ORIGINAL ARTICLE

Bali Medical Journal (*Bali MedJ)* 2022, Volume 11, Number 3: 1250-1258
P-ISSN.2089-1180, E-ISSN: 2302-2914

Granulocyte colony-stimulating factor therapy as a bridging treatment for pediatric decompensated liver cirrhosis prior to liver transplantation: an open-label randomized clinical trial

Tri Hening Rahayatri[1,2*], Alida Harahap[3], Aryono Hendarto[4], Hanifah Oswari[4], Rianto Setiabudy[5], Akmal Taher[6]

[1]Doctoral Program in Medical Sciences, Faculty of Medicine, Universitas Indonesia, Cipto Mangunkusumo Hospital, Jakarta, Indonesia;
[2]Department of Pediatric Surgery, Faculty of Medicine, Universitas Indonesia, Cipto Mangunkusumo Hospital, Jakarta, Indonesia;
[3]Eijkman Institute for Molecular Biology, Jakarta, Indonesia;
[4]Department of Pediatrics, Faculty of Medicine, Universitas Indonesia, Cipto Mangunkusumo Hospital, Jakarta, Indonesia;
[5]Department of Pharmacology, Faculty of Medicine, Universitas Indonesia, Cipto Mangunkusumo Hospital, Jakarta, Indonesia;
[6]Department of Urology, Faculty of Medicine, Universitas Indonesia, Cipto Mangunkusumo Hospital, Jakarta, Indonesia;

*Corresponding author:
Tri Hening Rahayatri;
Doctoral Program in Medical Sciences, Faculty of Medicine, Universitas Indonesia, Cipto Mangunkusumo Hospital, Jakarta, Indonesia; Department of Pediatric Surgery, Faculty of Medicine, Universitas Indonesia, Cipto Mangunkusumo Hospital, Jakarta, Indonesia;
heni.thr16@yahoo.com

Received: 2022-07-15
Accepted: 2022-08-11
Published: 2022-09-23

ABSTRACT

Background: Decompensated cirrhosis (DC) in children is the main indication of liver transplantation (LT). The lack of access to liver transplantation and impending clinical complications while awaiting transplantation affect the morbidity and mortality in pretransplant patients. Granulocyte colony-stimulating factor (G-CSF) therapy has shown promising results in adult decompensated cirrhosis as a potential bridging treatment. Our study aimed to identify the effect of G-CSF on pediatric end-stage liver disease (PELD) score, liver function, CD34+ cell mobilization, nutritional status, survival, and short-term side effects in children awaiting LT.

Methods: This study was an open-label, randomized controlled trial that included patients with decompensated liver cirrhosis between 3 months and 12 years of age. The intervention group received 12 courses of G-CSF subcutaneous injection (5 µg/kg/day) plus standard medical treatment (SMT) for liver cirrhosis. Results were obtained regarding PELD scores, liver function, CD34+ cell mobilization, changes in leukocyte and neutrophil counts, nutritional status, survival, and side effects within three months.

Results: Thirty-five pediatric patients were randomized into the intervention (17 patients) and control (18 patients) groups. During the trial, 14 (82%) in the intervention group completed the treatment. The median ages of the patients in the intervention and control groups were 18 and 14.5 months, respectively. The study's primary outcome identified no statistically significant difference in PELD scores between the intervention and control groups after G-CSF treatment. Liver function tests that showed significant changes in the intervention group compared to the control group were from improvements in alanine aminotransferase (ALT) levels. Other liver function tests, nutritional status, and survival did not. CD34+ cell mobilization was increased in the intervention group compared with the control group, but there was no significant difference. Minor side effects of G-CSF were observed in the intervention group.

Conclusion: Multiple doses of G-CSF did not improve the PELD score, nutritional status, and survival after three months but significantly showed temporary improvement in ALT level.

Keywords: Granulocyte colony-stimulating factor, decompensated liver, liver cirrhosis, pediatric, bridging therapy, liver transplantation.

Cite This Article: Rahayatri, T.H., Harahap, A., Hendarto, A., Oswari, H., Setiabudy, R., Taher, A. 2022. Granulocyte colony-stimulating factor therapy as a bridging treatment for pediatric decompensated liver cirrhosis prior to liver transplantation: an open-label randomized clinical trial. *Bali Medical Journal* 11(3): 1250-1258. DOI: 10.15562/bmj.v11i3.3527

INTRODUCTION

Liver cirrhosis is one of the main indications of pediatric liver transplantation (LT).[1] In Indonesia, deceased-donor LT is unavailable due to cultural, religious, and legal issues. Donor scarcity and the limited access to liver transplantation contribute to the unavoidable deaths of patients needing LT.[2] Malnutrition is a dominant clinical concern that must be managed before liver transplantation, as malnutrition affects morbidity and mortality in pretransplant patients.[3] Therefore, it is essential to create a bridging therapy to prolong the survival of these patients.[4]

Granulocyte colony-stimulating factor (G-CSF) is a cytokine whose regenerative role in damaged tissues has been comprehensively explored.[5,6] In adult patients, the use of G-CSF for acute-on-chronic liver failure has shown hopeful results.[4,6,7] G-CSF administration elicits the mobilization of CD34+ hematopoietic stem cells (HSCs) from the bone marrow to the peripheral blood.[5] G-CSF has been used clinically in pediatric populations to treat febrile, post-chemotherapy and congenital neutropenia.[8–11] However, in pediatric patients with decompensated liver cirrhosis, the use of G-CSF has not yet been reported. Therefore, this study is a pilot study of G-CSF treatment as an optimizing therapy prior to pediatric LT.[12] Here, we present an analysis of the effect of G-CSF on liver function, PELD score, CD34+ cell mobilization, changes

in leukocyte and neutrophil counts, nutritional status, survival, and side effects within three months in pediatric patients with decompensated liver cirrhosis.

PATIENTS AND METHODS

We conducted a prospective, open-label, randomized study of individuals enrolled since September 2019 with decompensated liver cirrhosis. This study is part of a larger clinical study regarding G-CSF administration.[12] Subjects were recruited from inpatient and outpatient settings of one center at dr. Cipto Mangunkusumo General Hospital (RSCM), Jakarta, Indonesia. Subjects' data were obtained through history from parents or guardians, physical examinations, and laboratory and radiology data. Before the intervention, parents or guardians were informed of the overall procedure, including G-CSF administration protocol and standard treatment, alongside possible side effects.

Patients

A total of 53 pediatric patients with cirrhosis and malnutrition were recruited. Eleven patients refused to participate, and seven were excluded due to active infections. In all, 35 patients were enrolled. The sample size was calculated to be approximately 17 in each group by estimating an AST interval of 75 after G-CSF administration, an a error of 0.05, and b an error of 0.1, with 80% power, assuming a dropout of 20%.

Inclusion criteria were clinically decompensated liver cirrhosis regardless of etiology, confirmed through biopsy and/or imaging. Other inclusion criteria were age between 3 months and 12 years, PELD scores between 10 and 25, a conscious state, and a compromised nutritional status (undernourished or severely malnourished). Malnutrition was evaluated by measuring the mid-arm muscle circumference, mid-arm circumference (MAC), and weight. Patients were classified as normal, undernourished, or severely malnourished, as plotted on the growth curve of the World Health Organization for children younger than five years (WEIGHT/HEIGHT) and the CDC growth curve for patients older than five years. Based on these classifications, seven enrolled patients were considered undernourished, whereas 28 patients were severely malnourished.

The exclusion criteria included malignancy, history of any transplantation procedure, acute liver failure, organ failure other than the liver, hepatic encephalopathy, severe infection, and refusal to participate.

Randomization

Block randomization was performed through a computer-generated system to assign eligible patients to one of the two groups. The principal investigator was not involved in the randomization. Before randomization, informed consent was obtained with the acknowledgment that patients may be allocated to either the control or the intervention group.

Treatment

Subjects within the intervention group received G-CSF (Leucogen^{Ò}, Kalbemed Pharmaceutical, Indonesia) with a dosage of 5 microgram/kg body weight for five consecutive days, followed by an intermittent interval of the same dosage every three days up to a total of 12 doses. The intervention and control groups both received the standard treatment of 30 – 50 mg/kg/day of ursodeoxycholic acid for three days, vitamin A 1 x 5000 – 25,000 International Units (IU), vitamin E 1 x 50 mg, vitamin C 1 x 800 – 5000 IU, injection of vitamin K 0.2 mg/kg/month, and enteral nutrition based on subjects nutritional evaluation. All patients received standard nutritional management, which comprised high medium-chain triglyceride milk (MCT) formula supplement. In cases where oral feeding was challenging, patients received feeding through a nasogastric tube. Baby patients were encouraged to receive breastmilk in addition to the MCT formula. In the presence of ascites, an additional 1–6 mg/kg of furosemide or 1–6 mg/kg of spironolactone was administered, and peritoneal fluid drainage was conducted if needed.

CD34 assessment

At baseline and day 30, peripheral venous blood samples were obtained and stored in a 3-mL ethylenediamine tetraacetic acid anticoagulated tube for stem cell enumeration. The blood sample was transferred to two tubes. One tube containing 100mL of whole blood was processed without staining to determine the baseline. The other tube, which contained 100mL of whole blood, was stained and mixed with 2-mL blocking reagent (to block non-C34-specific antibodies), 3-mL antibody, and 1.5-mL propidium iodide. The tubes were incubated for 30 min at 4°C and wrapped in aluminum foil to prevent direct exposure to light. CD34 staining was performed using a fluorochrome-conjugated monoclonal antibody technique. The enumeration was performed based on the International Society of Hematotherapy and Graft Engineering protocol. CD34+ cell count was calculated based on the number of cells detected *via* flow cytometry that were specifically stained with the CD34+ antibody. The following formula was used to calculate the percentage of CD34+ cells:[13]

$$\text{CD34 stem cells (\%)} = \frac{\text{CD34 positive events}}{\text{CD34 positive events}} \text{ X } 100$$

Data collection and follow-up

Physical examination, anthropometric data (weight, height, MUAC), peripheral blood count, neutrophil count, liver function tests, procalcitonin level, nutritional evaluation, and PELD score were conducted at baseline. The PELD score was repeatedly calculated using an online calculator developed by the Organ Procurement and Transplantation Network on days 0, 30, and 90.

The patients were observed for three months and evaluated for anthropometric, laboratory, and subjective assessments on days 30, 60, and 90. Flow cytometry detected mobility of CD34+ cells in the peripheral blood at baseline and day 30. Laboratory examinations were conducted before the study and on days 0, 6, 18, 30, 60, and 90. On days 6 and 18, peripheral blood cell and neutrophil counts were performed. Throughout the study, clinical and laboratory evaluations observed potential adverse effects of G-CSF were observed.

Questionnaire

A manual prospective chart review evaluated the short-term side effects of G-CSF injection. At each visit, the patients

and their parents were interviewed and questioned regarding previously reported side effects of G-CSF. Other subjective parameters were also obtained, including overall quality of life and sleep quality. Throughout the study, the parents were asked to communicate if there were any other symptoms not included in the questionnaire.

Outcomes

The primary outcome of the study was a change in PELD score. The secondary outcomes were liver function tests, CD34+ cell mobilization, leukocyte and neutrophil changes, and nutritional status. Subjective data from the patients (if eligible) and their parents were also obtained, alongside survival within three months.

Statistical analysis

Baseline data were described using descriptive statistics. Normally distributed data were expressed as means ± standard deviation and as medians and ranges for skewed data. The Kolmogorov–Smirnov test was used to determine normality distribution. Comparisons between the two groups were conducted using the independent t-test for normally distributed data and the Mann–Whitney U test for unpaired skewed data. Repeated measures were analyzed using the ANOVA test to compare trends between the intervention and control groups. Two-tailed statistical analyses were conducted, with $P<0.05$ considered statistically significant. An intention-to-treat analysis analyzed both groups. A per-protocol analysis was also conducted for both groups. Twenty-eight patients were included in the per-protocol analysis; seven patients were excluded from the sample size due to failure of protocol completion due to death before a 3-month study period. Data analyses were performed using the SPSS software version 20.

RESULTS

Fifty-three pediatric patients with cirrhosis and malnutrition were recruited for the study, and 35 patients were finally enrolled (Figure 1). In all, 17 and 18 patients were included in the intervention and control groups, respectively. During the study, 14 (82%) patients in the intervention group completed 12 doses of G-CSF. One patient skipped five injections due to recurrent fever and was admitted to the emergency room due to hematemesis and melena. Another patient skipped three injections due to fever and urinary tract infection. One patient was out of town at the time of his injection schedule and skipped one treatment. Table 1 summarizes the baseline characteristics of both groups.

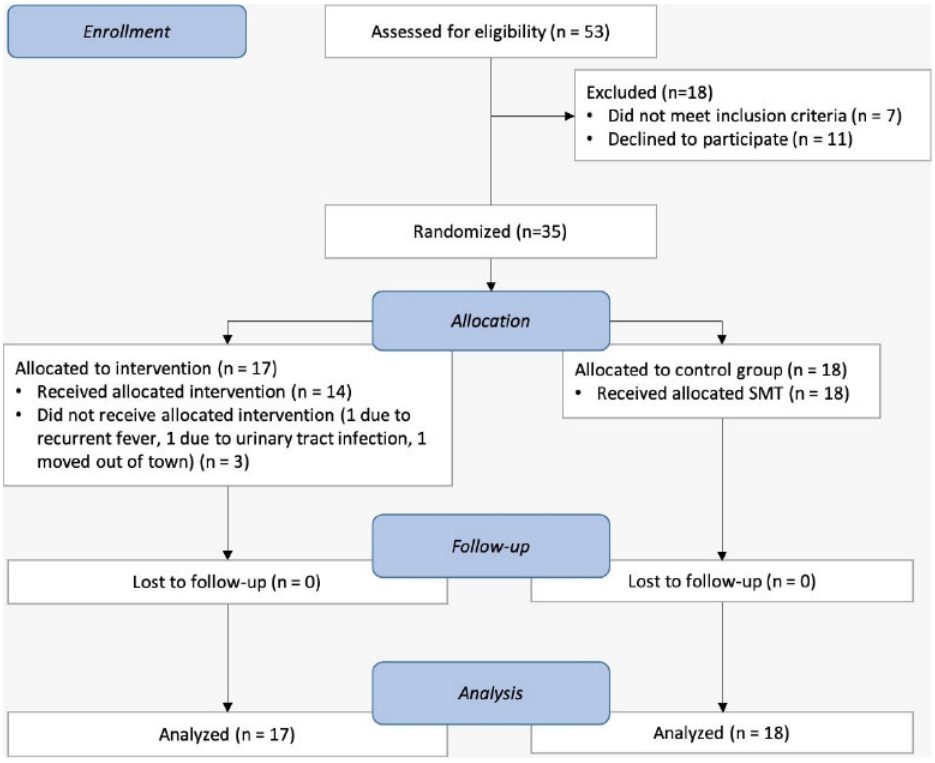

Figure 1. Study design and flow diagram of patient selection. (G-CSF: granulocyte colony-stimulating factor; SMT: standard medical therapy)

Primary outcome

The primary outcome of the study was the PELD score. Neither the control nor the intervention group exhibited any statistical difference in the PELD score (Table 2).

Secondary outcomes

The secondary outcomes observed in this study include liver function parameters, CD34+ cell mobilization, change in leukocyte and neutrophil count, nutritional status, adverse effects, and 3-month survival (Table 3).

Liver function

The intervention group showed significant improvement in alanine aminotransferase levels after three months of G-CSF administration. On the contrary, other liver function tests (AST, GGT, total bilirubin, and albumin) showed no statically significant difference between the two groups. Although a trend toward AST improvement was observed in the intervention group compared with the control group, the difference was not statistically significant (Figure 2). Based on the per-protocol analysis, all the liver function tests (AST, ALT, GGT, albumin, and total bilirubin) showed no statistically significant difference between the two groups.

Bone marrow cell mobilization

The parameter to evaluate bone marrow HSC mobilization was CD34+ expression in the peripheral blood circulation. An increase in CD34+ cells was apparent on day 30 in both groups. There was an increase in CD34+ cells in the intervention

Table 1. Baseline characteristics of the study population.

	Intervention group (n = 17)	Control group (n = 18)	p-value
Sex (male/female)[a]	8:9	9:9	
Diagnosis[a]			
Biliary atresia	10	10	
Alagille syndrome	4	5	
PFIC type III	2	0	
Caroli disease	0	2	
Choledochal cyst Type IV	1	1	
Nutritional status[a]			
Undernourished	4	3	
Severely undernourished	13	15	
Age (months)[b]	18 (7–136)	14.5 (5–120)	0.585
MAC (centimeters)[b]	11.5 (9.5–16)	10.75 (8.5–12)	0.047
PELD score[c]	17.35 ± 4.12	18.56 ± 3.88	0.381
Hemoglobin (g/dL)[c]	9.14 ± 1.26	8.9 ± 1.30	0.582
Leukocyte count ($\times 10^3$/mL)[b]	8.67 (3.08–22.15)	9.2 (3.19–25.35)	0.552
Neutrophils (%)[c]	50.16 ± 12.11	55.24 ± 10.87	0.200
Platelet count ($\times 10^3$/mL)[c]	170.59 ± 77.90	221.5 ± 111.65	0.129
Prothrombin time (second)[c]	12.92 ± 1.72	13.38 ± 1.77	0.442
INR	1.12 (0.97–1.71)	1.22 (1.05–1.63)	0.203
Procalcitonin (ng/mL)[b]	0.48 (0.07–17.34)	0.25 (0.09–1.6)	0.208
CD34+ cells[b]	10.7 ± 10.9	7.95 ± 7.54	0.700
AST (U/L)[b]	239 (68–644)	160 (56–948)	0.338
ALT (U/L)[b]	141 (18–419)	95 (24–567)	0.192
Albumin (g/dL)[c]	2.89 ± 0.49	2.97 ± 0.40	0.616
Total bilirubin (mg/dL)[c]	21.14 ± 7.04	22.06 ± 7.68	0.712
GGT (U/L)[b]	108 (37–844)	138 (25–1624)	0.987

Notes: [a]n; [b]normally distributed data; [c]skewed data; PELD = pediatric end-stage liver disease; PFIC = progressive familial intrahepatic cholestasis; MAC = mid-arm circumference; INR = international normalized ratio; GGT = gamma-glutamyl transferase; AST = aspartate aminotransferase; ALT = alanine aminotransferase.

Table 2. Primary outcome.

	ITT				PER PROTOCOL			
	Intervention Group (n = 17)	Control Group (n = 18)	p-value*	p-value**	Intervention Group (n = 15)	Control Group (n = 13)	p-value*	p-value**
PELD score[a]								
D-0	18 (10-23)	18.50 (12-25)	0.518		16.80 ± 4.07	18.46 ± 3.69	0.776	
D-30	18 (12-39)	19 (11-29)	0.466		17(12–39)	19 (11–26)	0.578	
D-90	17 (10-27)	18.33 (10-29)	0.508	0.843	17.27 ± 3.83	17.46 ± 4.41	0.379	0.677

Notes: *p-value for independent *t*-test (normally distributed data)/Mann–Whitney test (skewed data); **p-value for ANOVA test; [a]skewed data

group three times higher than in the control group, although the difference was insignificant (p=0.66).

Leukocyte and neutrophil counts

Changes in the leukocyte and neutrophil counts were significantly different in the intervention group compared to the control group (p<0.001). Both peaked on day 18 and then decreased until day 90. On day 30, the leukocyte count decreased close to the baseline level after twelve completed doses of G-CSF treatment. In contrast, the neutrophil count on day 60 had decreased to the same level as day six and had returned to baseline by day 60 (Figure 3).

Nutritional status

Overall, 75% of the patients in both groups were severely malnourished at baseline. This striking result was most obvious on day 30, when three patients exhibited an improved nutritional status after G-CSF administration. After two months of intervention, an additional patient became well-nourished; however, one patient experienced a further decline in nutritional status. On day 90, one well-nourished patient's nutritional status declined to be undernourished. Moreover, within three months of standard therapy, two severely malnourished patients

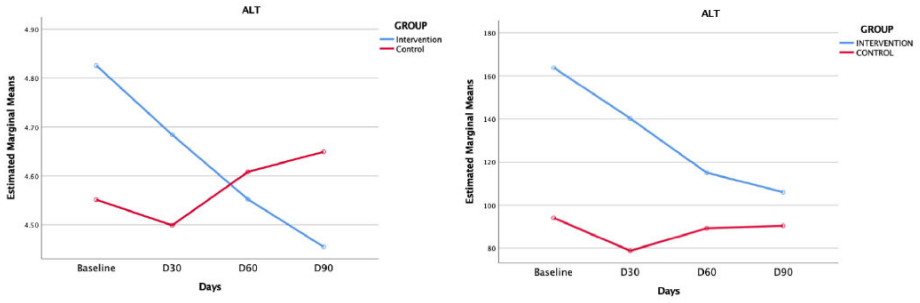

Figure 2. Repeated measures ANOVA test showed improvement in the ALT level (U/L) based on ITT and repeated measures ANOVA test as Per Protocol.

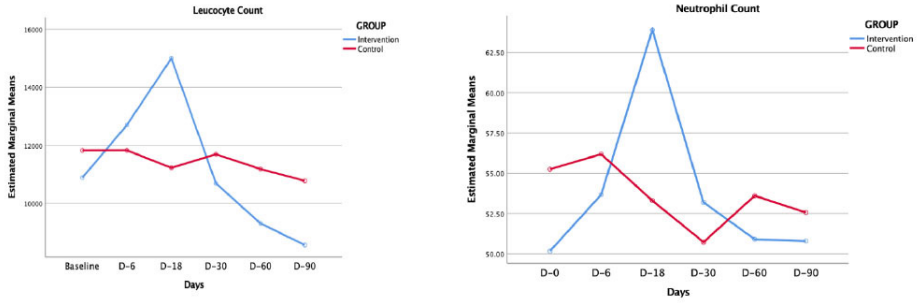

Figure 3. Repeated measures ANOVA test showed an increase in leukocyte ($\times 10^3$/mL) and neutrophil (%) counts after G-CSF administration, with a peak on day 18.

showed improvement within the control group. The differences between the groups were not statistically significant.

Infection events

Within the duration of the study, 11 patients experienced infections from both the intervention and control groups. Four patients were admitted to the hospital for an infection from the intervention group, while in the control group, seven patients were admitted to the hospital for infections.

Adverse effects

Minor adverse effects due to the administration of G-CSF have been reported. The most frequently reported adverse effect was the presence of low-grade fever, as reported by seven patients, four of whom developed a fever after the first dose of G-CSF. Other adverse effects mentioned by patients and guardians include bone pain in one patient. Ten patients in the intervention group experienced improved sleep quality, and their guardians reported two patients to have had a better mood after the G-CSF administration.

Survival

During the study, seven patients died, with five patients from the intervention group and two from the control group. The two patients in the intervention group had completed their injection protocol before their deaths and had died due to rupture of esophageal varices and hepatic encephalopathy. The five deaths reported from the control group were one due to refractory hypovolemic shock resulting from rupture of esophageal varices, two due to sepsis from pneumonia, and two due to respiratory failure. A Kaplan–Meier survival analysis showed no statistical difference between the groups (p=0.236).

DISCUSSION

The only definitive cure for liver cirrhosis remains liver transplantation; nevertheless, it is a challenging procedure. In many parts of the world, the acceptability of LT programs is limited. Only one active LT center in Indonesia is available, limited to living donor transplantations, despite being the fourth largest population globally.[2] Delayed LT in cirrhosis patients have decreased their survival. To prolong the survival of patients awaiting LT, bridging therapy is necessary to improve their general condition. This study aimed to find a bridging therapy for pediatric cirrhosis patients awaiting LT. This report is one part of a larger clinical trial that will measure cytokine changes after G-CSF administration.

G-CSF has been broadly studied and has been used for acute-on-chronic liver failure. Many previous studies have identified the benefits of G-CSF treatment.[7,14,15] However, several randomized controlled trials have shown contrary results that revealed no significant benefit in using G-CSF for decompensated liver cirrhosis.[16,17] In all previously mentioned studies, G-CSF administration was conducted on adult patients. Only one study has shown a beneficial outcome in pediatric patients with acute-on-chronic liver failure.[18]

G-CSF treatment has been postulated to beneficially mobilize pluripotent HSC from the bone marrow to the systemic circulation. Hematopoietic stem cells are then potentially engrafted into the liver, where they stimulate the differentiation of hepatocyte lineage cells.[19]

In our study, the only liver function test that showed improvement was ALT. AST showed a trend toward improvement, although the trend was not statistically significant. The transaminase enzymes influence the transfer of amino acids to ketoglutaric acid. Alanine aminotransferase is a more sensitive marker compared to aspartate transaminase.[20] Though not clinically apparent, improvements in these markers indicate a probable transient optimization of patients awaiting LT.

In our study, there was no significant improvement in the PELD score. This result contradicts that of Sharma et al.,[18] who demonstrated early survival benefits through improvements in the PELD score. At our center, the priority of organ transplantation among waiting list patients uses the PELD score to evaluate their 90-day mortality risk based on liver function and growth status.

The mobilization of autologous bone marrow-derived stem cells by G-CSF administration stimulates hepatic progenitor cell activity and HSC to differentiate into hepatocytes, restore

Table 3. Secondary outcomes.

	ITT				Per Protocol			
	Intervention Group (n = 17)	Control Group (n = 18)	p-value*	p-value**	Intervention Group (n = 15)	Control Group (n = 13)	p-value*	p-value**
AST[a]								
Baseline	239 (68–644)	160 (56–948)	0.338		239 (68–644)	146 (56–787)	0.088	
D-30	225 (53–658)	164 (57–948)	0.291		245 (53–658)	161 (57–275)	0.076	
D-60	202 (55–372)	178.5 (52–948)	0.961		200 ± 94.90	172.08 ± 84.23	0.643	
D-90	152 (52–336)	181 (62–948)	0.668	0.086	180 ± 100.04	166.31 ±66.23	0.110	0.289
ALT[a]								
Baseline	141 (18–419)	95 (24–567)	0.192		155 (18–419)	61 (24–435)	0.043	
D-30	99 (14–317)	99.5 (23–494)	0.541		140.13 ± 85.63	78.69 ± 42.66	0.011	
D-60	111 (16–233)	101 (27–494)	0.729		115.07 ± 59.19	89.23 ± 56.17	0.516	
D-90	87.23 (±52.16)	91.62 (±41.11)	0.704	0.023	106.07 ± 61.59	90.38 ± 46.29	0.057	0.221
Albumin[b]								
Baseline	2.89 ± 0.49	2.97 ± 0.40	0.240		2.93 ± 0.51	2.98 ± 044	0.480	
D-30	3.03 ± 0.48	2.95 ± 0.49	0.833		3.02 ± 0.51	3.03 ± 0.50	0.791	
D-60	2.95 ± 0.43	2.90 ± 0.40	0.748		2.93 ± 0.45	2.96 ± 0.37	0.510	
D-90	2.98 ± 0.44	2.84 ± 0.49	0.371	0.571	2.96 ± 0.46	2.87 ± 0.52	0.506	0.854
Total bilirubin[b]								
Baseline	21.14 ± 7.03	22.06 ± 7.68	0.790		20.73 ± 7.16	19.30 ± 7.00	0.621	
D-30	21.69 ± 8.81	21.78 ± 8.00	0.386		20.75 ± 8.57	19.70 ± 8.18	0.368	
D-60	23.40 ± 9.86	21.51 ± 8.10	0.371		22.68 ± 9.96	19.33 ± 8.22	0.551	
D-90	22.55 ± 10.53	23.34 ± 8.30	0.525	0.168	21.72 ± 10.64	21. 86 ± 9.07	0.906	0.262
GGT[a]								
Baseline	108 (37–844)	138 (25–1624)	0.987		314.33 ± 307.75	176.62 ± 144.57	0.001	
D-30	125 (35–797)	135 (24–1279)	0.882		281.47 ± 270.68	154.85 ± 143.75	0.005	
D-60	131 (29–670)	125 (26–1513)	0.869		216.00 ± 184.02	148.00 ± 138.86	0.164	
D-90	130 (23–729)	119.50 (11–1513)	0.754	0.629	226.07 ± 224.71	144.23 ± 151.42	0.199	0.314

*p-value for independent *t*-test (normally distributed data)/Mann–Whitney test (skewed data) comparing parameters between intervention and control; **p-value for ANOVA test represent significance in the trend of parameter from baseline to Day-90; [a]skewed data; [b]normally distributed data; AST = aspartate aminotransferase; ALT = alanine aminotransferase; GGT = gamma-glutamyl transferase; PELD = pediatric end-stage liver disease

function, enhance liver function and improve fibrosis.[21] CD34+ is known as a mobilization marker of bone marrow stem cells. Liver regeneration is initiated by anti-inflammatory cytokine activity initiated by paracrine properties of bone marrow stem cells in the peripheral blood.[22] In our study, the level of CD34+ cells in peripheral circulation was three times higher in the intervention group compared to the control group, representing the mobilization of bone marrow stem cells in children with decompensated cirrhosis. However, the difference was not statistically significant (p=0.66). The finding of CD34 positivity was similar to the findings in other studies, in which adults with decompensated cirrhosis and pediatric patients with acute liver failure were treated with G-CSF.[18,21,23,24] Moreover, other studies have reported that healthy bone marrow function at baseline is required for effective treatment with growth factors,[25] which is not evident in patients with decompensated cirrhosis.

There was a significant increase in leukocyte and neutrophil counts in the intervention group, with a peak observed on day 18, which paralleled the G-CSF administration. In our study, no adverse events were recorded during the highest peak of leukocyte count, thus, contrary to the concerns that G-CSF causes potentially fatal leukocytosis, our studies confirmed the safety of G-CSF administration to children. Neutrophil plays an important role in tissue repair and infection in the liver. However, continuous and chronic parenchymal damage renders the reduction of neutrophil function and its immuno-modularity role, known as cirrhosis-induced immunodeficiency. Hence fluctuating levels of immune cells can be noticeable. During this period, patients did not present with any clinically remarkable symptoms of infection, despite the temporary neutropenia.[26,27]

The main problems of decompensated cirrhosis pediatric patients awaiting LT are malnutrition and infection.[28,29] We evaluated the nutritional factors, including changes in body weight, height, and MAC, between the groups. Both groups presented a similar profile of nutritional characteristics. The control group consisted of 3 undernourished and 15 severely malnourished patients, whereas the intervention group consisted of 4 undernourished and 13 severely malnourished patients. All patients received standard nutritional management, consisting of a high medium-chain triglyceride (MCT) formula supplement. If oral feeding was challenging, patients were fed through a nasogastric tube. Babies were encouraged to consume breast milk in addition to the MCT formula. Vitamins A, D, E, and K were adequately given. Our study showed no significant improvements in nutritional status, contrasting with the previous findings of Verma et al., who demonstrated a substantial increase in MAC.[24] The infection rates were identified based on the hospitalizations of patients during the study period. The intervention and control groups consisted of 4 and 7 patients, respectively, that were admitted due to infection. The infection rates were lower in the intervention groups. Additional studies must be conducted to evaluate the significance of G-CSF treatment in reducing infection rates in pediatric patients with decompensated cirrhosis.

The reported mortality rate in the control group was higher than in the intervention group, although the difference was not statistically significant. The reported deaths of patients in our study were unlikely to be related to the G-CSF administration. Within the intervention group, both deaths occurred more than ten days after the G-CSF administration due to esophageal variceal bleeding. The peak concentration of G-CSF is reached in 4 h, and its half-life is approximately 8 h,[19] with a clearance rate of 0.6 mL/min/KgBW.[30] The deaths that occurred did not chronologically correspond to the G-CSF administration, but this finding requires further evaluation.

Throughout the duration of our study, the parents of patients in the intervention group reported subjective improvements after G-CSF administration. The improvements were noted as less irritability, enhanced quality of sleep, and significant mood improvement, with still yet unclear reasons.

STUDY LIMITATIONS

Several limitations restricted the outcomes of the study. The limited sample size of our study could have resulted in the limited statistical significance of our results. The variability in etiology resulting in liver cirrhosis within our sample size is limited. Most patients enrolled in this study were diagnosed with liver cirrhosis due to biliary causes, and the lack of patients with non-biliary-related cirrhosis in Indonesia limits the scope of our study population. Therefore, further studies may be conducted with more variable etiologies of cirrhosis to widen the applications of this treatment. The authors have missed the opportunity to identify the effects of G-CSF compared to various liver cirrhosis etiologies. It is unknown how diseases such as Caroli disease and choledochal cysts are affected by G-CSF treatment as their progression of cirrhosis differs from other etiologies, such as biliary atresia. While the authors have reported subjective improvement in patients with G-CSF treatment, the evaluation method seems subjective and difficult to evaluate. An objective method to evaluate the subjective improvements must be assessed. General improvement in the quality of life is associated with cytokine levels, which will be evaluated and reported in our future study. As our study is considered the earliest study that explores the effect of G-CSF in children with decompensated liver disease, the effect of G-CSF on synthetic liver enzymes is not well-understood. Further studies regarding stimulation of liver regeneration through changes in a systemic milieu as observed through cytokine changes need to be conducted.

CONCLUSION

The effect of granulocyte colony-stimulating factor (G-CSF) in children with decompensated liver cirrhosis awaiting LT was reported in this study. Multiple doses of G-CSF did not significantly improve the pediatric end-stage liver disease score. The significant improvement after G-CSF treatment was limited to the alanine aminotransferase levels, but this finding did not represent a remarkable clinical

outcome. Other parameters, including liver function tests, nutritional status, and survival after three months, showed no significant improvement. Mobilization of CD34+ cells was achieved with G-CSF, although the effect was insignificant.

FUNDING

This research was funded by the Ministry of Research Technology and Higher Education (NKB-180/UN2.RST/HKP.05.00/2020).

ETHICAL APPROVAL

The patients' parents obtained written informed consent approval for experimentation with human subjects. A written informed consent approval of publication without disclosing personal information was obtained from every legal guardian of the research participant. The ethics committee approved the study protocol of our institution (reference 19-07-0943), and all procedures used in this study adhered to the tenets of the Declaration of Helsinki of 1977. The trial sponsor was the Indonesian Ministry of Research and Technology (NKB-180/UN2.RST/HKP.05.00/2020), registered at ClinicalTrials.gov (number NCT04113317).

CONFLICT OF INTEREST

There is no conflict of interest or competing interest reported by the authors.

CLINICAL TRIALS REGISTRATION

NCT04113317 at Clinicaltrials.gov.

AVAILABILITY OF DATA AND MATERIALS

Digital raw data and materials are available from the corresponding author upon request, should they be required to be reviewed.

AUTHORS' CONTRIBUTIONS

Conceptualization: Tri Hening Rahayatri, Aria Kekalih, Alida Harahap, Aryono Hendarto, Hanifah Oswari, Zakiudin Munasir, Rianto Setiabudy, Akmal Taher; Methodology: Tri Hening Rahayatri, Aria Kekalih, Alida Harahap, Hanifah Oswari, Rianto Setiabudy, Akmal Taher; Formal analysis and investigation: Tri Hening Rahayatri, Aria Kekalih; Writing - original draft preparation: Tri Hening Rahayatri; Writing - review and editing: Tri Hening Rahayatri, Aria Kekalih, Alida Harahap, Aryono Hendarto, Hanifah Oswari, Zakiudin Munasir, Rianto Setiabudy, Akmal Taher; Funding acquisition: Tri Hening Rahayatri; Supervision: Aryono Hendarto, Hanifah Oswari, Zakiudin Munasir, Rianto Setiabudy, Akmal Taher

REFERENCES

1. Robert H. Squires VN, Rene R, Ekong U, Hardikar W, Emre Sukru, Mazariegos GV. Evaluation of the pediatric patient for liver transplantation: 2014 practice guideline by the American Association for the Study of Liver Diseases, American Society of Transplantation and the North American Society for Pediatric Gastroenterology, Hepatology and Nutrition. Hepatology. 2014;60.
2. Oswari H, Rahayatri TH, Soedibyo S. Pediatric living donor liver transplant in indonesia's national referral hospital. Transplantation. 2020;104:1305-7.
3. Zhang QK, Wang M-L. The management of perioperative nutrition in patients with end stage liver disease undergoing liver transplantation. Hepatobiliary Surg Nutr. 2015;4:336-44.
4. Prajapati R, Arora A, Sharma P, Bansal N, Singla V, Kumar A. Granulocyte colony-stimulating factor improves survival of patients with decompensated cirrhosis: a randomized-controlled trial. Eur J Gastroenterol Hepatol. 2017;29:448-55.
5. Philips C, Augustine P, Ahamed R, Rajesh S, George T, Valiathan G, et al. Role of granulocyte colony-stimulating factor therapy in cirrhosis, 'inside any deep asking is the answering'. J Clin Transl Hepatol. 2019;7:371-83.
6. Yang Q, Yang Y, Shi Y, Lv F, He J, Chen Z. Effects of granulocyte colony-stimulating factor on patients with liver failure: a meta-analysis. J Clin Transl Hepatol. 2016;4:90-6.
7. Chavez-Tapia N, Mendiola-Pastrana I, Ornelas-Arroyo V, Norena-Herrera C, Vidana-Perez D, Delgado-Sanchez G, et al. Granulocyte-colony stimulating factor for acute-on-chronic liver failure: systematic review and meta-analysis. Ann Hepatol. 2015;14:631-41.
8. Skokowa J, Dale DC, Touw IP, Zeidler C, Welte K. Severe congenital neutropenias. Nat Rev Dis Primers. 2017;3:17032. doi: 10.1038/nrdp.2017.32.
9. Rodríguez ZN, Tordecilla CJ, Campbell BM, Joannon SP, Rizzardini LC, Soto AV, et al. Usefulness of G-CSF in pediatric high risk cancer patients with fever and neutropenia. Rev Chilena Infectol. 2005;22(3):223-7.
10. Badr M, Hassan T, Sakr H, Karam N, Rahman D, Shahbah D, et al. Chemotherapy-induced neutropenia among pediatric cancer patients in Egypt: risks and consequences. Molecular and Clinical Oncology. 2016;5(3):300-6.
11. Krishnankutty B, Advani S, Achreckar S, Thomas D. Granulocyte colony-stimulating factor (filgrastim) in chemotherapy-induced febrile neutropenia. Indian J Med Paediatr Oncol. 2010;31(3):125.
12. Rahayatri TH. Granulocyte-colony stimulating factor (G-CSF) as optimizing therapy for pediatric liver transplantation. ClinicalTrials.gov: Fakultas Kedokteran Universitas Indonesia; 2019.
13. Alvarez-Larran A, Jover L, Marin P, Petriz J. A multicolor, no-lyse no-wash assay for the absolute counting of CD34+ cells by flow cytometry. Cytometry. 2002;50(5):249-53. doi: 10.1002/cyto.10129.
14. Simonetto DA, Shah VH, Kamath PS. Improving survival in ACLF: growing evidence for use of G-CSF. Hepatol Int. 2017;11(6):473-5. doi: 10.1007/s12072-017-9834-x.
15. Duan XZ, Liu FF, Tong JJ, Yang HZ, Chen J, Liu XY, et al. Granulocyte-colony stimulating factor therapy improves survival in patients with hepatitis B virus-associated acute-on-chronic liver failure. World J Gastroenterol. 2013;19(7):1104-10. doi: 10.3748/wjg.v19.i7.1104.
16. Newsome PN, Fox R, King AL, Barton D, Than N-N, Moore J, et al. Granulocyte colony-stimulating factor and autologous CD133-positive stem-cell therapy in liver cirrhosis (REALISTIC): an open-label, randomised, controlled phase 2 trial. The Lancet Gastroenterology & Hepatology. 2018;3(1):25-36. doi: 10.1016/s2468-1253(17)30326-6.
17. Philips CA, Augustine P, Rajesh S, Ahamed R, George T, Padsalgi G, et al. Granulocyte colony-stimulating Factor Use in Decompensated Cirrhosis: Lack of Survival Benefit. J Clin Exp Hepatol. 2020;10(2):124-34. doi: 10.1016/j.jceh.2019.05.003.
18. Sharma S, Lal SB, Sachdeva M, Bhatia A, Varma N. Role of granulocyte colony stimulating factor on the short-term outcome of children with acute on chronic liver failure. J Clin Exp Hepatol. 2020;10(3):201-10. doi: 10.1016/j.jceh.2019.10.001.
19. Rathi S, Hussaini T, Yoshida EM. Granulocyte colony stimulating factor: A potential therapeutic rescue in severe alcoholic hepatitis and decompensated cirrhosis. Ann Hepatol. 2020. doi: 10.1016/j.aohep.2020.04.011.
20. Kwo PY, Cohen SM, Lim JK. ACG linical guideline: evaluation of abnormal liver chemistries. Am J Gastroenterol. 2017;112(1):18-35. doi: 10.1038/ajg.2016.517.
21. Sharma M, Rao PN, Sasikala M, Kuncharam MR, Reddy C, Gokak V, et al. Autologous mobilized peripheral blood CD34(+) cell infusion in non-viral decompensated liver cirrhosis. World J Gastroenterol. 2015;21(23):7264-71. doi: 10.3748/wjg.v21.i23.7264.
22. Burdon TJ, Paul A, Noiseux N, Prakash S, Shum-Tim D. Bone marrow stem cell derived paracrine factors for regenerative medicine: current perspectives and therapeutic potential. Bone Marrow Res. 2011;2011:207326. doi: 10.1155/2011/207326.
23. Gaia S, Olivero A, Smedile A, Ruella M, Abate ML, Fadda M, et al. Multiple courses of G-CSF

in patients with decompensated cirrhosis: consistent mobilization of immature cells expressing hepatocyte markers and exploratory clinical evaluation. Hepatol Int. 2013;7(4):1075-83. doi: 10.1007/s12072-013-9473-9.
24. Verma N, Kaur A, Sharma R, Bhalla A, Sharma N, De A, et al. Outcomes after multiple courses of granulocyte colony-stimulating factor and growth hormone in decompensated cirrhosis: A randomized trial. Hepatology. 2018;68(4):1559-73. doi: 10.1002/hep.29763.
25. Anand L, Bihari C, Kedarisetty CK, Rooge SB, Kumar D, Shubham S, et al. Early cirrhosis and a preserved bone marrow niche favour regenerative response to growth factors in decompensated cirrhosis. Liver Int. 2019;39(1):115-26. doi: 10.1111/liv.13923.
26. Liu K, Wang FS, Xu R. Neutrophils in liver diseases: pathogenesis and therapeutic targets. Cell Mol Immunol. 2021;18(1):38-44. doi: 10.1038/s41423-020-00560-0.
27. Albillos A, Lario M, Álvarez-Mon M. Cirrhosis-associated immune dysfunction: Distinctive features and clinical relevance. Journal of Hepatology. 2014;61(6):1385-96. doi: 10.1016/j.jhep.2014.08.010.
28. Cameron R, Kogan-Liberman D. Nutritional considerations in pediatric liver disease. Pediatr Rev. 2014;35(11):493-6. doi: 10.1542/pir.35-11-493.
29. Mouzaki M, Bronsky J, Gupte G, Hojsak I, Jahnel J, Pai N, et al. Nutrition Support of Children With Chronic Liver Diseases: A Joint Position Paper of the North American Society for Pediatric Gastroenterology, Hepatology, and Nutrition and the European Society for Pediatric Gastroenterology, Hepatology, and Nutrition. J Pediatr Gastroenterol Nutr. 2019;69(4):498-511. doi: 10.1097/mpg.0000000000002443.
30. Sveikata A, Gumbrevicius G, Sestakauskas K, Kregzdyte R, Janulionis V, Fokas V. Comparison of the pharmacokinetic and pharmacodynamic properties of two recombinant granulocyte colony-stimulating factor formulations after single subcutaneous administration to healthy volunteers. Medicina (Kaunas). 2014;50(3):144-9. doi: 10.1016/j.medici.2014.08.001.