Research Article

Role of Granulocyte Colony Stimulating Factor (G-Csf) in Neonatal Sepsis with Neutropenia

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ABSTRACT

Randomized controlled double blind trial was done at NICU, Department of Pediatrics, MLB Medical College, Jhansi to determine whether adjunctive therapy with G-CSF could reverse sepsis associated neonatal neutropenia and improve neonatal survival compared with conventional therapy. 52 neonates with sepsis and absolute neutrophil count(ANC)<1800/mm3. The G-CSF group (n=26) received G-CSF single daily dose of 10 mg/kg/day subcutaneously for 3 days along with conventional therapy while control group (n=26) received conventional therapy (antibiotic and supportive care) alone. Hematological parameters [ANC, total leukocyte count (TLC)] on day 0,1,3,7 & 14 of study entry, neonatal survival and duration of hospital stay were compared between two groups. Basic demographic profile among two groups were comparable. By day 3, ANC among G-CSF group was significantly higher 8292±605.21 compared to control group 1544.04±250.49(p value <0.0001). Mortality rate was significantly lower in G-CSF group than in control group 11.53% vs 46.15%. Duration of hospital stay in G-CSF group was 18.32±4.42 compared to 24.57±9.06 in control group(p value 0.0027). G-CSF can increase the neutrophil count, decrease mortality rate and reduce duration of hospital stay in critically ill septic neutropenic neonates. Further studies are required to confirm our results and establish the adjunctive therapy in neonatal sepsis.

KEY WORDS: absolute neutrophil count(ANC), granulocyte colony stimulating factor(G-CSF), neonatal sepsis, neutropenia.

INTRODUCTION:

Bacterial sepsis is one of the major causes of mortality in newborns. Bacterial sepsis occurs in 0.1 to 1% of term newborn[1] and is upto 50 times more common in extremely low birth weight infants[2]. Mortality from neonatal sepsis depends upon the virulence of the organism, the gestational age of neonate and the particular combination and severity of patient's concomitant illness[1,3].

Neutropenia is a common association of neonates with sepsis and is associated with increased risk of death[4]. Compared to adults, the unique susceptibility of neonates to sepsis associated neutropenia is due to a smaller neutrophil storage pool, reduced capacity of neutrophil to be mobilized from bone marrow and a slower regeneration of neutrophil from bone marrow[5]. In septic neonates smaller neutrophil storage pool is due to shortened neutrophil half life from 6.3 hrs to less than 4 hrs[6], a limited ability to augment production of neutrophil proliferative pool, defective production of cytokines and a rapid depletion of infant's neutrophil storage pool during bacteremia[7,8].

Immaturity of neutrophil functioning also coexist in neonates and these neonates fails to mount an adequate immune response during overwhelming bacterial sepsis.

Thus, in addition to conventional therapy for neonatal sepsis with antibiotic medications and supportive care, several new modes of immunotherapy such as granulocyte transfusion and intravenous immunoglobulin administration have been used to reduce mortality without any proven positive results[9].

Intravenous immunoglobulin failed to create a major impact in reducing sepsis related mortality and now the attention is more on the potential enhancement of phagocytic immaturity using the hematopoietic colony stimulating factor[10].

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rhG-CSF has been considered to enhance stochastic entry into granulocytic pathway, increase rapid egress of immature neutrophil into peripheral circulation[11] and also improve the killing capacity of mature neutrophils[12]. In addition in newborn with sepsis, short term therapy with G-CSF increased the neutrophil count and improved survival. G-CSF therapy in very low birth weight (VLBW) infants was demonstrated to be safe and tolerance is good. Clinical trials in neonates have been preceded by extensive in vitro and animal studies because of the concern about acute and long term toxicities of such agents in neonates[10].

Serious side effects of G-CSF application have not been reported[13,14].

MATERIALS AND METHODS:

The aim of this study was to determine whether the adjunctive therapy with (G-CSF) could reverse sepsis associated neonatal neutropenia and improve neonatal survival compared with conventional therapy.

Patient selection:

A prospective randomized controlled double blind trial was conducted in the Neonatal Intensive Care Unit (NICU) of MLB Medical College, Jhansi, UP, India from September 2012 to Aug 2013 with the approval of the Institutional ethics committee.

An informed consent had been taken from each of the participating neonate's parents. Neonates with clinical signs of sepsis associated with neutropenia, a positive sepsis screen based on absolute neutrophil count (ANC), Total Leukocyte count, immature to total neutrophil ratio, micro-ESR, C-reactive protein (CRP)[15] and confirmed by at least one positive blood culture in 1st 28 days of life were included in the study.

Neutropenia was defined as absolute neutrophil count <1800 cells/mm3 with minor modification of criteria of Manroe et al[16] and Mouzinho et al[17].

Inclusion Criteria:

I) Neonates of age <28 days.
II) Newborns with clinical criteria recommended by National Neonatology Forum for sepsis.
III) Absolute neutrophil count (ANC) <1800 cells/mm3.
IV) Any one of the following hematological parameter.
   a. Total leucocyte count <5000cells/mm3
   b. I/T ratio >0.2
   c. C-reactive protein (CRP) > 1mg/dl
   d. Micro ESR – increased >15mmHg on 1st hr

Diagnosis was confirmed by blood culture by Bactec method.

Exclusion Criteria:

The neonates with the following anomalies were not included in the study :-

- Major congenital anomalies
- Cyanotic congenital heart disease
- Stigma of intrauterine infection
- Intraventricular hemorrhage grade III and IV
- Inborn errors of metabolism

Treatment protocol:

Included infants were randomized to receive either (G-CSF) or conventional therapy using a randomly generated (computer generated) predetermined schedule. Investigator were divided in two teams- blinded and unblinded. Those collecting data and following babies in study group were blinded. A computer generated random number table was followed and an allocation number was assigned to each random number. Corresponding allocation numbers were also present concealed in the cover of each medication, which was not accessible to blinded team. The unblinded team administered the medication following randomization.

Prior to study, maternal characteristics, approximate gestational age, anthropometry, vitals of newborn, serum biochemistry, CRP, blood sugar and electrolytes were recorded. Gestational age was confirmed by New Ballard Score.

Both the G-CSF group and control group were treated with appropriate conventional therapeutic interventions including antibiotics, oxygen, intravenous fluid, vasopressor drugs (dopamine or dobutamine) and other standard interventions deemed necessary for the clinical treatment of neonate independent of study drug usages. Antibiotic regimens were modified subsequently according to blood culture report and sensitivity pattern.

In addition, neonates in study group received G-CSF (filgramstin) at a single daily dose of drug 10mg/kg/day for 3 days subcutaneously diluted in 5% dextrose with dilution not less than 5mg/ml[18,19].

Included infants showed no signs of disturbance in respiration, heart rate or blood pressure during administration of study medicine. Routine examinations were performed daily and vitals and all systems were closely monitored until discharge.

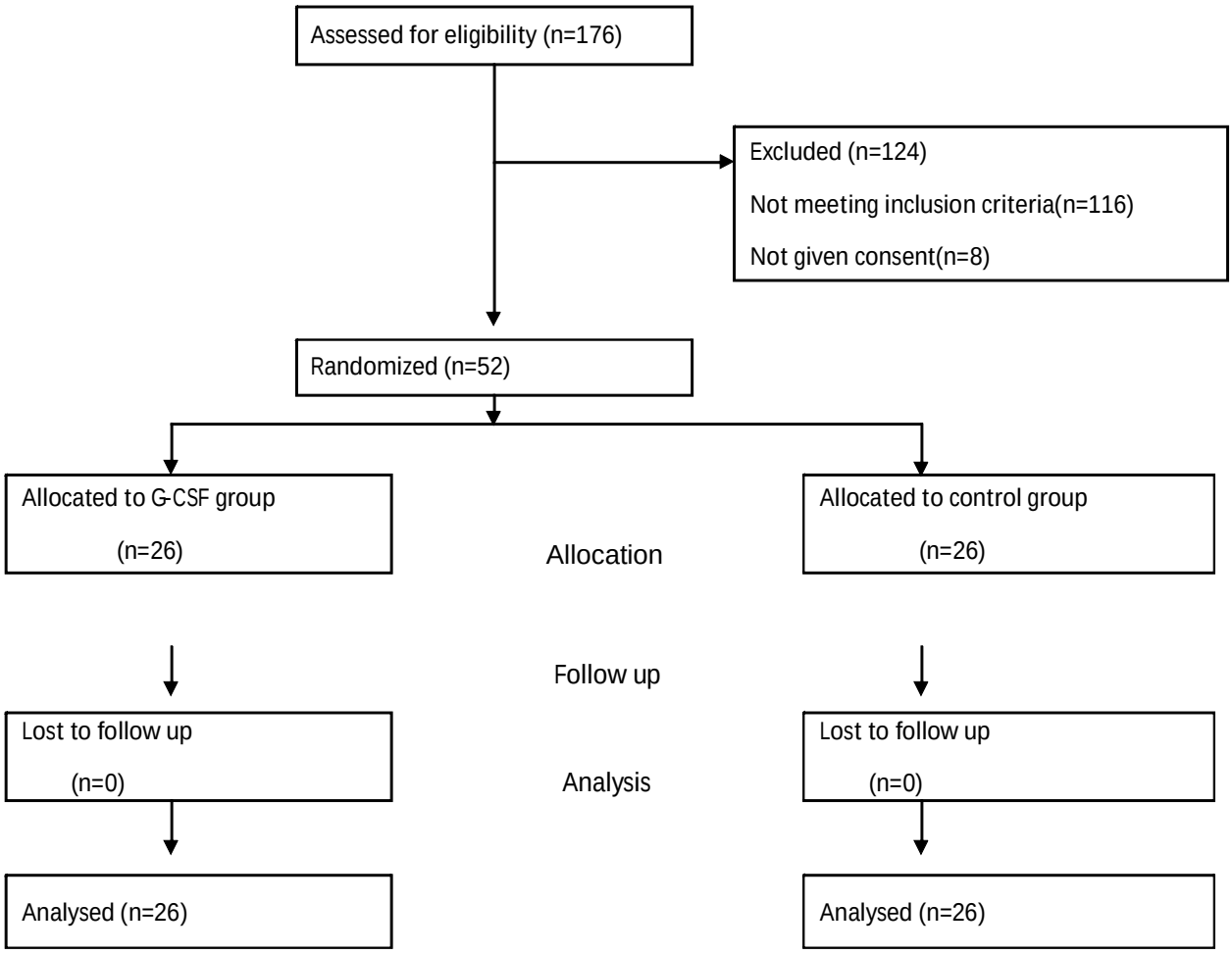

Figure 1: Flow chart for randomization and follow up.

Complete blood counts were obtained by counter autoanalyzer machine at study entry and after treatment on day 1, 3, 7 and 14. Absolute neutrophil count was obtained by manual counting from peripheral blood smear.

Statistical Analysis*:*

Data were expressed as numbers (%), and mean± SD. p value <0.05 was taken as significant. Fisher's exact t-test & Chisquare test used for comparing the two groups in regard to get significance.

RESULTS:

A total of 52 neonates with sepsis and absolute neutrophill count (ANC) <1800cells/mm3 were enrolled and randomized to receive either G-CSF (n=26) plus conventional therapy or conventional therapy (n=26) alone. All babies completed the study. The demographic clinical characteristics of both groups showed no significant difference.

Clinical hospital course:

All neonates tolerated G-CSF well and in each case no adverse reactions were identified with regard to cardiovascular performance, electrolyte balance, skin reactions, irritability or worsening of respiratory function. Patients in both the groups received conventional neonatal intensive care unit treatment for severe sepsis which included antibiotics for 10 days or more, oxygen inhalation, vasopressors and blood transfusions as and when required.

Hematological Indices

Absolute neutrophill count (ANC):

At study entry the ANC in the G-CSF group was 1479.2±269.85 compared to 1483.3±242.54 in other group which was statistically nonsignificant (p=NS).

By day 1 (After 24 hrs) of starting intervention, the G-CSF group had significantly

Table 1: Demographic characteristics of study patients.

S.No.	Variable	GCSF group (n=26) (%)	Control group (n -25) (%)	p value
1)	Birth weight (mean ±SD)	1411.15 ±204.01	1500.38 ±306.35	0.2222 (ns)
2)	Gestational age (mean ±SD) weeks	31.15 ±1.95	31±1.87	0.7734 (ns)
3)	Gender			
	Male	17 (65.38%)	18 (69.23%)	>0.05 (ns)
	Female	9 (34.62%)	8 (30.77%)	>0.05 (ns)
4)	Intrauterine growth retardation	6 (23.08%)	5 (19.23%)	>0.05 (ns)
5)	Number with			
	Early sepsis	24 (92.3%)	25 (96.15%)	>0.05 (ns)
	Late sepsis	2 (7.7%)	1 (3.85%)	>0.05 (ns)
6)	Maternal characteristics			
a)	Received antenatal care	10 (38.46%)	11 (42.31%)	>0.05 (ns)
b)	H/o premature rupture of membrane	7 (26.92%)	6 (23.08%)	>0.05 (ns)
c)	Cesarean delivery	12 (46.15%)	13 (50.0%)	>0.05 (ns)
d)	Twin pregnancy	4 (15.38%)	4 (15.38%)	>0.05 (ns)
e)	Pregnancy induced hypertension	4 (15.5%)	3 (11.5%)	>0.05 (ns)
7)	Bacteria of sepsis			
	E.Coli	10 (38.46%)	10 (38.46%)	>0.05 (ns)
	Klebsiella	10 (38.46%)	12 (46.15%)	>0.05 (ns)
	Enterobactor	2 (7.7%)	1 (3.85%)	>0.05 (ns)
	Candida	4 (15.38%)	3 (11.54%)	>0.05 (ns)

compared to the control group. ANC in G-CSF group was 4817.92±575.06 as compared to 1551.65±251.22 in control group with p value <0.0001 (Hs). Also in G-CSF group, ANC at day 1 increased by 3 fold as compared to day 0 (4817.92±575.06 vs 1479.20±269.86) which is highly significant with p value <0.0001 (HS) while in control group no significant increase in ANC at day 1 as compared to day 0(1551.65±251.22 vs 1489.38±242.54) with p value 0.3676 (ns).

By day 3, the G-CSF group had ANC of 8292±605.21 as compared to 1544.03±250.49 in the control group with a p value <0..0001 (HS). Also in G-CSF group ANC increased by 5 fold as compared to day 0 [8292.00±605.21 vs 1479.20±269.86, p value <0.0001 (HS)] while in control group 2 fold increase [2544.03±250.49 vs 1489.38±242.54, with p value 0.4279 (ns)] occured.

By day 7 the G-CSF group had ANC of 9281.82±437.27 as compared to 5223.52±369.09 in control group [p value <0.0001 (HS)]. Also there was 7 fold vs 4 fold increase in ANC compared to respective day 0 with p value in both group <0.0001 (HS).

The timing of changes in the ANC in the G-CSF group occurs sooner and remains longer than in conventionally treated group.

Mortality:

There were 11.53% (3/26) deaths in the G-CSF group as compared to 46.15% (12/26) in the control group which was significantly low (p value 0.014)

Causes of death among both groups were septic shock, DIC, and respiratory failure.

Table 2: Absolute neutrophil count.

	Day 0	Day 1	Day 3	Day 7
G-CSF group (n=26)	1479.2 ± 269.8592	4817.92 ± 575.0616	8292 ± 605.2118	9281.826 ± 437.2726
Control group(n=26)	1489.385 ± 242.5417	1551.654 ± 251.2256	2544.038 ± 250.494	5223.52 ± 369.0955
t-value	0.14313	26.53962	44.4463	36.16316
p-value	0.8868 (ns)	<0.0001 (HS)	<0.0001 (HS)	<0.0001 (HS)

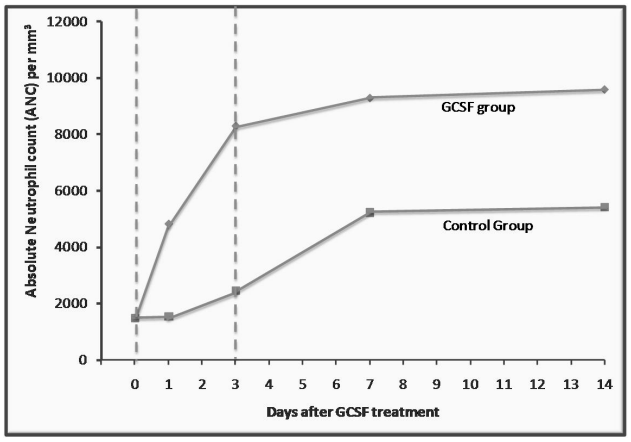

Figure 2: The timing of changes in the ANC in the G-CSF group occurs sooner and remains longer than in conventionally treated group.

Duration of hospital stay:

Duration of hospital stay in G-CSF group was 18.32±4.42 days as compared to 24.57±9.06 days in control group which was significantly low with p value 0.0027 (s).

DISCUSSION:

Our study demonstrated that preterm babies with sepsis and neutropenia who were treated with G-CSF for 3 day along with conventional care had a significantly lower mortality than the control group.

G-CSF increased the ANC and the increase in ANC in the G-CSF treated group was statistically significantly higher at day 1 than that the conventionally treated control group in which the increase only achieved significance at 7 days after entry. The neonatal response followed a predictable pattern of timing similar to observations made in adult patients in sepsis and pneumonia[20].

Overall, favorable prognosis in neonatal septicemia depends on effective host mechanism which again depends on normal hematological indices[21]. Neutropenia, when associated with neonatal sepsis, worsens the prognosis[22]. An immaturity in the quantitative and qualitative aspects of phagocytic immunity contributes to a state of relative immunodeficiency in newborn infants. GCSF is a physiological regulator of myelopoiesis and an activator of mature effective neutrophil function. It supports the clonal growth of neutrophil progenitors, primes neutrophils to increased expression of chemotactic receptors, and enhances antibody dependent cellular cytotoxicity[23]. Compared to adults, newborns do not seem to generate GCSF effectively. Estimates suggest that when sepsis is associated with severe neutropenia, mortality exceeds 50%. Relative neutropenia, though, is a low-risk group in developed countries; in developing countries with resource-limited settings, sepsis-related neonatal neutropenia is a significant cause of neonatal mortality and morbidity[24]. In addition, in developing countries, the microbiological organisms causing septicemia are different from those in developed nations; in developing countries organisms are mostly gram negative such as Klebsiella and Pseudomonas, E.coli[25].

There have been studies on the use of GCSF both as an adjunctive to treatment in neutropenic septicemic neonates and also its prophylactic use in preterm neonates, but all these studies are heterogeneous with regard to patient selection, duration of intervention, dosage and route of intervention, and outcome criteria. Duration of intervention varies widely in studies, mostly between 5 and 7 days.

It was chosen in the present study to discontinue the G-CSF therapy after 3 daily doses because the magnitude of the ANC response begins to plateau in adults after 4 days of treatment and we found that neonatal cell count had entered into normal range by then, thereby reducing cost of treatment and any possibility of side effect.

We have utilized the subcutaneous route for drug administration instead of continuous intravenous infusion as used in previous studies for which special infusion sets are needed thereby reducing cost of treatment and maintaining proper dilution of drug, and can be easily administered.

G-CSF receptors are expressed on variety of hematopoietic cells including neutrophils, monocytes, lymphocytes, platelets, leukemic cells and nonhematopoietic cells like endothelial cells, neurons and glial cells. So we also examined the effect of G-CSF on other leukocytes like lymphocytes and monocytes and found that there was no statistically significant effect on these cells.

Among the different studies, most are with positive outcomes[18,21]. Our study showed remarkable results both in terms of mortality and duration of NICU stay with the use of GCSF. So, further studies are required to confirm our results and establish this adjunctive therapy in neonatal sepsis.

CONCLUSIONS:

Neutropenia increases risk of death in neonatal sepsis and G-CSF may be used as adjunctive therapy. G-CSF improves survival and reduces duration of hospital stay when used as adjunctive therapy.

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