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***Clinical Trials Study***

**Autologous mobilized peripheral blood CD34+ cell infusion in non-viral decompensated liver cirrhosis.**

Sharma M *et al.* Autologous CD34+ cell infusion in cirrhosis

Mithun Sharma, Padaki Nagaraja Rao, Mitnala Sasikala, Mamata Reddy Kuncharam, Chima Reddy, Vardaraj Gokak, BPSS Raju, Jagdeesh R Singh, Piyal Nag, D Nageshwar Reddy

**Mithun Sharma, Padaki Nagaraja Rao, Vardaraj Gokak, BPSS Raju, D Nageshwar Reddy**, Department of Gastroenterology and Hepatology, Asian Institute of Gastroenterology, Asian Healthcare Foundation, Hyderabad 500082, India

**Mitnala Sasikala, Mamata Reddy Kuncharam, Chimpa Reddy,** Asian Healthcare Foundation, Hyderabad 500082, India

**Jagdeesh R Singh, Piyal Nag**, Department of Interventional Radiology, Asian Institute of Gastroenterology, Hyderabad 500082, India

**Author contribution**: Sharma M, Sasikala M, Rao PN and Reddy DN designed research; Sharma M, Raju BPSS, Vardaraj Gokak, Singh JR, Nag P, Sasikala M, Kuncharam MR, Reddy C, Rao PN and Reddy DN performed research; Sharma M analyzed data; Sharma M and Sasikala M wrote the paper.

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**Correspondence to:** **Mithun Sharma, MD**, Department of Gastroenterology and Hepatology, Asian Institute of Gastroenterology, 6-3-661 Red Rose Café lane, Somajigudda, Hyderabad 500082, India. drmithunsharma@gmail.com

**Telephone:** +91-40-23378888

**Fax:** +91-40-23324255

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**Abstract**

**AIM:** To study the effect of mobilized peripheral blood autologous CD34 positive (CD34+) cell infusion in patients with non-viral decompensated cirrhosis.

**METHODS:** Cirrhotic patients of non-viral etiology were divided into 2 groups based on their willingness to be listed for deceased donor liver transplant (DDLT) (control, *n =* 23) or to receive autologous CD34+ cell infusion through hepatic artery (study group, *n =* 22). Patient in study group were admitted in hospital and received granulocyte colony stimulating factor injections @ 520 μgm per day for 3 consecutive days to mobilize CD34+ cells from the bone marrow. On day 4, leukapheresis was done and CD34+ cells were isolated using CliniMAC magnetic cell sorter. The isolated CD34+ cells were infused into the hepatic artery under radiological guidance. The patients were discharged within 48 h. The control group received standard of care treatment for liver cirrhosis and were worked up for DDLT as per protocol of the institute. Both groups were followed up every weekly for 4 wk and then every month for 3 mo.

**RESULTS:** In control and study group, the cause of cirrhosis was cryptogenic in 18 (78.2%) and 16 (72.72%) and alcohol related in 5 (21.7%) and 6 (27.27%), respectively. The mean day 3 cell count (cells/μL) was 27.00 ± 20.43 with a viability (%) of 81.84 ± 11.99. and purity of 80%-90%. Primary end point analysis revealed that at 4 wk, the mean serum albumin in the study group increased significantly (2.83 ± 0.36 *vs* 2.43 ± 0.42, *P =* 0.001) when compared with controls. This improvement in albumin was however not sustained at 3 mo. However, at the end of 3 mo there was a statistically significant improvement in serum creatinine in the study group (0.96 ± 0.33 *vs* 1.42 ± 0.70, *P =* 0.01) which translated into a significant improvement in the Model for End-Stage Liver Disease score (15.75 ± 5.13 *vs* 19.94 ± 6.68, *P =* 0.04). On statistical analysis of secondary end points, the transplant free survival at the end of 1 mo and 3 mo did not show any significant difference (*P =* 0.60) when compared to the control group. There was no improvement in aspartate transaminase, alanine transaminase, and bilirubin at any point in the study population. There was no mortality benefit in the study group. The procedure was safe with no procedural or treatment related complications.

**CONCLUSION:** Autologous CD 34+ cell infusion is safe and effectively improved liver function in the short term and may serve as a bridge to liver transplantation.

**Key words:** CD34 cell infusion; Stem cell; Cirrhosis; Model for End-Stage Liver Disease; Liver transplantation

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**Core tip:** Cirrhosis of liver is a chronic progressive disease with high morbidity and mortality without liver transplantation. Alternative stem cell based therapies have shown promising results. In our study, we used autologous CD34 positive (CD34+) cell infusion in decompensated cirrhotic patients of non-viral etiology and is the first study which has compared them with controls who were selected from waiting list of liver transplantation. The results shows improvement in albumin at 1 mo and Model for End-Stage Liver Disease score at 3 mo. Though many questions still remain unanswered, stem cell therapy is a promising treatment modality and serve currently as a bridge to liver transplantation.

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**INTRODUCTION**

The increasing prevalence of cirrhosis[1] coupled with paucity of donor tissues available for liver transplantation as well as high financial burden, have paved the way for finding alternative therapeutic options for this potentially life threatening condition. Cell based regenerative therapies, including adult hemopoetic stem cells (HSC) and mesenchymal stem cell (MSC) based therapies, have evolved as the fore runner in the search for an alternative to whole-organ transplantion[2,3]. HSCs are cells that are capable of differentiating into multiple cell lineages including hepatocytes[4]. HSCs differentiating into hepatocytes, was first demonstrated by Peterson *et al*, who showed that following a noxious insult to the murine liver, pluripotent stem cells in bone marrow contributed to liver regeneration[5]. A phase I study by Gordon *et al*[6] on 5 patients of decompensated cirrhosis paved the way for use of peripherally mobilized CD34 positive (CD34+) cells in humans. Mobilization of bone marrow derived stem cells induced by granulocyte colony stimulating factor (G-CSF) has shown to increase CD34+ cells along with increase in hepatocyte growth factor leading to hepatic progenitor cell proliferation in patients with alcoholic steatohepatitis[7]. However, the degree of hepatic engraftment in human liver is highly variable. A recent study by Garg *et al*[8] has shown that bone marrow stimulation with G-CSF in acute on chronic liver failure patients leads to significant increase in CD 34+ cells in liver tissues after 4 wk of G-CSF injection. Coinciding with this rise of CD 34+ cells in liver tissues, there was a significant decrease in peripheral CD34+cells, possibly due to migration and settlement of the CD34+ cells in the liver. This study therefore paved the way to explore whether direct infusion of CD34 cells into the hepatic artery in patients with cirrhosis would help in liver regeneration and improve prognosis. With the background of these studies, we designed a research protocol to prospectively study the effect of transfusion of mobilized autologous CD34+ cells into the hepatic artery of cirrhotic patients. This is the first research study which have used autologous transfusion of mobilized blood CD34+ cells in decompensated cirrhotics of non-viral etiology and have compared them with patients receiving standard of care for cirrhosis

This research protocol was designed to assess the effects of autologous CD34+ hematopoietic cell infusion in patients with decompensated cirrhosis of liver.The primary endpoint of the study was improvement in the Model for End-Stage Liver Disease (MELD) score along with improvement in synthetic function of the liver as measured by serum albumin and INR at 3 mo with autologus CD34+ cell infusion compared with standard of care. The secondary endpoints were decrease in ascites as measured by ultrasonography and requirement of therapeutic paracentesis and improvement in transplantation free survival.

**MATERIALS AND METHODS**

***Patient selection***

The study protocol was approved by the by the Institutional Review Board, Institutional Ethics committee and Institutional Committee for Stem Cell Research (ICSCR). This study was performed as per 2007 guidelines of Indian Council of Medical research (ICMR) and department of Biotechnology (DBT) India utilizing autologous minimally manipulated hematopoietic stem cells under permitted area of research.

Between July 2012 to June 2013, 100 cirrhotic patients who attended the Hepatology unit of the Institute were screened. All patients who required a liver transplantation were counseled for either living donor liver transplantation (LDLT) or deceased donor liver transplantation (DDLT). The patients who did not have the option of a living donor and who were unwilling for DDLT either due to the long waiting time or due to immediate financial constraints were counseled about autologous CD34+ cell infusion as a research tool which is being evaluated as an alternative or bridge to liver transplantation. These patients were considered as the study group. On the other hand, those patients who were willing only for DDLT were included in the institutional liver transplantation waiting list for DDLT. These patients were considered as the control group.

Patients aged between 18-70 years, with clinically diagnosed hepatic cirrhosis, having a MELD score of > 14 and unwilling for immediate liver transplantation, with life expectancy of at least 3 mo (based on MELD score) and ability to give informed consent were included in the study group. All enrolled patients were counseled in detail about the nature of the research protocol to be followed and possible outcomes. An informed consent was obtained from all patients.

Patients with liver tumors or history of any other malignancy, active infections including HIV, HBV, HCV, severe cardiac and pulmonary co-morbidities unrelated to cirrhosis, recent gastrointestinal bleed, acute kidney injury or hepatorenal syndrome, portal vein thrombosis, pregnancy, lactation, and inability to give informed consent were excluded from the study.

Patients of decompensated chronic liver disease between 18-70 years, who were enrolled in the waiting list during the study period for DDLT were considered as control group. All other inclusion criteria were same as the study group except that they were willing and eligible for DDLT and unwilling to be a part of the CD34+ cell infusion research protocol. Informed consent was taken from the control group for enrollment in the DDLT list as well as for regular follow up and comparison with autologous CD34 + cell infusion patients.

Patients in the study population were admitted to the hospital and a complete clinical examination was done. Investigations included complete blood counts, liver function test, serum creatinine, blood urea, alpha fetoprotein, coagulation profile, trans abdominal ultrasonography of the abdomen with doppler study of the spleno-portal axis, contrast enhanced computed topography scan for all patients except those with serum creatinine > 1.5 mg/dL, screening for hepatitis B and C, HIV, syphilis, cytomegalovirus. All other tests required to ascertain the cause of cirrhosis was done on case to case basis.

These patients in the control group underwent complete clinical and laboratory work up as above in addition to all other test required for listing in the DDLT program as per liver transplantation protocol of the Institute.

Refractory ascites was defined as ascites unresponsive to sodium restricted diet (less than 2 g/d) and high dose diuretic treatment (spironolactone 400 mg/d and frusemide 160 mg/d) or therapy limited by the complications of diuretics.

***Treatment protocol***

Patients in the study group were admitted to the hospital and received daily subcutaneous injections of human granulocyte-colony-stimulating factor (G-CSF Neupogen, Filgrastim, Roche @ 520 μgm/d) for mobilization of CD34+ cells from the bone marrow for 3 consecutive days. This was based on an initial observation (*n =* 5) that the peak CD34+ cell levels was achieved on day 3 followed by a steady decrease on day 4 and 5. Following G-CSF injections, daily monitoring of blood for complete blood counts, coagulation profile, creatinine and liver function test were done. Any adverse effects were recorded. The peripheral concentration of CD34+ cells were measured daily prior to leukapheresis to ensure satisfactory levels (> 2 cells/μL). On day 4, leukapheresis was done using MCS-3P magnetic cell separator (Hemaneics, USA) and 60-120 mL of peripheral blood was collected. Peripheral blood mononuclear cells (PBMCs) were isolated from the leukapheresis products in the clean room. Mono nuclear cells were isolated employing Hi-Sep method (HiSep LSM1077, LS001, Himedia). The mono nuclear cells were washed with phosphate buffer saline (PBS) and diluted with CliniMACS buffer (Miltenyi Biotech, GmbH). The cells were centrifuged and incubated with CD34+ monoclonal antibodies directly labelled to micro beads (MACS, Miltney Biotech, GmbH171-01, Bergisch, Galdbach, Germany) for 30 min. After incubation the cells were washed with CliniMACS buffer and placed on a CliniMACs cell separator. The labelled cells were isolated using high gradient magnetic field and eluted from the column. At the end of the separation, the cells were counted under microscope and viability was assessed by trypan blue dye exclusion method. Purity of the cells were assessed by flowcytometer. The CD34+ cells were diluted with 10 mL of PBS with 2% human serum albumin in a sterile tube and were immediately infused through the hepatic artery under radiological guidance by the interventional radiologist. The patients were kept under observation for 24 h post procedure and discharged on the subsequent day. During the hospital stay, all clinical parameters and any adverse events were recorded.

***Follow up***

Following discharge from the hospital, the patients were followed up every weekly for one month and thereafter at end of 3 mo. During each visit, ascites was evaluated by ultrasonography and need for therapeutic paracentesis due to ascites causing respiratory embarrassment was recorded. Laboratory tests at each visit included complete blood count, liver function test, coagulation profile, creatinine, blood urea, alpha feto-protein and doppler ultrasonography of whole abdomen.

The control group was followed up with similar protocol for 3 mo.

***Statistical analysis***

The clinical and laboratory data at baseline between the study population and the control group and at 1 mo and end of 3 mo were analyzed. The values are expressed as mean with standard deviation and as median with range wherever deemed appropriate. For categorical variables, Fischer’s exact test was used. Data was analyzed using online graphpad software 2014 and a *P* value (two tailed) of < 0.05 was considered to be statistically significant. The statistical analysis was per protocol analysis.

**RESULTS**

Of the 100 patient screened for eligibility, 55 patients were excluded. Thirty patients did not meet the inclusion criteria and 25 patients refused to be a part of the study. Twenty two patients were enrolled in the study population. At 1 mo, in the study group, all 22 patients were analyzed for response to CD34+ cell infusion. Between 1 mo to 3 mo, 2 patents despite being clinically stable decided to undergo lLDLT, 1 patient died of hepatorenal syndrome and 1 patient was lost to follow up. In the control group, 23 patients were enrolled and all patients were analyzed at 1 month. In between 1 mo to 3 mo, 3 patients died – 2 due to sepsis and 1 due to hepatorenal syndrome, 1 patient underwent DDLT and 2 patient were lost to follow up. In the final analysis, there were 17 and 18 patents in control and study group respectively at 3 mo (Figure 1).

The baseline characteristics between the control group and the study population were identical (Table 1). In the control and study group, the cause of cirrhosis was cryptogenic in 18 (78.2%) and 16 (72.72%) and alcohol related in 5 (21.7%) and 6 (27.27%), respectively. The MELD score in both the groups were similar. The median period of alcohol abstinence in the control group was 7 mo (range, 5-11 mo) and in the study group ~~it~~ was 6 mo (range, 4-12 mo). Among the patients of cryptogenic cirrhosis, 7 patients in the control group and 6 in the study group were having type 2 diabetes mellitus with mean glycosylated hemoglobin (HBA1c) level of 6.45 (range, 5.3-8.3)and 6.63 (range, 5.1-9), respectively. Twenty two (95.6%) patients in the control group and 22 (100%) in the study group and had ascites. Refractory ascites requiring therapeutic paracentesis at least once every month for respiratory difficulty was 5 (22.7%) in the study group and 6 (21.7%) in the control group. In our population, the high maximum diuretic dose of 400 mg of spironolactone/d combined with frusemide 160mg/d could not be given to any patients due to development of complications of diuretic therapy. All of our refractory ascites patients were therefore refractory due to development of diuretic therapy related complications which limited their use at maximal dose. The mean number of therapeutic paracentesis in 3 mo period prior to enrollment was similar between the two groups. The presence of documented previous overt hepatic encephalopathy within last 3 mo was 6 (26%) and 7 (31.81%) in control and study population respectively.

***Mobilization of CD34+ cells after G-CSF***

The mean (± SD) baseline cell count (cells/µl) was 2.3 ± 2.56 with baseline viability (%) of 48.17 ± 23.95. The day 3 cell count (cells/μL) was 27.00 ± 20.43 while the viability (%) was 81.84 ± 11.99. The purity of cells as assessed by enumerating CD34+ cells on flow cytometer was 80%-90%.

***Clinical results after CD34 + cell infusion***

Following CD34+ cell infusion, patients were regularly monitored and clinical parameters were recorded. The liver and renal function tests during the course of stay in the hospital were normal. Following the procedure protocol Doppler ultrasound was done and there was no evidence of any portal vein or hepatic artery thrombosis. All patients were discharged from hospital as planned in the protocol after 24 h of the procedure.

Primary end point analysis revealed that at 4 weeks, the mean serum albumin in the study group increased significantly (2.83 ± 0.36 *vs* 2.43 ± 0.42, *P =* 0.001) when compared with controls. This improvement in albumin was however not sustained at 3 months. (Tables 2 and 3). Serum bilirubin, alanine aminotransferase (ALT), and aspartate aminotransferase (AST) did not show any statistically significant improvement at 4 wk and at end of 3 mo. However, at the end of 3 months there was a statistically significant improvement in serum creatinine in the study group (0.96 ± 0.33 *vs* 1.42 ± 0.70, *P =* 0.01) which translated into a significant improvement in the MELD score (15.75 ± 5.13 *vs* 19.94 ± 6.68, *P =* 0.04). Platelet count and INR showed some improvement but did not reach statistical significance at any point of time.

On statistical analysis of secondary end points, the transplant free survival with autologus CD34+ cell infusion at the end of 1 mo and 3 mo and did not show any significant difference (*P =* 0.60) when compared to the control group. The mean requirement of therapeutic paracentesis was not significantly different at the end of 3 months between the study and control groups (1.28 ± 0.75 *vs* 1.59 ± 1.06, *P =* 0.32) (Table 3). There was no patient in whom the requirement for therapeutic paracentesis increased after CD34 cell infusion.

***Mortality data***

In the study population, 1 patient developed sepsis with hepatorenal syndrome and died on 88th day post CD34+ cell infusion. In the control group, 3 patients died within the 3 mo: 2 died due to sepsis and 1 due to massive upper gastrointestinal bleed. The number of deaths between the study and the control group was not statistically significant at 3 months (*P =* 0.34)

***Adverse events***

The procedure of autologus CD34 + cells through hepatic artery was safe with no specific treatment or procedure related side effects or mortality. The commonest complain was slight discomfort at the peripheral catheter site and the pain score on the visual analog scale was always less than 4. One patient complained of chest discomfort 24 h after the procedure. However, the cardiac workup for ischemia was negative and it subsided within 1 h after administration of proton pump inhibitor.

**DISCUSSION**

Cell based regenerative therapies for chronic liver disease are a promising new alternative to whole organ liver transplantation[9], where the lack of donor tissues and high cost[10] acts as a major obstacles. The attention towards bone marrow derived stem cells for liver regeneration, started after a study by Theise *et al*[11] where Y chromosome positive liver cells were detected in autopsied women who received therapeutic bone marrow transplantation from male donors, suggesting existence of pluripotent stem cells among bone marrow cells. Previous studies[12,13,14] have demonstrated improvement in liver function with autologus CD34+ cell infusion in decompensatedcirrhosis of varied etiology. In our study, we used peripheral CD34+ autologous stem cell infusion and is the first study which has compared the results with controls in non-viral decompensated cirrhosis.

In our study population, mobilization of bone marrow derived CD34+ cells was done using G-CSF for 3 consecutive days instead of the conventional 5 d therapy used in most of the other studies[12,13,14]. This was based on the initial pilot study done at our Institute on 5 patients, wherein it was observed that the mean cell counts reached the peak after the third dose of G-CSF injection and then showed a downward trend on day 4 and day 5 in non-viral cirrhosis patients. The mobilization of CD34+ cells by use of G-CSF was safe despite the presence of decompensation and portal hypertension in all the patients. In contrast to previous reports[15,16], there was no case of splenic rupture during the CD34+ mobilization procedure. The blood counts were monitored in all patients daily for 5 consecutive days and also every weekly post procedure up to one month. The cell counts returned to baseline in all patients after the end of 1 month.

Liver stem cells are thought to be precursors to liver parenchymal cells or cholangiocytes[17-19]. The actual ability of these progenitor cells to differentiate into hepatocytes or cholangiocytes is not clear[20]. It has been suggested that conversion of stem cells to hepatocytes may occur *via* cell fusion[21,22]. Transplantation of stem cells by infusion into a peripheral or portal vein has shown successful engraftment and multiplication even in the setting of fibrosis in rodent models[23,24]. A recent human study on acute on chronic liver failure[8], has shown that G-CSF injections increases the homing of CD34+ cell in the liver. In our study, to improve hepatic homing of cells, which may involve chemo-attractants like stroma derived factor 1[25,26], the CD34+ cells were directly infused into the hepatic artery under radiological guidance. No procedural complications were observed in this process. Earlier studies had also safely used the hepatic artery for infusion[27]. Post procedure, there was no increase in portal vein or hepatic artery thrombosis nor was there any evidence of ischemic hepatitis.

The significant improvement in serum albumin which was observed at 1 mo coincides with the 4 wk period of maximal homing in of CD34+ cells in the liver[8]. The 3 mo improvement in the MELD score in the study population may indicate that the CD34+ cells which have homed in the liver may be exerting its peak regenerative effect at that time. This improvement in albumin and MELD score has been observed in previous studies[13,28]. Although studies in the past have shown improvement in bilirubin and transaminases, our study could not find any such statistical significance.

More patients in the study group (*n =* 2) underwent liver transplant. All of these patient had living donor liver transplantation and the cause of opting for LDLT after CD34+ cell infusion was purely based on availability of living donor, and wish for a curative treatment. The living donor as well as the family of the patients needed time for convincing themselves as well for arranging finances for a LDLT. In these patients, autologous CD34+ cell infusion served as a bridge to liver transplant. Only 1 patients in the control population underwent DDLT, reaffirming the paucity of deceased donor tissue for liver transplant. However, the mean duration to liver transplant was not statistically different in both the groups.

Survival analysis to assess the transplant free survival was not statistically feasible as the number of patients were low. However, the mortality was not statistically different in both the groups through the number of deaths in the control group was more (*n =* 3) than the study population (*n =* 1). The most common cause of death was hepatorenal syndrome and sepsis. This may suggest that the infusion of G-CSF causes restoration of neutrophil function[29,30] in decompensated cirrhosis, which has been attributed to development of sepsis and HRS in such patients. G-CSF infusion has also shown to improve the development of antibody to hepatitis B after hepatitis B vaccination in cirrhosis of non-viral etiology and thereby improve prognosis by preventing new hepatitis B infection in this patient population[31].

The limitations of this study was that 2 patients who enrolled for study group later decided to go for LDLT thereby causing bias in analysis of transplant free survival and overall mortality analysis. A larger study population would have given a clearer picture on the mortality data. Another limitation of the study was the lack of liver biopsy. Therefore, we could not objectively demonstrate the homing in and expansion of CD34+ cells in liver tissue.

The positives of this study however is that, this is the first study in a non-viral decompensated cirrhosis of diverse etiology wherein the effects of CD34+ cell infusion has been analyzed for 3 mo and compared with controls. The improvement in MELD at 3 months paves the way for identifying a potential window to delay transplantation. Long term data are required in this field.

In summary, autologous CD34+ cell infusion appears to be a safe and effective modality to delay the need for liver transplantation and thereby serve as a bridge to either DDLT or LDLT. The benefits of autologous CD34+ cell infusion indicates that there is a window during which a transplant may be still be required in few subjects. Whether repeated infusion of stem cells can further delay the need for transplant merits evaluation in further trials.

**COMMENTS:**

***Background***

Cirrhosis of liver is a chronic liver disease with high morbidity and mortality. The most common causes of cirrhosis are alcohol, hepatitis B, hepatitis C and non-alcoholic fatty liver related. The only curative option for cirrhosis of liver is a liver transplantation. However, the paucity of donor organs available for liver transplantation, coupled with the high costs involved in the surgery has paved the way for search for alternative cell based therapies. Stem cell therapy has emerged as a fore-runner in this research effort and initial studies have shown promising results in cirrhosis of both viral and non-viral etiologies.

***Research frontiers***

Studies in the past have shown improvement in liver function by using stem cell from various sources. In our study, mobilization of patient’s own stem cells from the bone marrow and subsequent isolation of these cells was done in the stem cell laboratory. The stem cell isolates were then infused back into the patient with the expectation that they will differentiate in the liver into liver cells and help in regeneration of the diseased liver.

***Innovations and breakthroughs***

Previous studies have compared the effect of autologus stem cell infusion in non-viral decompensated cirrhotic patients. This study is the first in this subset of patients wherein we have compared them with controls. The controls were the patients waiting for liver transplantation. The authors directly infused the isolated cells into the hepatic artery of the patient with the hope that it will increase the availability of stem cells delivered to the liver. We found that there was improvement in albumin at 1 mo. At 3 mo there was significant improvement in the MELD score and creatinine.

***Applications***

This study suggests that autologous infusion of CD34+ cells in patients with decompensated cirrhosis of non-viral etiology can be used safely to improve liver function in the short term.

***Terminology***

Decompensated cirrhosis means a cirrhotic patient in whom due to the failing function of the liver, there is development of either jaundice or ascites (fluid accumulation in the abdomen). Liver transplantation is a surgical procedure in which the whole liver from a deceased person or a part of the liver from a related living donor is transplanted into a patient having cirrhosis of liver after his own diseased liver is removed. Stem cells are the master cells of the human body which have the ability to differentiate into various tissue cell typesincluding hepatocytes. CD34+ cells are stem cells of hemopoetic origin which are recognized in the laboratory by the presence of the marker CD34.

***Peer-review***

In this study, the authors have compared the use of autologous CD34+ cells in patients of decompensated liver cirrhosis of non-viral etiology with patients receiving standard of care in the waiting list for liver transplantation. This idea is very good work, especially in the number of cases. The author needs to follow up the long term data of these patients to detect any long term side effects.

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Granulocyte colony stimulating factor adjuvant role on the immunological response to hepatitis B vaccine in patients with cirrhosis: a double blind randomized placebo controlled trial. *Hepat Mon* 2014; **14**: e15447 [PMID: 24910704 DOI: 10.5812/hepatmon.15447] |

**P-Reviewer:** Eshraghian A, El-Hawary AK, Zhao HT **S-Editor:** Yu J

**L-Editor:** **E-Editor:**

Assessed for eligibility (*n* = 100)

**Excluded (*n* = 55)**

Not meeting inclusion criteria (n=30)

Refused to participate (*n* = 25)

Offered Liver transplantation or CD34 infusion

Agreed for DDLT = Control group (*n =* 23)

Unwilling for DDLT, Willing for CD34 infusion = Study group (*n* = 22)

**1 mo – 3 mo**

Died (*n* = 3)

Underwent DDLT (*n* = 1)

Lost to follow up (*n* = 2)

**1month – 3 months**

Died (n=1)

Underwent LDLT (*n* = 2)

Lost to follow up (*n* = 1)

Control group (*n* = 23)

Study Group

(*n* = 18)

**3 mo analysis**

**Figure 1 Consort flow diagram.** LDLT: Living donor liver transplantation; DDLT: Deceased donor liver transplantation.

**Table 1 Baseline characteristics between control and study group (**mean ± SD**)**

**------------------------------------------------------------------------------------------------------------------**

|  |  |  |  |
| --- | --- | --- | --- |
| Parameter | Control group (*n =* 23), | Study group (*n =* 22), | *P* value |
| Age (yr) | 47.35 ± 12.54 | 48.91 ± 9.25 | 0.64 |
| Sex ratio (M:F)  | 20:3 | 16:6 | 0.072 |
| Hemoglobin (g/dL) | 9.29 ± 1.86 | 9.15 ± 1.60 | 0.79 |
| Platelet count (Lakhs/mm3) | 0.92 ± 0.27 | 1.1 ± 0.72 | 0.24 |
| Total bilirubin(mg/dL) | 4.78 ± 4.06 | 3.55 ± 2.12 | 0.21 |
| AST (IU/mL) | 101.61 ± 174.41 | 67.14 ± 55.99 | 0.30 |
| ALT (IU/mL) | 77.87 ± 125.54 | 32.45 ± 17.07 | 0.10 |
| Albumin (mg/dL) | 2.7 ± 0.35 | 2.55 ± 0.35 | 0.16 |
| INR | 1.72 ± 0.53 | 1.80 ± 0.52 | 0.62 |
| Creatinine(mg/dL) | 1.08 ± 0.38 | 1.02 ± 0.29 | 0.56 |
| MELD score | 18.73 ± 5.29 | 18.28 ± 3.50 | 0.74 |

AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; IU: International unit; INR: International normalized ratio; MELD: Model for end-stage liver disease score.

**Table 2 Comparison between control and study group at 1 mo (**mean ± SD**)**

|  |  |  |  |
| --- | --- | --- | --- |
| Parameter | Control group (*n =* 23) | Study group (*n =* 22) | *P* value |
| Platelet count (Lakhs/mm3) | 0.87 ± 0.23 | 0.94 ± 0.41 | 0.48 |
| Total bilirubin(mg/dL) | 4.63 ± 3.16 | 3.37 ± 1.91 | 0.11 |
| AST (IU/mL) | 80.78 ± 74.98 | 68 ± 24.98 | 0.45 |
| ALT (IU/mL) | 55.13 ± 61.34 | 41.73 ± 20.56 | 0.34 |
| Albumin (mg/dL) | 2.43 ± 0.42 | 2.83 ± 0.36 | ***0.001*** |
| INR | 1.78 ± 0.65 | 1.67 ± 0.49 | 0.50 |
| Creatinine(mg/dL) | 1.24 ± 0.50 | 1.01 ± 0.32 | 0.06 |
| MELD score | 19.42 ± 6.52 | 15.73 ± 3.35 | 0.02 |

AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; IU: International unit; INR: International normalized ratio; MELD: Model for end-stage liver disease score.

**Table 3 Comparison between control and study group at 3 mo (**mean ± SD**)**

|  |  |  |  |
| --- | --- | --- | --- |
| Parameter | Control group (*n =* 17) | Study group (*n =* 18) | *P* value |
| Platelet count (Lakhs/mm3) | 0.91 ± 0.36 | 1.16 ± 0.71 | 0.21 |
| Total bilirubin(mg/dL) | 5.89 ± 7.13 | 3.82 ± 3.30 | 0.27 |
| AST (IU/mL) | 70.29 ± 43.57 | 48.39 ± 15.79 | 0.05 |
| ALT (IU/mL) | 49.18 ± 66.59 | 39.44 ± 19.02 | 0.55 |
| Albumin (mg/dL) | 2.65 ± 0.53 | 2.83 ± 0.33 | 0.24 |
| INR | 1.88 ± 0.48 | 1.61 ± 0.30 | 0.05 |
| Creatinine(mg/dL) | 1.42 ± 0.70 | 0.96 ± 0.33 | **0.01** |
| MELD score | 19.94 ± 6.68 | 15.75 ± 5.13 | **0.04** |
| Frequency of tap in last 3 mo | 1.59 ± 1.06 | 1.28 ± 0.75 | 0.32 |

AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; IU: International unit; INR: International normalized ratio; MELD: Model for end-stage liver disease score; tap: Therapeutic paracentesis.