Therapeutic effect of autologous bone marrow cells injected into the liver under the guidance of B-ultrasound in the treatment of HBV-related decompensated liver cirrhosis

LEI LI[1*], YANHUI SI[1*], MINGRONG CHENG[2], LIN LANG[3], AIJUN LI[4] and BAOCHI LIU[1,2]

[1]Department of Surgery, Shanghai Public Health Clinical Center, Fudan University, Shanghai 201508; [2]Henuo Medical Clinic, Shanghai New Hongqiao International Medical Center, Shanghai 201100; [3]Department of General Surgery, The First Affiliated Hospital of Zhengzhou University, Zhengzhou, Henan 450052; [4]Department of Hepatobiliary Surgery, Oriental Hepatobiliary Surgery Hospital Affiliated to Naval Military Medical University, Shanghai 200433, P.R. China

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Abstract. The 5-year mortality rates associated with decompensated liver cirrhosis (DLC) can reach 50%, which suggests that this condition poses a serious health risk. In previous studies conducted by our group, autologous bone marrow nucleated cells (ABMNCs) were used to treat HIV-positive patients with DLC through the right omental vein; however, trauma and poor compliance were encountered. In the present study, the percutaneous liver approach to inject ABMNCs under the guidance of B-ultrasound was employed for the treatment of DLC. A total of 108 patients with DLC were retrospectively divided into the routine drug treatment group (control group; 30 cases), the right omental vein infusion of ABMNCs group (observation group 1; 38 cases) and the B-ultrasound-guided liver injection of ABMNCs group (observation group 2; 40 cases). After treatment, the liver synthesis (prothrombin time, albumin and ascites) and secretion functions (total bilirubin) in observation groups 1 and 2 were significantly improved compared with those of the control group (P<0.01) and the bone marrow function was also significantly improved compared with that of the control group (P<0.01). While, the bone marrow function (white blood cell, platelet, and hemoglobin) in observation group 1 was significantly improved compared with that of observation group 2 at the end of treatment (P<0.01). After a 1-year follow-up, the case fatality rate was 2.5% (1/40) in observation group 2, which was significantly lower than the 20% fatality rate (6/30) recorded in the control group (P<0.05). The injection of ABMNCs into the liver under the guidance of B-ultrasound was significantly better than conventional drug therapy in treating DLC. This approach has obvious advantages such as no hospitalization, minimal trauma, rapid recovery and good compliance, all of which make it worthy of application in primary hospitals.

Correspondence to: Dr Baochi Liu, Department of Surgery, Shanghai Public Health Clinical Center, Fudan University, 2901 Caolang Road, Jinshan, Shanghai 201508, P.R. China
E-mail: liubaochi200227@aliyun.com

*Contributed equally

Abbreviations: ABMNCs, autologous bone marrow nucleated cells; ALB, albumin; DLC, decompensated liver cirrhosis; HGB, hemoglobin; PLT, platelet; PT, prothrombin time; TB, total bilirubin; WBC, white blood cell

Key words: minimally invasive treatment, DLC, bone marrow stem cells, surgical treatment, nucleated cell therapy

Introduction

Liver cirrhosis is a chronic, progressive and diffuse liver disease that includes liver cell degeneration, apoptosis, necrosis, connective tissue regeneration, and the destruction of ultimately normal vascular anatomy and liver lobule structure under the action of hepatitis B virus (HBV) (1). HBV has been identified as the main cause of liver cirrhosis in China, accounting for 40-60% of all cases of liver cirrhosis (2). After the destruction of the liver structure, blood vessels are blocked, and liver function is in the decompensated stage (3). Following this, serious complications, including gastroesophageal varices, hepatic encephalopathy, ascites and hepatorenal syndrome, can occur (4). According to modern medicine, once liver cirrhosis develops, the liver enters an irreversible chronic pathological state (5). In patients with liver cirrhosis, the main focus is on the etiology and palliative treatment, such as liver protection, anti-inflammatory, anti-fibrosis, anti-virus, immune regulation and symptomatic support treatment (6). In the decompensated stage, interventions to induce diuresis, increase colloid osmotic pressure and reduce portal vein pressure are used to treat ascites, hypoproteinemia, portal hypertension and other pathological changes (7). However, the overall curative effect is generally poor, and electrolyte disorders, heart failure, upper gastrointestinal bleeding and other complications are still commonly found (8). Therefore, this condition can pose a serious threat to the life of patients.

The treatment of decompensated liver cirrhosis (DLC) is mainly based on protecting liver function and alleviating

symptoms; however, the curative effect is usually poor (9). Liver transplantation is finally considered for end-stage liver disease, although suitable donor organs are scarce (10). In addition, the cost of liver transplantation is high, and the associated complications and risk of rejection of liver transplantation are relatively common (11). Stem cells are undifferentiated cells with the potential to differentiate into various cell types. The bone marrow contains hematopoietic stem cells, mesenchymal stem cells, endothelial progenitor cells and other undifferentiated cells (12). Once these undifferentiated cells from the bone marrow are transplanted into the liver with hepatocyte injury, they can differentiate into hepatocytes in the liver microenvironment, or they can secrete certain cytokines to promote the repair of damaged hepatocytes and improve liver function (13). The emerging bone marrow stem cell transplantation for the treatment of DLC has the advantage of being a simple and low pain procedure, which is characterized by repeatability, weak immunogenicity and low treatment costs (14). This approach can ameliorate the clinical symptoms, as well as liver function and Child-Pugh score, of patients with DLC to varying degrees (15). Therefore, it is expected to be a novel approach for the treatment of DLC. In a previous study, our team used an intravenous injection of autologous bone marrow nucleated cells (ABMNCs) administered through the right omental vein to treat patients with DLC and HIV, revealing marked improvements in the liver function and survival time of patients (16). However, due to the need for surgical catheterization, great trauma and poor compliance were observed. In order to overcome these limitations, ABMNCs were injected into the liver under the guidance of ultrasound in the present study. This was compared with the efficacy of injecting the ABMNCs into the portal vein through the infusion port, which was established as a channel via right omental vein cannulation, and the liver synthesis, secretion and bone marrow function were compared in two different treatment methods. The results of the different treatments are summarized below.

Materials and methods

Clinical data. A total of 108 patients with DLC, including 69 males and 39 females, with an average age of 47.53±8.82 years (range, 27-75 years), diagnosed at Shanghai Public Health Clinical Center (Shanghai, China), Shanghai Oriental Hepatobiliary Hospital (Shanghai, China) and Shanghai New Hongqiao International Medical Center (Shanghai, China) between January 2018 and December 2021, were included in this retrospective analysis. Patients were divided into the control group (n=30), observation group 1 (n=38) and observation group 2 (n=40) according to different treatment methods. The control group included 17 male and 13 female patients with an average age of 46.47±9.19 years (range, 27-75 years) and average disease course of 3.76±1.38 years (range, 1-10 years), while the Child-Pugh grades were as follows: Grade B (n=18) and grade C (n=12). Observation group 1 included 26 male and 12 female patients with an average age of 47.53±8.51 years (range, 27-69 years) and an average course of disease of 3.65±1.52 years (range, 1-10 years), while the Child-Pugh grades were as follows: Grade B (n=20) and grade C (n=18). Observation group 2 included 26 male and 14 female patients with an average age of 47.53±9.21 years (range, 27-75 years) and an average disease course of 3.81±1.53 years (range, 1-10 years), while the Child-Pugh grades were as follows: Grade B (n=24) and grade C (n=16). Written informed consent form was signed by all patients and the present study was approved by the Ethics Committee of the Shanghai Public Health Clinical Center (Shanghai, China; approval no. 2018-S035-02). There were no significant differences in baseline data, such as age, sex, course of disease and liver function classification among the three groups. The inclusion criteria were as follows: i) Consistent diagnostic criteria for DLC; ii) clear history of HBV infection; iii) no other serious basic diseases (chronic respiratory or circulatory systems diseases, among others); and iv) no cell biology-related treatment used before hospitalization. The exclusion criteria were: i) Cirrhosis due to other causes (other than HBV or alcoholic cirrhosis); ii) combined with hepatic coma, severe hepatitis (such as, within 10 days, bilirubin levels >10 times the upper limit of normal or prothrombin activity <40%) and primary liver cancer; iii) participation in other clinical trials; iv) combined with other serious systemic diseases; v) allergic to drugs in this test; vi) blood and immune diseases; vii) malignant tumors; viii) spontaneous peritonitis; ix) uncontrollable gastrointestinal bleeding; x) hepatorenal syndrome; and xi) intellectual disability or mental illness.

Diagnostic criteria. The diagnostic criteria for DLC (17) were: i) Formation of liver cirrhosis identified through computed tomography, color Doppler ultrasound or liver biopsy; ii) liver cirrhosis diagnosed using liver hardness scan; iii) albumin (ALB) level <35 g in liver function test; iv) gastroscopy showing signs of esophageal and gastric varices; v) platelet (PLT) count $<100x10^9$/l; vi) esophagus-gastric varices by gastroscopy; vii) prothrombin time (PT) over the normal value for 3 sec; and viii) ascites formation. DLC was diagnosed if any three of the diagnostic criteria i)-v) and any one of the diagnostic criteria vi)-viii) were met.

Therapeutic method. The control group underwent routine basic treatment, which mainly included bed rest, nutritional support, supplementation of coagulation factors, correction of water and electrolyte disturbances, prevention of nosocomial infections, liver protection (reduced glutathione), diuresis (furosemide), ALB input, antiviral therapy such as lamivudine and entecavir (for patients with active virus replication), prevention of ectopic gut bacteria (lactulose), hepatoprotective therapy (reduced glutathione) and immunomodulators (thymosin α1).

For observation group 1, infusion of ABMNCs through right omental vein intubation was performed. After general anesthesia or local anesthesia, a small 4-cm incision was made into the upper abdomen with a scalpel according to our previous operation methods (9) and the infusion port of the right omental vein was established. After the operation, 100 ml bone marrow was collected from the anterior superior iliac crest and ~10 ml ABMNCs were separated using density gradient centrifugation (1,000 x g for 10 min at room temperature). The total number of nucleated cells was $5\text{-}8x10^9$ cells/l as measured using an automatic blood cell analyzer (Ulite URIT-2981; URIT Medical Electronic Co., Ltd.) and ABMNCs were infused through the infusion port. After the separation of

nucleated cells, the bone marrow plasma and red blood cells were reinfused from the peripheral vein. The same procedure was repeated twice at 1 and 3 months after treatment.

For observation group 2, infusion of ABMNCs was performed under the guidance of B-ultrasound. In general, the patient was placed in the left lying position. Subsequently, under the guidance of B-ultrasound (Mindray DC-N3S; Nanjing Beden Medical Co., Ltd.), the ABMNCs (collected in the same manner as in observation group 1) were injected into the liver through the sixth intercostal puncture at the axillary front. The gradual diffusion of ABMNCs from the puncture point to the surrounding area could be observed using the real-time B-ultrasound. Furthermore, after separation, the bone marrow plasma and red blood cells were reinfused from the peripheral vein (steps A-C; Fig. 1). The same procedure was repeated twice at 1 and 3 months after treatment.

All three groups of patients were followed up for 1 year; the indicators of liver synthesis, secretion function and bone marrow function were observed in the two groups, and the mortality rate of each group was compared.

Retention of blood samples and detection of indicators. Prior to treatment, and at 1, 3, 6 and 12 months after treatment, ~6 ml fasting cubital venous blood was drawn and ~3 ml venous blood was taken, placed at room temperature for 20 min and then centrifuged for 10 min using a centrifuge with a centrifugal radius of 15 cm and a speed of 1,000 x g at room temperature. The serum was separated for further analysis stored in the refrigerator at -70˚C. The serum total bilirubin (TB) and ALB levels were detected using an automatic biochemical analyzer (ARCHITECT C16000; Abbott Pharmaceutical Co. Ltd.). An automatic coagulation analyzer [PUN-2045A; BOOPU (Changzhou) Biotechnology Co., Ltd.] was used to detect the PT of anticoagulated whole blood. An automatic blood cell analyzer (SE9000; Sysmex Corporation) was used to determine the white blood cell (WBC) count, PLT count and hemoglobin (HGB) levels in whole coagulated blood.

Determination of ascites. The knee flexion and push-up positions were used, and fluid that accumulated between the anterior abdominal wall and the bowel was detected by B-ultrasound. The abdomen as a spheroid of the radius was r. The spine was parallel to the bed surface, and the ascites were deposited in the anterior part of the abdominal cavity, becoming the part of the spheroid; the depth of ascites was d. The abdominal circumference (C) was measured;, the formula was V=1/3 [πd x d(3r-d)] (note: r=C/2π), and the abdominal liquid volume was calculated.

Figure 1. B-ultrasound-guided ABMNC infusion procedure. (A) Bone marrow was collected from the patient in the supine position. (B) ABMNCs were transfused through the right 6th intercostal space under the guidance of a B-ultrasound in the left lying position. (C) The hyperechoic area was where the ABMNCs were injected, and ultrasound of the liver after rib blockage was performed for the anechoic area after injection. ABMNCs, autologous bone marrow nucleated cells.

Statistical analysis. SPSS 20.0 (IBM Corp.) statistical software was used for data analysis. Normally distributed data are presented as the mean ± standard deviation. Comparisons among multiple groups were performed by mixed analysis of variance followed by Sidak's post hoc test. Non-normally distributed data are presented as the median (25th percentile, 75th percentile), and the pairwise comparisons of medians were performed using Friedman's test followed by Nemenyi test for comparisons among the paired data, after the comparisons among multiple groups were performed using Dunn's post hoc test for pairwise comparisons after the Kruskal-Wallis test. After 1 year of follow-up, the patients with DLC died of liver failure were observed in two groups, the χ^2 test was used to assess fatality rate. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Impact of ultrasound-guided ABMNC injection on liver synthesis in patients with DLC. No significant difference was observed in serum PT, ALB and ascites levels among the three groups before treatment ($P>0.05$; Tables I-III; Fig. 2A-C).

Table I. Serum prothrombin time levels (sec) in the three groups before and after treatment.

Group	Before	1 month	3 months	6 months	12 months
Observation 1	18.35±1.65	17.13±1.56	15.98±1.37	14.90±1.53	14.18±1.73
Observation 2	18.45±1.80	17.16±1.75	15.93±1.40	15.15±1.57	14.34±1.73
Control	18.73±2.12	19.20±2.35	20.50±2.41	20.89±1.91	19.40±3.83

Data are presented as the mean ± standard deviation. Time, F=49.148, P<0.001; intergroup, F=32.221, P<0.001; interaction, F=49.282, P<0.001. The comparison of prothrombin time levels was performed by mixed analysis of variance among the observation 1, observation 2 and control, and followed by Sidak's post hoc test among before, 1, 3, 6 and 12 months groups.

Table II. Serum albumin levels (g/l) in the three groups before and after treatment.

Group	Before	1 month	3 months	6 months	12 months
Observation 1	26.94±6.04	31.37±7.43	33.14±7.90	34.9±8.37	34.17±10.85
Observation 2	29.45±4.25	35.05±3.45	37.13±3.20	39.5±2.30	40.24±2.24
Control	29.00±4.39	28.77±3.13	25.90±7.74	26.96±5.97	24.81±7.75

Data are presented as the mean ± standard deviation. Time, F=177.145, P<0.001; intergroup, F=62.909, P<0.001; interaction, F=68.059, P<0.001. The comparison of albumin levels was performed by mixed analysis of variance among the observation 1, observation 2, control, and followed by Sidak's post hoc test among before, 1, 3, 6 and 12 months groups.

Table III. Number of ascites (ml) in the three groups before and after treatment.

Group	Before	1 month	3 months	6 months	12 months	χ^2 (Nemenyi test)	P-value
Observation 1	1,500 (1,000-2,000)	500 (0-1,000)	0 (0-500)	0	0	65.419	<0.001
Observation 2	1,750 (1,000-1,500)	1,000 (0-1,500)	500 (0-1,000)	0 (0-500)	0 (0-500)	126.538	<0.001
Control	1,500 (1,000-2,500)	2,000 (1,500-2,500)	2,000 (1,500-2,500)	2,000 (2,000-3,000)	2,000 (2,000-3,000)	68.000	<0.001
χ^2 (Kruskal-Wallis test)	1.716	39.471	60.704	70.722	72.458		
P-value	0.424	<0.001	<0.001	<0.001	<0.001		

Data are presented as the median (25th percentile, 75th percentile). The comparison of ascites levels was performed using the Dunn post hoc test used for pairwise comparisons after Kruskal-Wallis test among the observation 1, observation 2 and control groups. Comparisons among the before, 1, 3, 6 and 12 months groups were performed using Friedman's test followed by Nemenyi test.

However, at each time point from 1 to 12 months after treatment, the PT levels in observation group 2 and observation group 1 were decreased, and the ascites were also significantly decreased (P<0.01), whereas the serum ALB levels were significantly increased compared with before treatment (P<0.01). In addition, the ALB level in observation group 1 was significantly lower than that in observation group 2 from 1 to 12 months after treatment (P<0.01).

Impact of ultrasound-guided ABMNC injection on the liver secretions in patients with DLC. No significant difference in serum TB levels was observed among the three groups before treatment (all P>0.05; Table IV; Fig. 3). The serum TB levels in observation group 1 and observation group 2 at each time point from 1 to 12 months after treatment were significantly lower than those before their respective treatments and in the control group (P<0.01), whereas the TB levels did not significantly differ between observation group 1 and observation group 2 at each time point (P>0.05).

Impact of ultrasound-guided ABMNC injection on the bone marrow function in patients with DLC. No significant difference was observed in the WBC, PLT and HGB levels among the three groups before treatment (P>0.05; Tables V-VII;

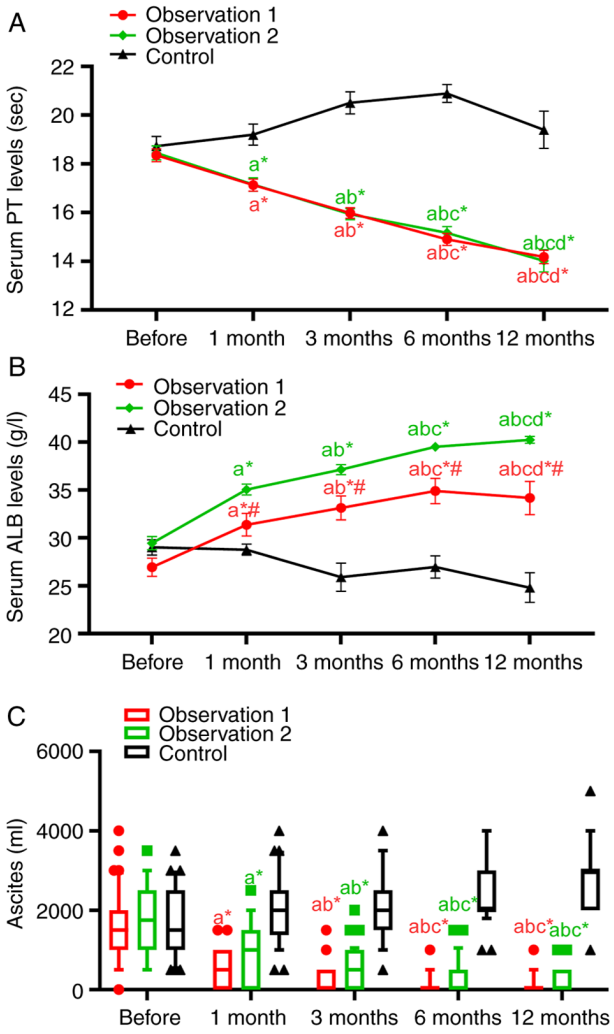

Figure 2. Effect of ultrasound-guided ABMNC infusion on liver synthesis in patients with DLC. Effect of ABMNCs on the (A) PT levels, (B) ALB levels and (C) ascites volume in patients with DLC. [a]P<0.01 vs. before; [b]P<0.01 vs. 1 month; [c]P<0.01 vs. 3 months; [d]P<0.01 vs. 6 months; [*]P<0.01 vs. control; [#]P<0.01 vs. observation group 2. The comparison of PT or ALB levels among the control, observation 1, observation 2, before, 1, 3, 6 and 12 months groups was performed using mixed analysis of variance followed by Sidak's post hoc test. The comparison of ascites levels among the observation 1, observation 2 and control groups was performed using the Kruskal-Wallis test. Comparisons among before, 1, 3, 6 and 12 months were performed using Friedman's test followed by Nemenyi test. ABMNCs, autologous bone marrow nucleated cells; ALB, albumin; DLC, decompensated liver cirrhosis; PT, prothrombin time.

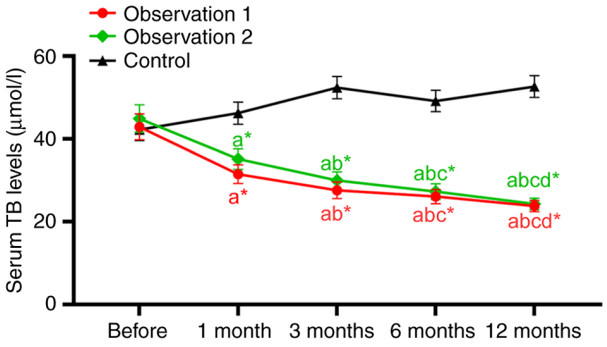

Figure 3. Effect of ultrasound-guided autologous bone marrow nucleated cell infusion on the TB levels in patients with decompensated liver cirrhosis. [a]P<0.01 vs. before; [b]P<0.01 vs. 1 month; [c]P<0.01 vs. 3 months; [d]P<0.01 vs. 6 months; [*]P<0.01 vs. control. The comparison of TB levels among the control, observation 1, observation 2, before, 1, 3, 6 and 12 months groups was performed using mixed analysis of variance followed by Sidak's post hoc test. ABMNCs, autologous bone marrow nucleated cells; TB, total bilirubin.

Fig. 4). The WBC, PLT and HGB levels in observation group 1 and 2 were significantly higher than those before treatment and in the control group from 3 to 12 months after treatment (P<0.01). Furthermore, the levels of WBC, PLT and HGB in observation group 1 were significantly higher than those in observation group 2 at 12 months after treatment (P<0.01), whereas the WBC levels in observation group 2 were higher at 1 month after treatment than those in observation group 1 (P<0.01).

Mortality and complications of ultrasound-guided ABMNC injection in patients with DLC. After 1 year of follow-up, six patients died of liver failure in the control group, resulting in a mortality rate of 20% (6/30) and one patient died of liver failure in observation group 2, resulting in a mortality rate of 2.5% (1/40), which was significantly lower than that in the control group (P<0.05). A total of eight patients in observation group 2 had bleeding at the liver puncture site, which stopped spontaneously in ~5 min (data not shown).

Discussion

Patients with viral hepatitis may have different clinical outcomes (such as persisting disease, chronic active hepatitis, cirrhosis or liver cancer) (18); however, some patients develop post-hepatitis cirrhosis with progressive, diffuse and fibrotic liver lesions. The specific manifestations are diffuse degeneration and necrosis of hepatocytes, followed by fibrous tissue proliferation and nodular regeneration of hepatocytes. These three changes are repeated and staggered, resulting in the gradual remodeling of the hepatic lobule structure and blood circulation pathway, where the liver deforms and hardens, finally resulting in cirrhosis of the liver (8). Global mortality forecast data in 2002 showed that 929,000 individuals died of liver diseases caused by chronic HBV and hepatitis C virus (HCV) infection, including 446,000 deaths from cirrhosis (HBV-related deaths, 235,000; HCV-related deaths, 211,000) (19). Carriers of HBV and patients with chronic hepatitis B in China account for ~80% of the total number of patients with hepatitis B worldwide. There are also several cases of liver cirrhosis after hepatitis B (20). Accordingly, the present study focused on HBV cirrhosis.

To the best of our knowledge, there is no specific drug for DLC and blocking the factors that cause further damage to liver cells is the basic treatment for post-hepatitis cirrhosis (12). Anti-HBV drugs, such as entecavir and tenofovir, can control the massive replication of HBV but cannot clear HBV, thus long-term antiviral treatment is required (21). Hepatocyte-protecting drugs such as glutathione, ganning tablets, silymarin, inosine and vitamins C, E and B groups can improve liver cell metabolism, prevent fatty degeneration and protect liver cells (22). For hypoalbuminemia and ascites, although treatments such as ALB infusion and diuretics have a certain curative effect, they cannot solve

Table IV. Serum total bilirubin levels (μmol/l) in the three groups before and after treatment.

Group	Before	1 month	3 months	6 months	12 months
Observation 1	42.9±19.77	31.48±14.12	27.58±12.55	26.05±11.02	23.77±8.51
Observation 2	44.89±20.56	35.11±15.20	29.95±12.69	27.26±11.77	24.29±8.57
Control	42.2±14.51	46.20±14.64	52.36±14.29	49.15±13.40	52.64±13.06

Data are presented as the mean ± standard deviation. Time, F=46.994, P<0.001; intergroup, F=18.254, P<0.001; interaction, F=40.971, P<0.001. The comparison of serum total bilirubin levels was performed by mixed analysis of variance among the observation 1, observation 2, control, and followed by Sidak's post hoc test among before, 1, 3, 6 and 12 months groups.

Table V. Comparison of white blood cell counts ($x10^9$/l) in the three groups before and after treatment.

Group	Before	1 month	3 months	6 months	12 months
Observation 1	3.20±1.72	3.62±1.16	3.76±0.99	4.08±1.10	4.25±0.99
Observation 2	3.14±0.76	4.51±1.35	3.43±0.98	3.27±0.93	3.33±0.82
Control	3.05±0.72	3.01±0.65	2.84±0.99	2.78±0.28	2.58±0.28

Data are presented as the mean ± standard deviation. Time, F=21.019, P<0.001; intergroup, F=8.570, P<0.001; interaction, F=32.397, P<0.001. The comparison of white blood cell counts was performed by mixed analysis of variance among observation 1, observation 2, control, and followed by Sidak's post hoc test among before, 1, 3, 6 and 12 months groups.

Table VI. Comparison of platelet counts ($x10^9$/l) in the three groups before and after treatment.

Group	Before	1 month	3 months	6 months	12 months
Observation 1	44.3±13.51	47.87±13.68	49.5±12.79	51.68±13.50	55.00±13.09
Observation 2	42.82±12.66	44.63±13.08	45.79±12.67	47.45±12.02	49.00±13.27
Control	39.30±9.82	39.97±9.47	38.64±9.48	38.11±8.70	38.44±7.08

Data are presented as the mean ± standard deviation. Time, F=27.772, P<0.001; intergroup, F=6.043, P<0.001; interaction, F=12.900, P<0.001. The comparison of platelet counts was performed by mixed analysis of variance among the observation 1, observation 2, control, and followed by Sidak's post hoc test among before, 1, 3, 6 and 12 months groups.

the fundamental problem of DLC, thus DLC gradually leads to liver failure (23,24). The present study revealed that the routine drug treatment for DLC, which was administered for 1 year, led to no significant changes in liver function and blood indexes. Additionally, six patients in the control group died of liver failure, with a fatality rate of 20%, thus suggesting that the conventional drug therapy for DLC had little effect. Liver transplantation is an effective method for the treatment of DLC; however, due to the shortage of liver donors, high cost and the need for the life-long application of immunosuppressive drugs, several patients are unable or unwilling to undergo liver transplantation. Stem cells, which are a type of cell with multi-directional differentiation potential, can differentiate into hepatocytes *in vitro* and *in vivo* under appropriate conditions (25). In 2000, Japanese researchers used rat bone marrow stem cells and added different concentrations of hepatocyte growth factor to induce culture *in vitro*, revealing that bone marrow stem cells could differentiate into liver-like cells (26,27). In addition, in a previous study, HBV was used to infect immunodeficient mice and generate a mouse model of liver cirrhosis. Subsequently, the model mice were injected with human-derived bone marrow mesenchymal stem cells intraperitoneally, which then entered the liver through the spleen vein reaching the portal vein. This approach was found to be effective for the treatment of liver cirrhosis (28). Consequently, bone marrow stem cell transplantation has been considered an effective method for treating liver cirrhosis.

In 2009, our group started splenectomy and portal vein infusion of ABMNCs for the treatment of patients with DLC and acquired immunodeficiency syndrome, achieving good results (16). Subsequently, this method was applied to patients with DLC and HBV, achieving further improved results. Patients with DLC generally had coagulation dysfunction, hypoalbuminemia and ascites, and were at a high risk of splenectomy. The small incision in the upper abdomen and the implantation of the infusion port through right gastric vein cannulation were relatively simple to perform, but there was still a risk of bleeding at the surgical incision site. Therefore, B-ultrasound-guided percutaneous transhepatic infusion of ABMNCs was employed in this study and the results

Table VII. Comparison of hemoglobin levels (g/l) in the three groups before and after treatment.

Group	Before	1 month	3 months	6 months	12 months
Observation 1	97.48±23.20	97.02±20.19	106.2±18.64	112.08±16.82	115.15±12.99
Observation 2	94.74±20.86	95.02±19.39	99.47±19.12	102.84±18.11	104.13±17.19
Control	95.23±20.97	93.73±19.70	92.54±17.51	91.26±14.82	90.16±16.36

Data are presented as the mean ± standard deviation. Time, F=43.586, P<0.001; intergroup, F=25.077, P<0.001; interaction, F=4.266, P=0.017. The comparison of hemoglobin levels was performed by mixed analysis of variance among the observation 1, observation 2, control, and followed by Sidak's post hoc test among before, 1, 3, 6 and 12 months groups.

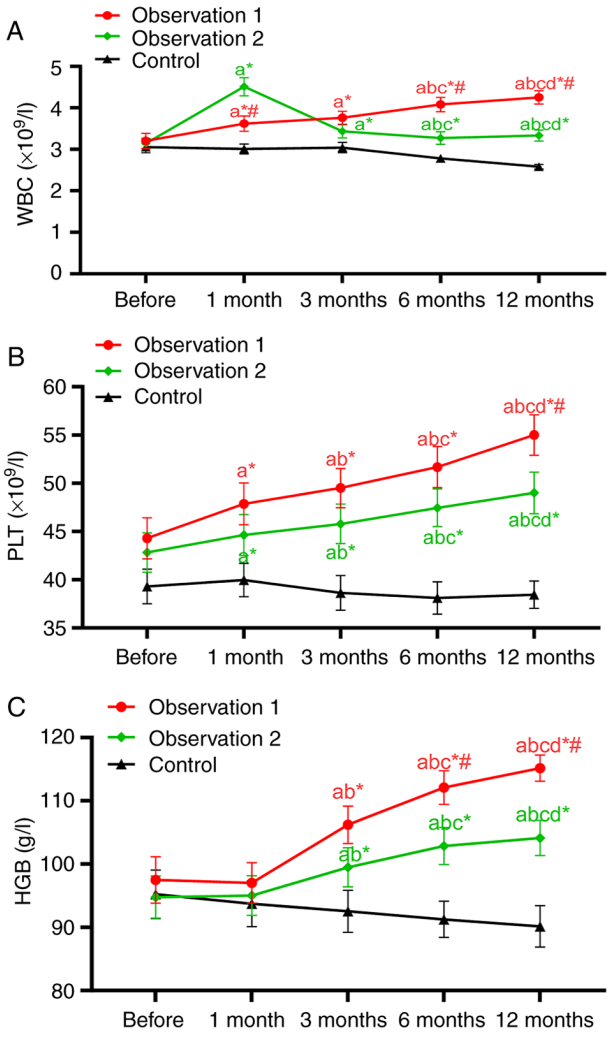

Figure 4. Effect of ultrasound-guided ABMNC infusion on the bone marrow function in patients with DLC. Effect of ABMNCs on (A) WBC count, (B) PLT levels and (C) HGB levels in patients with DLC. [a]P<0.01 vs. before; [b]P<0.01 vs. 1 month; [c]P<0.01 vs. 3 months; [d]P<0.01 vs. 6 months; [*]P<0.01 vs. control; [#]P<0.01 vs. observation group 2. The comparison of WBC, PLT or HBG levels among the control, observation 1, observation 2, before, 1, 3, 6 and 12 months groups was performed using mixed analysis of variance followed by Sidak's post hoc test. ABMNCs, autologous bone marrow nucleated cells; DLC, decompensated liver cirrhosis; HGB, hemoglobin; PLT, platelet; WBC, white blood cell.

demonstrated that the liver function in the control group was not significantly improved 12 months after treatment. Such patients with DLC are likely to gradually develop liver failure within 2 years if liver transplantation is not performed and only drugs such as those for liver protection (reduced glutathione) and diuresis (furosemide) are given (16). The present study demonstrated that the serum ALB, PT, serum TB and ascites levels were improved in observation group 2 at 1, 3, 6 and 12 months after treatment compared with the control group. Compared with those in observation group 2, the ALB levels in the observation group 1 were markedly lower, which may be related to the fact that these patients with minimally invasive surgery did not need to be hospitalized and did not need to receive intravenous infusion of ALB. In the present study, ABMNCs were used instead of bone marrow stem cells, since nucleated cell therapy does not require sorting or complex stem cell culture, and as the current therapeutic effects were relatively positive, this may be used in clinical practice. The recovery of WBC, PLT and HGB levels in observation group 1 was greater than that in the control group and observation group 2, indicating that the surgical trauma in observation group 1 was not conducive to an improvement in liver function, whereas the minimally invasive approach was conducive to the recovery of liver function. It was hypothesized that after improving liver cirrhosis, the hyperactivity of spleen function could be reduced, thereby improving peripheral blood indicators. Animal experiments may confirm this hypothesis in the future. After the liver function gradually improved, the liver function could be maintained at normal levels for 1-12 months, as the ABMNCs had no rejection effect. The 1-year mortality rate of patients undergoing ABMNC therapy was 2.5%, indicating that this treatment may reduce the mortality rate of patients with DLC compared with conventional drug therapy. After the ABMNCs enter the liver, the bone marrow stem cells may gradually differentiate into functional liver cells or secrete certain cytokines to promote the reconstruction of liver function (29). Usually, within 1 week, the patients felt less abdominal distension and the ascites gradually decreased. After 1 month, the liver function was markedly improved. Although ABMNC transplantation does not cause a rejection reaction and ABMNCs can be transformed into functional liver cells, they cannot continue to proliferate, therefore ABMNCs need to be infused again after 1 month. Liver function is generally close to normal after three infusions.

B-ultrasound-guided percutaneous transhepatic infusion of ABMNCs does not require hospitalization, is not associated with surgical scars and is almost non-invasive. Direct transplantation of ABMNCs into the liver parenchyma has the advantages

of no invasiveness, minimal trauma, mild pain and no need for hospitalization, which is in line with the treatment concepts of modern minimally invasive medicine. The liver is adjacent to the diaphragm, and the right 5th to 9th intercostal space is generally chosen for puncturing the liver. ABMNCs were infused with a 7-gauge lumbar anesthesia needle and most patients did not bleed after infusion. Even if there was a small amount of bleeding at the liver puncture site, compression of the bleeding site by the diaphragm promoted hemostasis. For patients with a large number of ascites, few platelets and coagulation dysfunction, there was a possibility of bleeding at the site of liver puncture. The bleeding could enter the ascites for ~10 min, thus increasing the risk of hemorrhagic shock. Therefore, for patients with DLC with coagulation dysfunction, more ascites and thrombocytopenia, a small incision in the upper abdomen could be made and the right gastroepiploic vein cannula could be inserted into the infusion port to infuse ABMNCs, thus avoiding liver puncture and the risk of intra-abdominal bleeding. After injection of ABMNCs, bright spots could be seen on the B-ultrasound apparatus and the movement trajectory of ABMNCs in the liver could be clearly seen, having a similar effect to the ultrasound contrast agent. The brightness of the liver tended to increase as the ABMNCs gradually diffused and distributed into the liver through the hepatic sinusoids. After 10-20 min, the brightness of the liver in the B-ultrasound examination gradually returned to normal, which could be due to the fact that, after the ABMNCs settled into the liver, the ABMNCs gradually fused with the liver tissue.

The present study had some limitations. The follow-up time was too short to analyze the 5-year survival of patients. In addition, the mechanisms behind the changes in clinical indicators could not be determined in the human body, so the specific mechanism of nucleated cells still remains unclear. For example, how the nucleated cells can improve the synthesis and secretion function of the liver and how to improve the function of bone marrow is unclear, as the methodology of human experiments is still limited, and detailed studies in animal experiments are needed. In order to address the aforementioned shortcomings, the progression of these same patients will be monitored in future clinical works and the follow-up time will be extended to >5 years. Furthermore, animal experiments will be performed to further explore the mechanism of action.

In conclusion, B-ultrasound-guided injection of ABMNCs in the liver was more effective than the conventional drug therapy for the treatment of DLC. Furthermore, this approach had an effect comparable with the infusion of ABMNCs through the infusion port in the treatment of DLC, with no need for hospitalization and less trauma, thus being worthy of promotion and application in primary hospitals for fast recovery and high patient acceptance.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

BL conceived and designed the experiments. BL and MC confirm the authenticity of all the raw data. LL and MC performed the experimental work and analyzed the data. LLi, YS and AL participated in the experiments. All authors read and approved the final manuscript.

Ethics approval and consent to participate

This study was approved by the Ethics Committee of the Shanghai Public Health Clinical Center (Shanghai, China; approval no. 2018-S035-02). Written informed consent was obtained from all participants for the use of their samples for detection and publication of their relevant data.

Patient consent for publication

All participants in this study provided written informed consent for the use of their samples and publication of their data.

Competing interests

The authors declare that they have no competing interests.

References

1. Gines P, Krag A, Abraldes JG, Sola E, Fabrellas N and Kamath PS: Liver cirrhosis. Lancet 398: 1359-1376, 2021.
2. Xiong J, Wang J, Huang J, Sun W, Wang J and Chen D: Non-alcoholic steatohepatitis-related liver cirrhosis is increasing in China: A ten-year retrospective study. Clinics (Sao Paulo) 70: 563-568, 2015.
3. Iwaisako K, Taura K, Koyama Y, Takemoto K and Asagiri M: Strategies to detect hepatic myofibroblasts in liver cirrhosis of different etiologies. Curr Pathobiol Rep 2: 209-215, 2014.
4. Hanada S, Kumashiro R, Kaji R, Harada M and Sata M: Additional benefit of lamivudine treatment as a preventive therapy for hepatic encephalopathy in patients with decompensated liver cirrhosis associated with hepatitis B. Int J Mol Med 10: 647-648, 2002.
5. Cheng NC, Ko JY, Tai HC, Horng SY and Tang YB: Microvascular head and neck reconstruction in patients with liver cirrhosis. Head Neck 30: 829-835, 2008.
6. Falleti E, Cmet S and Cussigh AR, Salvador E, Bitetto D, Fornasiere E, Fumolo E, Fabris C and Toniutto P: Recurrent and treatment-unresponsive spontaneous bacterial peritonitis worsens survival in decompensated liver cirrhosis. J Clin Exp Hepatol 11: 334-342, 2021.
7. Benmassaoud A, Freeman SC, Roccarina D, Plaz Torres MC, Sutton AJ, Cooper NJ, Iogna Prat L, Cowlin M, Milne EJ, Hawkins N, *et al*: Treatment for ascites in adults with decompensated liver cirrhosis: A network meta-analysis. Cochrane Database Syst Rev 1: CD013123, 2020.
8. Sola E and Gines P: Challenges and management of liver cirrhosis: Pathophysiology of renal dysfunction in cirrhosis. Dig Dis 33: 534-538, 2015.
9. Hagel S, Bruns T, Herrmann A, Stallmach A and Schmidt C: Abnormal glucose tolerance: A predictor of 30-day mortality in patients with decompensated liver cirrhosis. Z Gastroenterol 49: 331-334, 2011.

10. Zhu Z, Liu Y, Wu W, Huang D, Guo Y, Zheng H, Wang N, Xu Z, Li X, Qin J, *et al*: Liver transplantation reverses hepatic myelopathy in hepatitis B-Related decompensated liver cirrhosis: Case report and review of the literature. Transplant Proc 54: 158-160, 2022.
11. Kaido T and Uemoto S: Current status and issues of liver transplantation for decompensated liver cirrhosis. Nihon Shokakibyo Gakkai Zasshi 114: 35-42, 2017 (In Japanese).
12. Shi M, Li YY, Xu RN, Meng FP, Yu SJ, Fu JL, Hu JH, Li JX, Wang LF, Jin L and Wang FS: Mesenchymal stem cell therapy in decompensated liver cirrhosis: A long-term follow-up analysis of the randomized controlled clinical trial. Hepatol Int 15: 1431-1441, 2021.
13. Bai YQ, Yang YX, Yang YG, Ding SZ, Jin FL, Cao MB, Zhang YR and Zhang BY: Outcomes of autologous bone marrow mononuclear cell transplantation in decompensated liver cirrhosis. World J Gastroenterol 20: 8660-8666, 2014.
14. Pan XN, Zheng LQ and Lai XH: Bone marrow-derived mesenchymal stem cell therapy for decompensated liver cirrhosis: A meta-analysis. World J Gastroenterol 20: 14051-14057, 2014.
15. Wu CX, Wang D, Cai Y, Luo AR and Sun H: Effect of autologous bone marrow stem cell therapy in patients with liver cirrhosis: A meta-analysis. J Clin Transl Hepatol 7: 238-248, 2019.
16. Liu B, Cheng M, Chen X, Li L, Si Y, Wang S, Wang Y and Shi Y: Autologous bone marrow cell transplantation in the treatment of HIV patients with compensated cirrhosis. Biosci Rep 40: BSR20191316, 2020.
17. Yoshiji H, Nagoshi S, Akahane T, Asaoka Y, Ueno Y, Ogawa K, Kawaguchi T, Kurosaki M, Sakaida I, Shimizu M, *et al*: Evidence-based clinical practice guidelines for liver cirrhosis 2020. Hepatol Res 51: 725-749, 2021.
18. Teng D, Zuo H, Liu L, Dong J and Ding L: Long-term clinical outcomes in patients with viral hepatitis related liver cirrhosis after transjugular intrahepatic portosystemic shunt treatment. Virol J 15: 151, 2018.
19. Perz JF, Armstrong GL, Farrington LA, Hutin YJ and Bell BP: The contributions of hepatitis B virus and hepatitis C virus infections to cirrhosis and primary liver cancer worldwide. J Hepatol 45: 529-538, 2006.
20. Zheng X, Wang J and Yang D: Antiviral therapy for chronic hepatitis B in China. Med Microbiol Immunol 204: 115-120, 2015.
21. Peng CY, Chien RN and Liaw YF: Hepatitis B virus-related decompensated liver cirrhosis: Benefits of antiviral therapy. J Hepatol 57: 442-450, 2012.
22. Plaz Torres MC, Best LM, Freeman SC, Roberts D, Cooper NJ, Sutton AJ, Roccarina D, Benmassaoud A, Iogna Prat L, Williams NR, *et al*: Secondary prevention of variceal bleeding in adults with previous oesophageal variceal bleeding due to decompensated liver cirrhosis: A network meta-analysis. Cochrane Database Syst Rev 3: CD013122, 2021.
23. Ashour AA, Atta MA, Sadek KW, Obaid KR, Ashour MA, Ashour A, Danjuma MI, Doi SA and ElZouki AN: Albumin administration in patients with decompensated liver cirrhosis: A meta-analytic update. Eur J Gastroenterol Hepatol 33: 479-486, 2021.
24. Grungreiff K, Gottstein T, Reinhold D and Blindauer CA: Albumin substitution in decompensated liver cirrhosis: Don't Forget Zinc. Nutrients 13: 4011, 2021.
25. Palakkan AA, Nanda J and Ross JA: Pluripotent stem cells to hepatocytes, the journey so far. Biomed Rep 6: 367-373, 2017.
26. Pan HC, Chen YJ, Lin JP, Tsai MJ, Jenq CC, Lee WC, Tsai MH, Fan PC, Chang CH, Chang MY, *et al*: Proteinuria can predict prognosis after liver transplantation. BMC Surg 16: 63, 2016.
27. Philips CA and Augustine P: Still 'dwelling in the possibility'-critical update on stem cell therapy for acute on chronic liver failure. World J Stem Cells 12: 1124-1132, 2020.
28. Wang D, Zhang H, Liang J, Gu Z, Ma X, Huang J, Lin J, Hou Y, Lu L and Sun L: Effect of allogeneic bone marrow-derived mesenchymal stem cells transplantation in a polyI:C-induced primary biliary cirrhosis mouse model. Clin Exp Med 11: 25-32, 2011.
29. Zhang Z, Liu J, Liu Y, Li Z, Gao WQ and He Z: Generation, characterization and potential therapeutic applications of mature and functional hepatocytes from stem cells. J Cell Physiol 228: 298-305, 2013.