Namvar G, Shahbazi A, Williams R. Astrocyte swelling in hepatic encephalopathy: molecular perspective of cytotoxic edema. Metab Brain Dis 2020;35(4):559–578. doi:10.1007/s11011-020-00549-8, PMID:32146658.
[20] Perego J, Bourbon C, Chasson L, Laprie C, Spinelli L, Camosseto V, *et al*. Guanabenz Prevents d-Galactosamine/Lipopolysaccharide-Induced Liver Damage and Mortality. Front Immunol 2017;8:679. doi:10.3389/fimmu.2017.00679, PMID:28659918.