European Review for Medical and Pharmacological Sciences 2016; 20: 2908-2915

Autologous hematopoietic stem cell transplantation in patients with end-stage liver disease: a 5-year follow-up study of 48 patients

L. LUO[1], Y.-T. YAO[1], H. XUE[1], L.-Y. LUO[1], H.-B. ZOU[1], G. WANG[1], G.-M. XIANG[1], L.-L. WEI[1], M.-Z. YANG[1], T. ZHANG[2], P. XIE[2], G. XU[3], S.-P. DENG[1], X.-L. HUANG[1]

[1]Department of Hepatobiliary Surgery and Cell Transplantation Center, Sichuan Academy of Medical Sciences and Sichuan Provincial People's Hospital, Chengdu, China
[2]Department of Radiology, Sichuan Academy of Medical Sciences and Sichuan Provincial People's Hospital, Chengdu, China

Le Luo and *Yutong Yao* have controbuted equally to this work, and therefore should be considered as co-first authors

Abstract. – OBJECTIVE: To investigate the long-term therapeutic effect of autologous hematopoietic stem cell transplantation in patients with End-stage Liver Disease (ESLD).

PATIENTS AND METHODS: Forty-eight ESLD patients underwent autologous CD34+ stem cell transplantation were retrospectively reviewed. Changes in clinical and biochemical data, complications, and quality of life were monitored at 3, 6, 12, 36, and 60 months following the stem cell transplantation. Liver biopsies were obtained for histopathological analysis using Ishak system.

RESULTS: Marked improvement in clinical and biochemical data was observed during the long-term follow-up. Serum albumin was significantly increased ($p<0.001$), while total serum bilirubin, prothrombin time (PT), and international normalized ratio (INR) were all significantly decreased ($p<0.001$). Ishak inflammation and fibrosis scores were significantly decreased with the increased time ($p<0.001$). The number of patients with ascites, model of end-stage liver disease (MELD) score, Child-Pugh class, and indocyanine green (ICG) score were all markedly reduced with increased time. Meanwhile, the quality of life score of the patients was significantly increased ($p<0.001$). Six patients died during the 5-years follow-up, and complications occurred in 17 patients. The incidence of complications was significantly associated with mortality of the patients ($p<0.05$).

CONCLUSIONS: The study provided the evidence that autologous CD34+ stem cell transplantation could offer a long-term therapeutic benefit to patients with ESLD. The complications occurred during the process was significantly associated with survival of the patients. Future studies on a large cohort of patients are needed to confirm the long-term effect of stem cell therapy on ESLD.

Key Words: End-stage liver disease (ESLD), Hematopoietic stem cells, Long-term outcome, Liver function, Quality of life, Complications.

Introduction

End-stage liver disease (ESLD) is a medical dilemma that associates with poor prognosis of the patients[1]. Currently, the only available radical treatment is liver transplantation, which, however, is hampered due to the limited donor organs, operative risk, potential immunological rejection, and social and economic concerns[2]. That makes the development of new regenerative therapies for ESLD patients an urgent task. Increasing attention has been focused on stem cell therapy as a prospective alternative for liver regeneration[3,4]. Stem cells with hepatic differentiation potential are theoretically eligible for liver cell replacement[5].

Different types of stem or progenitor cells have been used for liver regeneration, including CD34 positive (CD34+) cells from peripheral blood induced by granulocyte colony-stimulating factor (G-CSF)[4]. Stem cells transplantation has demonstrated to be effective in liver regeneration with the improved liver function[6]. CD34+ cells collected from peripheral blood after G-CSF induction have been administered via a hepatic artery, and the results showed improved serum levels of bilirubin and albumin during a short observation period of 60 days[7]. The improvements in serum albumin, Child-Pugh score, and accumulation of ascites have also been reported in patients with

Corresponding Author: Xiaolun Huang, MD; e-mail: huangxiaolun@med.uestc.edu.cn
Hua Xue, MD; e-mail: xh81@hotmail.com

alcoholic liver cirrhosis during the 3-months follow-up, with CD34+ cells administered via a hepatic artery[8]. Furthermore, our previous study has also indicated that transplantation of autologous hematopoietic stem cells improved liver function and histology in ESLD patients during the 12-months observation, while administration route of either hepatic artery or portal vein showed no significant difference[9]. However, most of these studies are based on a comparatively shorter time intervals. Further studies with longer follow-up have been suggested to be helpful in exploring the role of stem cell transplantation for treatment of ESLD[10]. Therefore, in this study, the 5-year survival of the patients with ESLD that underwent CD34+ stem cells transplantation was retrospectively analyzed.

Patients and Methods

Patients

This study retrospectively analyzed ESLD patients underwent autologous hematopoietic stem cell infusion at Sichuan Province People's Hospital during 2010. All patients were diagnosed with decompensated liver cirrhosis according to the guidelines defined by the Chinese Society of Hepatology and Chinese Society of Infectious Diseases[11]. Patient with prothrombin time (PT) change less than 5 seconds following anticoagulation injection were recruited into this study. Patients were excluded if they were: pregnant or lactating women, or those with hepatocellular carcinoma, bacterial peritonitis, human immunodeficiency virus (HIV) infection or any other life-threatening infection; recurrent gastrointestinal hemorrhage; portal thrombosis with main portal trunk obstruction, sponge blood vessel, superior mesenteric vein occlusion, or severe intestinal congestion; ascites and resistance to diuretic therapy or developed abdominal compartment syndrome; hepatic encephalopathy; hypersensitive to granulocyte-colony stimulating factor (G-CSF), or with incomplete follow-up data. This study was approved by the Ethics Committee of Sichuan Academy of Medical Sciences & Sichuan Provincial People's Hospital and informed consent was obtained from the patients.

Preparation and Transplantation of CD34+ Hematopoietic Stem Cells

Pre-transplantation preparation and transplantation of $CD34^+$ hematopoietic stem cells isolated were performed as described in our previous study[9]. Briefly, patients underwent regular hepatoprotective therapy and nutrition support therapy. α1-thymosin (Patheon Italia SPA, Monza, Italy) was given for immune enhancement, somatostatin (Merck Serono SA, Aubonne Branch, Geneva, Switzerland) and Propranolol (Tianjin Lisheng Pharmaceutical Co., Ltd., Tianjin, China) were administrated for portal vein pressure. Patients received G-CSF (5 μg/kg per day, Kyowa Hakko Kirin Co., Ltd., Chiyoda-ku, Tokyo, Japan) for 3-5 days. Mononuclear cells (100-200 mL) were collected from the circulating blood using the COM.TEC cell separator (Fresenius Hemocare, Fresenius Hemocare, Bad Homburg, Germany). Anticoagulant acid citrate dextrose solution (ACD-A) was added as the anticoagulant. The number of circulating CD34+ hematopoietic stem cells was evaluated using flow cytometry (FACScalibur, BD Inc., San Jose, CA, USA). The collected $CD34^+$ cells ($1.79\pm1.66\times10^6$) were infused the next day through either hepatic artery or portal vein. The portal vein pressure was monitored during the process. Anticoagulant (low molecular weight heparin, Sanofi-Aventis, Paris, France) was administrated at postoperative day 3 to prevent portal vein thrombosis. Patients were well informed about the procedures.

Clinical and Laboratory Analysis

Clinical examination was done for all patients. Laboratory investigations included routine blood test (hemoglobin, white blood cell count, and platelet count), liver function test (ALT, AST, albumin, and total bilirubin), coagulation profile (PT, aPPT, and INR), infectious profile, blood urea, and serum creatinine levels. Ultrasound and computed tomography (CT) scan were done before and after stem cell transplantation. Child-Pugh score, Model of end-stage liver disease (MELD) score, and indocyanine green (ICG) score were used to assess the severity of liver disease. Liver biopsy was guided by ultrasound or during surgery. The formalin-fixed and paraffin embedded specimens (4 μm) were stained with hematoxylin and eosin (HE) and Gomori methenamine silver. The results were reviewed by two experienced pathologists; the inflammatory activity and fibrosis were assessed using the Ishak system[12]. The chronic liver disease questionnaire was used to measure health-related Quality of Life (QOL) of the patients[13].

Follow-up

All patients were followed-up from the date of stem cells transplantation to the death of patients

Table I. Clinical characteristic of patients with ESLD following autologous CD34+ stem cells transplantation.

Variable	Patients with ESLD (n=48)
Sex male n (%)	38 (79%)
Age (years)	46 (12-76)
Body weight (kg)	62.13±9.85
Body height (cm)	165.3±6.7
BMI (kg/m2)	22.65±2.78
Aetiology	
Hepatitis B cirrhosis	34 (71%)
Hepatitis C cirrhosis	4 (8%)
Alcoholic cirrhosis	8 (17%)
Autoimmune cirrhosis	1 (2%)
Wilson disease	1 (2%)
Child-Pugh class	
A	7
B	23
C	18

ESLD: End stage liver disease; BMI: Body mass index.

or July 2015. The primary outcome was the survival of patients during the 5-years follow-up. The secondary outcomes were the changes in Child-Pugh score, MELD score, ICG score, Ishak inflammatory and fibrosis scores, HRQL score, and the incidence of complications, as well as their prognostic relevance.

Statistical Analysis

Continuous variables were expressed as mean and standard deviation (SD), and categorical variables were expressed as number and percentage. Comparison analysis was done repeated measures ANOVA or generalized estimating equations. Survival analysis was performed by Kaplan-Meier method. Univariate analysis was performed by Cox regression analysis. All *p*-values were 2-tailed and $p<0.05$ was considered statistically significant. Statistical analysis was done using the SPSS software version 17.0 (SPSS Inc., Chicago, IL, USA).

Results

Clinical Characteristics of the Patients

A total of 48 patients with ESLD were recruited into the study. The median age of the patients was 46 years (range: 12 to 76 years), and 38 (79%) were males (Table I). The mean body mass index (BMI) of the patients was 22.65±2.78 kg/m^2. Thirty-four patients had Hepatitis B cirrhosis, 8 had alcoholic cirrhosis, 4 had Hepatitis C cirrhosis, and each one had autoimmune cirrhosis and Wilson disease. Seven patients were diagnosed as Child-Pugh class A, 23 as class B, and the other 18 patients as class C.

Biochemical and Histological Findings

Changes in the laboratory data of 48 patients who received stem cell transplantation were summarized in Table II. There was a marked improvement in biochemical parameters of the patients during the follow-up. Serum albumin level was significantly increased with increased time ($p<0.001$), while serum levels of total bili-

Table II. Changes in cliniccal data of patients underwent autologous CD34+ stem cells transplantation during the 5 years follow-up.

Variable	T0 (n=48)	T1 (n=46)	T2 (n=45)	T3 (n=44)	T4 (n=43)	T5 (n=42)	*p*-value
Albumin (g/L)	30.4±5.7	31.1±4.4	33.6±4.2	35.9±4.1	37.4±3.6	37.7±3.7	<0.001
Bilirubin (μmol/L)	38±18.2	34.1±15.1	32.7±13.1	26.6±9.2	24.8±9.1	22.4±8.3	<0.001
PT (s)	15.2±2.7	14.5±2.5	13.8±1.7	13±1	12.9±0.9	12.6±0.8	<0.001
INR	84.3±49	82.1±41	74.7±23.1	72.5±26.4	69.2±10.8	72.4±12.2	<0.001
Creatinine (μmol/L)	26.9±6.4	24±6.4	20.7±6.1	16.7±4.4	15.1±3.6	14.7±3.9	>0.05
Ascites n (%)#	40 (83.3%)	33 (68.8%)	30 (63.8%)	17 (37.0%)	10 (22.2%)	8 (18.2%)	<0.001
MELD score	13.06±3.57	11.76±3.72	10.7±2.89	9.62±3.16	8.79±1.89	8.16±2.03	<0.001
Child-Pugh score	8.89±1.93	7.95±1.96	7.14±1.47	6.02±1.00	5.66±0.86	5.57±0.76	<0.001
Child-Pugh class#	7/23/18	11/27/10	15/30/2	28/18/0	37/8/0	39/5/0	<0.001
ICG score	27.24±6.63	24.21±6.61	21.04±6.43	16.71±4.50	15.02±3.60	14.45±4.03	<0.001
QOL score	141±18	148±18	156±18	167±21	174±16	175±19	<0.001
Ishak inflammation score	9.92±1.77	9.25±1.97	8.74±1.79	7.39±1.31	6.46±1.50	5.92±1.23	<0.001
Ishak fibrosis score	5.05±0.93	4.77±1.21	4.46±1.00	4.21±0.91	4.03±0.76	3.81±0.82	<0.001

PT: Prothrombin time; INR: International normalized ratio; MELD: Model for end stage liver disease; ICG: Indocyanine green; QOL: Quality of life. T0: Pri-transplantation; T1, T2, T3, T4, and T5: post-transplantation month 3, 6, 12, 36, and 60. #: Data were analyzed by generalized estimating equations, while all others were analyzed by repeated measures ANOVA.

Table III. Postoperative complications during the 5 years follow-up.

Follow-up (months)	3	6	12	36	60	Total
Upper gastrointestinal hemorrhage	4	1	3	1	1	10
Hepatorenal syndrome	1	1	1	0	0	3
Hepatic encephalopathy	2	1	0	0	0	3
Portal thrombosis	1	1	0	0	0	2
Hypohepatia	1	1	1	0	1	4

PT: Prothrombin time; INR: International normalized ratio; MELD: Model for end stage liver disease; ICG: Indocyanine green; QOL: Quality of life. T0: Pri-transplantation; T1, T2, T3, T4, and T5: post-transplantation month 3, 6, 12, 36, and 60. #: Data were analyzed by generalized estimating equations, while all others were analyzed by repeated measures ANOVA.

rubin, prothrombin time (PT), and international normalized ratio (INR) were all significantly decreased during the observation (p<0.001 for each). Serum level of creatinine was found also to be decreased gradually, but the difference was not statistically significant. For histopathological analysis, the liver biopsies were tested with HE and silver stainings (Figure 1A). Ishak inflammation and fibrosis scores were significantly decreased during the increased time (p<0.001, Figure 1B).

Clinical Data and Performance Tests

The MELD score, Child-Pugh class, ICG score, and some patients with ascites were all markedly reduced with increased time post-transplantation, and the quality of life score of the patients was significantly increased (Table II, Figure 2).

Outcome Analysis

Six patients died post-transplantation during the follow-up, 3 patients died from hepatorenal syndrome, 2 from hypohepatia, and the other one from hepatic encephalopathy (Figure 3A). The 5-year survival rate of the patients was about 84.7%. Complications occurred in 17 (35.4%) patients. The most common long-term complications were upper gastrointestinal hemorrhage, occurred 10 times in 8 patients, followed by hypohepatia in 4 patients, hepatorenal syndrome and hepatic encephalopathy in 3 patients, and portal thrombosis in 2 patients (Table III). The prevalence of complications was significantly higher in patients of death group than that of the survival group (p<0.001, Supplemental Table I). Univariate analysis showed a significant correlation between the presence of complications and mortality of the patients (p<0.05, Table IV).

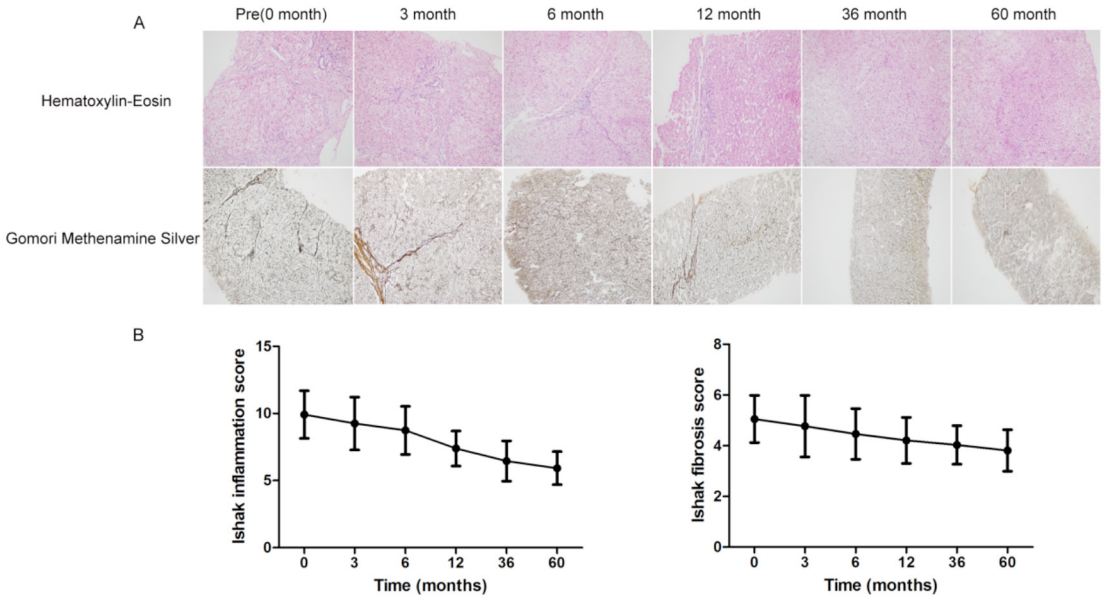

Figure 1. Histopathological analysis of liver biopsies during the different time points of follow up. (A) HE and Gomori methenamine silver stainings; and (B) Inflammation and fibrosis scores using Ishak system.

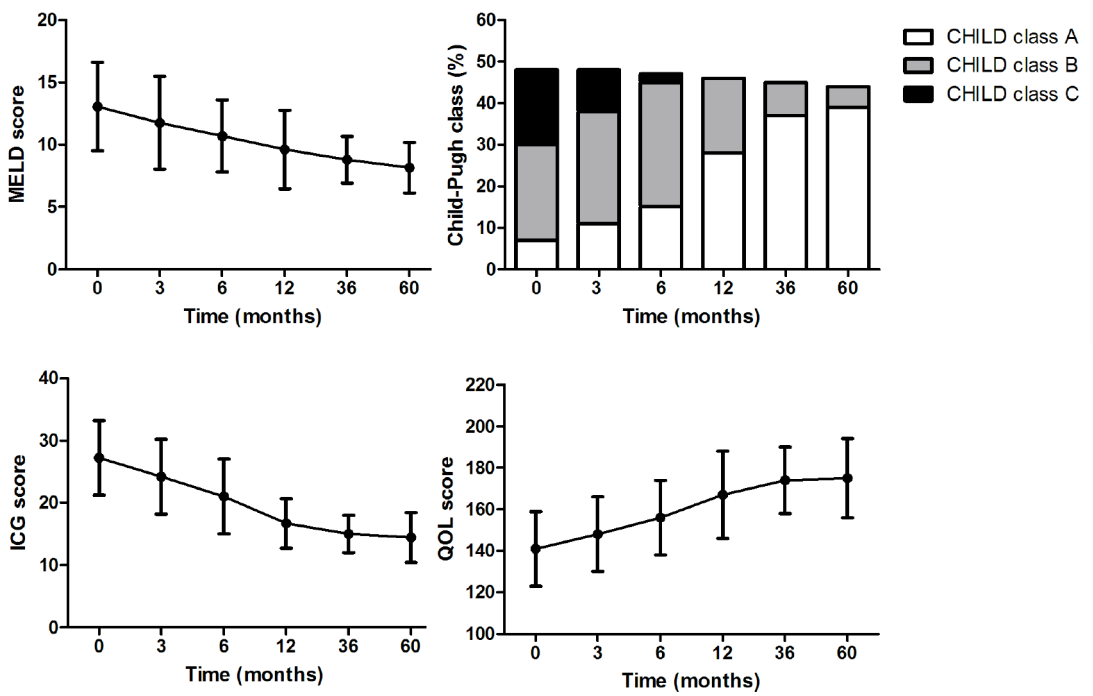

Figure 2. Changes in MELD score, Child-Pugh class, ICG score, and Quality of life (QOL) score of the patients following autologous CD34+ stem cell transplantation.

Discussion

Hepatic cirrhosis is known as the end-stage of chronic liver diseases. Most of the patients with hepatic cirrhosis die from life-threatening clinical complications, like ascites, hepatic encephalopathy, and variceal hemorrhage, occurring at their earlier ages[14]. The encouraging advances in stem cell research have paved the way for their successful use in ESLD therapy[15].

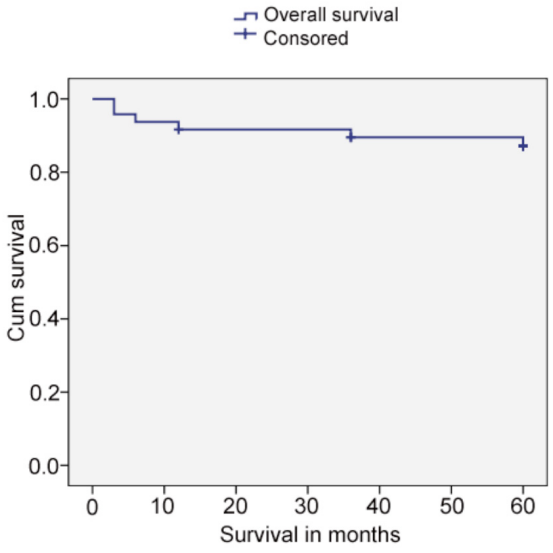

Figure 3. Survival analysis of patients underwent CD34+ stem cell transplantation during the long term follow-up.

Table IV. Univariate analysis of prognosis factors in patients with ESLD following autologous CD34+ stem cells transplantation.

Univariate analysis	Exp(B) (95.0% CI)	*p*-value
Gender	1.368 (0.16-11.717)	0.775
Age	0.962 (0.902-1.027)	0.247
BMI	1.022 (0.766-1.364)	0.882
Etiology	1.552 (0.851-2.833)	0.152
Ascites	0.39 (0.071-2.129)	0.277
MELD score	1.155 (0.918-1.452)	0.218
Child-Pugh score	0.975 (0.633-1.502)	0.910
ICG score	1.055 (0.922-1.207)	0.438
QOL score	0.989 (0.944-1.036)	0.635
Ishak inflammation score	0.982 (0.6-1.609)	0.943
Ishak fibrosis score	1.237 (0.484-3.165)	0.657
Complications	1.644 (1.123-2.407)	0.011

PT: Prothrombin time; INR: International normalized ratio; MELD: Model for end stage liver disease; ICG: Indocyanine green; QOL: Quality of life. T0: Pri-transplantation; T1, T2, T3, T4, and T5: post-transplantation month 3, 6, 12, 36, and 60. #: Data were analyzed by generalized estimating equations, while all others were analyzed by repeated measures ANOVA.

Supplemental Table 1. Comparative analysis of clinical and biochemical data of ESLD patients in survival and death groups.

Survival group	Death group	p-value	
Sex, male n (%)	33 (78%)	5 (83.3%)	>0.05
Age (years)	48.90±10.9	43.0±16.11	>0.05
BMI (kg/m2)	22.63±2.91	22.82±1.75	>0.05
Aetiology			
Hepatitis B cirrhosis	31 (73.8%)	3 (50%)	
Hepatitis C cirrhosis	3 (7.1%)	1 (16.7%)	
Alcoholic cirrhosis	7 (16.7%)	1 (16.7%)	>0.05
Autoimmune cirrhosis	1 (2.4%)	0 (0%)	
Wilson disease	0 (0%)	1 (16.7%)	
Child-Pugh class			
A	7 (16.7%)	0 (0%)	
B	19 (45.2%)	4 (66.7%)	>0.05
C	16 (38.1%)	2 (33.3%)	
Albumin (g/L)	30.65±5.79	28.77±4.70	>0.05
Bilirubin (μmol/L)	36.40±16.72	49.35±25.16	>0.05
PT (s)	15.20±2.88	15.05±1.75	>0.05
INR	1.31±0.25	1.29±0.16	>0.05
Creatinine (μmol/L)	80.83±41.73	108.75±86.45	>0.05
Ascites n (%)	36 (85.7%)	4 (66.7%)	>0.05
MELD score	12.83±3.54	14.66±3.70	>0.05
Child-Pugh score	8.90±1.87	8.83±1.94	>0.05
ICG score	26.67±6.13	28.77±8.66	>0.05
QOL score	141.38±17.89	137.83±22.82	>0.05
Ishak inflammation score	9.90±1.62	9.83±2.23	>0.05
Ishak fibrosis score	5.00±0.937	5.17±0.75	>0.05
Complications			
Upper gastrointestinal hemorrhage	7 (16.67%)	1 (16.67%)	
Hepatorenal syndrome	0 (0%)	3 (50%)	
Hepatic encephalopathy	2 (4.76%)	1 (16.67%)	**<0.001**
Portal thrombosis	2 (4.76%)	0 (0%)	
Hypohepatia	2 (4.76%)	1 (16.67%)	

ESLD: End stage liver disease; BMI: Body mass index; PT: Prothrombin time; INR: International normalized ratio; MELD: Model for end stage liver disease; ICG: Indocyanine green; QOL: Quality of life.

Increasing evidences have suggested an important role of stem cells as an attractive alternative in the treatment of liver diseases[16]. Clinical studies[8,9,17,18] on the therapeutic application of stem cells demonstrated the feasibility and safety of CD34+ stem cells transplantation for ESLD therapy. The long-term follow-up data of 18 months have obtained from five patients infused with CD34+ adult stem cells, and the study showed a beneficial effect of stem cell therapy in the short and over long term observation[19]. However, other studies also indicated that transplantation of bone marrow-derived mesenchymal stem cells did not improve liver function and survival of the patients, but even aggravated liver fibrosis[20]. Meanwhile, most of the previous studies focused on the shorter-term therapeutic effect of stem cell transplantation, and its application has been suggested to need further exploration with large cohorts and long follow-ups. Therefore, in this study, we analyzed 48 ESLD patients underwent autologous bone marrow-derived CD34+ hematopoietic stem cell transplantation during 2010. There were marked improvements in terms of clinical and biochemical data during the 5 years follow-up. Serum albumin levels were found to significantly increase with the increased time, while serum levels of bilirubin were obviously decreased post-transplantation, corroborating the results of our previous study[9].

MELD and Child-Pugh scores are commonly used to assess liver function and are associated with postoperative morbidity and complication[21,22]. How-

ever, these recipient-derived methods are suggested to poorly predict the mortality in liver transplant recipients[22,23]. Indocyanine green (ICG) is a commonly used ophthalmic dye that has been clinically approved as a sensitive indicator of liver function[24]. The findings of our study showed that infusion of autologous CD34+ stem cells resulted in a continual decrease in MELD score, Child-Pugh score, and ICG score in our patients during the observation period of 5 years. However, there was no significant correlation of MELD score, Child-Pugh score, or ICG score with the survival of the patients. Meanwhile, quality of life score of the patients was found to improve significantly with the increased time. All these results indicated a long-term therapeutic benefit of autologous CD34+ stem cells in our ESLD patients.

Autologous bone marrow cell infusions have been reported to improve the cases of liver fibrosis[25]. However, Kim et al[26] also indicated that there was no significant change in grade or stage of liver fibrosis following autologous bone marrow infusion. The long-term inflammation and fibrosis of hepatic biopsies were, thus, evaluated using Ishak's system, a revised version of histological activity index that describes activity grade and fibrosis stage as two separate items[12]. Our results showed that both Ishak inflammation and fibrosis scores were significantly decreased during the long term observation, which further corroborated the results of clinical data and performance tests, demonstrating the long-term beneficial effect of stem cell therapy in ESLD patients.

The previous study of liver failure patients by Peng et al[27] indicated that transplantation of autologous marrow mesenchymal stem cells caused no significant improvement in mortality of patients during 192 weeks of follow-up. In regards to long-term prognosis of patients in our study, 6 patients died during the long-term observation. The 5-years survival rate of our patients was about 84.7%. Hepatic cellular cancer has been reported in liver failure patients underwent autologous bone marrow mesenchymal stem cell transplantation during the long term follow-up[27]. However, in our study, no patient suffered from hepatic cellular cancer, and the common complications observed were upper gastrointestinal hemorrhage, hepatorenal syndrome, hepatic encephalopathy, portal thrombosis, and hypohepatia. The development of complications was significantly associated with the increased mortality of our patients. However, given the small number of patients recruited, multivariate analysis was not performed, and a large cohort study with long-term follow-up are still needed to figure it out further.

Conclusions

The findings of our study provided the evidence that autologous CD34+ stem cell transplantation offered a long-term therapeutic benefit to patients with ESLD. The prevalence of complications was significantly associated with survival of the patients. Future studies on a large cohort of patients are still needed to further confirm the long-term therapeutic benefits of stem cell transplantation.

Acknowledgement

This work was financially supported by the National Basic Research Program of China (973 Program, No. 2015CB964703) and Science and Technology Achievements Transformation Project of Sichuan Province (No. 14010127).

Conflicts of interest

The authors declare no conflicts of interest.

References

1) Si-Tayeb K, Lemaigre FP, Duncan SA. Organogenesis and development of the liver. Dev Cell 2010; 18: 175-189.
2) Francoz C, Belghiti J, Durand F. Indications of liver transplantation in patients with complications of cirrhosis. Best Pract Res Clin Gastroenterol 2007; 21: 175-190.
3) Hassan GM, Sliem H, Ellethy AT, Salama ME. Chronic liver disease: stem cell therapy. J Gastroenterol Hepatol Res 2013; 2.
4) Takami T, Terai S, Sakaida I. Stem cell therapy in chronic liver disease. Curr Opin Gastroenterol 2012; 28: 203-208.
5) Puglisi M, Saulnier N, Piscaglia A, Tondi P, Agnes S, Gasbarrini A. Adipose tissue-derived mesenchymal stem cells and hepatic differentiation: old concepts and future perspectives. Eur Rev Med Pharmacol Sci 2011; 15: 355.
6) Pan XN, Zheng LQ, Lai XH. Bone marrow-derived mesenchymal stem cell therapy for decompensated liver cirrhosis: a meta-analysis. World J Gastroenterol 2014; 20: 14051-14057.
7) Gordon MY, Levicar N, Pai M, Bachellier P, Dimarakis I, Al-Allaf F, M'Hamdi H, Thalji T, Welsh JP, Marley SB, Davies J, Dazzi F, Marelli-Berg F, Tait P, Playford R, Jiao L, Jensen S, Nicholls JP, Ayav A, Nohandani M, Farzaneh F, Gaken J, Dodge R, Alison M, Apperley JF, Lechler R, Habib NA. Characterization and clinical application of human CD34+ stem/progenitor cell populations mobilized into the blood by granulocyte colony-stimulating factor. Stem Cells 2006; 24: 1822-1830.

8) Pai M, Zacharoulis D, Milicevic MN, Helmy S, Jiao LR, Levicar N, Tait P, Scott M, Marley SB, Jestice K, Glibetic M, Bansi D, Khan SA, Kyriakou D, Rountas C, Thillainayagam A, Nicholls JP, Jensen S, Apperley JF, Gordon MY, Habib NA. Autologous infusion of expanded mobilized adult bone marrow-derived CD34+ cells into patients with alcoholic liver cirrhosis. Am J Gastroenterol 2008; 103: 1952-1958.

9) Huang XL, Luo L, Luo LY, Xue H, Wei LL, Yao YT, Zou HB, Huang XB, Zhu YF, Zhang T, Xie P, Yang MZ, Deng SP. Clinical outcome of autologous hematopoietic stem cell infusion via hepatic artery or portal vein in patients with end-stage liver diseases. Chin Med Sci J 2014; 29: 15-22.

10) Margini C, Vukotic R, Brodosi L, Bernardi M, Andreone P. Bone marrow derived stem cells for the treatment of end-stage liver disease. World J Gastroenterol 2014; 20: 9098-9105.

11) Chinese Society of Hepatology CMA, Chinese Society of Infectious Diseases CMA. The guidelines of prevention and treatment for chronic hepatitis B. Zhonghua Gan Zang Bing Za Zhi= Zhonghua Ganzangbing Zazhi= Chinese Journal of Hepatology 2005; 13: 881.

12) Brunt EM. Grading and staging the histopathological lesions of chronic hepatitis: the Knodell histology activity index and beyond. Hepatology 2000; 31: 241-246.

13) Younossi Z, Guyatt G, Kiwi M, Boparai N, King D. Development of a disease specific questionnaire to measure health related quality of life in patients with chronic liver disease. Gut 1999; 45: 295-300.

14) Bataller R, Brenner DA. Liver fibrosis. J Clin Invest 2005; 115: 209-218.

15) Chen Z, Qi L, Zeng R, Li H, Dai L. Stem cells and hepatic cirrhosis. Panminerva Med 2010; 52: 149-165.

16) Di Campli C, Nestola M, Piscaglia A, Santoliquido A, Gasbarrini G, Pola P, Gasbarrini A. Cell-based therapy for liver diseases. Eur Rev Med Pharmacol Sci 2003; 7: 41-44.

17) Salama H, Zekri A, Bahnassy AA, Medhat E, Halim HA, Ahmed OS, Mohamed G, Al Alim SA, Sherif GM. Autologous CD34+ and CD133+ stem cells transplantation in patients with end stage liver disease. World J Gastroenterol 2010; 16: 5297-5305.

18) Salama H, Zekri A-R, Zern M, Bahnassy A, Loutfy S, Shalaby S, Vigen C, Burke W, Mostafa M, Medhat E, Medhat E, Alfi O, Huttinger E. Autologous hematopoietic stem cell transplantation in 48 patients with end-stage chronic liver diseases. Cell Transplant 2010; 19: 1475-1486.

19) Levičar N, Pai M, Habib N, Tait P, Jiao L, Marley S, Davis J, Dazzi F, Smadja C, Jensen S, Nicholls JP, Apperley JF, Gordon MY. Long-term clinical results of autologous infusion of mobilized adult bone marrow derived CD34+ cells in patients with chronic liver disease. Cell Prolif 2008; 41: 115-125.

20) Wu XZ, Chen D. Helicobacter pylori and hepatocellular carcinoma: correlated or uncorrelated? J Gastroenterol Hepatol 2006; 21: 345-347.

21) Eaton JE, Thackeray EW, Lindor KD. Likelihood of malignancy in gallbladder polyps and outcomes following cholecystectomy in primary sclerosing cholangitis. Am J Gastroenterol 2012; 107: 431-439.

22) Oberkofler CE, Dutkowski P, Stocker R, Schuepbach RA, Stover JF, Clavien PA, Béchir M. Model of end stage liver disease (MELD) score greater than 23 predicts length of stay in the ICU but not mortality in liver transplant recipients. Critical Care 2010; 14: R117.

23) Chung I, Park M, Ko J, Gwak M, Kim G, Lee SK. Which score system can best predict recipient outcomes after living donor liver transplantation? Transplant Proc 2012; 44: 393-395.

24) Feng HL, Li Q, Wang L, Yuan GY, Cao WK. Indocyanine green clearance test combined with MELD score in predicting the short-term prognosis of patients with acute liver failure. Hepatobiliary Pancreat Dis Int 2014; 13: 271-275.

25) Maeda M, Takami T, Terai S, Sakaida I. Autologous bone marrow cell infusions suppress tumor initiation in hepatocarcinogenic mice with liver cirrhosis. J Gastroenterol Hepatol 2012; 27: 104-111.

26) Kim JK, Park YN, Kim JS, Park MS, Paik YH, Seok JY, Chung YE, Kim HO, Kim KS, Ahn SH, Kim DY, Kim MJ, Lee KS, Chon CY, Kim SJ, Terai S, Sakaida I, Han KH. Autologous bone marrow infusion activates the progenitor cell compartment in patients with advanced liver cirrhosis. Cell Transplant 2010; 19: 1237-1246.

27) Peng L, Xie DY, Lin BL, Liu J, Zhu HP, Xie C, Zheng YB, Gao ZL. 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.