

Enhanced therapeutic effects of umbilical cord mesenchymal stem cells after prolonged treatment for HBV-related liver failure and liver cirrhosis

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Abstract

Background: This study aimed to investigate the therapeutic effect of umbilical cord mesenchymal stem cells (UCMSCs) on HBV-related liver failure and liver cirrhosis and to compare the different efficacies of UCMSCs after different treatment courses.

Methods: This was an observational study that retrospectively considered a three-year period during which 513 patients who received stem cell infusion met the criteria of hepatic failure and liver cirrhosis were identified from databases of the Third Affiliated Hospital of Sun Yat-sen University. Eligible patients were categorized into the liver failure group and liver cirrhosis group. The two groups were divided into different subgroups according to the times of stem cell therapy. In the liver failure group, group A received more than 4 weeks and group B received less than 4 weeks. In the liver cirrhosis group, patients who received more than 4 weeks of stem cell therapy belonged to group C, and group D received less than 4 weeks. The patients were followed up for 24 weeks. The demographics, clinical characteristics, biochemical factors, and MELD scores were recorded and compared among different groups.

Results: A total of 64 patients met the criteria of liver failure, and 59 patients met the criteria of liver cirrhosis. After UCMSC treatments, the levels of ALT, AST, and TBIL at all postbaseline time points were significantly lower than those at baseline in the liver failure group and liver cirrhosis group; the PTA and MELD scores only gradually improved in the liver failure group. Four weeks after UCMSC treatment, patients with prolonged treatment with UCMSCs had higher TBIL decline levels than patients who terminated treatment with UCMSCs. After more than 4 weeks of UCMSC treatment, there was no statistically significant difference in the levels of change for ALT, AST, TBIL, PTA value and the MELD score between patients with liver failure with prolonged treatment with UCMSCs and patients with liver cirrhosis with prolonged treatment with UCMSCs at all observation weeks. However, the median decline and cumulative decline in the TBIL level of patients with liver failure with a standard 4-week treatment course were higher than those of patients with liver cirrhosis with a standard 4-week treatment course.

Conclusion: Peripheral infusion of UCMSCs showed good therapeutic effects for HBV-related liver failure and liver cirrhosis. Prolonging the treatment course can increase the curative effect of UCMSCs for end-stage liver disease, especially for patients with cirrhosis.

1. Introduction

Liver failure and cirrhosis are the results of chronic liver damage caused by many factors, including alcohol, drugs, and hepatitis virus (HBV, HCV, etc.), among which hepatitis B virus (HBV) infection is the most common cause of liver failure and cirrhosis, with the characteristics of high mortality and high economic burden(1). HBV-related end-stage liver disease mainly includes liver failure and decompensated cirrhosis. Due to its rapid progression and poor prognosis, liver transplantation is considered the most effective treatment for patients with HBV-related end-stage liver disease(2). However, the application of liver transplantation was limited by the shortage of donor liver, the risk of transplantation and the long-term use of immunosuppressants after transplantation (3). Therefore, the majority of gastroenterologists/hepatologists have been looking for new treatment methods to treat patients with HBV-related end-stage liver disease.

Twenty years ago, Theise *et al.*(4) reported that Y chromosome-positive hepatocyte-like cells exist in the livers of women who had received allogeneic bone marrow transplantations from male donors and concluded that pluripotent stem cells may exist among bone marrow cells. Stem cell therapy has been applied in the treatment of end-stage liver disease, and its clinical efficiency is satisfactory (5). Among most basic or clinical studies, there is evidence that MSCs are safe and effective in the treatment of liver failure and cirrhosis. With the deepening of the research, scholars have also put forward a series of conjectures about the influencing factors of MSCs in the treatment of liver failure and cirrhosis, such as the type

of MSCs, the time of infusion, the method of infusion, and the dosage of infusion (6, 7). Previous research in our department shows that allogeneic bone marrow-derived MSCs are safe and effective for patients with HBV-related acute-on-chronic liver failure (ACLF) (8, 9). However, autologous mesenchymal stem cells often lead to delayed treatment due to culture. Ethical issues and uncertainty about the possibility of malignant differentiation *in vivo* also limit their clinical significance. Fortunately, allogeneic mesenchymal stem cells can overcome this limitation and can be widely used in the clinic as “ready-made” immune privilege therapy agents. Embryonic stem cells (ESs) and induced pluripotent stem cells (iPSs) have been shown to be the cells most capable of producing a large number of functional liver-like cells (HLCs) in mice and humans (10). MSCs from the umbilical cord performed as well as those from the bone marrow compared to other types of adult stem cells, although no study showed complete and sustainable performance in the outcome measures(11).

Nevertheless, almost all studies have only focused on liver failure or cirrhosis. Is there a difference in the efficacy of umbilical cord mesenchymal stem cells (UCMSCs) between patients with HBV-related liver failure and patients with liver cirrhosis in real-world evidence? Is there a difference in the efficacy of UCMSCs with different treatment courses in patients with HBV-related liver failure or cirrhosis? We conducted this research to try to answer the two questions above.

2. Methods

2.1 Study Population

The data of patients with HBV-related liver failure or liver cirrhosis who received UCMSCs at the Third Affiliated Hospital of Sun Yat-sen University between February 2014 and December 2015 were collected. All study procedures adhered to the tenets of the Declaration of Helsinki, and informed consent was obtained from all patients. The study was approved by the Human Ethics Committee of The Third Affiliated Hospital, Sun Yat-sen University, Guangzhou, China. Patients with the following were deemed eligible for enrollment in this study: A. HBsAg positive more than 6 months; B. meet the 2009 APASL diagnostic criteria for hepatitis B liver failure and cirrhosis(12); and C. age between 16 and 60 years. Patients were excluded for the following reasons: A. severe complications in the last two months, such as esophageal and gastric vein rupture and hemorrhage, septicemia, etc.; B. other autoimmune diseases; C. infection with other hepatitis viruses; D. impairment of other important organ functions; E. pregnant or lactating woman; F. human liver or liver transplantation; G. alcoholic liver disease; and H. liver cancer.

2.2 Study Design

All patients with HBV-related end-stage liver disease who were treated with UCMSCs participated in the study. According to the diagnosis, the patients were divided into two groups: the liver failure group and the liver cirrhosis group. The liver failure group was then categorized into group A and group B. Group A included patients with liver failure treated with UCMSCs for more than 4 weeks; group B included patients with liver failure who received UCMSCs for less than or equal to 4 weeks. In the liver cirrhosis group, patients who received more than 4 weeks of UCMSC therapy belonged to group C, and group D included patients with liver cirrhosis treated with UCMSCs less than or equal to 4 weeks. The patients were followed up for 24 weeks. Data of all patients were collected at baseline and at 1, 4, 12 and 24 weeks after off-therapy. All patients in the study received standard clinical treatments (including albumin supplementation, coagulation correction, S-adenosylmethionine infusion, antiviral treatment and necessary anti-infective treatment) for approximately one week in advance. One week later, all patients received the related dose of stem cell infusion after knowing the relevant information of stem cell treatment and signing the informed consent. The levels of alanine aminotransferase (ALT), glutamic-oxaloacetic transaminase (AST), total bilirubin (TBIL), prothrombin activity (PTA), and MELD scores were analyzed to comprehensively evaluate the infusion effect of different doses of stem cells.

2.3 UCMSC preparation and transfusions

The processing of the umbilical cords and preparation of UCMSCs were performed at the GMP Stem Cell Laboratory Facility of the biotherapy center in The Third Affiliated Hospital of Sun Yat-sen University, Guangzhou, China. Fresh human umbilical cords were obtained and collected in phosphate-buffered saline at 4°C after birth with the consent of parents. The specific details of the isolation, culture and characterization of UCMSCs were described in previous research(13). Bacterial, fungal and viral monitoring (including hepatitis B virus [HBV], hepatitis C virus [HCV], human immunodeficiency virus [HIV] and cytomegalovirus) was performed in all umbilical cords and prior to injection to control the quality. Patients receiving less than or equal to 4 doses of UCMSC transfusions received them at 1, 2, 3 and 4 weeks after being recruited. Patients receiving more than 4 doses of UCMSC transfusions received them at 1, 2, 3, 4, 5, 6, 7 and 8 weeks after being recruited. As reported in previous research (ClinicalTrials.gov, NCT02223897)(14), approximately 1.0×10^6 UCMSCs per kilogram of body weight suspended in 100 mL of normal saline solution were infused intravenously through the vein of the forearm each time. Patients were allowed to be discharged after one day of observation without any adverse events.

2.4. Statistical Analysis

SPSS software (version 22.0; SPSS, Inc., Chicago, IL) was used to perform the statistical analyses. The Shapiro–Wilk test of normality was performed. Normally distributed variables were analyzed with ANOVA test, while non-normally distributed variables were analyzed with nonparametric tests. Continuous variables were expressed as the mean \pm standard deviation (SD) or median (interquartile) depending on the result of normality testing and were analyzed using a t test or Wilcoxon test, as appropriate. Sex was expressed as the number of patients (percentage) and tested with the χ^2 test or Fisher's exact test, as appropriate. All analyses were performed as two-sided tests with a 0.05 level of significance.

3. Results

3.1 Patient characteristics

A total of 513 patients signed informed consent forms and received UCMSC treatment. According to diagnostic criteria and matching treatment medication records, only 123 patients were enrolled and eligible for efficacy analysis in the present study. Of the patients, 64 were included in the liver failure group and 59 in the cirrhosis group. The patients were divided into four groups: prolonged treatment liver failure (group A, N=37), standard treatment liver failure (group B, N=27), prolonged treatment liver cirrhosis (group C, N=28) and standard treatment liver cirrhosis (group D, N=31). The baseline characteristics of the 123 patients in this study are shown in Table 1 and Table 2. These variables were generally similar among group A and group B, and no significant differences were observed in the baseline characteristics between group C and group D (Table 1). However, the difference between group A and group C was statistically significant; similarly, the difference between group B and group D was statistically significant (Table 2).

3.2 Therapeutic effect of UCMSCs in the liver failure group and liver cirrhosis group

The therapeutic effect of UCMSCs varied based on the week of observation in the liver failure group and liver cirrhosis group. The levels of ALT, AST, TBIL, and the MELD score at all postbaseline time points were significantly lower than those at baseline in the liver failure group, and the value of PTA was gradually increased after UCMSC treatment in the liver failure group (Fig. 1A). In the liver cirrhosis group, the levels of ALT, AST, and TBIL progressively decreased after UCMSC treatments. However, no significant differences were observed in the PTA value and MELD score among all observation weeks (Fig. 1B). The decline in TBIL varied significantly according to the treatment regimen. The median serum TBIL decline in the liver failure group was significantly greater than that in the liver cirrhosis group from week 4 to week 12 ($P=0.003$, Fig. 2A). However, no apparent difference was found at the other three time points (Fig. 2A). Similarly, no statistically significant changes in the levels of ALT, AST, PTA value or MELD score were found between the two groups at any of the four time points.

3.3 Comparative analysis of the therapeutic effect of UCMSCs between group A and group B

To understand whether the course of treatment affects the efficacy of UCMSCs, we compared the outcome of group A with that of group B in patients with liver failure. The median serum TBIL decline showed no difference between group A and group B at W0-W1 (Fig. 2B). The median serum TBIL decline in group A was significantly lower than that in group B at W1-W4 (Fig. 2B). However, patients treated with UCMSCs for more than 4 weeks (group A) had higher TBIL decline levels than patients who received UCMSCs for less than or equal to 4 weeks (group B) at W4-W12 and W12-W24 (Fig. 2B). When the cumulative decline in TBIL was compared between the two groups at different time periods, no significant difference was found between the two groups at any observation week except week 4. The cumulative decline in TBIL gradually increased in group A after four weeks of treatment, and no significant differences were maintained thereafter in either group (Fig. 3A). The levels of decline in ALT and AST of patients in group A were higher than those in group B after 4 weeks of UCMSC treatment, and the PTA value and MELD score differed before week 4 (Table 3).

3.4 Comparative analysis of the therapeutic effect of UCMSCs between group C and group D

Similarly, we compared the outcome of group C with that of group D in liver cirrhosis patients to understand the treatment course and the efficacy of UCMSCs. The median serum TBIL decline in patients treated with UCMSCs for more than 4 weeks (group C) was higher than that in group D at W4-W12 (Fig. 2C). The cumulative decline in TBIL in group C gradually increased from week 4 and was higher than that of group D (Fig. 3B). Similarly, the levels of decline in ALT and AST in patients in group C were also higher than those in group D after 4 weeks of UCMSC treatment, and the PTA value and MELD score were significantly different before week 4 (Table 3).

3.5 Comparative analysis of the therapeutic effect of UCMSCs between group A and group C

To investigate the therapeutic effect of UCMSCs for more than 4 weeks in patients with liver failure and patients with liver cirrhosis, we compared the outcome of group A with that of group C. The median serum TBIL decline, ALT, AST, PTA value and MELD score at all postbaseline time points were not significantly different between group A and group C (Fig. 2D) (Table 3). However, the cumulative decline in TBIL of patients in group A was higher than that of group C at week 24 (Fig. 3C).

3.6 Comparative analysis of the therapeutic effect of UCMSCs between group B and group D

Meanwhile, the outcomes of patients with liver failure who received UCMSCs for less than or equal to 4 weeks in group B were compared with those of patients with liver cirrhosis in group D to learn more about the therapeutic effect of UCMSCs. The median decline in TBIL was not different between the two groups at any observation week except week 4-week 12 (Fig. 2E). At week 4-week 12, the median decline in TBIL in group B was higher than that in group D ($P= 0.003$, Fig. 2E). The cumulative decline in TBIL of patients in group B was higher than that of group D at week 12 and week 24 (Fig. 3D). The levels of ALT, AST, PTA and MELD scores at all postbaseline time points were not significantly different between group B and group D (Table 3).

4. Discussion

Although the gold standard therapy for HBV-related end-stage liver disease is liver transplant, there are discrepancies between liver supply and liver demand. Stem cell therapy is an alternative approach to liver transplantation for HBV-related liver failure and liver cirrhosis. Previous research from our department shows that allogeneic bone marrow-derived MSCs are safe and effective for patients with HBV-related acute-on-chronic liver failure (ACLF)(8, 9). However, ethical issues, uncertainty about malignant differentiation and culture *in vivo* limit its wider uptake and clinical significance. Umbilical cord mesenchymal stem cells (UCMSCs) have shown great potential in regenerative medicine due to their extensive sources, multilineage differentiation potential, low immunogenicity and self-renewal ability(15). In the CCl₄-induced acute liver injury model, significant hepatoprotective effects of UCMSCs with decreased levels of hepatocellular necrosis and lobular neutrophilic infiltration were found(16). A recent study (17) found that UCMSC treatment can disrupt

the development of the inflammatory cascade by inhibiting monocyte activation, and peripheral infusion of human UCMSCs rescues acute liver failure lethality in monkeys. In this study, UCMSCs were used to treat patients with HBV-related liver failure. We found that the levels of ALT, AST, TBIL, and the MELD score gradually decreased and the value of PTA gradually increased after UCMSC treatments. This finding is consistent with previous reports showing that UCMSC transfusions significantly increased the survival rates in patients with ACLF; reduced the model for end-stage liver disease scores; increased serum albumin, cholinesterase, and prothrombin activity; and increased platelet counts(7). Yan *et al.*'s study(18) demonstrated that UCMSCs could deliver GPX1 to exert its hepatocyte protective effects by detoxifying CCl₄ and H₂O₂ and reducing oxidative stress and apoptosis of hepatocytes. miR-455-3p-enriched exosomes derived from UCMSCs could attenuate macrophage infiltration and local liver damage and reduce the serum levels of inflammatory factors, thereby improving liver histology and systemic disorders(19). Certainly, when MSCs are used to treat liver disease, the trans differentiation of MSCs into hepatocytes was determined, and MSCs also secrete various bioactive molecules to promote liver regeneration(20). Therefore, it was not unexpected that UCMSCs would have such a good therapeutic effect on liver failure in this study.

The characteristics of MSCs are continuous self-renewal, proliferation, multipotent differentiation, and immunomodulatory activities. UCMSCs possess not only the common characteristics of MSCs but also more stable biological characteristics, relatively easy accessibility, abundant source, and no ethical issues, making UCMSCs a good choice for the treatment of liver fibrosis(21). In this study, the therapeutic effect of UCMSCs on liver cirrhosis was also investigated. Our results reveal that the treatment of MSCs significantly improves liver function in patients with liver cirrhosis as evidenced by the levels of ALT, AST, and total bilirubin. However, it has no beneficial effects in terms of PTA and the MELD score. This finding is consistent with previous reports of systematic reviews and meta-analyses of the therapeutic efficacy of MSCs against liver disease(22). In addition, ascites in patients with decompensated liver cirrhosis could be improved by UCMSCs(6). In a CCl₄-induced rat liver fibrosis model(23), UCMSCs could differentiate into functional hepatocytes that improved both biochemical and histopathologic changes. In addition, secretomes from UCMSCs also reduced the liver expression of multiple fibrotic factors, collagens, metalloproteinases, TGFβ, and smad proteins in the TGFβ signaling pathways(24). Furthermore, UCMSCs also have a significant immune modulatory effect on immune cells(25). Therefore, UCMSCs can be a simple and effective treatment for the management of fibrotic liver diseases.

The dosage and duration of MSCs are two important issues. Using too many intraportal or intrahepatic BM-MNCs is counterproductive, and it seems more beneficial when they are enriched to reduce the cell number(11). In this study, we mainly focused on whether prolonging the treatment course can improve the curative effect of UCMSCs. Before 4 weeks of UCMSC treatment, the median decline and cumulative decline in TBIL were not significantly different between patients with prolonged treatment and patients with a standard 4-week treatment course. Moreover, the median decline and cumulative decline in TBIL of liver patients with a standard 4-week treatment course were higher than those of liver patients with prolonged treatment. Surprisingly, the data indicate that the median decline and cumulative decline in TBIL of prolonged treatment patients caught up or even surpassed the decline levels of patients with a standard 4-week treatment course after prolonging the treatment course of UCMSCs. Our results show that extending the treatment course can be an option to improve the efficacy of UCMSCs. At present, there are few clinical trials that have investigated the relationship between efficacy and MSC number. A recent meta-analysis(26) finding suggested that the number of cells injected was an important factor influencing the efficacy of autologous MSC therapies, and BMSCs $> 4 \times 10^8$ were more beneficial to patients. However, some clinical trials with controls using adult stem cells show that cell number might play a role in the result and that too many cells might be deleterious(11). Our results show that increasing the dosage of MSCs by extending the course of treatment may be one of the most appropriate methods of using UCMSCs to treat liver disease. In the future, large-scale and prospective studies are required to identify prolonged strategies for patients receiving UCMSC therapies.

The last issue we are concerned about is which UCMSCs are more suitable for liver failure or liver cirrhosis? The results of this study showed that only the median TBIL decline of patients with liver failure was greater than that of patients with liver cirrhosis after 4 weeks of UCMSC treatment; no statistically significant differences in the levels of ALT, AST, and the PTA value or the MELD score were found between patients with liver failure and patients with liver cirrhosis at all observation weeks. However, the median decline and cumulative decline in the TBIL level of patients with liver failure with a standard 4-week treatment course were higher than those of patients with liver cirrhosis. Pseudolobule formation and destruction of the liver parenchyma may limit the trans differentiation of UCMSCs into hepatocytes. Apart from this, liver sinusoidal endothelial cells (LSECs) may play an important role in this difference. LSECs are the first cells affected by liver injury or chest rating the liver response to damage. LSECs govern regenerative process initiation, and aberrant LSEC activation in chronic liver injury induces fibrosis. Sustaining hepatic injury can lead to the loss of the LSEC phenotype and protective properties, promote angiogenesis and vasoconstriction and contribute to inflammation and fibrosis(27). Shubham *et al.* (28) found that cellular and functional loss of liver endothelial cells correlates with poor hepatocyte regeneration in acute-on-chronic liver failure. Therefore, standard 4-week UCMSC treatment for liver cirrhosis failed to achieve the same satisfactory effect as treatment for liver failure; the prolonging strategies for patients with liver cirrhosis may be a choice after weighing the advantages and disadvantages.

There were some limitations to this study. First, this study was a retrospective study. Second, this study was based on data from only one health center, yielding a rather small cohort and possible examiner bias. Third, the study period was only 24 weeks; a longer time could provide additional insights. In addition, the number of patients evaluated for treatment efficacy was relatively small. In the future, large-scale and prospective studies are required to identify the therapeutic effect of UCMSCs in HBV-related end-stage liver disease.

In conclusion, peripheral infusion of UCMSCs showed good therapeutic effect for HBV-related liver failure and liver cirrhosis; prolonging the treatment course can increase the curative effect of UCMSCs for end-stage liver disease, especially for patients with cirrhosis. The long-term efficacy should be further assessed in a randomized, large-scale, double-blind and well-controlled trial.

Declarations

Ethical Approval and Consent to participate

This is a retrospective cohort study. This study procedures adhered to the tenets of the Declaration of Helsinki, and informed consent was obtained from all patients. The study was approved by the Human Ethics Committee of The Third Affiliated Hospital, Sun Yat-sen University.

Consent for publication

Not applicable.

Availability of supporting data

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

Competing interests

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Authors' contributions

YJ, XS, KZ, QX, GL, and YY contributed to the experimental design, data analysis and interpretation, manuscript writing, and manuscript revision. HC, XY and HS contributed to the data collection and processing. All authors approved the final version of the manuscript.

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Abbreviations

HBV, hepatitis B virus; ALT, alanine aminotransferase; UCMSCs, umbilical cord mesenchymal stem cells; ACLF, acute-on-chronic liver failure; TBIL, total bilirubin; PTA, prothrombin activity; MELD, model for end-stage liver disease.

References

1. Marcellin P, Kutala BK. Liver diseases: A major, neglected global public health problem requiring urgent actions and large-scale screening. *Liver Int* 2018;38 Suppl 1:2-6.
2. Sundaram V, Jalan R, Wu T, Volk ML, Asrani SK, Klein AS, Wong RJ. Factors Associated with Survival of Patients With Severe Acute-On-Chronic Liver Failure Before and After Liver Transplantation. *Gastroenterology* 2019;156:1381-1391.
3. Durand F, Levitsky J, Cauchy F, Gilgenkrantz H, Soubrane O, Francoz C. Age and liver transplantation. *J Hepatol* 2019;70:745-758.
4. Theise ND, Nimmakayalu M, Gardner R, Illei PB, Morgan G, Teperman L, Henegariu O, et al. Liver from bone marrow in humans. *Hepatology* 2000;32:11-16.
5. Xue R, Meng Q, Dong J, Li J, Yao Q, Zhu Y, Yu H. Clinical performance of stem cell therapy in patients with acute-on-chronic liver failure: a systematic review and meta-analysis. *J Transl Med* 2018;16:018-1464.
6. Zhang Z, Lin H, Shi M, Xu R, Fu J, Lv J, Chen L, et al. Human umbilical cord mesenchymal stem cells improve liver function and ascites in decompensated liver cirrhosis patients. *J Gastroenterol Hepatol* 2012;27 Suppl 2:112-120.
7. Shi M, Zhang Z, Xu R, Lin H, Fu J, Zou Z, Zhang A, et al. Human mesenchymal stem cell transfusion is safe and improves liver function in acute-on-chronic liver failure patients. *Stem Cells Transl Med* 2012;1:725-731.
8. Peng L, Xie DY, Lin BL, Liu J, Zhu HP, Xie C, Zheng YB, et al. Autologous bone marrow mesenchymal stem cell transplantation in liver failure patients caused by hepatitis B: short-term and long-term outcomes. *Hepatology* 2011;54:820-828.
9. Lin BL, Chen JF, Qiu WH, Wang KW, Xie DY, Chen XY, Liu QL, et al. Allogeneic bone marrow-derived mesenchymal stromal cells for hepatitis B virus-related acute-on-chronic liver failure: A randomized controlled trial. *Hepatology* 2017;66:209-219.
10. Huang P, He Z, Ji S, Sun H, Xiang D, Liu C, Hu Y, et al. Induction of functional hepatocyte-like cells from mouse fibroblasts by defined factors. *Nature* 2011;475:386-389.
11. AdiwinataPawitan J. Exploring the Most Promising Stem Cell Therapy in Liver Failure: A Systematic Review. *Stem Cells Int* 2019;1.

12. Sarin SK, Kedarisetty CK, Abbas Z, Amarapurkar D, Bihari C, Chan AC, Chawla YK, et al. Acute-on-chronic liver failure: consensus recommendations of the Asian Pacific Association for the Study of the Liver (APASL) 2014. *Hepatol Int* 2014;8:453-471.
13. Deng Y, Yi S, Wang G, Cheng J, Zhang Y, Chen W, Tai Y, et al. Umbilical cord-derived mesenchymal stem cells instruct dendritic cells to acquire tolerogenic phenotypes through the IL-6-mediated upregulation of SOCS1. *Stem Cells Dev* 2014;23:2080-2092.
14. Zhang YC, Liu W, Fu BS, Wang GY, Li HB, Yi HM, Jiang N, et al. Therapeutic potentials of umbilical cord-derived mesenchymal stromal cells for ischemic-type biliary lesions following liver transplantation. *Cytotherapy* 2017;19:194-199.
15. Qiao Y, Xu Z, Yu Y, Hou S, Geng J, Xiao T, Liang Y, et al. Single cell derived spheres of umbilical cord mesenchymal stem cells enhance cell stemness properties, survival ability and therapeutic potential on liver failure. *Biomaterials* 2020;227:21.
16. Yun JW, Ahn JH, Kwon E, Kim SH, Kim H, Jang JJ, Kim WH, et al. Human umbilical cord-derived mesenchymal stem cells in acute liver injury: Hepatoprotective efficacy, subchronic toxicity, tumorigenicity, and biodistribution. *Regul Toxicol Pharmacol* 2016;81:437-447.
17. Guo G, Zhuang X, Xu Q, Wu Z, Zhu Y, Zhou Y, Li Y, et al. Peripheral infusion of human umbilical cord mesenchymal stem cells rescues acute liver failure lethality in monkeys. *Stem Cell Res Ther* 2019;10:84.
18. Yan Y, Jiang W, Tan Y, Zou S, Zhang H, Mao F, Gong A, et al. hucMSC Exosome-Derived GPX1 Is Required for the Recovery of Hepatic Oxidant Injury. *Mol Ther* 2017;25:465-479.
19. Shao M, Xu Q, Wu Z, Chen Y, Shu Y, Cao X, Chen M, et al. Exosomes derived from human umbilical cord mesenchymal stem cells ameliorate IL-6-induced acute liver injury through miR-455-3p. *Stem Cell Res Ther* 2020;11:020-1550.
20. Liu WH, Song FQ, Ren LN, Guo WQ, Wang T, Feng YX, Tang LJ, et al. The multiple functional roles of mesenchymal stem cells in participating in treating liver diseases. *J Cell Mol Med* 2015;19:511-520.
21. Yin F, Wang WY, Jiang WH. Human umbilical cord mesenchymal stem cells ameliorate liver fibrosis in vitro and in vivo: From biological characteristics to therapeutic mechanisms. *World J Stem Cells* 2019;11:548-564.
22. Zhao L, Chen S, Shi X, Cao H, Li L. A pooled analysis of mesenchymal stem cell-based therapy for liver disease. *Stem Cell Res Ther* 2018;9:72.
23. Zhang GZ, Sun HC, Zheng LB, Guo JB, Zhang XL. In vivo hepatic differentiation potential of human umbilical cord-derived mesenchymal stem cells: Therapeutic effect on liver fibrosis/cirrhosis. *World J Gastroenterol* 2017;23:8152-8168.
24. An SY, Jang YJ, Lim HJ, Han J, Lee J, Lee G, Park JY, et al. Milk Fat Globule-EGF Factor 8, Secreted by Mesenchymal Stem Cells, Protects Against Liver Fibrosis in Mice. *Gastroenterology* 2017;152:1174-1186.
25. Mansour MF, Greish SM, El-Serafi AT, Abdelall H, El-Wazir YM. Therapeutic potential of human umbilical cord derived mesenchymal stem cells on rat model of liver fibrosis. *Am J Stem Cells* 2019;8:7-18.
26. Wu CX, Wang D, Cai Y, Luo AR, Sun H. Effect of Autologous Bone Marrow Stem Cell Therapy in Patients with Liver Cirrhosis: A Meta-analysis. *J Clin Transl Hepatol* 2019;7:238-248.
27. Lafoz E, Ruat M, Anton A, Oncins A, Hernández-Gea V. The Endothelium as a Driver of Liver Fibrosis and Regeneration. *Cells* 2020;9.
28. Shubham S, Kumar D, Rooge S, Maras JS, Maheshwari D, Nautiyal N, Kumari R, et al. Cellular and functional loss of liver endothelial cells correlates with poor hepatocyte regeneration in acute-on-chronic liver failure. *Hepatol Int* 2019;13:777-787.

Tables

Table 1. Patient demographics and baseline characteristics between group A and group B, and group C and group D.

Factors	Liver Failure		Liver Cirrhosis			
	Group A	Group B	P value	Group C	Group D	P value
Sex (M/F)	34/3	22/5	0.266	26/5	23/5	1.000
Age (years)	40(35-46.5)	42(33-50)	0.581	47.5(37.5-56.5)	44(35-60)	0.665
ALT (U/L)	88(46.5-312)	77(46-336)	0.703	48(23.25-75.75)	42.5(25.25-89)	0.716
AST (U/L)	109(68-173.5)	103(68-147)	0.760	73(45.25-131.5)	65(47.5-92)	0.268
TBIL (mmol/L)	486(346-550.5)	381(219-495)	0.053	415(85.25-488.5)	149(75.7-306.75)	0.027
PTA	35(25.5-40.5)	34(31-47)	0.438	40.5(29.75-53)	44.5(34.75-59.25)	0.161
MELD	78(76.5-81)	77(75-82)	0.411	76(73-78)	72(67.75-76)	0.005

ALT, alanine aminotransferase; AST, glutamic-oxaloacetic transaminase; TBIL, total bilirubin; PTA, prothrombin time activity; MELD, model for end-stage liver disease.

Table 2. Patient demographics and baseline characteristics between group A and group C, and group B and group D.

Factors	> 4 weeks		≤ 4 weeks			
	Group A	Group C	P value	Group B	Group D	P value
Sex (M/F)	34/3	23/5	0.275	22/5	26/5	1.000
Age (years)	40(35-46.5)	47.5(37.5-56.5)	0.035	42(33-50)	44(35-60)	0.435
ALT (U/L)	88(46.5-312)	48(23.25-75.75)	0.004	77(46-336)	42.5(25.25-89)	0.005
AST (U/L)	109(68-173.5)	73(45.25-131.5)	0.054	103(68-147)	65(47.5-92)	0.002
TBIL (mmol/L)	486(346-550.5)	415(85.25-488.5)	0.043	381(219-495)	149(75.75-306.75)	0.000
PTA	35(25.5-40.5)	40.5(29.75-53)	0.033	34(31-47)	44.5(34.75-59.25)	0.006
MELD	78(76.5-81)	76(73-78)	0.006	77(75-82)	72(67.75-76)	0.000

ALT, alanine aminotransferase; AST, glutamic-oxaloacetic transaminase; TBIL, total bilirubin; PTA, prothrombin time activity; MELD, model for end-stage liver disease.

Table 3. Comparative analysis of the therapeutic effect of UCMSCs among different groups at different time points.

Outcome	Group	W0-W1		W1-W4		W4-W12		W12-W24	
		Value	P value	Value	P value	Value	P value	Value	P value
ALT	Group A	37.5(5.25-214)	NS ^{AB}	5(-6-42)	NS ^{AB}	8(-1.25-23.5)	0.002 ^{AB}	-3(-11-5)	NS ^{AB}
	Group B	24(9-260)	0.042 ^{BD}	22.5(6.2-45.3)	NS ^{BD}	-6(-15-6)	NS ^{BD}	0.5(-4-5.5)	NS ^{BD}
	Group C	19(2-48)	NS ^{CD}	-0.5(-9.3-10.25)	0.041 ^{CD}	2.5(-0.5-11)	0.014 ^{CD}	-4(-12-60)	NS ^{CD}
	Group D	12.5(-0.5-98.5)	NS ^{AC}	9.5(-0.75-9.5)	NS ^{AC}	-6(-24.5-3.5)	NS ^{AC}	5.5(0.25-7)	NS ^{AC}
AST	Group A	17(-5.5-60.25)	NS ^{AB}	-1(-17-11)	0.010 ^{AB}	26(6-53.5)	0.006 ^{AB}	9(-5-20)	0.032 ^{AB}
	Group B	24(-10-52)	NS ^{BD}	16(-1-43.75)	NS ^{BD}	1(-15-22)	NS ^{BD}	3(-0.25-14.5)	NS ^{BD}
	Group C	13(4-28)	NS ^{CD}	-7.5(-16.75-17)	NS ^{CD}	26(7.5-40.25)	0.000 ^{CD}	-3(-15-9)	NS ^{CD}
	Group D	4(-4-33.75)	NS ^{AC}	7.5(-7-21.25)	NS ^{AC}	4(-11.5-9)	NS ^{AC}	8.5(3.25-17)	NS ^{AC}
PTA	Group A	4(-1.75-6)	0.019 ^{AB}	-1(-7-3.5)	0.033 ^{AB}	-7(-15.5-0.5)	NS ^{AB}	-3(-5.7-2.1)	NS ^{AB}
	Group B	-2(-4-3)	NS ^{BD}	-5(-12-1)	NS ^{BD}	-15(-22.5-4.75)	NS ^{BD}	-4(-7.2-1.9)	NS ^{BD}
	Group C	5(-4-7)	0.014 ^{CD}	-1(-7-2.5)	0.008 ^{CD}	-7(-11.75-1.5)	NS ^{CD}	-2(-4.1-1.9)	NS ^{CD}
	Group D	-2(-8-1)	NS ^{AC}	-10(-19-4)	NS ^{AC}	-4(-14.5-5)	NS ^{AC}	-3(-5.2-2.6)	NS ^{AC}
MELD	Group A	-1(-2-1)	0.044 ^{AB}	1(-1-2.5)	0.000 ^{AB}	6.11±5.32	NS ^{AB}	2.14±1.71	NS ^{AB}
	Group B	1(-1-2)	NS ^{BD}	4(2-6)	NS ^{BD}	6.86±5.01	NS ^{BD}	1.96±1.64	NS ^{BD}
	Group C	0(-1-2)	NS ^{CD}	1(-1-3)	0.011 ^{CD}	4(0.5-7)	NS ^{CD}	2.4(0.1-5.3)	NS ^{CD}
	Group D	2(0-4)	NS ^{AC}	4(1-6.5)	NS ^{AC}	0.5(-0.75-5.5)	NS ^{AC}	3.3(-0.5-4.9)	NS ^{AC}

P^{AB}, P values for comparison of group A and group B; P^{CD}, P values for comparison of group C and group D; P^{AC}, P values for comparison of group A and group C; P^{BD}, P values for comparison of group B and group D. NS, nonsignificant. ALT, alanine aminotransferase; AST, glutamic-oxaloacetic transaminase; TBIL, total bilirubin; PTA, prothrombin time activity; MELD, model for end-stage liver disease.

Figures

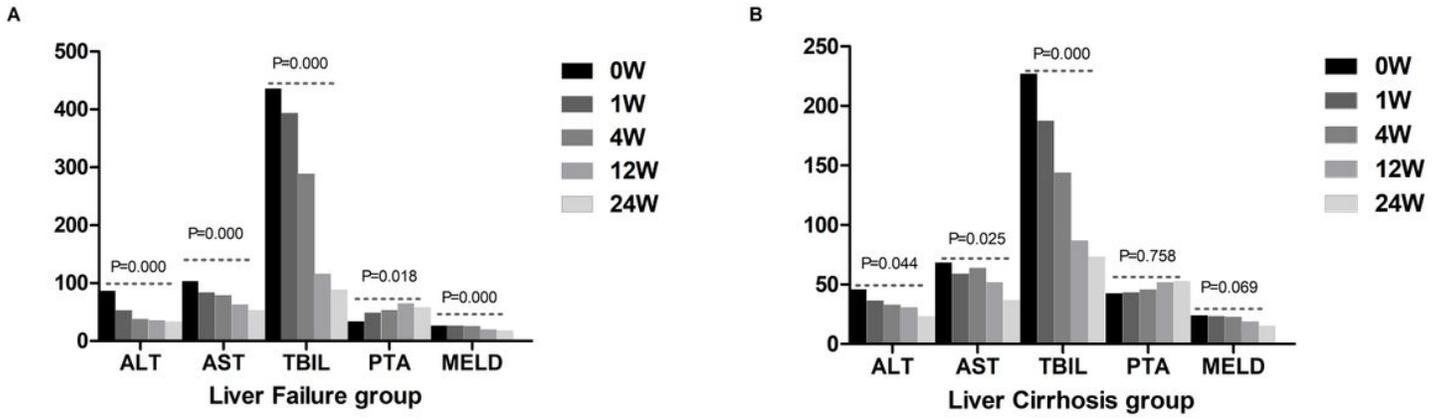


Figure 1

Comparison of the therapeutic effect of UCMSCs among different observation weeks in the liver failure group and liver cirrhosis group. ALT, alanine aminotransferase; AST, glutamic-oxaloacetic transaminase; TBIL, total bilirubin; PTA, prothrombin time activity; MELD, model for end-stage liver disease.

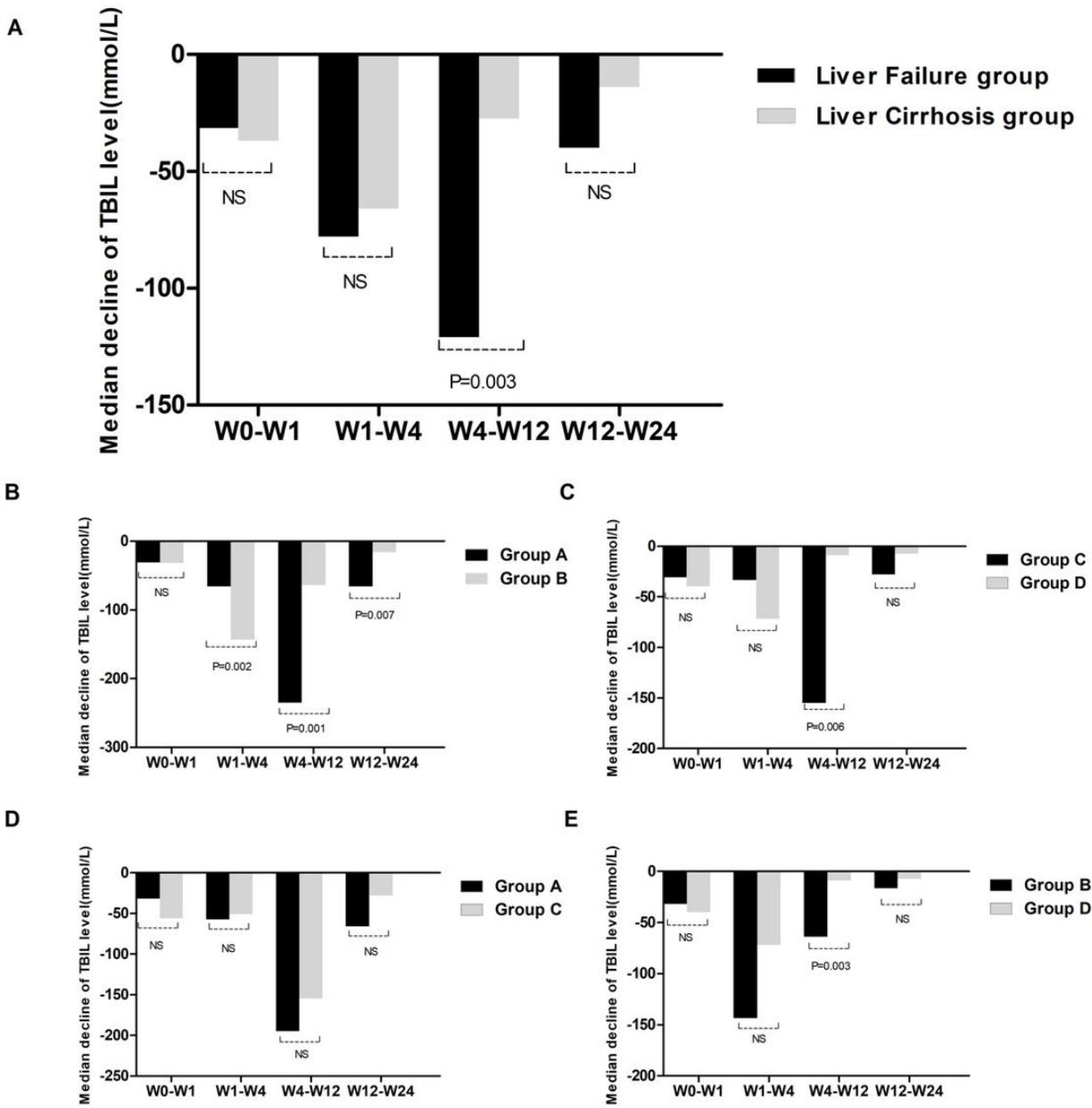


Figure 2

Comparison of the median declines in TBIL levels among different groups at different time points. TBIL, total bilirubin.

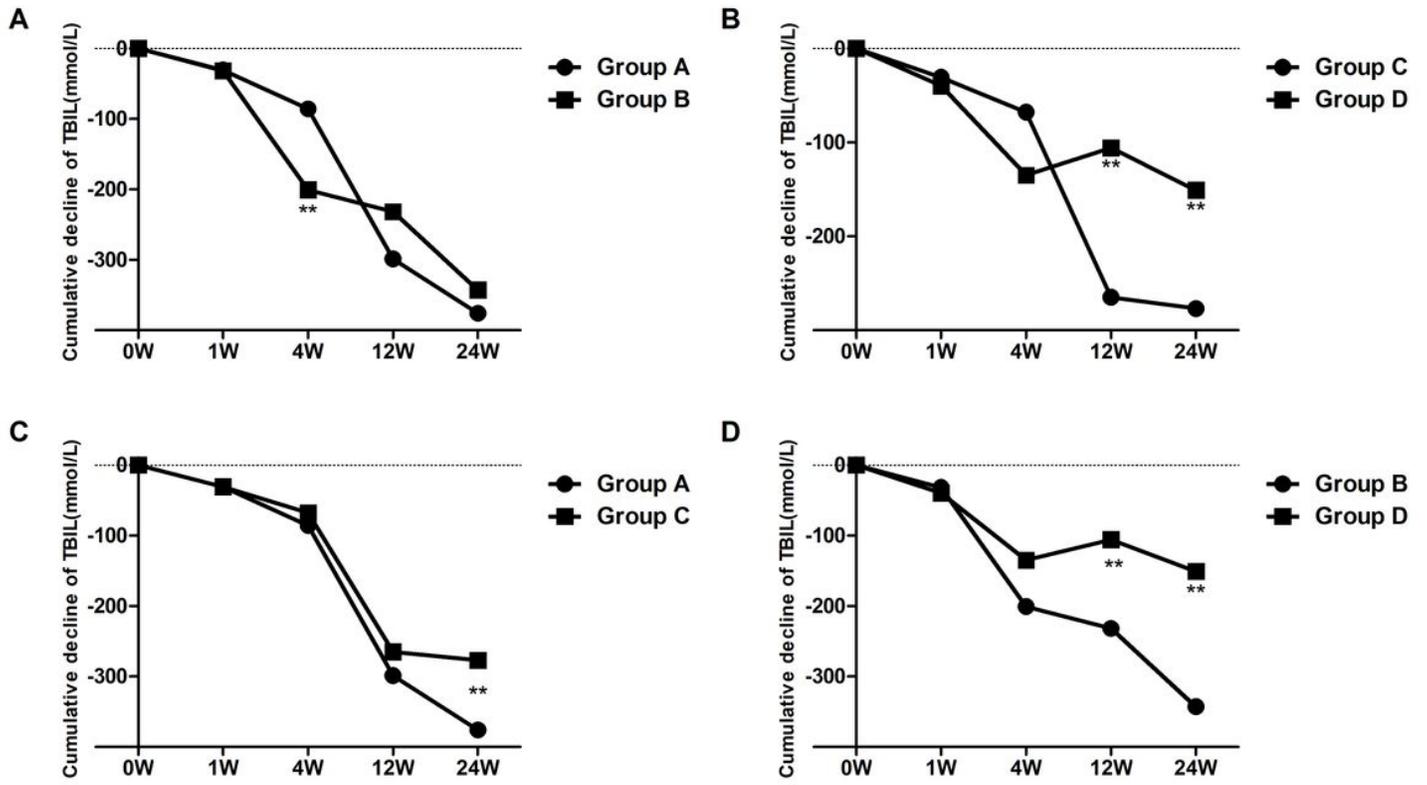


Figure 3

Comparison of the cumulative declines in TBIL levels among different groups at different time points. TBIL, total bilirubin.
 **P < 0.01.