Original Article

# Autologous Transplantation of Bone Marrow-derived Mononuclear and CD133+ Cells in Patients with Decompensated Cirrhosis

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**Abstract**

**Background:** Cirrhosis, the end stage of progressive hepatic fibrosis, is characterized by distortion of the hepatic architecture and the formation of regenerative nodules. Liver transplantation is one of the few available therapies for such patients. However, due to a severe shortage of organ donors, surgical complications, transplant rejection and the high cost of this procedure much interest has focused on research to find new treatment modalities for this disease. There is accumulating evidence for the contribution of bone marrow stem cells to participate in liver regeneration.

**Methods:** Here we report on six patients with end stage liver disease who were subjected to intraportal administration of autologous bone marrow-derived CD133+ in comparison to mononuclear cells in short-term (6 months) and long-term (24 months) follow up.

**Results:** There were no adverse effects in any of the patients during the short- and long-term follow up period. Moreover, there were no significant alterations of liver function parameters, liver enzymes, serum albumin, creatinine, serum bilirubin and/or liver volume after transplantation of both types of autologous cells in these patients.

**Conclusion:** Our study has shown both the safety and feasibility of this type of liver cell therapy and may be a bridge to liver transplantation.

The trial was registered with NIH clinical trials (www.clinicaltrials.gov) as identifier: NCT00713934.

**Keywords:** autologous transplantation, bone marrow, decompensated cirrhosis, liver

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## Introduction

In patients suffering from cirrhosis due to distortion of the hepatic architecture and the formation of regenerative nodules, liver transplantation is the main modality of treatment.[1] However, a serious shortage of organ donors, surgical complications, rejection and the high cost of this procedure has sparked tremendous interest in research to find new treatment modalities for this disease. In Iran the minimum number of patients needing liver transplants yearly is approximately 1000,[2] but at present, the majority of these patients die while waiting for transplantation because of the limitations of organ donors and liver transplant centers. Therefore, alternative methods such as cell therapy are necessary to increase the survival rate in patients who are on the liver transplant waiting list. Several sources of stem cells have been proposed for cell therapy. Bone marrow is the most accessible and interesting since it contains different stem cells that can generate a variety of cell types found in other tissues.[3–8] Additionally, the transplantation of bone marrow (BM)-derived mesenchymal and hematopoetic stem cells (MSCs and HSCs) reduce

**Table 1.** Patients' baseline and follow-up characteristics

| CD 133+ group | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Case 1 | | | | Case 2 | | | | Case 3 | | | |
| Age (yr) | 34 | | | | 39 | | | | 38 | | | |
| Gender | Female | | | | Male | | | | Female | | | |
| Etiology of cirrhosis | AIH* | | | | Cryptogenic | | | | Cryptogenic | | | |
| Edema | None | | | | None | | | | None | | | |
| Acites | Mild | | | | Mild | | | | Mild | | | |
| **Follow up** | **Baseline** | **3 m** | **6 m** | **24 m** | **Baseline** | **3 m** | **6 m** | **24 m** | **Baseline** | **3 m** | **6 m** | **24 m** |
| Serum albumin (g/L) | 3.3 | 4.1 | 3.5 | 3.1 | 3.5 | 3.7 | 3.1 | 3.9 | 3.7 | 3.1 | 3.3 | 4 |
| Total protein (g/L) | 7.3 | 8.4 | 9 | 6.6 | 7.1 | 7.4 | 6.6 | 6.9 | 6.9 | 6.9 | 7.3 | 7.6 |
| PT (second) | 19.1 | 20 | 14 | 16.8 | 16.2 | 17 | 18 | 24 | 17.3 | 13 | 12 | 13 |
| INR† | 2.2 | 2.8 | 1.2 | 1.7 | 1.5 | 1.9 | 2.1 | 3.7 | 1 | 1.2 | 1.2 | 1.2 |
| Cr (mg/dL) | 0.9 | 0.8 | 1.4 | 0.6 | 0.7 | 0.6 | 0.8 | 0.6 | 0.8 | 0.9 | | 1 |
| Total bilirubin (mg/dL) | 0.9 | 1.4 | 1.8 | 1.2 | 2.6 | 2.4 | 0.8 | 3.1 | 2.3 | 0.3 | 0.6 | 1.6 |
| Direct bilirubin (mg/dL) | 0.2 | 0.9 | 0.9 | 0.4 | 0.8 | 1 | 0.2 | 1.9 | 0.6 | 0.1 | 0.2 | 0.2 |
| AST (IU/mLl) | 184 | 90 | 55 | 37 | 61 | 45 | 32 | 58 | 43 | 88 | 59 | 44 |
| ALT (IU/mL) | 126 | 49 | 39 | 22 | 32 | 19 | 13 | 29 | 45 | 79 | 73 | 45 |
| Hb | 9.2 | 9.8 | 13.5 | 7.1 | 10.5 | 9 | 9.5 | 10.8 | 12.6 | 11.5 | 11.5 | 13.6 |
| WBC | 2500 | 3700 | 4000 | 2700 | 2400 | 3600 | 3000 | 2000 | 5400 | 5400 | 5400 | 5900 |
| AFP (ug/L) | 5.8 | 4.2 | 3.7 | NA | 4.6 | 3.3 | 4.1 | NA | 2.1 | 1.9 | 3.6 | 1.2 |
| HA (ug/mL) | 243.4 | 308.1 | NA | NA | 99.2 | 54.1 | 91.6 | NA | 48.3 | 64.8 | 53 | NA |
| HGF (ng/mL) | 2939 | 2276 | NA | NA | 2743.5 | 2576 | 2509 | NA | 1557.5 | 1962 | 997 | NA |
| TIMP-1 (ng/mL) | 2.2 | 2 | NA | NA | 2.6 | 2.5 | 1.9 | NA | 2.5 | 2.8 | 1.6 | NA |
| MELD** score | 15 | 20 | 14 | 13 | 25 | 17 | 15 | 25 | 6 | 4 | NA | 10 |
| **MNC group** | | | | | | | | | | | | |
| | **Case 1** | | | | **Case 2** | | | | **Case 3** | | | |
| Age (yr) | 29 | | | | 40 | | | | 34 | | | |
| Gender | Male | | | | Female | | | | Male | | | |
| Etiology of cirrhosis | Cryptogenic | | | | Hemochromatosis | | | | Cryptogenic | | | |
| Edema | None | | | | Mild | | | | Mild | | | |
| Acites | None | | | | Mild | | | | None | | | |
| **Follow up** | **Baseline** | **3 m** | **6 m** | **24 m††** | **Baseline** | **3 m** | **6 m** | **24 m** | **Baseline** | **3 m** | **6 m** | **24 m** |
| Serum albumin (g/l) | 4 | 4.1 | 4.2 | — | 2.6 | 4.3 | 4.8 | 3.6 | 2.9 | 3 | 2.6 | NA |
| Total protein (g/l) | 6.4 | 7.7 | 7.2 | — | NA | NA | 7.3 | 6.9 | NA | 5.8 | 5.6 | NA |
| PT (second) | 16.7 | 18.3 | 13 | — | 14.5 | 12 | 14.2 | 13 | 19.7 | 17 | 18 | 19 |
| INR† | 1.8 | 1.9 | 2.1 | — | 1.1 | 1.7 | 1.3 | 1.4 | 2.2 | 1.6 | 2.1 | 2.1 |
| Cr (mg/dl) | 1.1 | 0.7 | 1.3 | — | 0.6 | 0.7 | 0.7 | 0.7 | 1.2 | 0.7 | 1 | 0.9 |
| Total bilirubin (mg/dl) | 1.5 | 2.2 | 1.8 | — | 4.2 | 1.4 | 1.2 | 3 | 0.8 | 1.5 | 4.2 | 2 |
| Direct bilirubin (mg/dl) | 0.4 | 0.5 | 0.2 | — | 0.4 | 0.3 | 0.4 | 0.7 | 0.2 | 0.5 | 0.4 | 0.7 |
| AST (IU/ml) | 65 | 58 | 72 | — | 55 | 37 | 63 | 64 | 49 | 21 | 49 | 30 |
| ALT (IU/ml) | 33 | 28 | 45 | — | 47 | 30 | 60 | 57 | 49 | 18 | 40 | 21 |
| Hb | 10.8 | 11.7 | 11.9 | — | 14.5 | 13.5 | 12.9 | 13.1 | 10.2 | 11 | 11.6 | 10.9 |
| WBC | 4300 | 3800 | 4700 | — | 2400 | 3000 | 3300 | 2700 | 2600 | 1300 | 1800 | 3300 |
| AFP (ug/L) | 1.7 | 6 | NA | — | 2.6 | 0.62 | 4 | NA | 1.7 | 2.1 | NA | NA |
| HA (ug/ml) | 115.9 | 92.5 | 98.8 | — | 243 | NA | 195.1 | NA | 52.3 | 154.8 | 112.1 | NA |
| HGF (ng/ml) | 2965 | 2550 | 2225 | — | 1836 | 1729 | 1986 | NA | 2475.5 | 2254 | 997 | NA |
| TIMP-1 (ng/ml) | 2.1 | 1.9 | 1.3 | — | 1.7 | 1.9 | 1.2 | NA | 0 | 0 | 0 | NA |
| MELD** score | 17 | 17 | 19 | — | 10 | 14 | 10 | 15 | 27 | 14 | 20 | 17 |

*autoimmune hepatitis,**model for end stage liver disease, ,† international normalized ratio, †† patient died due to massive hemorrhage, NA: not determined AFP=alpha-feto ptotein; HGF=hepatocyte growth factor; TIMP-1= tissue inhibitor of metalloproteinase; MELD= Model for End-Stage Liver Disease

hepatic fibrosis, improve liver function, and increase the survival rate in animal models.[4,6,7]

Many clinical trials in humans have also shown the potential for bone marrow stem cells (BMSCs) to treat liver fibrosis.[9] Transplantation of CD34+ cells,[10–14] MSCs,[15,16] CD133+,[17,18] and mononuclear cells (MNCs)[19,20] have been used in clinical practice for the treatment of chronic liver disease in humans. CD133 expression is believed to represent a more stem cell-enriched subpopulation of the CD34+ cells.[21] Importantly, another rationale for using purified CD133+ cells has been to avoid injection of large numbers of leukocytes and their progenitors, which have limited plasticity and the presence of which, in large numbers, may give rise to an unwanted inflammatory response at the graft site.[22] However, there is no information on the injection of BM-CD133+ cells into the portal vein of patients undergoing decompensated cirrhosis, the outcomes of cell transplantation in comparison to the baseline and injection of BM-MNCs in order to determine the efficacy of this approach. The present investigation was carried out to study the safety, feasibility and clinical outcome of this approach.

## Patients and Methods

### Patients

The study was conducted with the approval of the Insti-

**Table 2.** Antibody characteristics

| Antibody name | Concentration | Cat No. | Company | Isotype control | Company |
|---|---|---|---|---|---|
| CD45/34, Dual | 1:100 | 341071 | BD | IgG1-FITC, IgG1-PE | Dako |
| CD105, PE | 1:100 | FAB10971P | R&D | IgG1-FITC, IgG1-PE | Dako |
| CD16, PE | 1:100 | 12-0168-73 | Ebioscience | IgG2a-FITC, IgG1-PE | Miltenyibiotec & Ebioscience |
| CD14, FITC | 1:100 | 130-080-701 | Miltenyiebiotec | IgG2a-FITC, IgG1-PE | Miltenyibiotec & Ebioscience |
| CD31, FITC | 1:100 | 555445 | BD | IgG1-FITC, IgG1-PE | Dako |
| VEGFR, PE | 1:100 | FAB357P | R&D | IgG1-FITC, IgG1-PE | Dako |
| CD33, PE | 1:100 | 555626 | BD | IgG1-FITC, IgG1-PE | Dako |
| CD29, PE | 1:100 | 557332 | BD | IgG1-FITC, IgG1-PE | Dako |
| CD38, FITC | 1:100 | 11-0389-73 | Ebioscience | IgG1-FITC, IgG1-PE | Dako |
| CD133, 293C2,PE | 1:100 | 130-090-853 | Miltenyiebiotec | IgG1-FITC, IgG2b-PE | Miltenyibiotec & Ebioscience |
| CD45, FITC | 1:100 | 130-080-202 | Miltenyiebiotec | IgG1-FITC, IgG2b-PE | Miltenyibiotec & Ebioscience |
| CD90, FITC | 1:100 | F7274 | Dako | IgG1-FITC, IgG1-PE | Dako |
| CD44, PE | 1:100 | 555479 | BD | IgG1-FITC, IgG2b-PE | Miltenyibiotec & Ebioscience |
| CD73, PE | 1:100 | 550257 | BD | IgG1-FITC, IgG1-PE | Dako |
| CD3, FITC | 1:100 | 130-080-401 | Miltenyiebiotec | IgG2a-FITC, IgG1-PE | Miltenyibiotec & Ebioscience |
| CD19, PE | 1:100 | 12-0199-73 | Ebioscience | IgG2a-FITC, IgG1-PE | Miltenyibiotec& Ebioscience |

tutional Review Board and Ethical Committee of Royan Institute. The trial was registered with National Institute of Health (NIH) clinical trials (www.clinicaltrials.gov) as identifier: NCT00713934. In this trial, six patients with decompensated cirrhosis, all of whom were on the liver transplant waiting list at the Shiraz Liver Transplant Research Center (Namazi Hospital, Shiraz, Iran) were enrolled. Written informed consent was signed by the patients after explaining all aspects of the study and its complications. Eligible patients were between 18 and 75 years of age with a clinical and paraclinical diagnosis of cirrhosis. The present subjects were patients with altered liver function tests (LFT) between 2× to 3× the normal value, liver cirrhosis as confirmed by ultrasonography and a percutaneous liver biopsy. Patients were excluded from the study if they had problems in organs other than the liver (e.g., heart or lungs), any degree of encephalopathy, refractory ascites, hepatocellular carcinoma or other malignancies, the presence of significant extrahepatic biliary disease, human immunodeficiency virus (HIV), hepatitis B (HBV) or hepatitis C (HCV) infection, active or chronic thrombosis of the portal or hepatic veins, active variceal bleeding, any type of systemic infection or spontaneous bacterial peritonitis, or evidence of active autoimmune liver disease (e.g., gamma globulin of more than 2× the upper limit of normal). Patients were randomly assigned into two groups: group 1 for administration of BM-CD133$^+$ cells and group 2 for administration of BM-MNCs. Table 1 shows the demographic data of each patient.

### Preparation of bone marrow cells

In six patients, approximately 276.2±31.5 mL of autologous BM was aspirated from the iliac crest under epidural anesthesia and collected into plastic bags that contained citrate phosphate dextrose anticoagulant (CPDA, Besat Co., Tehran) one day prior to cell implantation. MNCs were isolated under good manufacturing practice conditions by Ficoll-Hypaque (Lymphodex, Inno Train, H9L6114) density separation.

To enrich the CD133$^+$ cells, aspirated BM was incubated with CD133 monoclonal antibody directly labeled to microbeads and then continued based on the manufacturer's recommendations by a CliniMACS cell selection system (Miltenyi Biotech, Bergisch Gladbach, Germany).

At the end of separation, cells were washed twice with normal saline, counted and assessed for viability using trypan blue dye exclusion. The cells were suspended in 150 mL of normal saline that included 2% of the patient's own serum and kept at 4°C prior to transplantation.

### Flow cytometry analysis

Flow cytometry analysis of expressed cell surface antigens in both groups was performed by a flow cytometer (FACSCalibur, Becton Dickinson) and the purity of isolated BM-CD133$^+$ cells was calculated by the ISHAGE (International Society for Hematotherapy and Graft Engineering) method. Cells were adjusted to a volume of $1 - 2\times10^5$ cells/mL and were blocked with Fc receptor blocking reagent (Miltenyi Biotech, Germany) according to the manufacturer's instructions. They were subsequently stained for 30 minutes at 4°C with fluorochrome-labeled monoclonal antibodies (Table 2). Controls were appropriately diluted isotype-matched antibodies (Table 2). Data from 10,000 events were stored. List mode files were analyzed with computer software (WinMDI, v. 2.9).

### Transplantation of isolated cells

At 10 – 12 hr after BM harvest, an interventional radiologist administered the final MNC or CD133$^+$ cells via the portal vein by the percutaneous transhepatic method. All patients were sedated with intravenous midazolam and fentanyl. Under aseptic conditions and local anesthesia with lidocaine; the Seldinger technique was used with a right-sided percutaneous approach under color ultrasonography guidance, which allowed the advancement of an 18-gauge guidewire into the main portal vein. After the route was dilated by the standard method, a 5-F cobra catheter (Terumo, Leuven, Belgium) was introduced over the guidewire into the portal vein and positioned just proximal to the portal confluence. After intrahepatic administration of the

**Table 3.** Results of bone marrow cell preparation

| Patient No. | BM Vol. (mL) | BM cell count ($\times10^9$) | Cell count ($\times10^6$) | Viability (%) | Purity (%) | Total viable cells ($\times10^6$) |
|---|---|---|---|---|---|---|
| CD133 | | | | | | |
| 1 | 242 | 1.4 | 6 | 94 | 55 | 3.10 |
| 2 | 230 | 2.8 | 8 | 92 | 88.18 | 6.49 |
| 3 | 265 | 3.89 | 14 | 88 | 77.27 | 9.52 |
| Mean±SD | 245.7±17.8 | 2.7±1.3 | 9.3±4.2 | 91.3±3.1 | 73.5±16.91 | 6.4±3.2 |
| MNC | | | | | | |
| 1 | 277 | 4.8 | 1340 | 91 | — | 1220 |
| 2 | 312 | 6.75 | 1500 | 98 | — | 1470 |
| 3 | 297 | 4.32 | 1340 | 92 | — | 1230 |
| Mean±SD | 295.3±17.6 | 5.3±1.3 | 1390±90 | 93.7±3.8 | — | 1310±140 |

BM=bone marrow

**Table 4.** Flow cytometry analysis of MNCs

| Patient No. MNC | CD105 | CD16 | CD14 | CD31 | VEGFR | CD33 | CD29 | CD38 | CD133 | CD45 | CD34 | CD90 | CD44 | CD73 | CD3 | CD19 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 2.6 | 17.1 | 7.1 | 47.5 | 3.9 | 34.8 | 64.1 | 29.1 | 0.6 | 52.0 | 1.2 | 1.3 | 94.7 | 1.5 | 2.7 | 8.7 |
| 2 | 0.8 | 12.3 | 1.7 | 42.9 | 2.8 | 13.4 | 21.4 | 11.3 | 0.9 | 48.0 | 3.2 | 0.7 | 96.5 | 1.6 | 6.5 | 5.6 |
| 3 | 3.7 | 13.1 | 2.7 | 40.4 | 2.6 | 9.2 | 65.5 | 31.6 | 1.5 | 50.3 | 1.4 | 1.2 | 88.1 | 0.5 | 5.4 | 8.9 |
| Mean ±SD | 2.4 ±1.5 | 14.2 ±2.6 | 3.9 ±2.9 | 43.5 ±3.6 | 3.1 ±.0.7 | 19.1 ±13.7 | 50.3 ±2.1 | 24.0 ±11.1 | 1.0 ±0.4 | 50.1 ±1.9 | 2.0 ±1.2 | 1.1 ±0.3 | 93.1 ±4.42 | 1.1 ±0.5 | 4.9 ±1.9 | 7.7 ±1.9 |

MNC=mononuclear cell; VEGFR= vascular endothelial cell growth factor receptor

prepared cells, the portal catheter was removed. The procedure was successful in all six patients and no complications were noted.

### Follow up visits and outcome measures

Patients were admitted and observed at Namazi Hospital (Shiraz, Iran) for seven days. The following laboratory tests were performed at days 0, 1, 3, 5, and 7; weeks 2, 3, and 4; and months 2, 3, 4 and 6, in addition to 24 months post-transplantation for long-term follow up: complete blood counts, PT and international normalized ratio, serum albumin, urea, creatinine, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), serum total and direct bilirubin, and α-fetoprotein. Additionally at each visit, 10 mL of the patients' serum samples were collected and stored at -70°C in order to measure the serum levels of hepatocyte growth factor (HGF),tissue inhibitor of metalloproteinase 1( TIMP-1) and hyaluronic acid.

## Results

### Cell analysis

The mean volume of bone marrow aspirated from the iliac crest of the patients was 295.3±17.6 mL in the MNC group and 245.7±17.8 in the CD133 group. The mean number of viable MNCs transplanted into the patients' portal veins was 1.31±0.14 $\times10^9$ and the mean number of viable CD133+ cells was 6.4±3.2 $\times10^6$. The mean rate of viability of the CD133+ cells was 91.3±3.1% and mean purity was 73.5±16.9 (Table 3). The results of flow cytometry analysis in the MNC group have been summarized in Table 4.

### Patient characteristics and clinical outcomes

There were three patients (two male, one female) with a mean age of 34±6 years in the MNC group and three patients (one male, two female) with a mean age of 37±3 years in the CD133 group who were enrolled and underwent the procedure (Table 1). The paraclinical and serum levels of the liver related factors were determined (Table 1). All patients had low grade fevers (37.6 – 38.3°C) during the first 24 hours after injection, but no other complications (including bleeding, portal vein thrombosis or infection) were observed during the seven days they were inpatients. There were no adverse effects in the patients during the six months follow up. There were no hospital admissions for any cause (e.g., due to spontaneous bacterial peritonitis, gastrointestinal bleeding or intractable ascites) during the next six months of follow up.

In the six month follow-up one patient from each group had a detectable partial thrombosis (less than 25% of the lumen diameter) in the left branch of their portal veins, which was indistinguishable from the transfused cell mass by Doppler ultrasonography.

There were no significant changes in liver enzymes (ALT, AST, and ALP) and other laboratory data during the first week after the injection. Table 1 shows changes in laboratory data three and six months after the injection. As the charts reveal, there were no considerable differences in pre- and post-intervention values of AST, ALT, ALP, BR

(total and direct), Alb, INR, BUN, Cr, MELD score, HGF, TIMP-1, and hyaluronic acid in each group, separately or as a whole (Table 1).

Liver volume changed from a mean baseline of 1176±796 to 1092±827 $cm^3$. In the $CD133^+$ group, liver volume changed from 1421±1146 to 1387±1125 $cm^3$ and in the MNC group from 932±302 to 797±425 $cm^3$, which was not significantly different.

### Long-term follow-up

All patients were subjected to paraclinical analysis 24 months after cell transplantation in order to evaluate their clinical situation (Table 1). Total protein and Alb production levels were used to evaluate liver function and probable regeneration, as well as INR and BR (total and direct) synthesis during long-term survey. Also, to study the progression of inflammation and possible fibrosis, sera levels of ALT and AST were compared. Hemoglobin concentration, white blood cell count, and serum level of Cr were regarded as non-hepatic markers for systemic assessment of their general health condition. MELD scores of the patients were also compared with baseline values for better understanding of probable changes in clinical and paraclinical variants.

Serum levels of Cr, which is an essential index for renal perfusion and function, were within the desirable range. Hepatic enzymes (ALT and AST) and Alb changed after two years, however, nearly all of these alterations were favorable. Hemoglobin synthesis is a delicate indicator that reflects BM physiology and decreases during chronic and inflammatory diseases, but fortunately in these patients, there was no evidence of BM suppression.

## Discussion

According to the potential for stem cells to differentiate or their paracrine secretion, stem cell transplantation has become an attractive alternative therapeutic method for the treatment of patients with liver disease aiming, at least, at a temporary support of hepatic function until a liver becomes available for organ transplantation.

This phase I study has aimed to evaluate the feasibility and safety of autologous BM cell transplantation into the portal vein of patients with decompensated liver cirrhosis and compare the clinical outcome of MNCs and $CD133^+$ cell transplantations. Therefore, we infused MNCs and $CD133^+$ cells intraportally using a catheter under color ultrasonography guidance, which was feasible and not associated with serious local side effects. The portal vein was used for cell infusion since cell engraftment to the liver could be mostly guaranteed. Our results confirmed the feasibility and safety of MNCs and $CD133^+$ cell infusions into the portal veins of such patients.

Moreover, our results showed that in the MNC group, AST, and creatinine levels seemed to be reduced after three and six months, respectively and serum albumin increased after three months in which its significancy could be confirmed in a well designed double blind randomized clinical trial. However, there were no significant alterations of other liver function parameters, liver enzymes, serum bilirubin or liver volume after transplantation of autologous MNCs and $CD133^+$ cells in patients with decompensated liver cirrhosis.

Two years after transplantation, evidence has proven the safety and practicability of this therapeutic approach to end stage liver disease as a promising alternative for patients on waiting lists for liver transplantation, although one patient who received MNC was hospitalized 14 months after transplantation due to upper GI bleeding that originated from grade 4 gastroesophageal varices. Unfortunately the patient died after eight days due to massive hemorrhage. All hepatic and non-hepatic paraclinical parameters revealed liver decompensation. Esch et al. have reported that portal administration of autologous $CD133^+$ ($2.4 – 12.3\times10^6$) accelerated liver regeneration and is a novel therapy to support hepatic resection.[18] Moreover, in patients with malignant liver lesions, the combination of portal vein embolization (PVE) with $CD133^+$ administration substantially increased hepatic regeneration compared with PVE alone.[14] Additionally, Yannaki et al. demonstrated two cases treated with boost infusions of autologous mobilized $CD34^+$ to regenerate decompensated alcoholic cirrhosis livers. Three mobilization courses were performed in each patient and mobilized stem cells were collected and reinfused as a boost administered back to the respective patients (at least $5\times10^6$/kg). Both patients showed a lasting amelioration in the clinical course of the disease during the 30 months of follow-up.[17]

In contrast to these studies, the subjects of our study were patients with end stage liver cirrhosis.

Moreover, it has been demonstrated that MNC infusion improved patients with decompensated liver cirrhosis.[19,20] Lyra et al.[20] have shown that an MNC infusion $1.6 – 13.1\times10^8$ into the hepatic arteries of patients with advanced chronic liver disease was safe and feasible. A decrease in the mean serum bilirubin and INR levels and an increase in albumin levels were observed.[20] Moreover, it has been shown that after administration of $5.2\pm0.6\times10^9$ MNCs via the peripheral vein into patients with decompensated liver cirrhosis significant improvements in serum albumin levels, total protein, α-fetoprotein and Child-Pugh scores were observed at 24 weeks.[19]

We also evaluated serum levels of hepatocyte growth factor, TIMP-1 enzyme and hyaluronic acid as markers of liver fibrosis.[19] TIMP-1 showed a decrease in both groups, which can be an indicator of matrix metalloproteinase activation to improve fibrosis.[19] In our study, HGF levels decreased during therapy while Terai et al. have shown an elevated level of this factor.[19] Due to the small numbers of our

patients these differences were not statistically significant.

In conclusion, although this was a limited project and must be confirmed with a randomized blinded controlled trial; we found that infusion of autologous BM-derived cells through the portal vein was safe and had no adverse effects on patients' liver functions after 24 months of follow up. Although, elastogeram and CT scan analysis can inform more valuable data, which in future projects must be considered. There were no obvious differences between the groups who received MNC and $CD133^{+}$, thus MNC can be a good source for cell therapy while enrichment of CD133 is a very chargeable process. Based on these results and the reported studies, we suggest a protocol amendment in which boosts of stem cells can be administered intraportally. This could enhance the frequency of stem cells homing into the liver by potentially facilitating the engrafting mechanisms, thus providing a higher beneficial influence to the patients.

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