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Allogeneic Bone Marrow–Derived Mesenchymal Stromal Cells for Hepatitis B Virus–Related Acute-on-Chronic Liver Failure: A Randomized Controlled Trial

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Mortality from hepatitis B virus (HBV)–related acute-on-chronic liver failure (ACLF) is high due to limited treatment options. Preclinical and clinical investigations have proved that treatment with mesenchymal stromal cells (MSCs) is beneficial for recovery from liver injury. We hypothesized that the outcome of HBV-related ACLF would be improved by MSC treatment. From 2010 to 2013, 110 patients with HBV-related ACLF were enrolled in this open-label, nonblinded randomized controlled study. The control group (n = 54) was treated with standard medical therapy (SMT) only. The experimental group (n = 56) was infused weekly for 4 weeks with 1.0 to 10 × 10^5 cells/kg allogeneic bone marrow–derived MSCs and then followed for 24 weeks. The cumulated survival rate of the MSC group was 73.2% (95% confidence interval 61.6%-84.8%) versus 55.6% (95% confidence interval 42.3%-68.9%) for the SMT group (P = 0.03). There were no infusion-related side effects, but fever was more frequent in MSC compared to SMT patients during weeks 5-24 of follow-up. No carcinoma occurred in any trial patient in either group. Compared with the control group, allogeneic bone marrow–derived MSC treatment markedly improved clinical laboratory measurements, including serum total bilirubin and Model for End-Stage Liver Disease scores. The incidence of severe infection in the MSC group was much lower than that in the SMT group (16.1% versus 33.3%, P = 0.04). Mortality from multiple organ failure and severe infection was higher in the SMT group than in the MSC group (37.0% versus 17.9%, P = 0.02). *Conclusion:* Peripheral infusion of allogeneic bone marrow–derived MSCs is safe and convenient for patients with HBV-related ACLF and significantly increases the 24-week survival rate by improving liver function and decreasing the incidence of severe infections. (HEPATOLOGY 2017;66:209-219).

Acute-on-chronic liver failure (ACLF) occurs in patients with previously diagnosed or undiagnosed chronic liver disease and is characterized by acute hepatic insults such as jaundice and coagulopathy and complicated within 4 weeks by ascites and/or encephalopathy.[1] In Asia, ACLF is mainly caused by hepatitis B[2]; mortality is as high as 63%-72.3%.[3,4] Antiviral treatment can improve outcomes,

Abbreviations: ACLF, acute-on-chronic liver failure; ALB, albumin; ALT, alanine aminotransferase; BM-MSC, bone marrow-derived MSC; CD, cluster of differentiation; HBV, hepatitis B virus; HRS, hepatorenal syndrome; INR, international normalized ratio; MELD, Model for End-Stage Liver Disease; MSC, mesenchymal stromal cell; SMT, standard medical therapy; TBil, total bilirubin.

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but mortality remains at >50%.[5] Liver transplantation is the only therapy that has proven beneficial, but the rapid disease progression and lack of donors limit its application.[6] Therefore, it is urgent to find a safe and effective therapeutic approach to ACLF. Mesenchymal stromal cells (MSCs) are multipotent progenitor cells that can be isolated from various adult tissues, including bone marrow, adipose tissue, dental pulp, and spleen.[7] MSCs have enormous expansion potential in culture and can differentiate into various cell types that play important roles in tissue regeneration and repair.[8] The discovery of their paracrine properties and the fact that they migrate to injured sites (the "homing" function) expanded the spectrum of therapeutic applications.[9] Systemic infusion of MSCs has been determined to be useful in different models, including models of hepatic injury.[10,11] Fouraschen reported that MSCs could repair damaged hepatocytes and promote liver regeneration.[12] The mechanism is by colonization of the inflamed liver through the homing function and transdifferentiation to hepatocytes in the local microenvironment.[13] In addition, MSCs can regulate the local immune system and repair hepatocytes through their paracrine functions.[14] Shi's study showed that MSCs can inhibit the activation of stellate cells and thus reduce liver fibrosis, which is common among ACLF patients after recovery.[15] So infusion of MSCs might be a useful ACLF treatment because of their anti-inflammatory, immunoregulatory, cell-repairing, and antifibrosis effects.

In clinical practice, treatment of patients with end-stage liver disease with infusion of autologous bone marrow–derived MSCs (BM-MSCs) was safe and the short-term efficacy favorable,[16] but survival was not markedly improved.[17] Possible reasons might include impaired function of autologous MSCs (including slower growth with worse activity and faster aging),[18] advanced age of patients, and/or inadequate MSC doses. Allogeneic MSC treatment has potential advantages and might overcome the limitations of autologous MSC treatment, including as an "off-the-shelf" immunoprivileged therapeutic agent and having no treatment delay due to culturing (as is required for autologous MSC use).

To address these issues, we performed a prospective, randomized controlled clinical trial to evaluate the safety and efficacy of allogeneic BM-MSC infusion for patients with ACLF. The findings are likely to have implications for the development of cell-based therapies for ACLF and possibly other end-stage liver diseases.

Participants and Methods

STUDY DESIGN AND PARTICIPANTS

This study was a prospective, open-label, nonblinded randomized clinical trial. It was carried out in a single center in the south of China, with the purpose of evaluating the safety and efficacy of allogeneic BM-MSC infusion for the treatment of patients with hepatitis B virus (HBV)–related ACLF.

Using consensus recommendations of the Asian Pacific Association for the Study of the Liver 2009[1] and diagnostic and treatment guidelines for liver failure in China 2006,[19] patients with the following were deemed eligible for enrollment in this study: (1) ACLF, which is characterized by acute hepatic insult manifesting as jaundice (serum total bilirubin [TBil] $\geq$ 10 $\times$ the upper limit of normal, in micromoles per

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liter) and coagulopathy (international normalized ratio [INR] $\geq$ 1.5 or prothrombin activity <40%), complicated within 4 weeks by ascites and/or encephalopathy as determined by physical examination, in patients with previously diagnosed or undiagnosed chronic liver disease; (2) positive serum hepatitis B surface antigen for more than 6 months; (3) Model for End-Stage Liver Disease (MELD) scores ranging 17-30; and (4) age 16-60 years. Patients were excluded for the following reasons: (1) serious complications in the previous 2 months (e.g., gastrointestinal bleeding, serious infection such as sepsis); (2) concomitant autoimmune disease; (3) superinfection with other hepatitis viruses; (4) important organ dysfunctions (e.g., renal dysfunction; detailed definitions of organ dysfunction are presented in the Supporting Information) not due to liver disease or malignancies; (5) pregnancy and lactation; (6) liver tumor or regenerative nodules secondary to cirrhosis proven by ultrasound, computerized tomography, or magnetic resonance imaging; (7) bioartificial liver support therapy; or (8) previous liver transplantation.

This study conformed strictly to the ethics guidelines of the 1975 Declaration of Helsinki. The study protocol was approved by the ethics committee on clinical trials of the Third Affiliated Hospital of Sun Yat-sen University in 2010.

RANDOMIZATION AND MASKING

Patients were randomly allocated, in a 1:1 ratio, to receive standard medical treatment (SMT group) or allogeneic BM-MSC infusions (MSC group) by a computer-generated randomization sequence. The allocation message was kept in opaque sealed envelopes. The statistician who generated the randomization sequence and the allocation message was not involved in the rest of the study. The research assistant at the clinic was unaware of the participants' group assignment prior to allocation. This was an open-label study; both participants and the study team were unblinded to treatment allocation.

PATIENTS AND PROCEDURES

The Department of Infectious Diseases of the Third Affiliated Hospital of Sun Yat-sen University is the largest liver disease research center in south China, with a total of 232 beds; we treat about 400 inpatients with HBV-related ACLF each year. A total of 578 inpatients in this department were screened from October 11, 2010, to April 2, 2013, and 112 patients were eligible; among them, 110 patients who signed the consent form were enrolled. Participants were then assigned to the MSC or SMT group as described above.

All patients received standard medical treatment, including nutritional supplementation; administration of human serum albumin (10 g/day until serum albumin was 35 g/L), fresh frozen plasma (200-400 mL/day until the INR was <1.5), entecavir (0.5 mg/day; Squibb Pharmaceuticals Ltd., Shanghai, China), and *S*-adenosylmethionine (1.0 g/day, Abbott S.R.L., Italy); and appropriate treatment for complications such as infections (including of the respiratory tract, urinary tract, biliary tract, and digestive tract and spontaneous peritonitis), encephalopathy, gastrointestinal bleeding, and hepatorenal syndrome [HRS]). The MSC group received infusions of 1.0 to 10 $\times$ 10^5 cells/kg allogeneic BM-MSCs through the peripheral vein once a week for 4 weeks, in addition to SMT.

Observation and follow-up data were recorded immediately before the first infusion and at 1, 2, 3, 4, 8, 12, and 24 weeks afterward. To evaluate the safety of the therapy, we observed and recorded adverse reactions (e.g., fever, rash, and diarrhea), changes of white blood cell count and of hemoglobin, platelet, and creatinine levels and the incidence of hepatocellular carcinoma or extrahepatic malignant tumors. Levels of alanine aminotransferase (ALT), albumin (ALB), and TBil, along with INRs, MELD scores, survival times, and the incidence of liver failure–associated serious complications, such as infections, encephalopathy, gastrointestinal bleeding and HRS, were recorded by investigators and compared as an evaluation of short-term outcomes after allogeneic BM-MSC infusions. In our study, we defined dALT, dALB, dTBil, dINR, and dMELD score as the change of liver function between baseline and the most favorable value in a given period, to indicate the improvement of liver function. The groups were compared with regard to these changes occurring during the first 4 weeks and during the entire 24-week period. We defined severe infection as follows: infection caused by more than two pathogens (e.g., bacterial, fungal) or in more than 2 sites (e.g., respiratory tract, abdominal cavity, urinary tract, digestive tract, respiratory tract), needing intravenous antibiotics, or leading to unstable blood pressure requiring vasoactive pressor treatment.

ALLOGENEIC BM-MSCs: MARROW DONORS

Human bone marrow donated by healthy donors after informed consent, including an understanding of

the study, the inclusion and exclusion criteria, the process of bone marrow aspiration, and the consequences of bone marrow donation, was used to isolate and expand MSCs. All of the donors met all donor eligibility criteria for allogeneic blood donors, including negativity for anti–hepatitis A virus immunoglobulin M antibody, HBV antigen, anti–hepatitis C virus antibody, hepatitis D virus antigen, anti-hepatitis E virus immunoglobulin M/immunoglobulin G antibodies, anti–human immunodeficiency virus-1/2 antibodies, anti-human T lymphotropic virus I/II antibody, cytomegalovirus antigen, syphilis, bacteria, and fungi. The age of the donors was ≥18 and ≤25 years. Donors were in good health, without metabolic disease, hematologic diseases, allergic diseases, inherited diseases, autoimmune disease, or mental disorders.

COLLECTION, SEPARATION, AND CULTURE OF BM-MSCs

Bone marrow was obtained from the iliac crests of healthy volunteers and diluted 1:1 with phosphate-buffered saline. Following current good manufacturing practices, mononuclear bone marrow cells were isolated by Ficoll-Hypaque (1.077 g/mL; HuaJing bio-tech Co., Ltd. Shanghai, China) and centrifugation and then cultured at a cell density of 1×10^6/mL in low-glucose Dulbecco's modified Eagle medium (Hyclone, Logan, UT) supplemented with 10% fetal bovine serum; culture medium was replaced with fresh medium every 3 days. At 70%-80% confluence, MSCs were passaged following trypsin treatment. MSCs were harvested at passage 5-6 and frozen in liquid nitrogen (Supporting Fig. S1A). Prior to injection, cells were thawed and washed. Cells were negative for bacteria, endotoxin, HBV, hepatitis C virus, human immunodeficiency virus, syphilis, and fungus. Cell phenotype was assessed by flow-cytometric analysis and differentiation into adipocytes and osteocytes in culture was confirmed (Supporting Fig. S1B,C). The culture-expanded MSCs had a spindle-shape morphology and expressed the surface markers cluster of differentiation 29 (CD29), CD44, CD73, CD90, CD105, and CD166 but not the hematopoietic markers CD45 and CD34 (Supporting Fig. S1D).

ALLOGENEIC BM-MSC INFUSION

Allogeneic BM-MSCs in suspension at the appropriate dose were transferred into a 50-mL sterile syringe. The suspension tube was then washed with 10 mL of normal saline. Both the BM-MSC suspension and the washing fluid were transfused intravenously within 30 minutes using an infusion pump. During the infusion, the suspension was shaken slightly every 3 minutes to avoid adherence of BM-MSCs.

OUTCOME MEASURES

The primary outcome measures comprised survival time and survival status of patients after allogeneic BM-MSC infusions. The secondary outcome measures included (1) the incidence of adverse reactions (e.g., fever, rash, diarrhea), (2) the influence on liver function (including levels of ALT, ALB, TBil, INR, and MELD score), (3) the incidence of serious complications (including infections, encephalopathy, HRS, and gastrointestinal bleeding), and (4) causes of death.

STATISTICAL ANALYSIS AND SAMPLE SIZE

Clinical and biochemical data were expressed as frequencies, means ± standard deviation, or median and range, as appropriate. Frequencies were compared using the chi-squared test, and the quantitative data were compared using the Student t test (when values were normally distributed) or the nonparametric Mann-Whitney U test. Survival rates were calculated. Kaplan-Meier curves were delineated and compared using the log-rank test. Changes of liver functions (delta [d] values) were used to assess the severity of liver disease, as described above. All data were processed by SPSS 17.0 software (SPSS Inc., Chicago, IL), and a value of $P < 0.05$ was considered statistically significant. This study is registered at ClinicalTrials.gov (NCT01322906).

To determine the required sample size, the following criteria were taken into consideration: level = 0.05 (two-sided); statistical power = 0.8; proportion in control group = 0.5; mortality in control group = 54.7%, based on prior research[5]; and estimated treatment benefit = 50%. The required sample size was 102 (51 in each group). To accommodate an expected 10% dropout or lost-to-follow-up rate, the final enrollment was 112 patients.

Results

PROFILE AND GENERAL CHARACTERISTICS AT BASELINE

Of the 110 study participants, 56 received BM-MSC infusions. A total of 39 patients died by the end

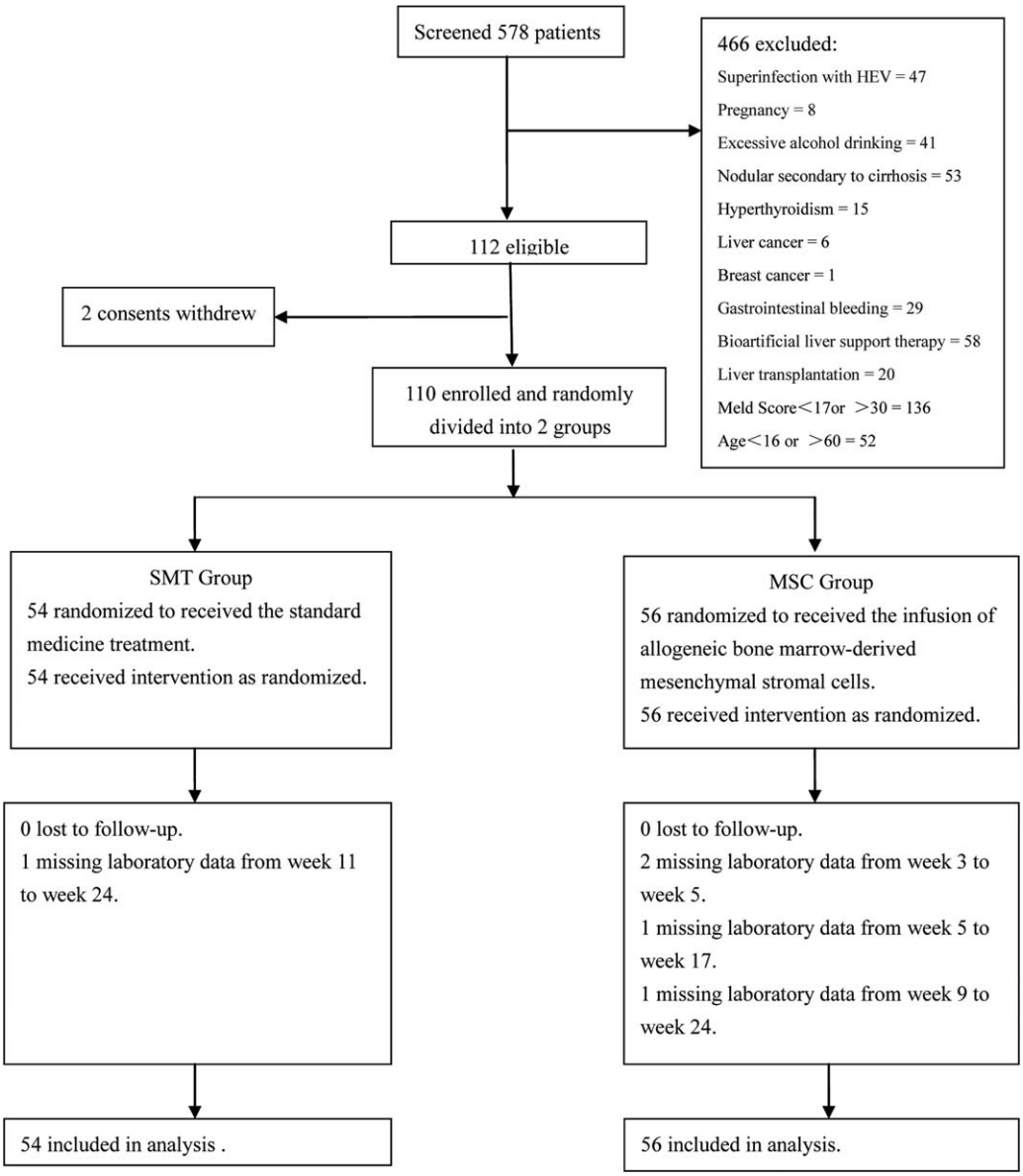

FIG. 1. Study profile. A total of 578 HBV-related ACLF patients were screened from October 2010 to April 2013, and 112 patients qualified for this study. In the end, 110 participants were enrolled and divided into two groups randomly (SMT group = 54, MSC group = 56). No participants were lost to follow-up. Abbreviation: HEV, hepatitis E virus.

of the study (24 in the SMT group and 15 in the MSC group). No patient was lost to follow-up in either group: however, 1 participant in the SMT group and 4 in the MSC group were missing some laboratory data (Fig. 1). In all, 96.4% (54/56) in the MSC group and 96.3% (52/54) in the SMT group were in the hospital for at least 4 weeks (or up to death, whichever came first). There were no significant differences in baseline clinical and biochemical profiles between the two patient groups (Table 1).

SURVIVAL

The SMT and MSC groups had 24-week cumulative survival rates of 55.6% (95% confidence interval 42.3%-68.9%) and 73.2% (95% confidence interval 61.6%-84.8%), respectively ($P = 0.03$ by log-rank test) (Fig. 2).

SAFETY

There were no adverse events during the first 6 hours after infusion. Fever was the most common adverse event

TABLE 1. Clinical and Biochemical Index of the Patients at Baseline

	SMT (n = 54)	MSC (n = 56)	*P*
Age (years)	42.8 ± 8.4	40.0 ± 9.9	0.12
Sex, male (%)	53 (98.2%)	51 (91.1%)	0.21
WBC ($\times 10^9$/L)	7.1 ± 2.6	6.1 ± 2.7	0.06
Hb (g/L)	107.8 ± 17.7	105.4 ± 16.3	0.46
PLT ($\times 10^9$/L)	111.4 ± 56.3	98.4 ± 42.3	0.18
Cr (μmol/L)	70.7 ± 15.2	67.2 ± 13.3	0.20
ALT (U/L)	151.0 ± 97.5	122.3 ± 91.9	0.12
ALB (g/L)	34.7 ± 4.4	35.9 ± 4.3	0.13
TBil (μmol/L)	457.3 ± 114.6	495.2 ± 164.4	0.16
INR	2.4 ± 0.7	2.3 ± 0.7	0.28
HBV DNA* (IU/mL)	5.1 ± 2.1	5.1 ± 1.8	0.84
MELD score	25.5 ± 3.5	25.1 ± 3.8	0.59
HBeAg-positive (%)	16 (29.6%)	25 (44.6%)	0.12
Cirrhosis (%)	19 (35.2%)	22 (39.3%)	0.53
Ascites (%)	44 (81.5%)	52 (92.9%)	0.07
Infection† (%)	51 (94.4%)	54 (96.4%)	0.68
Encephalopathy (%)	13 (24.1%)	7 (12.5%)	0.12
HRS (%)	0 (0.0%)	0 (0.0%)	—
Gastrointestinal bleeding (%)	0 (0.0%)	0 (0.0%)	—

*The titers of HBV DNA were determined logarithmically before analysis.
†Infection included spontaneous bacterial peritonitis (SMT, 22; MSC, 25), bile tract infection (SMT, 39; MSC, 40), bacterial pneumonia (SMT, 3; MSC, 5), fungal pneumonia (SMT, 1; MSC, 0), digestive tract fungal infection (SMT, 3; MSC, 2), urinary tract infection (SMT, 0; MSC, 1), and upper respiratory tract infection (SMT, 0; MSC, 1).
Abbreviations: Cr, creatinine; Hb, hemoglobin; HBeAg, hepatitis B e antigen; PLT, platelet count; WBC, white blood cell count.

during the 24-week follow-up period, often caused by respiratory infections, especially upper respiratory tract infection (Table 2). The incidence of fever in the MSC group was higher than that in the SMT group at 5-24 weeks (19.2% versus 2.4%, respectively, $P = 0.02$). However, patients usually recovered or significantly improved after treatment. There were no significant differences in white blood cell count, hemoglobin, platelets, and creatinine between the two groups after each infusion time point. No hepatocellular carcinoma or tumors in other organs were found in any study participant.

LIVER FUNCTION AND SERIOUS COMPLICATIONS

The specific changes of liver functions (including ALT, ALB, TBil, INR, and MELD score) at each time point are listed in Table 3: the levels of ALT and ALB in the MSC group had improved more significantly than the levels in the SMT group at week 1. The MELD score in the MSC group had decreased more dramatically than that in the SMT group at weeks 1 and 2. No difference was found at other time points in levels of ALT, ALB, and MELD score; and the levels of TBil and INR did not vary among all time points. The results of delta value comparison indicated that during the first 4 weeks the improvements of TBil and MELD score in the MSC group were significantly greater compared with those in the SMT group (151.2 ± 158.7 versus 84.0 ± 138.5, $P = 0.02$, Fig. 3C; 3.1 ± 4.3 versus 0.4 ± 5.5, $P = 0.00$, Fig. 3E). There were no dramatic

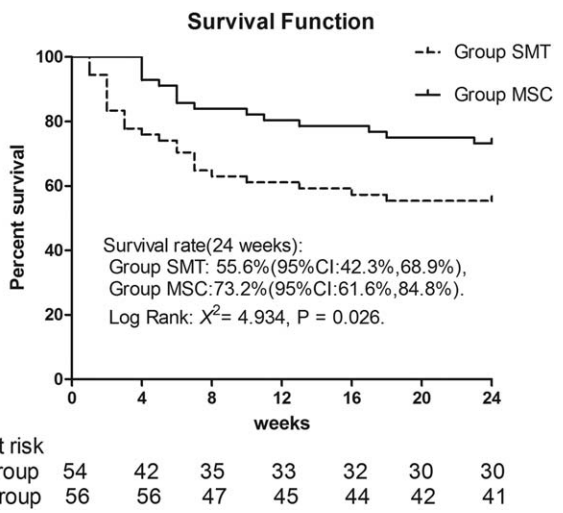

FIG. 2. Kaplan-Meier survival curves. The Kaplan-Meier curve was analyzed using the log-rank test. The solid line represents percent of surviving patients after MSC treatment, while the dashed line represents SMT treatment. The log-rank test revealed that there was a significant difference between the two survival curves ($P = 0.03$). Abbreviation: CI, confidence interval.

TABLE 2. Side Effects During Weeks 1-4 and weeks 5-24

	SMT (n = 54)	MSC (n = 56)	*P*
Fever			
1-4 weeks	12	15	0.58
5-24 weeks	1	10	0.02*
Rash			
1-4 weeks	3	5	0.72
5-24 weeks	4	3	0.70
Diarrhea			
1-4 weeks	7	7	0.94
5-24 weeks	0	0	–

*$P < 0.05$.

differences in dALT, dALB, and dINR between the two groups (Fig. 3A,B,D). As to the entire 24-week period, the decreases of TBil and MELD score in the MSC group were more significant than those in the SMT group (313.8 ± 233.8 versus 198.6 ± 223.4, $P = 0.01$, Fig. 3C; 9.4 ± 7.7 versus 4.7 ± 9.6, $P = 0.01$, Fig. 3E). No significant differences were found for dALT, dALB, and dINR between the two groups when considering the entire 24-week period (Fig. 3A,B,D).

No differences were observed in incidences of other serious complications (e.g., encephalopathy, HRS, gastrointestinal bleeding) between the SMT and MSC groups (Table 4). But the incidence of infection in the MSC group was much lower than that in the SMT group (25.0% versus 44.4%, $P = 0.03$).

In this study, 39 patients died: 24 in the SMT group and 15 in the MSC group. Of these 39, 30 had multiple organ failure (SMT group, 20; MSC group, 10), and the other causes of death were severe infections (SMT group, 18, including 6 severe infections only, 9 complicated with HRS, and 3 with encephalopathy; MSC group, 9, including 4 severe infections only, 3 with HRS, and 2 with encephalopathy), hepatic coma (SMT group, 7; MSC group, 4), and HRS (SMT group, 10; MSC group, 5). No differences were found between the two groups in the constituent ratio of death, but in the SMT compared with the MSC group more patients died from multiple organ failure (37.0% versus 17.9%, $P = 0.02$) and severe infections (33.3% versus 16.1%, $P = 0.04$) (Table 4).

Discussion

ACLF results in multiple organ failure and high short-term mortality, and the current standard treatment

TABLE 3. Levels of ALT, ALB, TBil, INR, and MELD Score in the Two Groups at Baseline and at Weeks 1, 2, 3, 4, 8, 12, and 24

	ALT			ALB			TBil			INR			MELD Score		
	SMT	MSC	*P*	SMT	MSC	*P*	SMT	MSC	*P*	SMT	MSC	*P*	SMT	MSC	*P*
Baseline	115.0 (73.0-221.3)	94.5 (61.5-145.3)	0.10	34.8 (31.9-36.6)	35.9 (32.9-38.2)	0.10	458.9 (387.3-539.0)	501.3 (405.7-565.6)	0.17	2.4 (1.9-2.8)	2.2 (1.8-2.7)	0.23	25.2 (22.8-27.8)	24.8 (22.4-28.3)	0.70
Week 1	89.5 (68.0-193.5)	71.5 (46.0-105.8)	0.01*	34.7 (30.9-36.9)	38.1 (34.3-41.3)	0.00*	467.9 (385.1-560.9)	475.7 (374.2-594.9)	0.92	2.5 (1.9-3.1)	2.2 (1.8-2.7)	0.08	26.6 (23.1-30.4)	24.1 (21.5-27.5)	0.01*
Week 2	61.5 (47.5-100.5)	61.0 (37.8-83.5)	0.12	36.9 (34.2-40.9)	38.0 (35.2-41.9)	0.08	472.0 (353.7-567.8)	395.3 (255.3-603.1)	0.17	2.3 (1.8-3.1)	2.2 (1.8-2.6)	0.25	25.8 (22.2-29.1)	24.1 (20.1-26.4)	0.02*
Week 3	55.0 (40.0-82.5)	47.5 (31.8-74.3)	0.27	38.2 (35.8-40.6)	37.5 (34.8-40.8)	0.56	431.6 (300.8-528.1)	370.1 (203.6-545.4)	0.37	2.1 (1.8-2.8)	2.2 (1.8-2.8)	1.00	24.5 (20.8-30.2)	24.3 (20.3-27.6)	0.37
Week 4	55.5 (37.0-72.5)	42.5 (27.8-64.3)	0.07	38.7 (35.8-41.8)	38.6 (34.8-41.8)	0.80	375.8 (188.4-533.7)	316.5 (154.0-514.8)	0.44	2.1 (1.6-3.0)	2.2 (1.8-2.8)	0.76	22.8 (19.6-29.0)	23.0 (18.2-27.7)	0.54
Week 8	42.0 (35.3-61.5)	42.5 (30.8-59.3)	0.64	37.2 (35.0-40.7)	38.3 (35.7-40.6)	0.82	133.0 (87.8-405.1)	170.9 (81.3-352.0)	0.66	1.8 (1.5-2.4)	2.0 (1.5-2.7)	0.66	18.4 (14.3-26.4)	19.1 (12.0-24.1)	0.77
Week 12	43.0 (34.3-53.0)	36.0 (26.0-54.0)	0.12	38.5 (34.5-40.5)	37.2 (34.5-39.0)	0.20	79.3 (43.0-176.7)	97.2 (49.5-199.9)	0.85	1.7 (1.4-2.0)	1.8 (1.2-2.1)	0.89	14.8 (11.5-19.6)	14.7 (9.7-20.4)	0.89
Week 24	43.0 (32.0-64.0)	38.0 (23.3-56.8)	0.19	39.6 (35.3-41.6)	37.8 (35.0-41.6)	0.47	30.1 (20.8-88.9)	43.9 (25.3-80.5)	0.25	1.5 (1.3-1.8)	1.4 (1.2-1.8)	0.34	11.3 (6.9-14.8)	11.0 (7.4-13.9)	0.94

*$P < 0.05$.

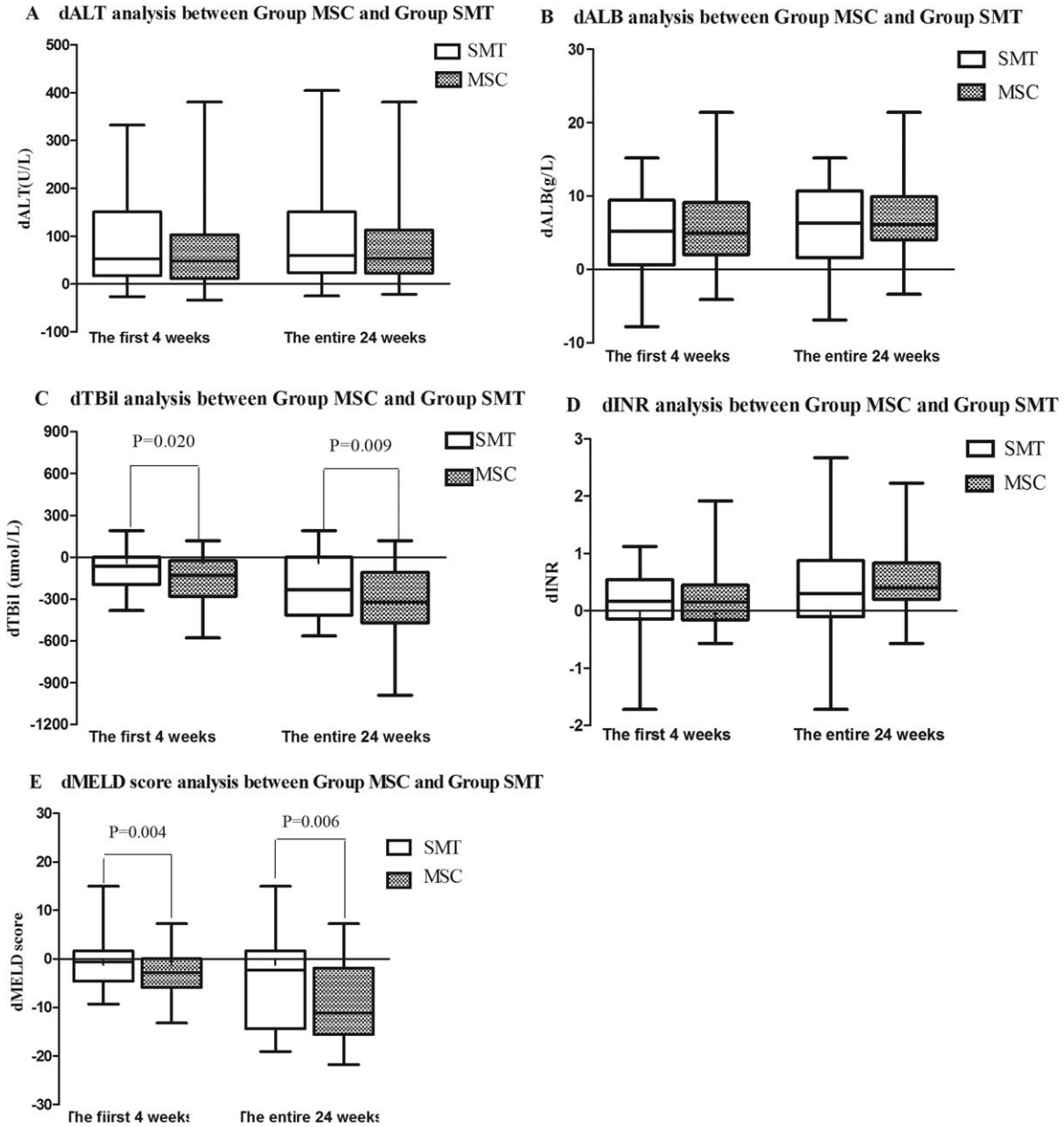

FIG. 3. Comparison of liver function alterations between the SMT and MSC groups. Liver functions of the study groups during two periods (the first 4 weeks and the entire 24 weeks) were compared. dALT, dALB, dTBil, dINR, and dMELD scores were defined as the change of liver function between baseline and the most favorable value in a given period. (A) No dramatic differences in dALT were observed in either time period. (B) No dramatic differences in dALB were observed in either time period. (C) The improvement of TBil in the MSC group was significantly greater compared with that in the SMT group (first 4 weeks, 151.2 ± 158.7 versus 84.1 ± 138.5, $P = 0.02$; entire 24 weeks, 313.8 ± 233.8 versus 198.6 ± 223.4, $P = 0.01$). (D) No dramatic differences in dINR were observed in either time period. (E) The improvement of the MELD score in the MSC group was significantly greater compared with that in the SMT group (first 4 weeks, 3.1 ± 4.3 versus 0.4 ± 5.5, $P = 0.00$: entire 24 weeks, 9.4 ± 7.7 versus 4.7 ± 9.6, $P = 0.01$). $P < 0.05$ indicates statistical significance.

remains far from satisfactory. Recent studies have shown that granulocyte colony-stimulating factor therapy can improve liver function and outcome of patients with ACLF or decompensated cirrhosis.[20-22] However, another study investigating reinfusion of $CD133^+$ stem/progenitor cells for end-stage liver disease found worsening liver function and creatinine levels in Child-Turcotte-Pugh C patients during the mobilization

TABLE 4. Serious Complications and Causes of Death

	SMT (n = 54)	MSC (n = 56)	*P*
Serious complications			
Infection*	24	14	0.03†
Encephalopathy	14	8	0.13
Hepatorenal syndrome	12	6	0.10
Gastrointestinal bleeding	3	1	0.36
Causes of death			
Severe infection‡	18	9	0.04†
Hepatic coma	7	4	0.31
Hepatorenal syndrome	10	5	0.14
Gastrointestinal bleeding	2	1	0.62
Multiple organ failure	20	10	0.02†

*Infections included spontaneous bacterial peritonitis (SMT, 20; MSC, 13), bile tract infection (SMT, 22; MSC, 13), bacterial pneumonia (SMT, 7; MSC, 6), fungal pneumonia (SMT, 2; MSC, 0), digestive tract fungal infection (SMT, 5; MSC, 2), urinary tract infection (SMT, 0; MSC, 1), and sepsis (SMT, 1; MSC, 1).
†$P < 0.05$.
‡Severe infection: infection caused by more than two pathogens (e.g., bacterial, fungal), or in more than two sites (e.g., respiratory tract, abdominal cavity, urinary tract, digestive tract, respiratory tract), needing intravenous antibiotics, or leading to unstable blood pressure requiring vasoactive pressor treatment.

procedure by granulocyte colony-stimulating factor.[23] The efficacy of granulocyte colony-stimulating factor therapy is still not clear. Therefore, novel therapeutic strategies for HBV-ACLF treatment are urgently needed.

The immunomodulatory and reparative functions of MSCs have been demonstrated to be therapeutically valuable for treating various diseases including autoimmune diseases,[24] diabetes,[25] myocardial infarction,[26] and organ transplantation rejection.[27] The present prospective randomized trial underlines the feasibility of MSC treatment for patients with ACLF.

This study demonstrated that MSCs improved the 24-week survival rate of patients with HBV-ACLF, which was likely due to the liver function improvement and a decrease of severe infections. Furthermore, this treatment was safe and without severe adverse reactions, suggesting the feasibility of development of cell-based therapies for HBV-ACLF and possibly other end-stage liver diseases with similar clinical features.

The survival benefit for the MSC group might have resulted from the improvement of liver function, including TBil and MELD score. In addition, the decreased incidence of serious complications such as severe infection contributed to the increased survival rate. Immune imbalance and systemic inflammatory reactions play key roles in the development of liver failure.[28,29] Higuchi et al. injected methylprednisolone intravenously for treatment in an acute liver failure rat model and found that the resulting high hepatic steroid concentration acted on inflammatory cells and suppressed the inflammatory response.[30] Short-term dexamethasone therapy in acute-on-chronic pre–liver failure (which was defined as a severe acute episode of chronic hepatitis B characterized by serum bilirubin of 171 μmol/L or more, ALT of 5 times or more the upper limit of normal, and prothrombin activity of >40%, having a potential for progression to ACLF) was effective at improving liver function and the survival rate of patients.[31] Although little is known about the mechanisms involved in the treatment of ACLF by MSCs, we speculate that the immunomodulation and anti-inflammation functions of BM-MSCs consequently alleviated the hepatic inflammation, improved liver function, decreased the incidence of fatal complications (especially severe infection), and enhanced the survival rate. This is consistent with the results of a preclinical animal study.[11]

In the current study, we compared liver function parameters at defined time points between groups, and the results show slight improvement in levels of ALT and ALB and in the MELD score (Table 3). But these results alone are not sufficient to assess the influence of MSC infusion on ACLF patients. As ACLF is a severe disease with high mortality, about 35% (39/110) of patients in our study died: data on these patients were not included in the analysis of the subsequent defined time points (following death), so the study information is incomplete. Using the delta value of liver functions can partially solve this problem, and by combining these 2 methods we can evaluate the influence of MSC infusion on the liver functions of ACLF patients more comprehensively. But we admit that this approach may also lead to a substantial bias, resulting in an overestimate of the effect of treatment.

In our study, no differences were found in ALB and ALT levels or in INRs; the reason may be that all patients received ALB supplements and plasma after admission. In clinical practice, we found that ALT elevated earlier than TBil in ACLF patients, and shortly thereafter, ALT declined while TBil increased. In our study, most patients were treated in other hospitals before admission to ours. When enrolled in our study, their ALT levels had already declined. That may be the main reason why no difference in ALT level was seen between our two study groups.

Our study indicated that allogeneic BM-MSC infusion for HBV-induced ACLF patients was safe without severe adverse reactions, which is consistent with

the observations of our previous study of treating end-stage liver disease patients with autologous BM-MSC infusions.[17] Although 18 of the patients developed fever and/or rash after BM-MSC infusions, the symptoms remitted when treated appropriately. In our study, fever occurred more frequently in the MSC group, often with upper respiratory tract infection. We speculate that this may have been a reaction to minimal residual phosphate-buffered saline as some patients' fever lasted less than 2 hours without any respiratory symptoms. Because MSCs do not express human leukocyte antigen class II or costimulatory molecules and have unique immunosuppressive properties, allogeneic MSCs may escape the recipient's immune surveillance when used for transplantation. A characteristic of ACLF is its rapid progression, and use of allogeneic MSCs may offer immediate availability without delay from the required cell culture of autologous MSCs. Moreover, systemic disease may affect the characteristics of autologous MSCs: MSCs isolated from multiple sclerosis patients have distinct gene expression profiles and decreased suppressive functions compared with their healthy counterparts.[32] Our group also demonstrated that MSCs derived from hepatitis B patients proliferated slowly and tended to undergo senescence.[18] It is thus evident that allogeneic MSCs, compared to autologous MSCs, have more potential advantages for the treatment of ACLF. HBV-ACLF is a disease with acute onset and rapid progression. Effective and timely intervention is of great importance for successful treatment. Allogeneic stem cell transplantation could potentially provide an alternative new therapy to meet these needs, particularly because allogeneic MSCs could be manufactured in standard production according to clinical requirements and then kept as convenient and safe frozen stock, ready for use.

Infection (especially spontaneous bacterial peritonitis and bile tract infection) is a common feature of ACLF, which complicates the natural history and is associated with significant morbidity and mortality.[33] In this study, severe infections and multiple organ failure were the main causes of death of HBV-ACLF patients. This study suggests that MSC infusions could decrease the occurrence of severe infection or death, most likely due to the immune repairing and immunoregulation functions of MSCs.[14,34,35]

There are some limitations in this study which we would like to point out to improve future investigations. Following our previous experience[5] (and the outcome of this study) that about 80% of deaths of ACLF patients occur within 12 weeks and that surviving patients will still be alive after 24 weeks, we used 24 weeks of follow-up to measure any curative effect. Nevertheless, 24 weeks was not long enough for complete evaluation of safety. Additionally, in our study, hospitalization time was not the same for all patients. Most patients were in hospital for at least 4 weeks, while there were a few patients discharged earlier than expected; once patients are discharged, the assessment of adverse effects/complications becomes less accurate. Also, as an open-label study, neither participants nor investigators were blinded to the intervention, so the interpretation of adverse events was inevitably prone to bias. Finally, the current study was conducted at a single center, which limits generalization of the results. The findings reported here could be further substantiated in a study with a more diversified patient population and a longer follow-up time in multiple centers.

In summary, peripheral infusion of allogeneic BM-MSCs is effective, safe, and feasible for treatment of patients with HBV-related ACLF. Our study clearly highlights the ability of allogeneic BM-MSCs to modulate the course of severe liver diseases. The current data support the need for future investigation of the use of allogeneic MSCs in a large-scale, well-designed, randomized, double-blind, placebo-controlled trial.

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