Infusion of autologous bone marrow mononuclear cells through hepatic artery results in a short-term improvement of liver function in patients with chronic liver disease: a pilot randomized controlled study

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Aim This randomized controlled study evaluated the effect of autologous infusion of bone marrow cells (BMC) in patients with hepatic cirrhosis.

Methods Thirty patients on the liver transplant waiting list were randomly assigned to receive BMC therapy or no treatment. They were followed up for 1 year. The study was nonblinded. Autologous mononuclear-enriched BMC were infused into the hepatic artery; liver function scores/tests were chosen as endpoints to assess efficacy. Statistical analysis calculated mean relative changes (RC) from baseline and fitted a random-effects model.

Results Mean age, baseline model for end-stage liver disease, and Child–Pugh score were similar in both groups. Child–Pugh score improved in the first 90 days in the cell therapy group compared with controls ($P = 0.017$, BMC group RC = – 8%, controls RC = +5%). The model for end-stage liver disease score remained stable in the treated patients (RC – 2 to +6%), whereas it increased during follow-up in the control group (RC +6 to +18%). Albumin levels improved in the treatment arm, whereas they remained stable among controls in the first 90 days ($P = 0.034$; BMC group RC = +16%, control group RC = +2%). Bilirubin levels increased among controls, whereas they decreased in the therapy arm during the first 60 days; INR RC differences between groups reached up to 10%. The changes observed did not persist beyond 90 days.

Conclusion Transplantation of autologous BMC into the hepatic artery improved liver function in patients with advanced cirrhosis in the first 90 days. However, larger studies are necessary to define the role of BMC therapy in cirrhotic patients. Repeated autologous BMC infusions or combination therapy with granulocyte-colony-stimulating factor might improve or sustain the treatment response. *Eur J Gastroenterol Hepatol* 22:33–42 © 2010 Wolters Kluwer Health | Lippincott Williams & Wilkins.

Introduction

Under physiological conditions, the human liver regenerates primarily through division of mature hepatocytes. When this organ is exposed to chronic insult as in viral hepatitis, the liver is repaired through the action of hepatic progenitor cells [1,2]. However, recent studies suggest that cell loss in the liver cannot be recovered exclusively through stem cells resident within the organ [3,4]. Thus, tissue damage might attract migratory stem cell populations from other organs such as the bone marrow. There is evidence that stem cells, particularly those in the bone marrow, have the ability to develop into endodermal, mesodermal, and ectodermal cell types [4–7]. The extent to which bone-marrow-derived cells repopulate the liver after injury, however, does not seem to be significant, and the therapeutic mechanism is not well known but could be through cell fusion and the delivery of specific substances to mature functional hepatocytes. Several studies suggest that cytokines and growth factors produced by infused bone marrow cells may support liver function and repair in adult animals without the infused cells forming new hepatocytes [7–9]. However, the specific cell types responsible for this liver regeneration process remain to be discovered.

Several investigators have recently evaluated the therapeutic potential of bone marrow mononuclear cell (BMC) transplantation into mice with acute and chronic liver diseases. Delivery routes have varied from peripheral venous injection to direct intralobular hepatic infusion. These studies have shown reduced hepatic fibrosis and improved liver function in different animal models [10–13].

Liver transplantation is the only available therapy for patients with chronic liver failure; however, there is a shortage of donated organs. Therefore, alternative methods are necessary to increase the survival of patients on the liver transplant waiting list. Positive results for cell therapy in animal models have led us and others to evaluate the feasibility and safety of bone marrow cell therapy in patients with chronic liver disease [14–17]. These clinical studies found that BMC therapy seemed to be safe and feasible, albumin levels increased in many patients, bilirubin levels decreased, the Child–Pugh score improved, and in at least one study, the model for end-stage liver disease (MELD) score improved. Terai et al. [18] followed up nine patients treated with BMCs for up to 15 months and found persistent improvement of albumin levels and other clinical parameters. In another study, Levicar et al. [19] followed up five patients after BMC infusion and found that the beneficial effects lasted for approximately 12 months. Both these studies had no controls.

Further investigations with randomized controlled studies are necessary to evaluate the efficacy of this modality of cell therapy in patients with chronic liver disease. In this randomized pilot study, we evaluated autologous bone marrow cell therapy in cirrhotic patients on a liver transplant list.

Materials and methods
Patient selection
We prospectively enrolled 30 patients with chronic liver disease who were on the waiting list for liver transplantation at the outpatient clinics of Hospital Sao Rafael, Salvador, Brazil, from January 2006 to April 2006. Written informed consent was obtained from all participants. The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki and was approved by the ethics committee of the institution. Eligible patients were men or women who understood the study procedures. They were also required to meet all the following inclusion criteria: age between 18 and 74 years, advanced chronic liver disease from different etiologies with indication for liver transplantation based on clinical, laboratorial, and radiological findings, absence of liver tumors, negative for human immunodeficiency virus infection, no history of malignant neoplasia except for nonmelanoma skin cancer, no history of heart failure, platelet count $\geq 30\,000/mm^3$, INR $\leq 2.4\,IU$, creatinine $\leq 2.5\,mg/dl$, absence of portal vein thrombosis, no

participation in other clinical trials; women could not be pregnant or lactating. Patients were excluded from the study if they were unable to be subjected to bone marrow aspiration or if they had, on the day of randomization, active hepatic encephalopathy or sepsis. Patients were randomly assigned to receive infusion of autologous BMCs or to be followed up as controls.

Study design and assessments
Potential patients underwent a medical history screening, physical examination, abdominal magnetic resonance imaging to exclude liver tumors, and laboratory tests. Thirty patients met all inclusion criteria and were moved forward to the next step, which was treatment allocation by randomization. The study was nonblinded. Baseline assessments at day 0 included complete clinical and laboratory evaluation. The first five patients randomly assigned to receive BMC therapy in the current study were also included in the analysis of a previous safety and feasibility study [16]. Laboratory tests consisted of complete blood count, serum bilirubin levels, prothrombin time, serum blood glucose, urea, creatinine, α-fetoprotein, total proteins and albumin levels, serum aminotransferase concentrations, alkaline phosphatase and γ-glutamyl transferase levels, and thyroid-stimulating hormone concentrations. Patients were followed up for adverse events with clinical and laboratory evaluation on days 1, 2, 3, 7, 15, 30, 45, 60, and every month thereafter up to 360 days. Patients were subjected to abdominal ultrasound every 6 months.

Therapy with bone marrow cells
Patients in the cell therapy arm were admitted to the hospital on the previous day and subjected to BMC infusion in the morning. First, patients were transferred to the day-hospital operating room and sedated with sulfentanyl and ketamine by an anesthesiologist. Approximately 50 ml of bone marrow were aspirated from the iliac crests by a hematologist. An enriched fraction of BMC was obtained by centrifugation of total bone marrow in a Ficoll-Hypaque gradient. Cell populations were analyzed by fluorescence-activated cell sorter analysis. The following antibodies from Becton Dickinson (Franklin Lakes, New Jersey, USA) were used for immunostaining: CD34-FITC and CD45-APC. Three-color immunofluorescence analysis was used for identification of CD34$^+$ population (hematopoietic progenitor cells) in BMC suspensions. The number of events acquired and analyzed for each patient was 100 000. Data acquisition and analysis were performed on a FacsCalibur device with CellQuest software (Becton Dickinson).

BMCs were prepared within approximately 2–3 h after bone marrow aspiration. The viability of the cells was determined by trypan blue exclusion. Mononuclear-enriched BMCs suspended in 20 ml of saline were slowly

infused into the hepatic artery of patients over a 20-min interval with the technique routinely used for arterial chemoembolization of liver tumors [20]. Patients were discharged from the hospital the next day.

Statistical analysis
Indicators of liver function [serum albumin, bilirubin, INR (prothrombin time), Child–Pugh, and MELD scores] were chosen as endpoints to assess efficacy. We chose baseline (day 0) and 30, 60, 90, 180, and 360 days after injection as evaluation time points. Chi-square tests (for categorical variables) and *t*-tests (for continuous variables) or Wilcoxon's tests (for ordinal variables) were used to compare patient characteristics at baseline. To determine the efficacy of cell therapy, we analyzed the data using boxplots, individual profiles of the patients, descriptive analyses of mean relative changes from baseline, and the random-effects model. Pearson's correlation was used to evaluate the correlation between the number of infused cells and the albumin levels.

Trends in liver function parameters [i.e. laboratory tests, Child–Pugh, and MELD scores] over time were explored by calculating means, standard deviations, and 95% confidence intervals for each evaluation time point. In addition, we calculated the relative mean changes with respect to the baseline level. Finally, we fitted a random-effects linear model to compare the response profiles of liver indicators between the treated and untreated groups. All statistical analyses were conducted using STATA statistical software (StataCorp. 2005. Stata Statistical Software: Release 8. College Station, Texas: StataCorp LP).

Results
The baseline characteristics of the patients enrolled into the study are outlined in Table 1. Sex distribution, mean age, bilirubin and albumin levels, MELD, and Child–Pugh scores were similar for both groups.

The etiology of the liver diseases encountered is described in Table 2. The majority of patients had alcoholic liver disease and/or hepatitis C. Both patients

Table 1 Patients' baseline characteristics

Characteristics	Controls ($n=15$)	Treatment arm ($n=15$)	*P* value
Age (years)			
Mean ± SD	50.0 ± 10.37	56.7 ± 9.23	0.075
Bilirubin levels (mg/dl)			
Mean ± SD	2.46 ± 1.62	1.97 ± 0.83	0.312
Albumin levels (g/dl)			
Mean ± SD	3.31 ± 0.78	2.93 ± 0.44	0.114
Child–Pugh score			
Mean ± SD	8.47 ± 1.60	8.6 ± 1.94	0.684
MELD score			
Mean ± SD	14.3 ± 3.66	13.3 ± 3.44	0.447
INR (prothrombin time)			
Mean ± SD	1.48 ± 0.27	1.50 ± 0.30	0.678

MELD, model for end-stage liver disease.

with hepatitis B were randomly assigned to the control group. One patient was kept on lamivudine and the other on adefovir. At baseline in the BMC therapy group, one patient with mild/moderate ascites was classified as Child–Pugh A, 10 as Child–Pugh B, and four as Child–Pugh C. Among controls, one patient with mild/moderate ascites was classified as Child–Pugh A, 11 as Child–Pugh B, and three as Child–Pugh C.

Only one patient from each group was on a regular therapeutic paracentesis program with albumin replacement every 4–8 weeks, on average, before the study was initiated. Baseline albumin levels for these patients were 3.2 g/dl (patient in control group) and 4.4 g/dl (patient in BMC therapy group).

The mean number of bone marrow cells infused was 3.78×10^8 ($\pm 2.69 \times 10^8$) and the median 3.00×10^8 (range, 0.88×10^8–11.2×10^8). The total number of infused cells for each patient, the etiology of the liver disease, and the age are shown in Table 2. The number of CD34$^+$ and CD45$^+$ cells infused was evaluated for 11 patients (Table 2). Six patients received blood products a few hours before or immediately before bone marrow aspiration and/or femoral artery catheterization because of coagulopathy and low platelet count. The type and amount of transfused blood product is shown in Table 2.

Five patients complained of mild pain at the sites of bone marrow puncture and/or arterial puncture, and four patients developed ecchymosis/hematoma at the site of the arterial puncture that was reabsorbed a few days later. Six months after BMC infusion, one patient with cirrhosis caused by alcoholic liver disease and hepatitis C was diagnosed with dilated cardiomyopathy, mild heart failure, and third-degree atrioventricular block; a cardiac pacemaker was implanted and the patient recovered well.

During follow-up, three patients died in the therapy group at 6, 8, and 10 months after randomization owing to complications of chronic liver disease, whereas in the control group, two patients died at 19 days and 10 months after randomization and one patient received a liver transplant because of progressive chronic liver failure at 45 days after randomization. No patients developed liver tumors.

Of the patients who were alive during the entire period of the study, only one control patient missed the visit at day 360. His last visit was at 240 days after randomization. He was later contacted by phone. The remaining patients attended all scheduled visits.

To determine the efficacy of cell therapy, we analyzed the data using boxplots, individual profiles of the patients, descriptive analyses of mean relative changes from baseline, and the random-effects model.

Table 2   Etiology of liver diseases of all patients and the number of infused cells and transfused blood products in the cell therapy arm

Patient no.	Age (years)	Etiology of liver disease cell therapy arm	Total number of cells infused	Number of CD34$^+$cells infused	Number of CD45$^+$cells infused	Transfused blood products
1	55	Cryptogenic cirrhosis	3.5×10^8	3.71×10^6	2.30×10^8	1 platelet apheresis
2	49	Alcoholic liver disease	4.8×10^8	3.46×10^6	2.81×10^8	None
3	66	Cryptogenic cirrhosis	3.4×10^8	NA	NA	None
4	41	Alcoholic liver disease + hepatitis C	2.4×10^8	2.52×10^6	2.05×10^8	None
5	70	Alcoholic liver disease	1.6×10^8	2.64×10^6	1.33×10^8	None
6	49	Alcoholic liver disease + hepatitis C	11.2×10^8	25.76×10^6	6.37×10^8	None
7	74	Cryptogenic cirrhosis	2.4×10^8	3.86×10^6	1.96×10^8	None
8	61	Alcoholic liver disease + hepatitis C	0.88×10^8	0.77×10^6	0.70×10^8	1 platelet apheresis
9	59	Alcoholic liver disease	2.3×10^8	3.04×10^6	1.68×10^8	None
10	50	Hemochromatosis	6.7×10^8	5.03×10^6	4.39×10^8	2 units of FFP
11	48	Hepatitis C	1.5×10^8	2.21×10^6	1.08×10^8	1 platelet apheresis
12	60	Alcoholic liver disease + hepatitis C	3.0×10^8	5.10×10^6	2.03×10^8	1 platelet apheresis + 8 CRYO units
13	54	Hepatitis C	3.7×10^8	NA	NA	1 platelet apheresis + 7 CRYO units
14	47	Primary biliary cirrhosis	2.4×10^8	NA	NA	None
15	57	Cryptogenic cirrhosis	6.9×10^8	NA	NA	None
		Etiology of liver disease control group				
16	54	Hepatitis C	–	–	–	–
17	50	Alcoholic liver disease	–	–	–	–
18	37	Alcoholic liver disease	–	–	–	–
19	64	Alcoholic liver disease	–	–	–	–
20	58	Alcoholic liver disease	–	–	–	–
21	50	Cryptogenic cirrhosis	–	–	–	–
22	55	Budd-chiari syndrome	–	–	–	–
23	54	Hepatitis C	–	–	–	–
24	37	Alcoholic liver disease	–	–	–	–
25	38	Alcoholic liver disease + hepatitis B	–	–	–	–
26	68	Chronic cholestatic liver disease	–	–	–	–
27	32	Hepatitis B	–	–	–	–
28	46	Alcoholic liver disease + hepatitis C	–	–	–	–
29	53	Hepatitis C	–	–	–	–
30	46	Alcoholic liver disease + hepatitis C	–	–	–	–

CRYO, cryoprecipitate; FFP, fresh frozen plasma; NA, not available.

Analysis of the Child–Pugh score over the first 3 months revealed a mild increase in the control group and a significant decrease in the treatment group. The Child–Pugh score improved in the therapy arm, particularly during the first 90 days after BMC therapy; this effect is clearly demonstrated by the boxplots as well as the mean relative changes from baseline, where the difference between groups reached 14% in the first 30 days (Fig. 1) (Table 3). Slopes obtained from the random-effects model were significantly different between the two groups ($P = 0.017$) (Table 4). At 12 months, there was a slight (nonsignificant) increase in the control group.

The MELD score significantly increased during the 12 months of follow-up in the control group ($P = 0.014$); this increase was greatest in the first 3 months ($P = 0.009$) (Table 4). In the treated patients, the MELD score remained stable. However, the difference between the slopes of the two groups did not reach statistical significance at 3 months ($P = 0.085$) or 12 months ($P = 0.189$) (Table 4). Boxplots of the MELD score also showed slight improvement after BMC therapy (Fig. 2). Evaluation of the mean relative changes from baseline reveal that the MELD score stabilized in the cell therapy group but increased in the control group; the difference between groups reached 14% after 60 days (Table 3).

Albumin levels increased significantly after BMC therapy, particularly in the first 90 days (Fig. 3); mean levels had increased by up to 16% at 90 days as compared with baseline levels (Table 3). In the control group, albumin levels decreased slightly during follow-up. The random-effects model showed that serum albumin levels at 3 months were significantly increased in the intervention group (0.14 g/dl per 30 days; $P = 0.001$), whereas they did not change in the control group (Table 4). The difference between the slopes of the two groups was statistically significant ($P = 0.034$). This difference had diminished at 12 months.

Fig. 1
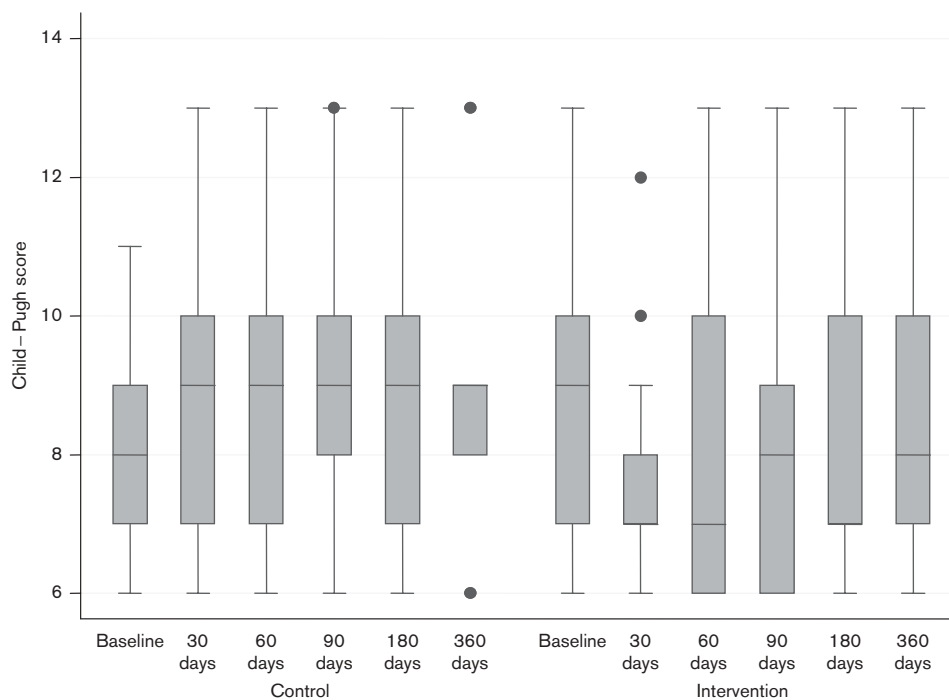
Boxplots of Child–Pugh scores and individual Child–Pugh scores from controls and patients of the intervention (bone marrow mononuclear cell therapy) group and their respective time points.

Table 3 Descriptive analysis of Child–Pugh and MELD scores, albumin levels, bilirubin, and INR (prothrombin time) levels in both the study groups during follow-up

Time point	Control (%)	Intervention (%)	Difference (%)
Mean relative change from baseline Child–Pugh score			
30 days	2	− 11	− 13
60 days	5	− 7	− 12
90 days	5	− 8	− 13
180 days	6	− 6	− 12
360 days	5	− 2	− 7
Mean relative change from baseline MELD score			
30 days	7	0	− 7
60 days	12	− 2	− 14
90 days	13	2	− 11
180 days	6	− 3	− 9
360 days	18	6	− 12
Mean relative change from baseline of albumin levels			
30 days	− 2	14	16
60 days	− 6	14	20
90 days	2	16	14
180 days	− 2	11	13
360 days	− 2	12	14
Mean relative change from baseline of bilirubin levels			
30 days	123	4	− 120
60 days	302	− 5	− 307
90 days	179	1	− 178
180 days	182	10	− 172
360 days	177	39	− 138
Mean relative change from baseline of INR (prothrombin time) levels			
30 days	1	− 1	− 2
60 days	9	− 1	− 10
90 days	7	1	− 6
180 days	1	− 4	− 5
360 days	9	1	− 8

MELD, model for end-stage liver disease.

To rule out the possibility that albumin levels could have been influenced by blood product transfusion, we compared the albumin levels of the six patients who received blood products during BMC therapy with those of nine patients who did not receive blood products. Albumin levels were similar in both of these subgroups at baseline ($P = 0.74$) and after BMC therapy ($P = 0.68$ at 30 days, $P = 0.83$ at 60 days and $P = 0.73$ at 90 days) (Table 5).

Of note, there was no correlation between the total number of cells infused, the number of CD34$^+$ cells, and the number of CD45$^+$ cells infused and the albumin levels (data not shown).

Total bilirubin levels of patients in the therapy arm were lower than baseline, especially in the first 2 months after BMC infusion (Fig. 4). Analysis of mean relative changes from baseline revealed stabilized bilirubin levels during the first 90 days after BMC therapy, whereas bilirubin levels were increased at all follow-up time points in controls (Table 3). The random-effects model showed that bilirubin levels increased significantly during the 12 months of follow-up in the control group ($P = 0.011$), whereas it remained unchanged in the treated patients. Despite this, the differences between the two slopes did not reach statistical significance at 3 months ($P = 0.099$)

Table 4   **Results from the random effects model at 90 and 360 days**

|	90 Days	Coefficient	95% Confidence interval	P value
CP score	Slope in control group	0.120	(−1.140 to 1.540)	0.185
	Slope in BMC group	−0.187	(−0.364 to −0.009)	0.039
	Comparison of slopes	−0.307	(−0.558 to −0.056)	0.017
MELD score	Slope of control group	0.633	(0.157 to 1.110)	0.009
	Slope of BMC group	0.040	(−0.437 to 0.517)	0.869
	Comparison of slopes	−0.593	(−1.268 to 0.081)	0.085
Albumin	Slope of control group	0.011	(−0.072 to 0.095)	0.790
	Slope of BMC group	0.139	(−0.056 to 0.223)	0.001
	Comparison of slopes	0.128	(0.010 to 0.246)	0.034
Bilirubin	Slope of control group	1.460	(0.222 to 2.698)	0.021
	Slope of BMC group	−0.015	(−1.253 to 1.222)	0.981
	Comparison of slopes	−1.475	(−3.225 to 0.275)	0.099
INR (prothrombin time)	Slope of control group	0.040	(−0.0004 to 0.080)	0.052
	Slope of BMC group	0.004	(−0.036 to 0.044)	0.851
	Comparison of slopes	−0.036	(−0.093 to 0.021)	0.215
	360 days			
CP score	Slope of control group	0.076	(−0.025 to 0.177)	0.140
	Slope of BMC group	0.010	(0.092 to 0.110)	0.854
	Comparison of slopes	−0.067	(−0.210 to 0.076)	0.361
Meld score	Slope of control group	0.345	(0.070 to 0.620)	0.014
	Slope of BMC group	0.084	(−0.191 to 0.359)	0.551
	Comparison of slopes	−0.261	(−0.650 to 0.128)	0.189
Albumin	Slope of control group	−0.002	(−0.049 to 0.045)	0.936
	Slope of BMC group	0.047	(−0.0002 to 0.094)	0.051
	Comparison of slopes	0.049	(−0.0177 to 0.115)	0.151
Bilirubin	Slope of control group	0.747	(0.169 to 1.325)	0.011
	Slope of BMC group	−0.015	(−1.253 to 1.223)	0.677
	Comparison of slopes	−0.624	(−1.44 to 0.193)	0.134
INR (prothrombin time)	Slope of control group	0.017	(−0.003 to 0.037)	0.102
	Slope of BMC group	0.0006	(−0.020 to 0.021)	0.955
	Comparison of slopes	−0.016	(−0.045 to 0.012)	0.265

CP, Child–Pugh; MELD, model for end-stage liver disease.

Fig. 2
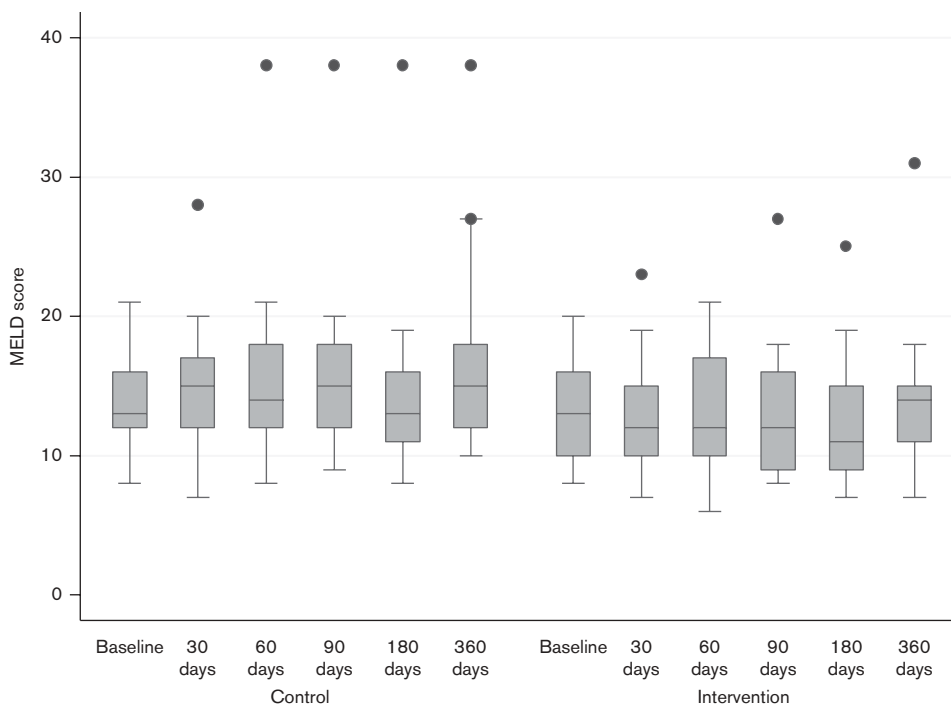
Boxplots and individual model for end-stage liver disease (MELD) scores of controls and patients of the intervention group at the evaluated time points.

Fig. 3
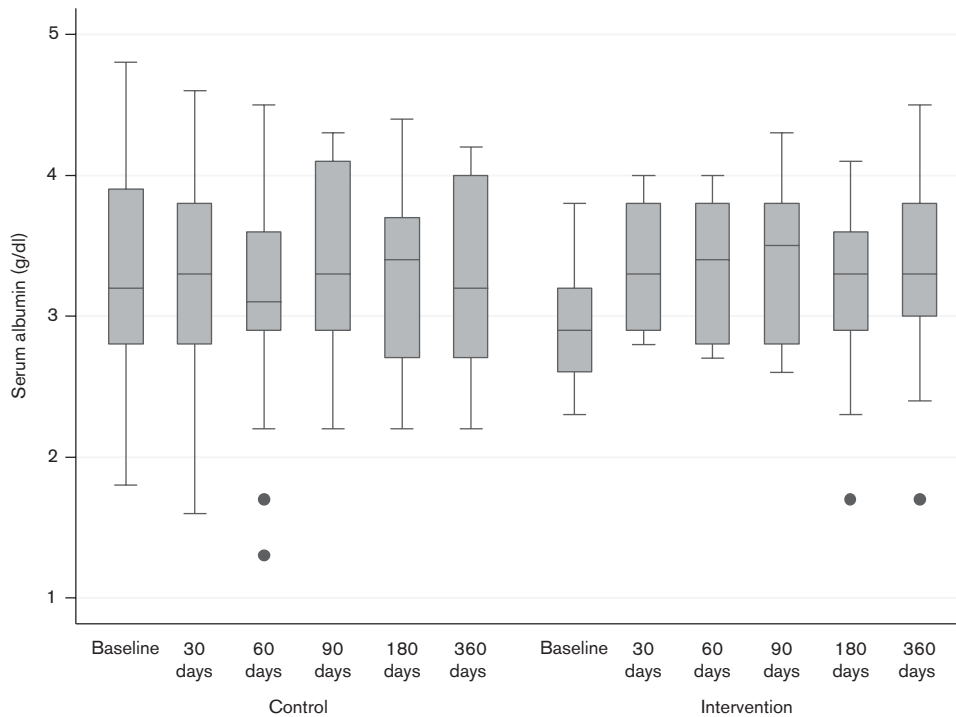
Boxplot analysis of albumin levels and individual albumin levels from controls and patients subjected to bone marrow mononuclear cell therapy.

Table 5   Comparison between albumin levels among patients who received blood products (subgroup 1) and patients who did not receive blood products during BMC therapy (subgroup 2)

Mean albumin level ± SD subgroup 1 (n=6)	Mean albumin level ± SD subgroup 2 (n=9)	P value
Baseline	Baseline	
2.83±0.40	2.98±0.50	0.74
30 days after BMC	30 days after BMC	
3.28±0.42	3.38±0.44	0.68
60 days after BMC	60 days after BMC	
3.20±0.52	3.46±0.48	0.83
90 days after BMC	90 days after BMC	
3.28±0.54	3.47±0.56	0.73

BMC, bone marrow mononuclear cell.

or 12 months ($P = 0.134$) (Table 4). Analysis of mean relative INR (prothrombin time) changes from baseline is shown in Table 3.

There were no significant differences between groups for other relevant laboratory parameters (white blood cells, hemoglobin, aspartate aminotransferase, alanine aminotransferase, γ-glutamyl transferase, and creatinine; data not shown).

Discussion

This is the first pilot randomized, controlled study to evaluate the efficacy of autologous BMC transplantation in patients with advanced chronic liver disease. We found a potential beneficial effect of this modality of cell therapy in the studied population; the Child–Pugh score improved, the MELD score and bilirubin levels stabilized, and albumin levels increased in the treated arm. The Child–Pugh score measures five variables, two of which (encephalopathy and ascites) are subjective; the fact that this study was not blinded might have influenced the evaluation of this score to some extent. Nevertheless, all other analyzed parameters were objective; the combination of results therefore suggests a beneficial effect for BMC-treated patients. It is notable that there were more patients with alcoholic liver disease in the control group, and that both patients with hepatitis B who were kept on lamivudine treatment were also assigned to the control group. Decompensated alcoholic cirrhosis might improve in some patients after they quit drinking, and occasionally patients with hepatitis B improve after drug therapy and are withdrawn from the liver transplant waiting list. If this happened in the present study, it would favor the control arm. In addition, only one patient from each group was on regular therapeutic paracentesis with albumin replacement, and blood product transfusion did not seem to influence albumin levels (Table 3). Therefore, the difference detected in albumin levels between groups was not related to albumin infusion or blood component transfusion.

The improvement of liver function detected in our study was partial and primarily occurred in the first 3 months after BMC therapy. Beyond this period, liver function

Fig. 4
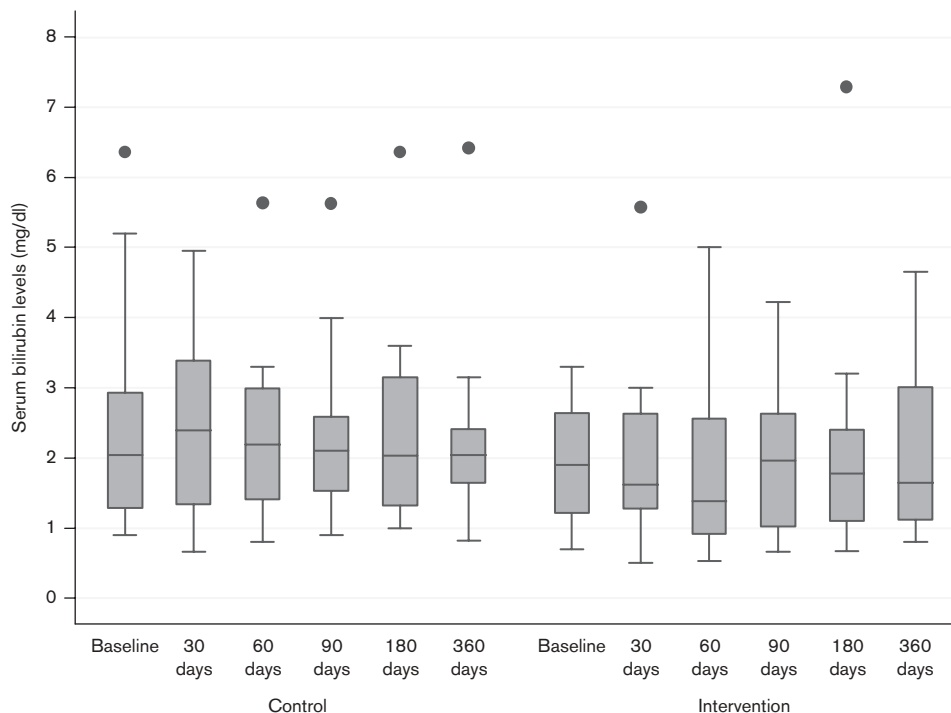
Boxplot analysis and individual slopes of bilirubin levels from the control and intervention groups and their respective time points.

seemed to return to baseline levels. The mechanisms underlying the improvement of liver function after infusion of mononuclear cells are not well understood; in addition, the cell types responsible for the observed hepatoprotective and putative regenerative effects remain to be determined. The pattern of response observed in our patients favors the hypothesis that BMC therapy benefits the liver through delivery of specific substances to mature functional hepatocytes rather than through transdifferentiation. As such, it is logical to expect that the duration of action of these substances should be temporary, as most infused mononuclear cells would not permanently reside in the liver. Several studies suggest that cytokines and growth factors produced by infused bone marrow cells may improve liver function and cause tissue regeneration in adult animals without formation of new hepatocytes from infused cells [7,8].

Mouse models of liver diseases have also shown decreased hepatic fibrosis after BMC infusion. We did not subject our patients from both groups to liver biopsies to evaluate the histological effects of this therapy because we thought this might be considered unethical. We were concerned about the development of complications after such an invasive procedure, even through transjugular access, especially considering that we were testing a new and perhaps still controversial therapeutic option. The development of complications might have prematurely jeopardized the analysis of this modality of therapy.

Our study did not show survival improvement, which is the ultimate goal of any therapy. Nevertheless, it should be noted that this is the first study to evaluate the efficacy of this new potential treatment for patients with decompensated cirrhosis; thus, future studies may tailor the treatment conditions to achieve the maximum therapeutic effect. In this regard, it is likely that repeated infusions of cells might be necessary to generate a greater beneficial effect. It is also important to evaluate adjuvant therapies such as injection of granulocyte-colony-stimulating factor (G-CSF) to mobilize BMCs. Gaia *et al.* [14] evaluated the feasibility and safety of BMC mobilization in eight patients with end-stage liver cirrhosis after G-CSF administration. Mobilization of bone marrow cells (monitored by the number of CD34$^+$ cells) was observed in all patients after G-CSF administration, which was well tolerated and free of adverse events. The Child–Pugh score decreased by 2 or more points in four patients, increased in one patient, and was unchanged (or decreased by less than 2 points) in three patients. Overall, the MELD score decreased from a median pretreatment value of 17.5 (range 11–20) to 14.5 (range 9–20) at the end of follow-up.

Some investigators have found that a significant proportion of myofibroblasts are of bone marrow origin in human liver fibrosis; concerns have thus been raised regarding bone marrow cell therapy in patients with hepatic

cirrhosis [6,7,21]. We, however, did not detect deterioration of liver function after BMC treatment, nor did we detect the development of hepatocellular carcinoma.

Another unresolved issue is the ideal administration route for BMCs. We chose infusion into the hepatic artery, because this technique is routinely used for arterial chemoembolization of liver tumors, and it is feasible and not associated with serious local side-effects [18]. In addition, the blood influx to the liver is mostly secured using the hepatic artery as the vascular access for cell infusion. For ethical reasons, we opted not to perform bone marrow aspiration and sham intrahepatic injections in our control group. It is worth mentioning that studies in animal models and in patients with cirrhosis have evaluated the infusion of BMCs using the peripheral vein with satisfactory results [17]. Thus, the best way to administer this therapy still remains to be defined.

We did not detect any major side-effects in the first month after BMC therapy. One patient developed third-degree atrioventricular block, mild heart failure and was implanted with a cardiac pacemaker at 6 months after therapy. In the authors' opinion, this event most probably was not related to BMC infusion. Intracoronary injection of mononuclear bone marrow cells in individuals with cardiac diseases has been evaluated in larger studies and has not been associated with a higher number of adverse events as compared with controls. In fact, several of these studies were associated with improved cardiac function and lower mortality [22–26]. In addition, in our study, the adverse event occurred at 6 months after BMC therapy; this was after the 3-month period of maximum benefit produced by the cell treatment.

In summary, this pilot randomized controlled study showed beneficial effects of autologous BMC transplantation on liver function for up to 90 days in patients with advanced chronic liver disease. Repeated autologous BMCs infusions or the association of cell therapy with G-CSF might be a promising treatment option for patients with advanced chronic liver disease. However, larger randomized, controlled, and double-blinded studies are necessary to define the role of BMC therapy in patients with liver disease and to determine new protocols to prolong the therapeutic effects [21,27].

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