

Granulocyte-colony stimulating factor in neonatal sepsis with leukopenia: A prospective cohort study

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Abstract

Objectives: To observe the duration for normalization of the Total Leucocyte Count (TLC) with adjuvant Granulocyte-Colony Stimulating Factor (G-CSF) treatment in leukopenic neonatal sepsis, and to compare the neutrophilic response to G-CSF in neutropenic vs non-neutropenic subgroups

Methods: This prospective cohort study was carried out at the Neonatal Intensive Care Unit at Military Hospital Rawalpindi (NICU) from 1st August 2015 to 25th January 2017. Fifty one newborns with sepsis and leucopenia were sampled judgmentally from a population of 5666 admitted to NICU during the study period. The sample was then divided into neutropenic (exposed) and non-neutropenic (non-exposed) subgroups on basis of the absolute neutrophil count (ANC). Adjuvant G-CSF was given to all subjects and stopped once TLC normalized. SPSS v22 was used to calculate mean G-CSF treatment duration and rise in ANC. A Pearson correlation coefficient and simple linear regression were computed to assess the relationship between pre-GCSF ANC and the duration of treatment with GCSF. Comparison of subgroups with respect to rise in ANC was done using independent samples T-test.

Results: The mean duration of G-CSF treatment was 1.82 ± 0.81 days (1.0 - 4.0). Neutropenic neonates constituted 49% (n=25). The Pearson correlation coefficient showed a positive but negligible and non-significant correlation between the two variables, $r = 0.070$, $n = 51$, $p = 0.625$. A non-significant regression equation was found ($F(1,49) = 0.242, p=0.625$) with an R^2 of 0.005. There was a 7.06 ± 4.5 fold rise in ANC in the neutropenic subgroup compared to the 4.5 ± 3.1 fold rise in the non-neutropenic subgroup ($p=0.04$).

Conclusions: The mean duration for recovery from leukopenia with G-CSF treatment in neonatal sepsis was less than 2 days and had no significant relationship with pre-GCSF absolute neutrophil count. The neutrophilic response was significantly higher in neutropenic compared to non-neutropenic neonates. As GCSF made no difference to the outcome in terms of mortality, its routine use is not recommended in leukopenic neonatal sepsis.

Keywords: Neonatal sepsis, neutropenia, Granulocyte Colony-Stimulating Factor.

(JPMA 68: 1613; 2018)

Introduction

The efforts of the global community to reduce child mortality through the Millenium Developmental Goals (MDG - 4) has resulted in a significant reduction in under-5 mortality from 12.7 million in 1990 to 6.3 million in 2013. When seen in terms of region and age categories however, a stark contrast is observed. Neonatal contribution to under-5 mortality in South Asia in 2013

was 54% which represents a 33% increase since 1990.¹ The most significant causes of mortality are prematurity, neonatal sepsis, intrapartum complications and congenital anomalies.¹ Neonatal sepsis is significant in being amongst those mortality contributors with the slowest progress from 2000 to 2013.²

Late onset neonatal sepsis is more common in developing countries associated with challenges in infection control and antibiotic stewardship.³ Antimicrobial resistance especially to Gram Negative organisms is becoming a significant problem

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necessitating use of drugs like colistin.⁴ Developing revised recommendations for effective second line antibiotics is likely to be a perpetual need due to ongoing bacterial resistance.⁵ There is also a high rate of community acquired sepsis which usually presents to NICUs as outborn or referral patients often requiring longer courses of antibiotics.^{6,7} Neonatal sepsis is diagnosed by clinical judgement,⁸ and laboratory tools [TLC, the C-reactive protein (CRP), the I:T ratio, procalcitonin and blood culture].⁹

A subpopulation of patients with neonatal sepsis consists of those with neutropenia. Neutropenia is seen in approximately 8% of all neonates admitted in NICU but can be as high as 49% in babies born to mothers with hypertension during pregnancy.¹⁰⁻¹² Upto 38% of patients with neonatal sepsis were associated with neutropenia with a mortality of upto 27%.¹¹ Sepsis induced neutropenia may be transient or resolve with treatment but in a critical infant with multi-system involvement it could be a sign of severe overwhelming sepsis.¹⁰ Despite infection control, antibiotic stewardship and improved care techniques,¹³ neonates may experience a prolonged NICU stay which is likely to contribute to higher rates of severe nosocomial sepsis and ventilator associated pneumonia.¹⁴ Treatment of neonates having neutropenic sepsis with G-CSF has met with variable success. In 1994 safety and efficacy studies showed that G-CSF was well tolerated at all gestational ages and was not associated with any recognized acute toxicity.¹⁵ Meta-analyses do not recommend routine use of G-CSF¹⁶ but some RCTs have found a benefit in neutropenic neonates with sepsis in terms of duration of haematological recovery and hospital stay.¹⁷ It has been observed that the neutrophilic response to G-CSF was greater in neutropenic patients compared to non-neutropenic ones.¹⁸

Available local studies on use of G-CSF in Pakistan are limited to adults and older children with febrile neutropenia or cancer. No local studies pertaining to neutropenia in neonatal sepsis were found.^{19,20} Treatment with G-CSF may be economically prohibitive in low income countries but potentially may offer an advantage to a subpopulation of neonates with neutropenic sepsis. The rationale of this study, therefore, was to explore the use of G-CSF in neutropenic neonates in terms of duration and quantum of haematological recovery in sepsis. The objectives were to observe the duration for

normalization of the Total Leucocyte Count (TLC) with adjuvant Granulocyte-Colony Stimulating Factor (G-CSF) treatment in leukopenic neonatal sepsis, and to compare the neutrophilic response to G-CSF in neutropenic vs non-neutropenic subgroups.

Patients and Methods

The study was a prospective cohort study carried out in NICU from 1st August 2015 to 25th January 2017. Newborns admitted in NICU with presumed or definitive neonatal sepsis along with leukopenia (defined as a TLC < 4000/ μ L) irrespective of the primary diagnosis were included by non-probability homogenous purposive (judgemental) sampling. Presumed sepsis was defined as clinical suspicion of sepsis (poor feeding, lethargy, sluggish neonatal reflexes, poor peripheral perfusion, and temperature instability) along with a C-Reactive Protein (CRP) ≥ 10 mg/ml. Definitive sepsis was defined as presumed sepsis with a positive blood culture. Neutropenia was defined as an Absolute Neutrophil Count (ANC) of ≤ 1500 / μ L. Babies with no clinical suspicion of sepsis, a CRP < 10 mg/ml and incidental leukopenia were not included. Approval of ethics committee of the hospital was obtained.

Fifty one neonates sampled (out of a total population of 5666 indoor neonates) during the defined study period were enrolled after informed consent from parents/guardians. All babies were given tailored supportive care and antibiotics guided by the NICU antibiogram. The TLC and pre-GCSF ANC were recorded with respective dates on a Microsoft Excel data collection proforma. The sample was then divided into neutropenic (exposed) and non-neutropenic (non-exposed) subgroups on basis of the ANC. Each patient was given G-CSF in a dose of 12 micrograms per kg per day subcutaneously in two divided doses irrespective of the ANC. Daily TLC with differential was done until TLC rose above 4000/microlitre at which point G-CSF was stopped. Duration of G-CSF treatment and post-GCSF ANC for each patient was documented. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) check list for observational studies was applied.²¹

SPSS version 22 was used to calculate the mean duration of G-CSF treatment and frequency/percentage of neutropenic patients. A Pearson correlation coefficient was computed to assess the relationship between pre-GCSF ANC and the duration of treatment with GCSF. A

simple linear regression was calculated to predict duration of G-CSF based on pre-G-CSF ANC. In order to study neutrophilic response to G-CSF, a new variable 'ANC rise' was computed using the Transform > Compute Variable option in SPSS to divide post-G-CSF ANC by pre-G-CSF ANC. ANC rise represents the fold change of the ANC after G-CSF treatment. An independent samples T-test was done comparing 'ANC rise' to neutropenic and non-neutropenic subgroups. Descriptive statistics for secondary outcomes such as duration of hospital stay (LOS), treatment outcome (death, discharge or left against medical advice), culture yield and demographic characteristics of the sample were analysed. Level of significance was taken as $p < 0.05$.

Results

The mean gestational age of the sample was 34.6 ± 3.0 weeks (range 28- 40). The mean weight was 2.1 ± 0.63 kg (1.25-4.0). There were 36 (70.6%) males and 15 (29.4%) females. The mean LOS was 14.63 ± 9.1 days (3 - 61) with 44 cases (86.3%) having an LOS ≤ 20 days. The positive culture yield was 11 (21.5%) all of which were Gram Negative organisms predominantly Klebsiella species ($n=6$). Other bacteria were Acinetobacter species ($n=2$), Pseudomonas aeruginosa ($n=2$) and Enterobacter species ($n=1$). In terms of overall outcome, 25 (49%) patients were discharged home, 23 (45.1%) babies expired and 03 (5.9%) Left Against Medical Advice. The contribution of neutropenic cases to the mortality was 13 (56.5%).

The mean TLC at trial entry was 2.96 ± 0.71 (range 1.1-3.9). The frequency of the sample with neutropenia was 25 (49%) and with non-neutropenia 26 (51%). The mean duration of G-CSF treatment in all patients of both subgroups was 1.82 ± 0.81 days (range: 1.0 - 4.0). The Pearson correlation coefficient computed to assess the relationship between pre-G-CSF ANC and the duration of treatment with G-CSF showed a positive but negligible and non-significant correlation between the two variables, $r = 0.070$, $n = 51$, $p = 0.625$. A simple linear regression was calculated to predict duration of G-CSF treatment based on pre-G-CSF ANC. A non-significant regression equation was found ($F(1,49) = 0.242$, $p=0.625$) with an R^2 of 0.005. The mean pre-G-CSF ANC was 1594.08 ± 556.85 (300-3060) while the mean post-G-CSF ANC was 8309.14 ± 5060.84 (2400-22704). The mean ANC rise was 5.88 ± 4.02 (1.13 -16.69) fold. The comparison of ANC subgroups (neutropenia and non-neutropenia) with mean ANC rise using the independent samples T-test showed a significant

mean difference for rise in ANC in neutropenic subgroup ($M=7.06$, $SD = 4.5$) compared to non-neutropenic subgroup ($M=4.5$, $SD = 3.1$); $t(49) = 2.114$, $p=0.040$ (Table). There was no observed adverse effect of G-CSF.

Table: Subgroup analysis of mean rise in ANC with G-CSF treatment.

Subgroups	N	Mean Rise in ANC	SD	Independent samples T-test			
				T	Df	Sig. (2 tailed)	Mean difference
Neutropenia	25	7.06	4.60	2.11	49	0.040	2.30
Non-neutropenia	26	4.76	3.1				

Discussion

The mean duration of G-CSF therapy was 1.82 ± 0.81 days. Only 10 (19.6%) patients required 3 days for rise of TLC to the cutoff value for stopping G-CSF where as only 01 (2.0%) patient required 4 days. This is similar to findings of Lee JA et al¹⁷ (2017) who retrospectively studied 30705 infants with neutropenia of whom 2142 (7%) had received G-CSF and found that the median time to haematological recovery was 2 days in both groups; however the G-CSF group showed a shorter adjusted time to haematological recovery (hazard ratio: 1.36, 95% confidence interval [CI]: 1.30-1.44).¹⁵ Chaudhuri J et al²² (2012) administered G-CSF for 3 days and Borjianyazdi L et al²³ for 5 days although no rationale was presented for selection of duration in either study. Chaudhuri J et al²² demonstrated a significant rise in ANC to the non-neutropenic range ($\geq 1500/\mu\text{L}$) at 72 hours, significantly higher in the G-CSF group ($p \leq 0.05$). In the pioneering study by Gillan ER et al (1994),¹⁵ 42 patients with presumed sepsis (of which only 2 had neutropenia) were studied for rise in ANC and G-CSF levels/C3bi expression at regular intervals after administration of G-CSF for 3 days. The rise in ANC was significant after 24 hours ($351\% \pm 89\%$) ($p \leq 0.05$) and was sustained at 96 hours. Maheshwari A,¹⁰ described the cut off for stopping G-CSF treatment as ANC of $\geq 5000/\mu\text{L}$. In our study the mean post G-CSF ANC (at which G-CSF was stopped), was 8309.14 ± 5060.84 (2400.0 to 22704.0). Considering that the mean G-CSF duration in our study was less than 2 days it can be extrapolated that the cutoff suggested by Maheshwari A was achieved/surpassed within 2 days of starting G-CSF.

Interestingly, the proportion of patients with neutropenia was 49% ($n=25$) out of the total sample of patients with sepsis and leukopenia ($n=51$). Statistics for rise in ANC after G-CSF treatment showed that the neutropenic group

experienced a 7.06 ± 4.5 fold rise in ANC compared to non-neutropenic group which showed a 4.5 ± 3.1 fold rise in ANC (Table). This differential neutrophilic response to G-CSF has been also observed in a study described in Avery's Diseases of the Newborn 9th edition which compared 14 VLBW infants preeclamptic neutropenic neonates with nonseptic neonates treated with G-CSF.¹⁸ The neutropenic group showed a 12 fold rise in ANC compared to a 2.5 fold rise in non-neutropenic group. Compared to our study showing 7 fold rise in ANC, the neutrophilic response of neutropenic patients was higher in this study (12.5 fold). However both studies had different sample characteristics with respect to gestation. Neutrophilic response in non-neutropenic patients has also been studied by Kucukoduk S, et al (2002)²⁴ who concluded that early G-CSF treatment in non-neutropenic preterm babies with sepsis is significant in reducing hospital stay.

Regarding secondary outcomes, the culture pattern is similar to those in NICUs in the developing world where Gram Negative organisms account for up to 63% of bacterial isolates.²⁵ The mean LOS was shorter than Borjanyazdi L et al (2013)²³ study i.e. 25 ± 6 days in the treatment group. Mortality was 23 (49%) for the sample while the contribution of neutropenic cases to the mortality was 13 (56.5%), which is significantly higher than other studies which show mortality in the range of 10% to 28%.^{11,22}

The strength of this study is the fact that it addresses a problem previously not studied in our local population in the specific context of neutropenic neonatal sepsis. The limitation of the study is the need to control for possible confounding factors such as the effect of varying gestational ages on the neutrophilic response, the possible contribution of factors other than sepsis to mortality and the possible variable effect of different bacterial species on the ANC.

Conclusions

The mean duration for recovery from leukopenia with G-CSF treatment in neonatal sepsis was less than 2 days and had no significant relationship with pre-GCSF absolute neutrophil count. Approximately half of hospitalized patients with neonatal sepsis and leukopenia may have neutropenia. After GCSF treatment, the neutrophilic response is significantly higher in neutropenic as compared to non-neutropenic neonates. As GCSF made

no difference to the outcome in terms of mortality, its routine use is not recommended in leukopenic neonatal sepsis.

Disclaimer: None to declare.

Conflict of Interest: None to declare.

Funding Sources: None to declare.

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