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# Safety and efficacy of double plasma molecular adsorption system with sequential low-volume plasma exchange in intermediate-stage hepatitis B virus-related acute-on-chronic liver failure

**Running Title: DPMAS+LPE in intermediate stage HBV-ACLF**

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## Availability of data and material

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

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**Conflicts of interest**

The authors declare that there are no conflicts of interest.

**Ethics approval**

The study protocol was approved by the Medical Ethics Committee of the Third Affiliated Hospital of Sun Yat-sen University (approval No. [2020]02-173-01).

**Patient consent**

All patients voluntarily signed an informed consent form approved by the medical ethics committee before participation.

**Clinical trial registration**

This study was registered on ClinicalTrials.gov (NCT 04597164).

**Author contributions**

All authors contributed to the concept and design. L.P. and W.X. had full access to all of the data in the study and took responsibility for the integrity of the data and the accuracy of the data analysis. W.X., S.Z., L.Y. and Q.L. contributed to the acquisition, analysis and interpretation of the data. W.X. drafted the manuscript. W.X. and L.P. obtained funding. L.P. and Q.L. took responsibility for the supervision of the study and the revision of the manuscript. All authors read and approved the final version of the manuscript.

**Abstract**

**Background:** Current evidence suggests that the mortality rate of intermediate-stage hepatitis B virus (HBV)-related acute-on-chronic liver failure (ACLF) remains high. We aimed to investigate the safety and efficacy of double plasma molecular adsorption system (DPMAS) with sequential low-volume

plasma exchange (LPE) treatment in intermediate-stage HBV-related ACLF.

**Methods:** This prospective study recruited intermediate-stage HBV-related ACLF patients and was registered on ClinicalTrials.gov (NCT 04597164). Eligible patients were randomly divided into a trial group and a control group. Patients in both groups received comprehensive medical treatment. Patients in the trial group further received DPMAS with sequential LPE. Data were recorded from baseline to week 12.

**Results:** 50 patients with intermediate-stage HBV-related ACLF were included in this study. The incidence of bleeding events and allergic reactions in the trial group was 12% and 4%, respectively, with no other treatment-related adverse events. The levels of TBIL and PT-INR, and MELD scores after each session of DPMAS with sequential LPE were significantly lower than those before treatment (all $p<0.05$). The 12-week cumulative liver transplantation-free survival rates in the trial and control groups were 52% and 24%, respectively ($p=0.041$). The 12-week cumulative overall survival rates in the trial and control groups were 64% and 36%, respectively ($p=0.048$). The Kaplan-Meier survival analysis revealed significant differences in liver transplantation-free survival ($p=0.047$) and overall survival ($p=0.038$) between the trial and control groups. COX regression analysis indicated that BUN ($p=0.038$), DPMAS with sequential LPE ($p=0.048$) and COSSH-ACLF II score ($p<0.001$) were significant risk factors for mortality.

**Conclusion:** DPMAS with sequential LPE treatment is safe and effective for patients with intermediate-stage HBV-related ACLF.

**Keywords:** double plasma molecular adsorption system, plasma exchange, hepatitis B virus, acute-on-chronic liver failure

## Introduction

According to the World Health Organization, an estimated 257 million people are infected with the hepatitis B virus (HBV) globally [1]. In China, twenty to thirty million people are reportedly diagnosed with chronic hepatitis B (CHB) [2] and the most common type of liver failure is HBV-related acute-on-chronic liver failure (ACLF) [3]. The clinical presentation of HBV-related ACLF includes gastrointestinal symptoms, jaundice, coagulation dysfunction, and complications such as hepatic encephalopathy (HE). Due to the lack of effective therapies, a high 90-day mortality rate of 50%-70% has been observed in patients with HBV-related ACLF [4-6]. Although liver transplantation remains the only curative approach for ACLF [7], it is limited by graft shortage, high costs, surgical complications, and organ rejection after transplantation. Fortunately, short-term and long-term survivals of patients with HBV-related ACLF can be improved by artificial liver support systems (ALSS) [8,9]. Accordingly, much emphasis has been placed on comprehensive pharmacological treatment and ALSS of patients with HBV-related ACLF by the Chinese Medical Association (CMA) [3] and the Asian Pacific Association for the Study of the Liver (APASL) [7].

Plasma exchange (PE) involves replacing the toxin-containing plasma of the patient with an equal amount of fresh plasma or fresh frozen plasma (FFP) and is the most common treatment ALSS approach against serious liver disease in China. Current evidence suggests that PE can improve the hepatic function and prognosis of patients with HBV-related ACLF [7,10]. The double plasma molecular adsorption system (DPMAS) is a patented ALSS technology pioneered by Jafron Biomedical in China that can adsorb bilirubin and bile acids by BS330 Disposable Plasma Bilirubin Adsorption Column [11] and medium and large molecular toxins by HA330-Ⅱ Disposable Hemoperfusion Cartridge [12]. DPMAS is well-tolerated

and can improve 12-week survival rates in HBV-related ACLF [13]. In recent years, combination treatment with DPMAS and low-volume (about 1000 mL per time) plasma exchange (LPE) has been reported as a novel therapeutic approach against HBV-related ACLF. Importantly, this approach can reduce treatment costs as it shares the same external circulating blood circuit and plasma separator. Meanwhile, it can reduce plasma consumption to ensure treatment feasibility in case of plasma shortage. This approach can reportedly maximize the removal of bilirubin, inflammatory mediators and other metabolites and reduce the risk of bleeding due to the minimum influence on coagulation function. There is a growing consensus that the combination of DPMAS and PE is more efficient and leads to a higher short-term survival rate than PE monotherapy in patients with HBV-related ACLF [14-16].

Current evidence suggests that worse hepatic function and more serious complications occur in intermediate-stage HBV-related ACLF leading to poorer outcomes with limited pharmacological treatment available compared to cases with early-stage disease. Few prospective studies have hitherto explored the combination of DPMAS and PE in treating intermediate-stage HBV-related ACLF. Therefore, we conducted a prospective study to explore the safety and efficacy of DPMAS with sequential LPE in patients with intermediate-stage HBV-related ACLF.

## Materials and methods

### Study design and participants

This prospective study recruited patients diagnosed with intermediate-stage HBV-related ACLF treated at the Department of Infectious Diseases of the Third Affiliated Hospital of Sun Yat-sen University (Guangzhou, China) between

January 2021 and August 2022. Eligible patients underwent randomization at a 1:1 ratio into a control group (comprehensive medical treatment) and a trial group (comprehensive medical treatment and DPMAS with sequential LPE). Randomization was performed according to a computer-generated number table. According to the European Association for the Study of the Liver and Chinese Group on the Study of Severe Hepatitis B (COSSH), the 90-day transplantation-free mortality rates of Grade 2 ACLF are 70.4% and 73.5%. The corresponding values for Grade 3 ACLF are 95.7% and 100% [5]. Accordingly, we assumed a mortality rate of 80% in the control group in this study. A nationwide prospective multicenter study in China indicated that PE-centered ALSS could improve survival by up to 61.6% [10]. We hence assumed a mortality rate of 40% in the trial group in this study. Then, 2 matched patient groups (n=25 per group) were established in this prospective study. All patients were followed up from baseline to 12 weeks.

According to the staging system for liver failure from CMA [3], the criteria for intermediate-stage of liver failure are as follows: (1) severe fatigue and gastrointestinal symptoms; (2) severe jaundice: serum total bilirubin (TBIL) ≥ 10 times the upper limit of normal; (3) coagulation dysfunction: prothrombin activity (PTA) ⩽ 30% and > 20%, or prothrombin time-international normalized ratio [PT-INR] ≥ 1.9 and < 2.6; and (4) presence of one liver disease-related complication or one extrahepatic organ failure. The inclusion criteria for this study were based on the consensus recommendations for ACLF from the CMA [3] and APASL [7]. The inclusion criteria were as follows: (1) hepatitis B surface antigen (HBsAg) positive or HBV deoxyribonucleic acid (DNA) positive > 6 months; (2) aged 18 to 65 years; (3) serum TBIL ≥ 10 times the upper limit of normal; (4) PTA

< 30% and ≥ 20%; and (5) presence of at least one of the following complications: ascites, bacterial infection, HE, hepatorenal syndrome (HRS).

The exclusion criteria were as follows: (1) patients with other active liver diseases, such as viral hepatitis A, C, D, E, alcoholic liver disease, drug-induced liver injury, etc.; (2) patients with hepatocellular carcinoma or other malignancy; (3) pregnancy or lactation; (4) patients with human immunodeficiency virus infection or congenital immune deficiency diseases; (4) patients with uncontrolled diabetes or autoimmune diseases; (5) patients with active bleeding events, such as gastrointestinal bleeding; (7) patients with unstable infarction due to cardio-cerebrovascular events; (7) patients with a history of organ transplantation or with organ dysfunctions not related to liver disease; or (8) patients that could not comply to the study requirements or were lost to follow-up.

This study was conducted in accordance with the Ethical Guidelines of the 1975 Declaration of Helsinki. The study protocol was approved by the Medical Ethics Committee of the Third Affiliated Hospital of Sun Yat-sen University (approval No. [2020]02-173-01). All patients voluntarily signed an informed consent form approved by the medical ethics committee before participation in this study. This study was registered on ClinicalTrials.gov (NCT 04597164).

**Treatment**

Patients in trial and control groups received comprehensive medical treatment, including antibiotics (if necessary), nucleot(s)ide analogues (NAs, including tenofovir disoproxil fumarate, tenofovir alafenamide, or entecavir), polyene phosphatidylcholine, compound glycyrrhizin, adenosyl methionine, ursodeoxycholic acid, glutathione, aspartate ornithine, diuretics, lactulose, albumin supplementation and fresh plasma.

Patients in the trial group further underwent DPMAS with sequential LPE three times at an interval of 2-3 days during the first 2 weeks in a specialized artificial liver treatment unit after enrolment. DPMAS and LPE were performed by applying a double-filtration technique with a membrane plasmapheresis apparatus with a plasma separator, extracorporeal blood circuit and dual lumen dialysis catheter according to the manufacturer's protocol. Unfractionated heparin sodium was used for anticoagulation; the specific dosage was determined based on the coagulation function of each patient. Adsorption products from Jafron Biomedical (HA330-Ⅱ Disposable Hemoperfusion Cartridge and BS330 Disposable Plasma Bilirubin Adsorption Column) were used in DPMAS. The total volume of plasma adsorption in DPMAS was 5000-6000 mL, with a plasma adsorption rate of 25-30 mL/min, a blood flow rate of 100-120 mL/min, and a treatment duration of about 3.5-4 hours. Subsequently, LPE treatment was performed for about 1 hour with the same membrane plasmapheresis apparatus, plasma separator, extracorporeal blood circuit and dual-lumen dialysis catheter used in DPMAS previously. The volume of fresh plasma or FFP used in LPE was 1000 mL, with a plasma exchange rate of 25-30 mL/min and a blood flow rate of 100-120 mL/min.

**Follow-up and data collection**

All patients were analyzed at baseline (before DPMAS with sequential LPE treatment for patients in the trial group) and followed up at 2, 4, 8 and 12 weeks post-treatment.

The demographic, clinical and laboratory data of all included patients were recorded at every predefined time point from baseline to 12-week follow-up. The demographic data included age, gender and body mass index (BMI). Clinical data included symptoms, signs, etiology for HBV activation, pre-existing chronic liver

diseases, complications, treatment, treatment-related adverse events (AEs), and treatment outcomes. Symptoms included nausea, vomiting, poor appetite, abdomen distention, fatigue, and jaundice. Signs included consciousness, body temperature and vital signs like heart rate, blood pressure and respiratory rate. Potential causes for HBV activation included the absence of NAs, withdrawal of NAs and others. Pre-existing chronic liver diseases included hepatitis and cirrhosis. Complications of ACLF included ascites, bacterial infection, HE and HRS. Bacterial infection was diagnosed based on infection-related symptoms, signs, laboratory indicators or imaging evidence. Treatment outcomes included recovery and unfavorable events such as death, liver transplantation and treatment abandonment. Laboratory data included complete blood count (white blood cell [WBC] count, neutrophil count, lymphocyte count, red blood cell count [RBC], serum hemoglobulin concentration, platelet count), biochemical parameters (serum aspartate aminotransferase, alanine aminotransferase [ALT], gamma-glutamyl transpeptidase, alkaline phosphatase, cholinesterase, TBIL, direct bilirubin, albumin, globulin, triglyceride, total cholesterol, potassium, sodium, chlorine, blood urea nitrogen [BUN], creatinine levels, and estimated glomerular filtration rate), coagulation function (prothrombin time [PT], PTA, PT-INR, fibrinogen, activated partial thromboplastin time), virological markers (HBsAg, hepatitis B e antigen, hepatitis B e antibody, HBV DNA load), alpha-fetoprotein, procalcitonin, abdominal ultrasound, computed tomography or magnetic resonance imaging.

The primary efficacy outcome was the cumulative survival rate. The secondary efficacy outcome was the model for end-stage liver disease (MELD) score changes. The MELD score was calculated using the following formula: MELD

score= 9.57 × ln (serum creatinine) (mg/dL) + 3.78 × ln (serum TBIL) (mg/dL) + 11.20 × ln (PT-INR) + 6.43 [17]. Child-Pugh-Turcotte (CPT) score and the new score from COSSH for patients with HBV-ACLF (COSSH-ACLF II score) were incorporated for analysis. CPT score was calculated by the sum of the five following items: for HE, none = 1 point, grade 1 and 2 = 2 points, grade 3 and 4 = 3 points; for ascites, none = 1 point, slight = 2 points, moderate = 3 points; for serum bilirubin: under 2 mg/ml = 1 point, 2 to 3 mg/ml = 2 points, over 3 mg/ml = 3 points; for serum albumin: greater than 3.5mg/ml = 1 point, 2.8 to 3.5mg/ml = 2 points, less than 2.8mg/ml = 3 points; for PT (sec prolonged): less than 4 sec = 1 point, 4 to 6 sec = 2 points, over 6 sec = 3 points [18]. COSSH-ACLF II score was calculated using the following formula: COSSH-ACLF II score = 1.649 × ln (PT-INR) + 0.457 × HE score (HE grade: 0/1, 1-2/2 and 3-4/3) + 0.425 × ln (neutrophil) ($10^9$/L) + 0.396 × ln (serum TBIL) (umol/L) + 0.576 × ln (serum BUN) (mmol/L) + 0.033 × age [19].

**Statistical analysis**

Normally distributed continuous data were expressed as mean ± standard deviation, whereas abnormally distributed continuous data were expressed as median (interquartile range). Categorical data were presented as count and percentage (%). The significance of differences between the two groups was assessed by the Student's *t*-test for continuous variables with a normal distribution or the Mann-Whitney test for continuous variables with a non-parametric distribution. Categorical data were compared by the Chi-square test or Fisher's exact test (if the expected value was <5). Univariate and multivariate COX regression analyses were applied to identify independent parameters for clinical outcomes. The Kaplan-Meier survival analysis and log-rank test were used

together to evaluate the effectiveness of a treatment or intervention on survival time. The Kaplan-Meier method provides an estimate of the survival function, while the log-rank test determines the significance of the difference in survival times between groups. A *P* value < 0.05 was statistically significant. Statistical analyses were conducted using IBM SPSS Statistics v19.0 and GraphPad Prism 5.

## Results

### Patient enrollment and baseline characteristics

The consort diagram and flow chart of our study are shown in **Figure 1.** A total of 50 patients (25 in the trial group and 25 in the control group) diagnosed with intermediate-stage HBV-related ACLF were included in this prospective study, with a mean age of 47.8 ± 8.8 years and male predominance (86%, n=43). Liver cirrhosis was observed in 27 (54%) patients. The baseline characteristics are shown in **Table 1**. Except for serum potassium level, no significant differences were found in other characteristics between the two groups (all $p$>0.05), indicating that these two groups were comparable at baseline.

### Safety of DPMAS with sequential LPE treatment

In the trial group, 25 patients received DPMAS with sequential LPE treatment. Blood parameters, such as WBC, hemoglobin, platelet, procalcitonin, PT and fibrinogen, were monitored before and after each session of DPMAS with sequential LPE. Dynamic changes in these parameters from baseline to 2 weeks follow-up are shown in **Figure 2**. Bleeding events occurred in 3 (12%) patients (1 patient with melena, 1 patient with bleeding at the catheter site, 1 patient with skin ecchymosis), and an allergic reaction was reported by 1 (4%) patient who experienced rash and pruritus. Details of AEs are shown in **Table 2**. All AEs were relieved by supportive and symptomatic treatment to ensure that DPMAS with

sequential LPE could be completed without interruption. Other treatment-related AEs, such as fever, infection, hypotension, thrombosis, or hemolysis, were not observed in the trial group.

**Treatment efficacy**

Key parameters related to liver function, such as ALT, TBIL, PT-INR and MELD score, were monitored before and after each session in the trial group. The levels of TBIL, PT-INR, and MELD scores after each session were significantly lower than before treatment (all $p<0.05$). The dynamic changes in these parameters in the trial and control groups from baseline to week 12 are shown in **Figure 3**.

In the trial group, 1 patient died at week 1, and 3 patients underwent liver transplantation (weeks 2, 5, and 5, respectively). In the control group, 1 patient died at week 1, and 3 patients underwent liver transplantation (weeks 6, 10, and 12, respectively). The 12-week cumulative rates of unfavorable events in the trial and control groups were 64% and 80%, respectively ($p=0.208$). The 12-week cumulative liver transplantation-free survival rates in the trial and control groups were 52% and 24%, respectively ($p=0.041$). The 12-week cumulative overall survival rates in the trial and control groups were 64% and 36%, respectively ($p=0.048$). More details on the cumulative rates of unfavorable events and survival are shown in **Table 3**. The Kaplan-Meier survival analysis revealed significant differences in liver transplantation-free survival ($p=0.047$) and overall survival ($p=0.038$) between the trial and control groups (**Figure 4)**.

**Independent variables associated with clinical outcomes**

The baseline characteristics in **Table 1** were analyzed to identify the factors associated with unfavorable events and death. COX regression analysis revealed that significant risk factors for unfavorable events included HE (hazard ratio

[HR]=3.27, 95% confidence interval [CI]: 1.29-8.32, *p*=0.008) and COSSH-ACLF II score (HR=2.45, 95% CI: 1.33-4.51, *p*=0.004) (**Table 4**). COX regression analysis revealed that significant risk factors for death included BUN (HR=0.40, 95% CI: 0.17-0.95, *p*=0.038), DPMAS sequential with LPE (HR=0.79, 95% CI: 0.62-0.99, *p*=0.048), and COSSH-ACLF II score (HR=7.49, 95% CI: 3.14-17.87, *p*<0.001) (**Table 4**).

**Discussion**

In this prospective study, mild AEs, including bleeding events and allergic reactions, were observed in the trial group during DPMAS with sequential LPE treatment and could be relieved by supportive and symptomatic treatment. These findings substantiated that DPMAS with sequential LPE had a relatively good safety profile. Meanwhile, the levels of TBIL and PT-INR, and MELD scores after each session were significantly lower than before treatment. Otherwise, patients in the trial group had better cumulative liver transplantation-free survival rates and overall survival rates than those in the control group. Overall, our findings suggest that DPMAS with sequential LPE is an effective therapeutic approach for patients with intermediate-stage HBV-related ACLF.

Moreover, we found that the WBC count increased after each session of DPMAS with sequential LPE and gradually returned to pre-treatment levels before the next session. Meanwhile, there was no increase in PCT level during treatment, indicating that leukocytosis was not induced by an infection but related to histocompatibility of the plasma separator, extracorporeal blood circuit and the two adsorption products, or the use of fresh plasma or FFP for LPE. Consistently, the hemoglobin levels and platelet count transiently decreased after each session and returned to pre-treatment levels before the next session, related to the

mechanical destruction by the plasma separator. Thrombocytopenia following ALSS treatment has been extensively reported in the literature [20, 21]. Current evidence suggests that heparin-induced thrombocytopenia should be avoided after administering unfractionated heparin for anticoagulation [22]. Accordingly, clinicians should monitor the changes in blood cells before and after ALSS therapy.

In the present study, the TBIL and PT-INR levels and MELD scores were significantly reduced after DPMAS with sequential LPE treatment in the trial group, exhibiting a decreasing trend during the treatment course (from baseline, 2 weeks, 4 weeks, 8 weeks, and 12 weeks). These results substantiated that DPMAS with sequential LPE treatment could improve the hepatic function of patients with intermediate-stage HBV-related ACLF. Compared with the control group, patients in the trial group had better cumulative liver transplantation-free survival rate (52% vs. 24%) and cumulative overall survival rate (64% vs. 36%) at 12 weeks. The Kaplan-Meier survival analysis revealed significance in liver transplantation-free survival ($p$=0.047) and overall survival ($p$=0.038) between the trial and control groups. The improved hepatic function and survival rates were in accordance with the literature [14-16, 23]. Indeed, it has been established that the levels of inflammatory cytokines are high in ACLF patients and determine the severity of ACLF [24]. Importantly, the combination of DPMAS and PE can effectively remove inflammatory cytokines, such as C-reactive protein, interleukin-6, and tumor necrosis factor-α [25].

In this study, COX regression analysis indicated BUN ($p$=0.038), DPMAS with sequential LPE ($p$=0.048) and COSSH-ACLF II score ($p$<0.001) were risk factors for mortality. In addition, we found that HE ($p$=0.008) and COSSH-ACLF II score

($p$=0.004) were risk factors for unfavorable events. Indeed, the COSSH-ACLF II score could accurately predict 4-week and 12-week mortality of HBV-ACLF patients [19]. Meanwhile, HE is a well-recognized indicator for the ACLF grading system based on consensus recommendations from the CMA [3], EASL [4] and APASL [7]. Besides, HE has been documented in many scoring systems for evaluating the severity of ACLF [5, 19, 26, 27]. Interestingly, HE was identified as an independent predictor of 90-day mortality in patients with HBV-related ACLF [28]. Moreover, a study found that HE was a strong independent risk factor for MELD score decline at one week, which was associated with a poor short-term prognosis in HBV-related ACLF [29].

There were several limitations in this study. First, this single-center study recruited a relatively small number of patients. Indeed, our findings should be validated in a multicenter cohort study to increase their robustness. Besides, no patients with HRS were included in our study. It is widely acknowledged that HRS is a common lethal complication of ACLF. Nonetheless, whether DPMAS with sequential LPE is safe and effective in HBV-related ACLF patients complicated with HRS remains unclear. Moreover, the patients in this study were followed up for only 12 weeks and further prospective studies are warranted to investigate the long-term effect of DPMAS with sequential LPE. However, it should be borne in mind that this is a rare prospective study on the safety and efficacy of DPMAS with sequential LPE treatment, providing novel insights into potential treatment options for patients with intermediate-stage HBV-related ACLF.

In conclusion, DPMAS with sequential LPE treatment is safe and effective for patients with intermediate-stage HBV-related ACLF. Accordingly, this therapeutic

approach has huge prospects for widespread clinical application to improve the short-term survival rate of this patient population.

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## Figure legend

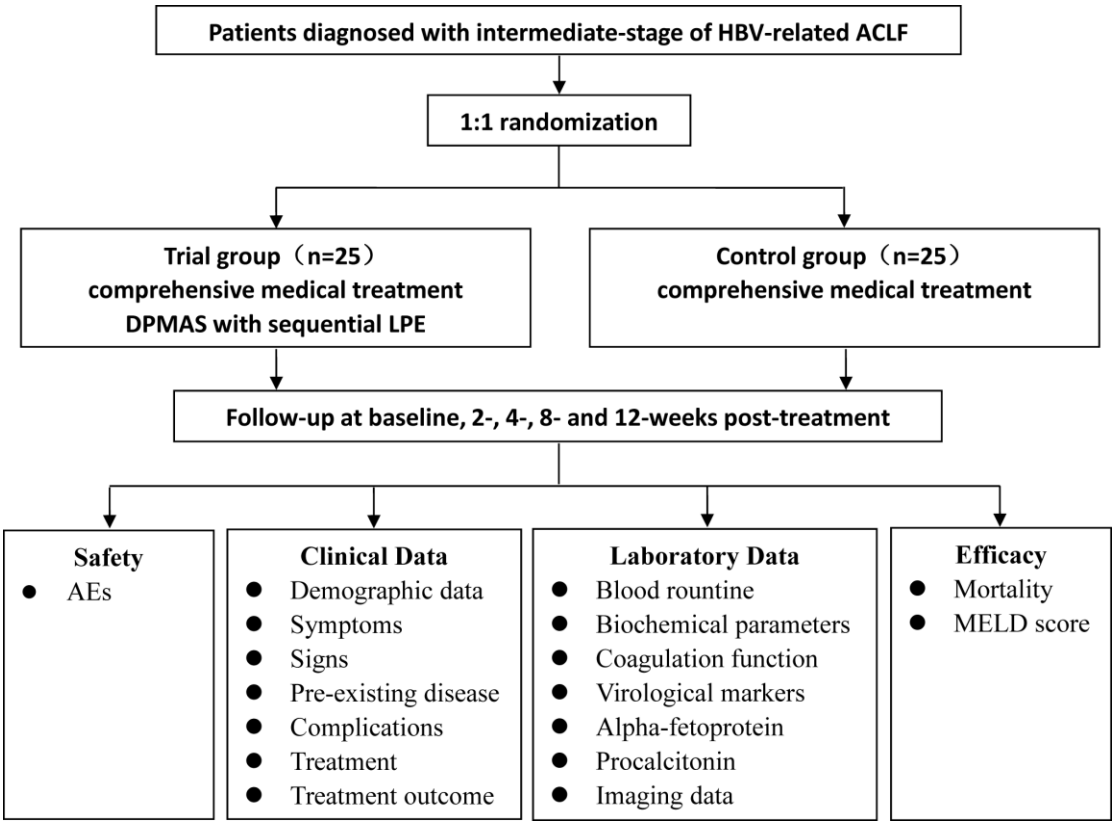


**Figure 1** The consort diagram and flow chart of analysis.

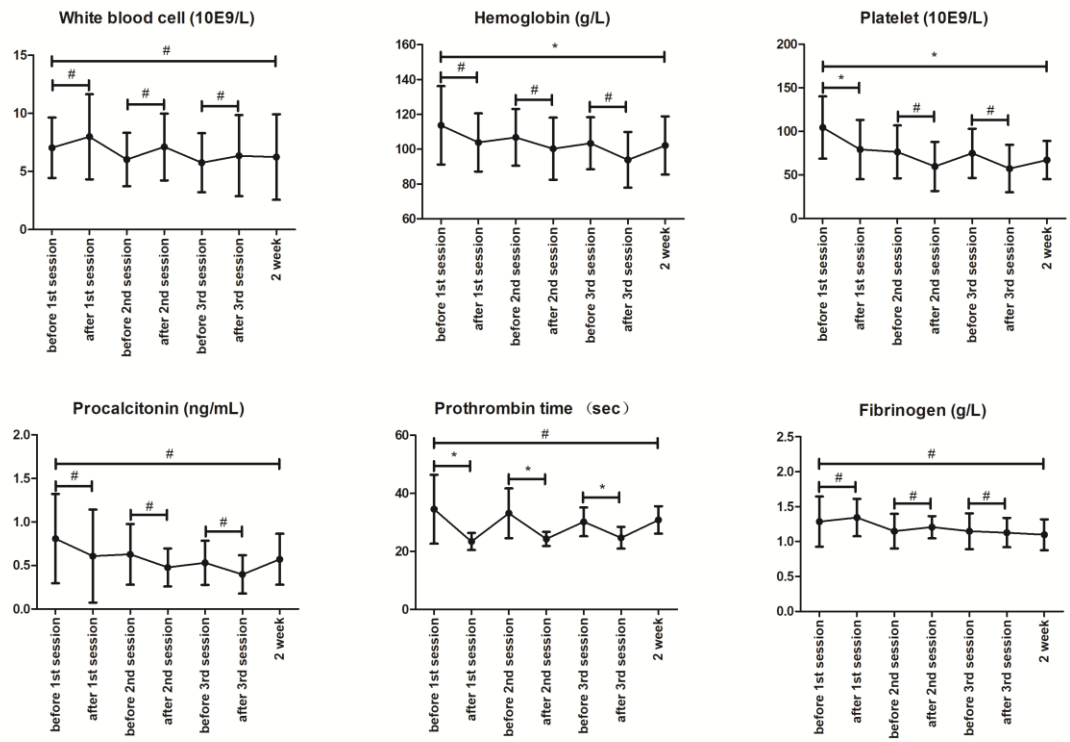


**Figure 2** Dynamic changes in white blood cell, hemoglobin, platelet, procalcitonin, prothrombin time and fibrinogen in the trial group from baseline to week 12. Dots and error bars indicate mean and standard deviation. #: $p > 0.05$; *: $p < 0.05$.

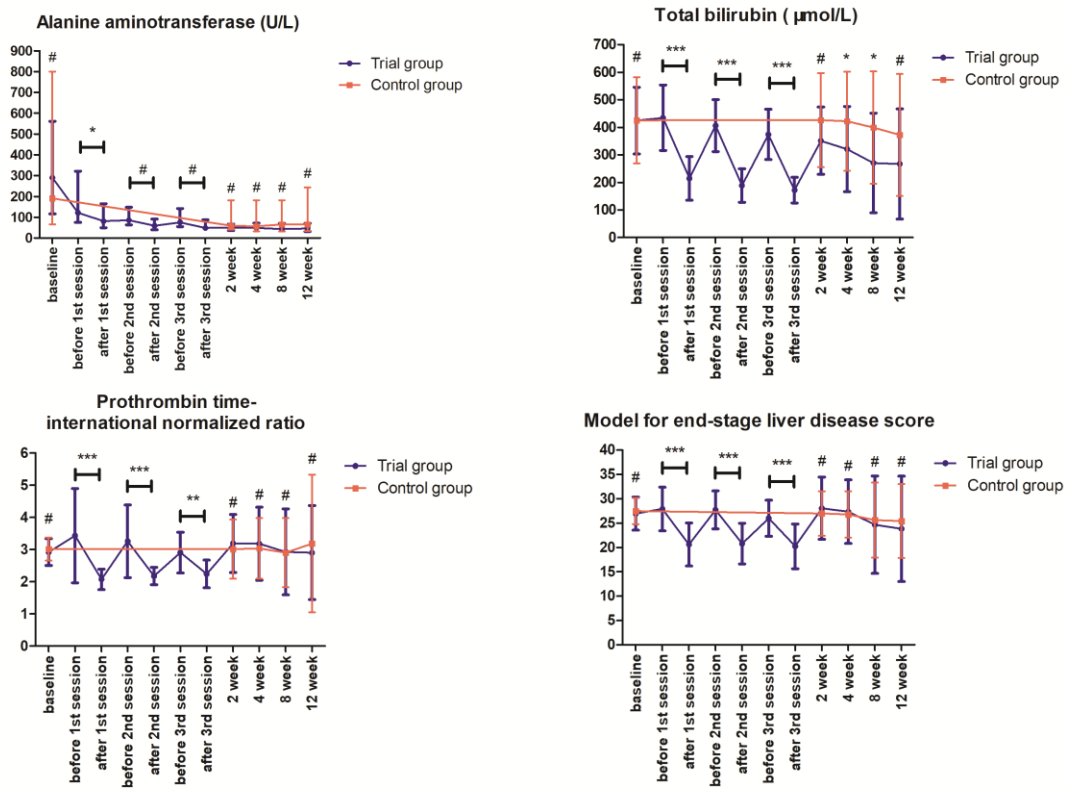


**Figure 3** Dynamic changes in alanine aminotransferase, total bilirubin, prothrombin time-international normalized ratio and model for end-stage liver disease score in the trial and control groups from baseline to week 12. For alanine aminotransferase, dots and error bars indicate median and interquartile range. For total bilirubin, prothrombin time-international normalized ratio and model for end-stage liver disease score, dots and error bars indicate mean and standard deviation. #: $p > 0.05$; *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$.

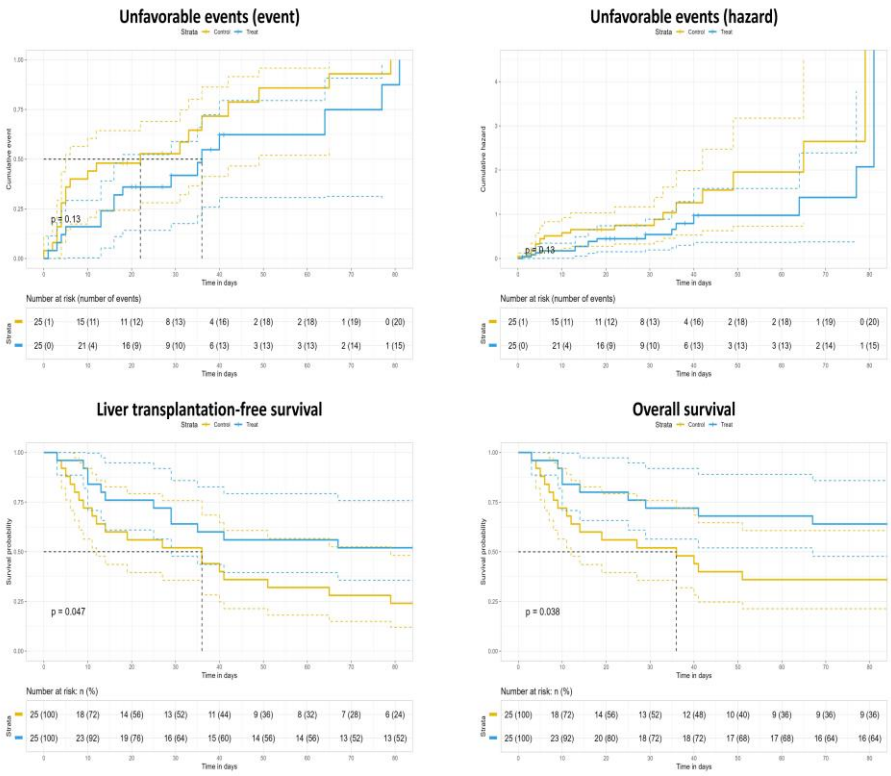


**Figure 4** Comparison of treatment using Kaplan-Meier Survival Estimates, including cumulative event and cumulative hazard of unfavorable events, and probability of liver transplant-free survival and overall survival.

**Table 1 Baseline characteristics of patients**

| Characteristic | Trial group ($n$ = 25) | Control group ($n$ = 25) | All ($n$ = 50) | $P$ value |
|---|---|---|---|---|
| Age, y | 49.1 ± 9.9 | 46.4 ± 7.5 | 47.8 ± 8.8 | 0.293 |
| Gender, male, n (%) | 22 (88%) | 21 (84%) | 43 (86%) | 0.684 |
| Body mass index, kg/m$^2$ | 22.5 ± 2.7 | 23.1 ± 3.1 | 22.8 ± 2.9 | 0.503 |
| Etiology for HBV activation | | | | 1.000 |
| Absence of NAs, n (%) | 21 (84%) | 22 (88%) | 43 (86%) | |
| Withdrawal of NAs, n (%) | 4 (16%) | 2 (8%) | 6 (12%) | |
| Others, n (%) | 0 (0%) | 1 (4%) | 1 (2%) | |
| Pre-existing chronic liver diseases | | | | 0.395 |

| | | | | |
|---|---|---|---|---|
| Hepatitis, n (%) | 13 (52%) | 10 (40%) | 23 (46%) | |
| Cirrhosis, n (%) | 12 (48 %) | 15 (60%) | 27 (54%) | |
| Complications | | | | |
| Ascites, n (%) | 19 (76%) | 21 (84%) | 40 (80%) | 0.725 |
| Bacterial infection, n (%) | 21 (84%) | 16 (64) | 37 (74%) | 0.107 |
| Peritonitis, n (%) | 11 (44%) | 9 (36%) | 20 (40%) | 0.564 |
| Pneumonia, n (%) | 6 (24%) | 3 (12%) | 9 (18%) | 0.463 |
| Biliary infection, n (%) | 5 (20%) | 5 (20%) | 10 (20%) | 1.000 |
| Hepatic encephalopathy, n (%) | 5 (20%) | 7 (28%) | 12 (24%) | 0.508 |
| Hepatorenal syndrome, n (%) | 0 (0%) | 0 (0%) | 0 (0%) | NA |
| Numbers of complications | 1.80 ± 0.65 | 1.76 ± 0.78 | 1.78 ± 0.71 | 0.844 |
| Period between admission to enrolment, d | 2 (1-7) | 2 (1-3) | 2 (1-3) | 0.301 |
| Period between enrolment to initiation of ALSS, d | 1 (0-2) | NA | NA | NA |
| NAs treatment after admission | | | | 0.106 |
| TDF, n (%) | 3 (12%) | 0 (0%) | 3 (6%) | |
| TAF, n (%) | 9 (36%) | 8 (32%) | 17 (34%) | |
| Entecavir, n (%) | 13 (52%) | 17 (68%) | 30 (60%) | |
| White blood cell, $\times 10^9$/L | 7.34 ± 2.89 | 7.43 ± 2.80 | 7.38 ± 2.82 | 0.915 |
| Neutrophil, $\times 10^9$/L | 4.99 ± 2.27 | 5.25 ± 2.90 | 5.12 ± 2.58 | 0.722 |
| Lymphocyte, $\times 10^9$/L | 1.32 ± 0.57 | 1.25 ± 0.60 | 1.28 ± 0.58 | 0.656 |
| Red blood cell, $\times 10^{12}$/L | 3.72 ± 0.69 | 3.43 ± 0.72 | 3.57 ± 0.71 | 0.163 |
| Hemoglobin, g/L | 114.4 ± 18.6 | 110.3 ± 18.2 | 112.3 ± 18.3 | 0.432 |
| Platelet, $\times 10^9$/L | 112.0 ± 35.7 | 103.5 ± 63.7 | 107.8 ± 51.3 | 0.561 |
| AST, U/L | 155 (108-37 | 170 (111-426) | 167.5 (108.75-410.5) | 0.721 |

| | | | | |
|---|---|---|---|---|
| | 9) | | | |
| ALT, U/L | 290 (127-559) | 191 (67-660) | 263 (70.75-562.75) | 0.985 |
| GGT, U/L | 74.6 ± 36.5 | 76.5 ± 53.3 | 75.6 ± 45.0 | 0.884 |
| ALP, U/L | 135.6 ± 38.8 | 140.7 ± 39.6 | 138.1 ± 38.9 | 0.656 |
| Cholinesterase, U/L | 3973.3 ± 1255.5 | 3968.2 ± 1639.5 | 3970.8 ± 1440.9 | 0.990 |
| Total Bilirubin, μmol/L | 424.3 ± 121.5 | 425.5 ± 156.8 | 424.9 ± 138.8 | 0.974 |
| Direct Bilirubin, μmol/L | 217.3 ± 68.3 | 220.1 ± 73.0 | 218.7 ± 70.0 | 0.892 |
| Albumin, g/L | 33.7 ± 4.8 | 33.5 ± 5.4 | 33.6 ± 5.0 | 0.938 |
| Globulin, g/L | 28.1 ± 5.8 | 29.2 ± 7.9 | 28.6 ± 6.9 | 0.576 |
| Albumin to globulin ratio | 1.25 ± 0.35 | 1.25 ± 0.44 | 1.25 ± 0.40 | 0.972 |
| Glucose, mmol/L | 6.72 ± 3.63 | 5.82 ± 4.02 | 6.28 ± 3.81 | 0.424 |
| Triglyceride, mmol/L | 0.93 ± 0.39 | 0.93 ± 0.46 | 0.93 ± 0.42 | 0.963 |
| Total cholesterol, mmol/L | 2.43 ± 1.03 | 2.29 ± 1.26 | 2.36 ± 1.14 | 0.700 |
| Potassium, mmol/L | 3.49 ± 0.46 | 3.83 ± 0.55 | 3.66 ± 0.53 | 0.020 |
| Sodium, mmol/L | 136.1 ± 5.0 | 136.3 ± 4.7 | 136.2 ± 4.8 | 0.860 |
| Chlorine, mmol/L | 99.8 ± 4.3 | 99.3 ± 6.0 | 99.6 ± 5.2 | 0.748 |
| Blood urea nitrogen, mmol/L | 3.84 ± 2.95 | 4.68 ± 2.77 | 4.26 ± 2.86 | 0.302 |
| Creatinine, μmol/L | 67.1 ± 22.1 | 67.5 ± 17.9 | 67.3 ± 19.9 | 0.945 |
| eGFR, ml/min | 105.8 ± 21.9 | 107.0 ± 18.4 | 106.4 ± 20.0 | 0.842 |
| Prothrombin time, sec | 30.5 ± 3.3 | 31.3 ± 3.1 | 30.9 ± 3.2 | 0.386 |
| Prothrombin activity, % | 25.8 ± 3.3 | 24.9 ± 2.9 | 25.4 ± 3.1 | 0.301 |
| PT-INR | 2.92 ± | 3.02 ± 0.36 | 2.97 ± 0.39 | 0.40 |

| | | | | |
|---|---|---|---|---|
| Fibrinogen, g/L | 0.42 1.31 ± 0.32 | 1.44 ± 0.35 | 1.38 ± 0.34 | 0 0.16 7 |
| APTT, sec | 71.4 ± 33.4 | 59.8 ± 7.5 | 65.6 ± 24.7 | 0.09 4 |
| HBsAg, log10 IU/mL | 3.05 ± 0.82 | 2.80 ± 0.77 | 2.92 ± 0.80 | 0.26 9 |
| Positive HBeAg, n (%) | 4 (16%) | 6 (24%) | 10 (20%) | 0.72 5 |
| HBV DNA, log10 IU/mL | 4.83 ± 1.75 | 4.66 ± 2.03 | 4.75 ± 1.87 | 0.76 1 |
| Alpha-fetoprotein, ng/mL | 74.38 (26.02-146) | 15.54 (9.42-44.51) | 29.51 (14.36-92.40) | 0.06 8 |
| Procalcitonin, ng/mL | 0.80 ± 0.55 | 0.77 ± 0.44 | 0.78 ± 0.49 | 0.84 2 |
| MELD score | 27.0 ± 3.4 | 27.5 ± 2.7 | 27.2 ± 3.1 | 0.54 8 |
| CPT score | 11.3 ± 1.3 | 11.3 ± 1.6 | 11.3 ± 1.4 | 1.00 0 |
| COSSH-ACLF Ⅱ score | 7.6 ± 0.7 | 7.8 ± 0.9 | 7.7 ± 0.8 | 0.45 5 |

HBV: hepatitis B virus; NAs: nucleot(s)ide analogues; ALSS: artificial liver support systems; TDF: tenofovir disoproxil fumarate; TAF: tenofovir alafenamide; AST: aspartate aminotransferase; ALT: alanine aminotransferase; GGT: gamma-glutamyl transpeptidase; ALP: alkaline phosphatase; eGFR: estimated glomerular filtration rate; PT-INR: prothrombin time-international normalized ratio; APTT: activated partial thromboplastin time; HBsAg: hepatitis B surface antigen; HBeAg: hepatitis B e antigen; DNA: deoxyribonucleic acid; MELD: model for end-stage liver disease; CPT: Child-Pugh-Turcotte; COSSH: Chinese Group on the Study of Severe Hepatitis B; ACLF: acute-on-chronic liver failure; NA: not applicable.

**Table 2 Details of treatment-related adverse events in the trial group**

| Patient | Adverse event | Time of occurrence | Severity | Period from occurrence to remission |
|---|---|---|---|---|
| 1 | Melena | Two hours after 2nd section of ALSS | Mild | Three days |
| 2 | Bleeding at the catheter site | One day before 2nd section of ALSS | Mild | One day |
| 3 | Skin ecchymosis | One day after 3rd section of ALSS | Mild | Three days |
| 4 | Rash and pruritus | During PE of 1st section of ALSS | Mild | Two hours |

ALSS: artificial liver support systems; PE: plasma exchange.

**Table 3 Cumulative rates of unfavorable events and survival**

| Parameters | Trial group ($n$ = 25) | Control group ($n$ = 25) | All ($n$ = 50) | $P$ value |
|---|---|---|---|---|
| Unfavorable events | | | | |
| 4 weeks | 9 (36%) | 13 (52%) | 22 (44%) | 0.254 |
| Death | 1 (4%) | 1 (4%) | 2 (4%) | |
| Liver transplantation | 1 (4%) | 0 (0%) | 1 (2%) | |
| Treatment abandonment | 7 (28%) | 12 (48%) | 19 (38%) | |
| 8 weeks | 13 (52%) | 18 (72%) | 31 (62%) | 0.145 |
| Death | 1 (4%) | 1 (4%) | 2 (4%) | |
| Liver transplantation | 3 (12%) | 1 (4%) | 4 (8%) | |
| Treatment abandonment | 9 (36%) | 16 (64%) | 25 (50%) | |
| 12 weeks | 16 (64%) | 20 (80%) | 36 (72%) | 0.208 |
| Death | 1 (4%) | 1 (4%) | 2 (4%) | |
| Liver transplantation | 3 (12%) | 3 (12%) | 6 (12%) | |
| Treatment abandonment | 12 (48%) | 16 (64%) | 28 (56%) | |
| Liver transplantation-free survival | | | | |
| 4 weeks | 18 (72%) | 13 (52%) | 31 (62%) | 0.145 |
| 8 weeks | 14 (56%) | 8 (32%) | 22 (44%) | 0.087 |
| 12 weeks | 13 (52%) | 6 (24%) | 19 (38%) | 0.041 |

| | | | |
|---|---|---|---|
| Overall survival | | | |
| 4 weeks | 19 (76%) | 13 (52%) | 32 (64%) | 0.077 |
| 8 weeks | 17 (68%) | 9 (36%) | 26 (52%) | 0.024 |
| 12 weeks | 16 (64%) | 9 (36%) | 25 (50%) | 0.048 |

**Table 4 Univariate and multivariate COX regression analysis of clinical outcomes**

| Outcomes | Variable | Univariate analysis HR (95% CI) | *P* value | Multivariate analysis HR (95% CI) | *P* value |
|---|---|---|---|---|---|
| Unfavorable events | | | | | |
| | Hepatic encephalopathy | 5.78 (2.57-12.99) | <0.001 | 3.27 (1.29-8.32) | 0.008 |
| | PT-INR | 3.38 (1.62-7.06) | <0.001 | 2.15 (0.99-4.67) | 0.052 |
| | COSSH-ACLF II score | 3.68 (2.15-6.32) | <0.001 | 2.45 (1.33-4.51) | 0.004 |
| LT+Death | | | | | |
| | COSSH-ACLF II score | 4.38 (2.53-7.60) | <0.001 | 4.38 (2.53-7.60) | <0.001 |
| Death | | | | | |
| | Blood urea nitrogen | 1.15 (1.02-1.29) | 0.018 | 0.40 (0.17-0.95) | 0.038 |
| | DPMAS with sequential LPE | 0.43 (0.19-0.98) | 0.045 | 0.79 (0.62-0.99) | 0.048 |
| | COSSH-ACLF II score | 3.73 (2.14-6.53) | <0.001 | 7.49 (3.14-17.87) | <0.001 |

HR: hazard ratio; CI: confidence interval; LT: liver transplantation; PT-INR: prothrombin time-international normalized ratio; HBsAg: hepatitis B surface antigen; COSSH: Chinese Group on the Study of Severe Hepatitis B; ACLF: acute-on-chronic liver failure; DPMAS: double plasma molecular adsorption system; LPE: low-volume plasma exchange.