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ORIGINAL ARTICLE

Therapeutic implications of granulocyte colony stimulating factor in patients with acute-on-chronic liver failure: increased survival and containment of liver damage

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Abstract
Background and purpose Mobilization of bone marrow-derived stem cells by granulocyte colony stimulating factor (G-CSF) supports hepatic regeneration and may augment clinical improvement in patients with acute-on-chronic liver failure (ACLF). The aim of this study is to assess the impact of G-CSF on complications and transplant-free survival in patients with ACLF.
Methods Thirty-two patients with ACLF defined by Asian Pacific Association for the Study of the Liver (APASL) criteria were openly randomized to control (group A) or intervention (group B) receiving G-CSF (5 μg/kg/day, for 6 consecutive days) in addition to standard medical therapy with antiviral drugs. The patients were followed for 90 days.
Results Simultaneous use of G-CSF and antiviral drugs in hepatitis B virus (HBV) ACLF significantly improved survival over antiviral drugs alone. Incidence of hepatorenal syndrome and hyponatremia were reduced due to use of G-CSF. Baseline parameters of the two groups of patients were comparable. Child–Turcotte–Pugh (CTP) and Model for End-Stage Liver Disease (MELD), disease severity scores improved in patients treated with G-CSF, with significant difference only for the CTP score at 90 days follow-up. In addition, mean white blood cell (WBC) count at day 15 was significantly higher in G-CSF group in absence of infection compared with control group.
Conclusions G-CSF therapy improved survival and clinical recovery in HBV-ACLF. G-CSF therapy also prevented renal failure and hyponatremia. We strongly recommend use of G-CSF therapy in addition to standard medical therapy.

Keywords Acute-on-chronic liver failure · Liver damage · Granulocyte colony stimulating factor (G-CSF)

Introduction

Acute-on-chronic liver failure (ACLF) results from an acute insult to the liver in patients with previously diagnosed or undiagnosed chronic liver diseases. Patients with ACLF display jaundice and coagulopathy, ascites, and/or encephalopathy with increasing mortality at 3 months mainly through multisystem organ failure [1]. Although the mechanisms underlying the pathogenesis of ACLF remain to be elucidated, uncoordinated reactions of the immune system seem to play an important role in progressive liver damage in ACLF in addition to impacts of viral and other etiological factors [2–6]. However, the critical inducers of distorted immunity in ACLF patients remain to be elucidated. With accumulated evidence regarding the pathogenesis and clinical course of ACLF, investigators have predicted that ACLF may pass through three stages: (1) an initial systemic inflammatory response syndrome (SIRS)

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stage, (2) a mixed inflammatory stage, and (3) a final compensated antiinflammatory response stage [7, 8]. In these complex circumstances, the levels of hepatocellular regeneration seem to be unable to compete with the extent of hepatocellular damage, finally resulting in high mortality in ACLF patients. At present, it appears that development of appropriate animal models of ACLF as well as more studies in ACLF patients regarding cellular and molecular mechanisms underlying its pathogenesis are warranted to tackle this intractable liver disease.

However, development of treatment and management of ACLF represents a medical emergency because the short-term and mid-term mortality from ACLF exceeds those of all comparable liver diseases, may be reaching 60 % within 3 months of initial symptoms [1]. At present, ACLF patients are mostly managed by conservative therapy on the basis of clinical and biochemical parameters, while a fraction of patients receive liver transplantation. These realities necessitate development of innovative or alternative therapies for ACLF with a global perspective.

From the scientific point of view, innovative approaches for treating ACLF may be based on three fundamental facets: (1) strategies to contain acute insult, (2) means to control cause of chronic liver diseases, and (3) targeting increased regeneration of hepatocytes so that management of acute insult and chronic etiologies may be accomplished successively. It is well accepted that liver transplantation is the best therapeutic approach for ACLF patients. However, it is not feasible in most clinical setups due to several inherent limitations, and almost impossible in most developing countries due to exacerbated burden of ACLF patients and lack of facilities. When liver transplantation is not a feasible option for management of ACLF patients, an alternate may be to induce regeneration of hepatocytes in these patients, so that they may proceed from the complex mixed inflammatory to compensated inflammatory response stage (transition from stage B to stage C).

To accomplish this goal, attention has been focused on the role of granulocyte colony stimulating factor (G-CSF), as it can mobilize stem cells in patients with advanced liver disease and restore liver functions. Garg et al. showed that G-CSF improves survival of patients with ACLF [9]. Duan et al. also published similar therapeutic impacts of G-CSF in Chinese ACLF patients with hepatitis B virus (HBV) background [10]. However, more clinical trials are required to develop greater insight in different racial groups and countries to assess the real potential of G-CSF for treating ACLF patients.

Bangladesh, a country of 160 million, harbors several million people with chronic liver diseases and is also burdened with almost all the features of acute insult that may cause ACLF. ACLF represents a major cause of hospital admission to hepatology departments in almost all academic and tertiary-level hospitals in Bangladesh [11–13]. The study presented herein represents a real-life situation where the role of G-CSF was evaluated in ACLF patients in Bangladesh. The outcome of this study may have implications for designing therapies including G-CSF for treatment of ACLF in other developing countries with similar socioeconomic conditions.

Methods

The study was conducted at the Hepatology Department of Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, Bangladesh. Written consent was obtained from either the patient or their nearest kin after explaining the nature and purpose of the study. The patients were followed up for 90 days, and data were analyzed using standard statistical procedures.

Patients

Thirty-two patients with ACLF aged 18 years or more were enrolled in the study. Diagnosis of ACLF was made on the basis of history of illness, clinical presentation, bilirubin level (>5.0 mg/dl), presence of coagulopathy [international normalized ratio (INR) > 1.5], presence of ascites (confirmed by physical assessment and ultrasonography), and encephalopathy, and confirmed according to the criteria provided in APASL guidelines [1].

Baseline data of the patients are presented in Table 1. The causes underlying chronic liver diseases were mostly chronic HBV infection (29 of 32 patients). One patient had been suffering from Wilson's disease and one from autoimmune hepatitis, and the cause could not be elucidated in one patient (cryptogenic). The nature of acute insult was activation of HBV [presence of high titers of IgM antibody to hepatitis B core antigen (anti-HBc IgM)] in 19 patients, hepatitis E virus (HEV) in 2 patients (anti-HEV IgM positive), herbal medicine in 5 patients (absence of acute infection related to all hepatotrophic viruses and history of intake of herbal drugs within last 2 weeks), and antitubercular drug in 1 patient. Although all sorts of measures were taken, the nature of the acute insult could not be ascertained in 4 patients. These patients were negative for markers of acute viral hepatitis, acute exacerbation of autoimmune hepatitis, and drug or alcohol intake that may cause acute hepatitis. Also, the clinical history of these patients was not suggestive of any specific etiological feature.

Study design and working hypothesis

The present clinical trial was designed as a prospective, controlled, and open study. The sample size was

Table 1 Patient baseline data per group

	Group A ($N = 16$)	Group B ($N = 16$)	p-Value
Age (years)	48 (22–62)	39 (18–55)	0.289 n.s.
Sex (male:female)	16:0	12:4	0.050 n.s.
Bilirubin (mg/dl)	20.4 (10–32)	19.5 (6.0–43.6)	0.724 n.s.
INR	2 (1.5–4.4)	2 (1.5–2.9)	0.843 n.s.
Ascites	16 (100 %)	16 (100 %)	1.000 n.s.
Encephalopathy	9	8	0.723 n.s.
MELD score	25.5 (21–35)	24.5 (21–32)	1.000 n.s.
CTP score	12 (10–14)	12 (10–13)	1.000 n.s.
Albumin	2.0 (1.3–3.0)	2.1 (1.1–3.8)	0.340 n.s.
Creatinine	1 (0.7–3.5)	1.1 (0.6–1.5)	0.722 n.s.
Esophageal varix	8	11	0.280 n.s.
WBC ($\times 10^3/mm^3$)	12.9 (4.8–20)	9.5 (2.0–17.0)	0.479 n.s.
Platelets ($\times 10^3/mm^3$)	175 (30–500)	175 (40–220)	0.724 n.s.

Control group: patients treated with conventional antiviral therapy; G-CSF group, patients treated with G-CSF in addition to conventional antiviral treatment

WBC white blood cells, *CTP* Child-Turcotte–Pugh

[$p < 0.05$ (*) significant differences, n.s: nonsignificant differences. Quantitative data [mean ± standard deviation (SD)] were analyzed by Student's t test. Qualitative data are presented as percentage/proportions and were analyzed by chi-squared test/Fisher exact test

determined based on the hypothesis that G-CSF therapy could improve the survival rate from an expected 25 % in the control group to 70 % in the treatment group, with power of 80 % and alpha error of 5 %. Based on this hypothesis and using the formula described in "Statistics" section, a number of 32 patients was determined.

The patients were divided into two groups: control (group A) and G-CSF group (group B) based on the therapy they received. Each group consisted of 16 patients, and grouping was done consecutively as per their visit to the hospital. Patients in the control group were provided with standard medical treatment of BSMMU, Dhaka, Bangladesh that included symptomatic treatment and general management of emergency patients.

General management of patients enrolled in the study

Patients were ensured nutritional support with palatable, locally cooked high-carbohydrate diet. Moderate restriction of animal protein was exercised in patients with hepatic encephalopathy. Ascites was managed with combination of furosemide (20 mg daily and above) and spironolactone (50 mg per day and above). Dose of diuretics was adjusted based on patient diuresis, body weight, electrolyte balance, and presence or absence of hepatic encephalopathy. For hepatic encephalopathy, we used syrup lactulose at 10–100 ml per day. In addition, rifaximin tablet (550 mg twice daily) was given. Intravenous ceftriaxone (1 g twice daily) was given as and when indicated. All patients included in the study received tenofovir tablet (300 mg daily) as standard practice. Other therapeutic interventions included use of intravenous fluid infusion (5 % dextrose in aqua or 5 % dextrose in normal saline, 1000–2000 ml per day) and oral proton pump inhibitor (20 mg twice daily).

Twenty-three patients received tenofovir at dosage of 300 mg daily, among whom 12 belonged to the control group and 11 the G-CSF group. Tenofovir therapy induced HBV DNA negativity in 21 patients, and 2 patients showed very low levels of HBV DNA at 90 days. Also, injectable antibiotics were given when indicated. Patients in the G-CSF group received traditional therapy similar to patients in the control group, plus G-CSF at dosage of 5 μg/kg/day subcutaneously for six consecutive days. All patients were followed up, and evaluation of follow-up was made at 0 (during admission), 7, 14, 30, 60, and 90 days after admission.

Statistics

The sample size was determined on the basis of the following formula:

$$n = \frac{p_1(1-p_1) + p_2(1-p_2)}{(p_1 - p_2)^2} \times (Z_\alpha + Z_\beta)^2,$$

where n is the sample size for each group, p_1 is the control group response (here 25 % or 0.25), p_2 is the treatment group response (here 70 % or 0.70), Z_α is the Z value (two tail) at definite level of significance, here 1.96 at 5 % level of significance, and Z_β is the Z value (one tail) at definite power, here 0.85 at 80 % power, resulting in a value of $n = 15.499$–16.

For statistical comparison of baseline data between groups, quantitative data (mean ± SD) were analyzed using Student's *t* test, while qualitative data are presented as percentage/proportions and were analyzed by chi-square test/Fisher exact test. CTP and MELD scores between groups were analyzed using Student's *t* test for intergroup comparisons of quantitative variables. The Wilcoxon rank-sum test was used to compare laboratory measurements obtained in the first and last visits in each group. Qualitative data are presented as percentage/proportions and were analyzed by chi-square test/Fisher exact test.

Results

Of the 32 patients, 16 with ACLF (control group) were managed by conventional therapy while 16 received G-CSF in addition to conventional therapy (G-CSF group). The two groups showed no statistical difference regarding age, gender, or baseline values of peripheral WBC, platelets, or other parameters (Table 1). The frequencies of patients with encephalopathy and ascites were similar in both groups. The CTP and MELD scores were also similar between groups ($p > 0.05$). Conventional therapy induced reduction of CTP score in patients of the control group when assessed at different time periods. CTP score at 90 days after therapy was significantly better in the G-CSF group compared with the control group (G-CSF group: reduction of CTP score from 11.9 ± 1.0 at 0 days to 7.6 ± 1.3 at 90 days; control group: reduction of CTP score from 12 ± 1.2 at 0 days to 9.2 ± 1.8 at 90 days) ($p < 0.05$) (Table 2). As shown in Table 3, The MELD score also was reduced more in the G-CSF therapy group, but the difference was not statistically significant during follow-up. G-CSF therapy improved patient survival. Data for survival and mortality of patients in group A and B are presented chronologically in Table 4. The survival benefit can be assessed based on the data for survival at 30 and 90 days after therapy commencement (Fig. 1). Patients of group A, who received conventional therapy, had survival benefit of 81.25 % at 30 days, which was reduced to 50 % at 90 days after therapy start. On the other hand, patients of group B, who received conventional therapy as well as G-CSF, had survival benefit of 87.5 % at both 30 and 90 days after treatment commencement. Taken together, these results show that, if patients receiving G-CSF survive for 30 days, it is likely that they will survive for 90 days or more (Table 4). However, mortality progressed in patients of group A receiving only conventional therapy after 30 days (Table 4).

Regarding cause of death, patients in the control group receiving traditional therapy showed hepatorenal syndrome (3 patients), variceal bleeding (2 patients), and electrolytic imbalance (3 patients) as complications related to death. Meanwhile, in the treatment group, hepatorenal syndrome (1 patient) and electrolytic imbalance (1 patient) were

Table 2 Child–Turcotte–Pugh (CTP) score per group at different follow-up times

Child–Turcotte–Pugh (CTP) score	Group A ($n = 16$) Mean ± SD	Group B ($n = 16$) Mean ± SD	*p*-Value
Pretreatment (0 days)	12.0 ± 1.2	11.9 ± 1.0	0.799 n.s.
Range (min–max)	(10–14)	(10–13)	
First follow-up (7 days)	11.1 ± 1.6	10.9 ± 1.1	0.683 n.s.
Range (min–max)	(9–14)	(9–12)	
Second follow-up (15 days)	10.3 ± 1.5	10.4 ± 1.4	0.195 n.s.
Range (min–max)	(7–13)	(8–13)	
	($n = 13$)	($n = 14$)	
Third follow-up (30 days)	9.8 ± 1.7	9.7 ± 0.7	0.841 n.s.
Range (min–max)	(6–12)	(9–11)	
Fourth follow-up (60 days)	9.3 ± 2.3	8.6 ± 1.1	0.317 n.s.
Range (min–max)	(6–13)	(6–10)	
	($n = 8$)	($n = 14$)	
Fifth follow-up (90 days)	9.2 ± 1.8	7.6 ± 1.3	0.025
Range (min–max)	(5–12)	(5–9)	
p-Value	<0.001	<0.001	–

Mean CTP score was significantly higher in group A (control) than group B (treated) only at the fifth follow-up assessment. Mean CTP score at fifth follow-up was statistically significant ($p < 0.05$) within group A and B as compared with each pretreatment value. Quantitative data (mean ± SD) were analyzed by Student's *t* test for intergroup comparisons. Wilcoxon rank-sum test was used to compare laboratory measurements obtained in the first and last visits in each group

Table 3 Model for End-Stage Liver Disease (MELD) score per group at different follow-up times

Model for End-Stage Liver Disease (MELD) score	Group A ($n = 16$)	Group B ($n = 16$)	p-Value
	Mean ± SD	Mean ± SD	
Pretreatment (0 days)	26.4 ± 4.6	25.3 ± 3.3	0.433 n.s.
Range (min–max)	(21–35)	(21–32)	
First follow-up (7 days)	24.4 ± 4.9	23.9 ± 6.1	0.800 n.s.
Range (min–max)	(16–33)	(17–43)	
Second follow-up (15 days)	21.3 ± 5.9	22.8 ± 7.8	0.544 n.s.
Range (min–max)	(21–30)	(14–46)	
	($n = 13$)	($n = 14$)	
Third follow-up (30 days)	19.2 ± 6.9	18.9 ± 5.6	0.901 n.s.
Range (min–max)	(10–43)	(9–31)	
Fourth follow-up (60 days)	18.1 ± 6.9	15.6 ± 5.6	0.397 n.s.
Range (min–max)	(10–43)	(14–36)	
	($n = 8$)	($n = 14$)	
Fifth follow-up (90 days)	16.6 ± 3.5	13.6 ± 4.1	0.098 n.s.
Range (min–max)	(12–22)	(8–24)	
p-Value	<0.001	<0.001	–

Mean MELD score was not significant between group A (control) and group B (treated) at any analysis time. Mean MELD score at fifth follow-up was statistically significant ($p < 0.05$) within group A and B as compared with each pretreatment value. Quantitative data (mean ± SD) were analyzed by Student's t test for intergroup comparisons. Wilcoxon rank-sum test was used to compare laboratory measurements obtained in the first and last visits in each group

Table 4 Chronological tabulation of survival and mortality of patients with ACLF up to 90 days after commencement of therapy

	Surviving	Dead
At 7 days after therapy commencement		
Group A	15	1
Group B	16	0
At 15 days after therapy commencement		
Group A	13	3
Group B	16	0
At 30 days after therapy commencement		
Group A	13	3
Group B	14	2
At 60 days after therapy commencement		
Group A	13	3
Group B	14	2
At 90 days after therapy commencement		
Group A	8	8
Group B	14	2

After 30 days, 13 out of 16 (81.3 %) patients were alive in group A and 14 (87.5 %) in group B ($p > 0.05$). Between 31 and 90 days, 8 (61.5 %) patients died in group A while no deaths were reported in group B ($p < 0.05$). Qualitative data are presented as percentage/proportions and were analyzed by chi-squared test/Fisher exact test

related to death. However, further studies are required to assess the impact of G-CSF on reduction of ACLF-related complications and their effects on patient survival.

Discussion

This open study included two groups of ACLF patients: one received all types of therapy traditionally provided in the only university hospital in Bangladesh, while the other received regular management plus G-CSF. The study was conducted over a period of 2 years, and patients were selected for the treatment group on the basis of their consent to receive either of the two lines of therapy. Originally, it was planned to carry out a study with 32 patients, i.e., 16 in each group. When the first patient attended the hospital and was accepted, after considering inclusion and exclusion criteria, the patient or his/her nearest kin was offered both treatment options after explaining the scope and limitations of both therapeutic procedures. On the basis of the choice by the patient or nearest kin, the first patient was enrolled into group A, and the next patient into group B. In certain circumstances, we had to wait for months to obtain patient consent for enrollment in the proper group.

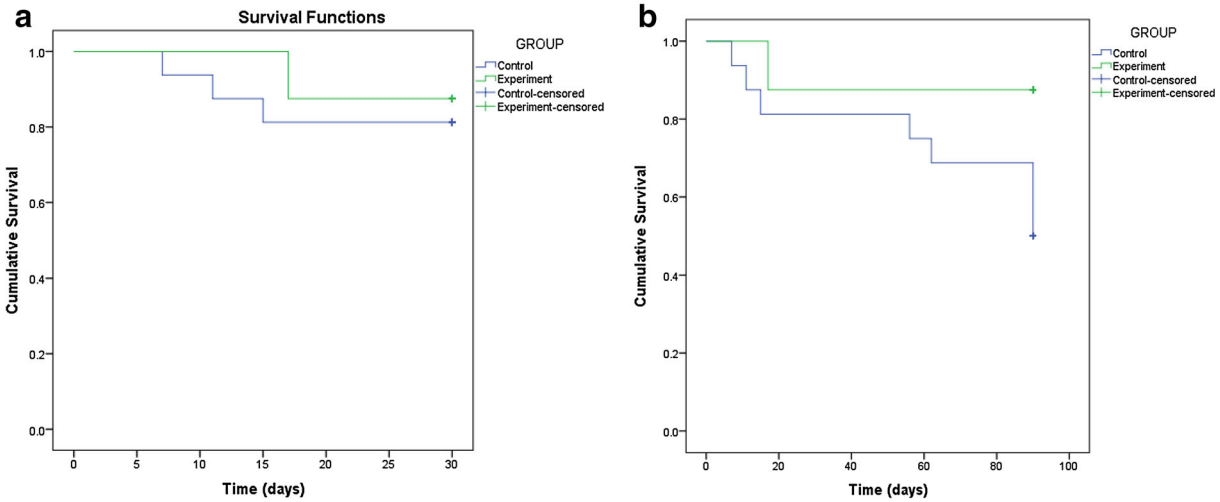

Fig. 1 (**a**) 30-day and (**b**) 90-day survival curves of patients with acute-on-chronic liver failure (ACLF). Control group represents patients in group A who received traditional therapy for ACLF. Experimental group represents patients of group B who received treatment with granulocyte colony stimulating factor (G-CSF) in addition to traditional therapy

The positive impact of G-CSF in patients with ACLF is a recent contribution to the context of management of ACLF. In 2012, Garg et al. noted reduction of CTP and MELD scores and prevention of development of sepsis, hepatorenal syndrome, and hepatic encephalopathy by administration of 12 doses of G-CSF in ACLF patients, comparing their data with ACLF patients receiving conventional therapy [9]. Subsequently, Duan et al. showed that CTP score, MELD score, and survival of ACLF patients were improved by administration of six doses of G-CSF [10]. The study presented here was carried out in Bangladesh, and the outcome of this prospective study confirms the beneficial effects of G-CSF in ACLF patients as described before [9, 10]. In addition, there are some notable points of this study. The first is that this study was conducted in Bangladesh, a developing country of 160 million with a huge burden of patients with chronic hepatitis and ACLF [11, 12]. Secondly, this prospective study checked the kinetics of CTP score and MELD score in at least five different points. Also, there was no drop out of patients in this study, and meaningful follow-up was achieved. The survival of ACLF patients undergoing this innovative therapy provided interesting data. Out of 16 patients in the control group receiving conventional therapy, 3 died within 30 days and 8 within 90 days. On the other hand, in the G-CSF group, 2 patients died within 30 days, a feature mostly similar to the survival of patients in the control group receiving conventional therapy. However, no patient receiving G-CSF died between 30 and 90 days. This is a very important and challenging issue in the context of treatment of ACLF. If this data regarding better survival of ACLF patients after 30 days can be reproduced in another clinical trial, it would provide a special impetus for use of G-CSF in these patients.

The study presented here has various limitations, such as relatively small sample size. Also, we used only one dosage of G-CSF. In fact, more clinical trials with different dosages of G-CSF should be carried out. Also, follow-up periods for G-CSF therapy of 60 [9] and 90 days [10] following commencement of therapy have been reported. Also, most patients had underlying HBV infection. This study took place over a period of 2 years. Finally, we could not check the mechanisms underlying the better therapeutic effect of G-CSF over conventional therapy, although data are now accumulating regarding the role of G-CSF in modulating the function of antigen-presenting dendritic cells and production of interferon-gamma in ACLF patients [12]. Moreover, information about regeneration of hepatocytes in ACLF patients by G-CSF must be presented.

A well-designed study that would include long-term follow-up and elucidation of the mechanism of action of G-CSF would cast more light on the real implications of G-CSF for management of ACLF. However, this study, along with already published studies, provides an ethical and scientific basis for use of G-CSF in ACLF patients.

Conclusions

Acute-on-chronic liver failure is a serious condition with high short-term mortality in absence of liver transplantation. Artificial liver support, augmentation of hepatocyte regeneration, cell-based therapy, immunotherapy, and gut microbiota modulation are emerging therapies to suppress

the injury. As an alternative to transplantation, G-CSF therapy offers multiple benefits as used in the present study. In addition to survival benefit, G-CSF prevents renal failure and hyponatremia, and augments clinical recovery in HBV-ACLF. The present study, although single center and with small sample size, strongly recommends G-CSF in addition to standard medical therapy in patients with ACLF to improve outcome in absence of liver transplantation. The added advantages of G-CSF in HBV-ACLF may likely be due to rapid viral clearance upon immune modulation, a novel concept that opens the window for further studies.

Compliance with ethical requirements

Conflict of interests Mamun Al Mahtab, Biplob Kumar Saha, Sheikh Mohammad Fazle Akbar, Sheikh Mohammad Noor-E-Alam, Ayub Al Mamun, Sharker Mohammad Shahadat Hossain, Mohammed Ashraful Alam, Ahmed Lutful Moben, Faiz Ahmad Khondaker, Forhadul Islam Chowdhury, Ruksana Raihan, Salimur Rahman, Ashok Kumar Choudhury, and APASL ACLF working party declare that they have no conflicts of interests.

Ethical approval This is a prospective study, and the study protocol was approved by the Institutional Review Board of BSMMU.

Informed consent in studies with human subjects All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2008. Written consent was obtained from either the patient or their nearest kin after explaining the nature and purpose of the study.

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