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Effect on Absolute Neutrophil Count Usingg-CSF in Neutropenic Septic Neonates and Its Relation With Outcome Specially Mortality

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ABSTRACT

Objective: To study effect of supplementing G-CSF to septic neonates with ANC<5000/cmm in terms of

1. Increase in Absolute Neutrophi Count (ANC)
2. Effect of increase ANC count on outcome especially mortality

Material And Method: Prospective observational study in division of neonatology of pediatrics Department, Pacific Medical College and Hospital, Udaipur from September 2022 to September 2023.

Inclusion Criteria:

1. Septic neonate
2. ANC <5000/cmm
3. Evidence of sepsis i.e., at least one positive blood culture in neonatal period

Methodology: Written consent from guardians of neonates were taken. Ethical clearance was taken, Necessary laboratory investigations were done and blood culture was taken by proper method. Antibiotic and appropriate treatment was initiated as per NICU unit protocol. Standard hematological techniques were employed for estimation of hematological parameters.

Result: The study was carried out in 40 septic neutropenic preterm neonates with ANC<5000/cmm. They were randomly assigned to treatment group(n=20) and control group (n=20). There was male preponderance with 60% of babies were male. Age of neonate at study entry was comparable between G-CSF and control group [6.5 $\pm$ 2.98 days Vs 6.0 $\pm$ 5.38 days (p>0.05)].

With administration of G-CSF, ANC rose to significantly higher level with G-CSF group as compared to control group on day 3 (4786$\pm$1089/cmm Vs 4212$\pm$754/cmm,p<0.05). The rise was sustained till day 5 (5008$\pm$1028/cmm Vs 4055$\pm$522/cmm, p<0.01). However by day 7, ANC was comparable between two groups. (5077$\pm$657/cmm Vs4652$\pm$779/cmm.p>0.05). ANC reached peak value on day 7 in G-CSF group. Mortality was significantly higher in control group compared G-CSF group(35%Vs 15%, p<0.05). Stepwise regression analysis revealed no significant correlation between G-CSF use and mortality. Only predictable factor affecting mortality significantly was ANC not recovering to >5000/cmm by day 7.Other factors like birth weight, IVH, meningitis,ANC recovering to >5000/cmm by day7 and blood culture sterility by day 6 were not found to correlate significantly with mortality. Clinical symptoms of sepsis like gastrointestinal symptoms and hypoglycemia were resolved earlier in G-CSF group as compared to control group (4.71$\pm$2.3 Vs 8.0$\pm$4.2 days,p<0.05) and(4.0$\pm$1.0 Vs 7.0$\pm$1.78 days, p<0.01 respectively).

Conclusion: G-CSF use in preterm septic neonates with Birth weight (900-1900 gm, mean 1345$\pm$289 gm) and Gestational age (28-36 weeks, mean 31.5$\pm$2.68 weeks) increased absolute neutrophil count and resulted in decrease in mortality. Mortality was more in patients in whom ANCdid not rise >5000/cmm by D7. However, studies with large number of patients are warranted before use of G-CSF can be recommended as standard therapy for preterm neutropenic septic neonates.

KEYWORDS: G-CSF, ANC, Sepsis, NeutropenicNeonate,Mortality

INTRODUCTION:

Human neonates appear capable of generating G/GM-CSF during infectious challenge, but not as responsively as adults.The observation that neonatal monocytes generates comparatively low quantities of G-CSF after inflammatory stimulus may contribute part of explanation for their defective upregulation of neutrophil production and function during infection[1].G-CSF has been shown to specifically promote granulocyte development and maturation by stimulating myeloid progenital proliferation, increasing bone marrowneutrophil storage pool, inducing peripheral neutrophilia[2].Sepsis is the commonest cause of neonatal sepsis and is responsible for 30 to 50% of total neonatal deaths in developing countries.[3]This study aims to find the relationship between increase ANC and its effects on outcome specialty on mortality with the use of G-CSF in septic neutropenic neonates.

MATERIAL AND METHOD:

Study was conducted in NICU, Department of Pediatrics, Pacific Medical College and Hospital, Udaipur.

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A total of 40 babies were studied. These were randomly assigned to treatment group(n=20) and control group(n=20). The period of study was twelve months from September 2022 to September 2023.

Inclusion Criteria:

1.Septic neonates
2.ANC<5000/cmm
3.Evidence of sepsis i.e positive blood culture in neonatal period

Those without consent for study were excluded from study. Basic maternal and neonatal data was recorded including basic neonatal characteristics like Birth weight, Birth length, Period of gestation, Apgar score etc. All babies were treated as per unit protocol. The babies in treatment group in addition received injection G-CSF 10 microgram/kg as slow intravenous infusion over a period of 2 hour once daily for a period of five consecutive days. All babies had hematological evaluation for Total Leucocyte Count, Absolute Neutrophil Count, Absolute Platelet Count on day 1,3,5,7 and 14 of study entry. Standard hematological technique were employed for the estimation hematological parameters.[4] Neonatal problems, and mortality were recorded for all cases. At the end of study, the data was collected and analyzed statistically by using students t-test, z-test of proportion and multiple regression analysis by using SPSS software.

RESULT:

A total of 40 babies were included who fulfilled the criteria of inclusion as per provision of study protocol. These were randomly assigned to the treatment group (n=20) and the control group(n=20). All babies had hematological evaluation on day 0,1,3,5,7 and 14. Various observations were as below

TABLE 1: PATIENT CHARATERSTIC

Characterstics	G-CSF (n=20)	Control(n=20)	p-value
Male/Female	11/9 1.22:1	13/7 1.95:1	
Birth weight (gm) Mean± SD Range <1000 1000-1499 1500-2000	1305±289 900-1900 1(5%) 14(70%) 5(25%)	1500±231 1100-1900 – 12(60%) 8(45%)	>0.05
Gestational age (weeks) Mean± SD Range	31.5+2.68 28-36	32.6+2.23 30-36	>0.05(NS)
AGA/SGA	15/5 3:1	15/5 3:1	

There was no significant difference between G-CSF and control group with reference to Birth weight, Gestational age and sex. Males outnumbered females in both the groups in ratio of 1.2:1 and 1.95:1 in G-CSF and control group respectively. Most babies in both groups were appropriate for Gestational age and had late onset sepsis.

TABLE 2: PREDOMINANT NEONATAL PROBLEMS

Neonatal problems	G-CSF (N=20)	Control (N=20)	p – value
Respiratory distress	12(60%)	12(60%)	>0.05(NS)
Feed intolerance	14(70%)	17(85%)	>0.05(NS)
NEC	2	---	>0.05(NS)
Hypoglycemia	10(50%)	9(45%)	>0.05(NS)
Thrombocytopenia	16(80%)	13(65%)	>0.05(NS)
Seizures	4(20%)	5(25%)	>0.05(NS)
Shock	3(15%)	5(25%)	>0.05(NS)
Assisted ventilation	3(15%)	6(30%)	>0.05(NS)
Meningitis	7	13	>0.05(NS)

Neonatal problems were almost similar in both G-CSF and control group. There was no significant difference.

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TABLE 3

Absolute neutrophil count (cells/c mm)			
Day after administration of G-CSF	G-CSF (n=20)	Control group (n=20)	p-Value
Day 0	3037±7.9 Range (1200-4060)	3761±402 Range (2660-4385)	<0.001(HS)
Day 1	4052±922	3755±676	>0.05(NS)
Day 3	4756±1089	4213±354	<0.05(S)
Day 5	5008±1028	4055±522	<0.05(S)
Day 7	5077±657	4652±779	>0.05(NS)
Day 14	4780±554	5067±733	>0.05(NS)

The initial absolute neutrophil count was significantly lower in G-CSF group as compared to control group 0.001). However, with administration of G-CSF the ANC rose to 4.52±922 cmm by day 1,4256±1084 cmm by day 3,5008± 1028 cmm by day 5 and 5077±657 cmm by day 7 when peak values were documented. These ANC values were significantly higher than in control group by day 3 through day 5. ByD7 however the ANC values had risen even in the control group.

TABLE 4: TIME TAKEN TO RESOLVE SYMPTOMS

Clinical Symptoms	Days taken to resolve symptoms after G-CSF treatment		P-Value
	G- CSF	Control	
1.Respiratory Distress Mean±SD Range	3.8±2.69 2-11	5.0±2.36 2-8	>0.05(NS)
2.Feed Intolerance Mean±SD Range	4.71±2.30 3-11	8.0±4.20 4-21	<0.05(S)
3.Hypoglycemia Mean±SD Range	4.0±1.0 3-5	7.0±1.78 4-21	<0.01(S)

In the G-CSF group feed intolerance and hypoglycemia resolved earlier than in control group. Mean duration to resolve feed intolerance was 4.71±2.30 days (Range 3-11 days) in G-CSF group as compared to 8,0±4.20 days (Range 4-20) in control group. Hypoglycemia resolved in 4.0±1.0 days (Range 3-5 days) in G-CSF group compared to 7.0±1.78 days (4.21 days) in control group

TABLE 5: MORTALITY

Cause	G-CSF (n=20)	Control (n=20)	p-Value
NEC (stage III)	1(33.3%)	0	>0.05(NS)
Septic shock	3(100%)	3(43%)	>0.05(NS)
Pulmonary Hemorrhage	---	3(43%)	>0.05(NS)
Intraventricular hemorrhage (Grade III&IV)	1(33.3%)	1(33.3%)	>0.05(NS)
Respiratory Failure	---	1(33.3%)	>0.05(NS)

In G-CSF group one patient died of NEC (stage III), one with refractory shock and one had intraventricular hemorrhage and died on day 4 of treatment of G-CSF i.e., had received 3 doses of G-CSF. In control group ,3 patients died of pulmonary hemorrhage, one with intraventricular hemorrhage and 2 with septic shock and one with recurrent apnea and respiratory failure.

TABLE 6: CLINICAL CHARACTERSTICS: SURVIVAL VS EXPIRED GROUP

Basic Characteristics	Mortality group(n=10)	Survivor group(N=30)	P-Value
Basic characteristic Birth weight (in grams)	1260,210 (900-1600g)	1510,250 (1000-1900g)	<0.05 (S)
Gestational age (in weeks)	31.5,2.36 (29-36 weeks)	32.26,2.54 (28-36 weeks)	>0.05(NS)

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Male Female AGA SGA	8(80%) 2(2%) 6 3 2:1	16(53) 14(47) 24 6 4:1	>0.05(NS) >0.05(NS) >0.05(NS) >0.05(NS)
Complications Septic shock Pulmonary hemorrhage Assisted ventilation Meningitis Necrotizing enterocolitis Intraventricular hemorrhage	 6(60%) 3(30%) 8(80%) 7(70) 1(10%) 2(20)	 2(6.7%) -- 1(3.3%) 13(43.3%) 1 --	 <0.01(S) <0.01(S) <0.01(S) >0.05(NS) >0.05(NS) <0.05(S)
ANC recovered to >5000by D7 ANc did not recover to >5000 till death	1(10%) 9(90%)	23(100) 0	<0.01(S) <0.01(S)
Blood culture Number of organism One More than one	 6(60%) 4(40%)	 22(73.3%) 8(26.7%)	 >0.05(NS) >0.05(NS)
G-CSF received	3(30%)	17(56.7%)	>0.05(NS)

There was no significant difference in gestational age,gender,ratio of AGA to SGA in mortality versus survival group.But there was difference in:
(i)Birth weight: Mortality group has lower birth weight than survival group.
(ii)Complications: Septic shock, Pulmonary hemorrhage, intraventricular hemorrhage, requirement of assisted ventilation was more than as compared in survival group.
(iii)There was significant differencein absolute neutrophil count in both groups. In 90% of mortality group patientsANC did not rise to >5000/cmm until death. ANC was>5000/cmm by D7 in 76.7% of survival group as compared to mortality group.

TABLE 7: FACTORS AFFECTING MORTALITY

Mortality rate	Birth Weight	G-CSF use	Septic shock	Pulmonary hemorrhage	Intraventricular hemorrhage	Meningitis	ANc not receiving by D7 to >5000/cmm	ANC recovering to >5000/cmm	Blood culture sterile by D6
Var 1	Var 2	Var3	Var4	Var5	Var6	Var7	Var8	Var9	Var10
75%	900-1100	75%	50%	25%	25%	75%	75%	25%	25%
27.8%	1200-1499g	55.5 %	11.1 %	11.1%	5.55%	44.4%	22.2%	66.6%	50%
22.2%	1500-1799g	33.3 %	22.2 %	--	--	55.55	22.2%	55.55%	22.2%
0%	1800-200g	44.4 %	22.2 %	--	--	44.45	--	55.55%	44,4%

Stepwise multiple regression analysis was done using SPSS software to know the relation between mortality (dependent variable,VAR1) and independent variables:birth weight (VAR2),G-CSF use(VAR3),septic shock(VAR4) ,pulmonary hemorrhage (VAR5),intraventricular hemorrhage(VAR6),meningitis (VAR7),ANC not responding to>5000/cmm by day 7(VAR8) and blood culture becoming by day 6 (VAR10).

Model Summary:

Model	R	R square	Significance (p value)
1	0.096	0.992	0.004

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Coefficients

	Unstandardized coefficients		Standardized coefficients		
Model	B	Std.errors	Beta	t	Sig.
(Constant)	1.820	2.493	.996	.730	.541
VAR00008	.986	.061		16.076	.004

a. Dependent Variable; VAR00001

Individual Variables

Model		Partial Correlation	Significance(p Value)
1	VAR2	-0.995	0.062
	VAR3	0.451	0.702
	VAR4	-0.905	0.279
	VAR5	0.683	0.522
	VAR6	0.126	0.919
	VAR7	-0.982	0.122
	VAR9	0.895	0.295
	VAR10	0.751	0.459

Only predictor variable was VAR8i.e.ANC not recovering to >5000/cmm by day7(p=0.004) which had a significant correlation withmortality.Other variables had no significant correlation with mortality(p>0.05).The model had overall p value 0.004 i.e. when all variables were considered together, they had significant effect on mortality. Neonatal infection rate is inversely related to low birth weight and gestational age[10].

DISCUSSION:

A total of 40 septic blood culture positive neonates with an absolute neutrophil count <5000/cmm, admitted in NICU, department of Pediatrics, Pacific Medical College Hospital, Udaipur formed the subjects of study. They were randomly assigned the treatment group (n=20) and control group(n=20).There was male preponderance with 60% of babies being male. There was no significant difference between neonates of G-CSF group and control group with regard to mean birth weight [1395±289gm Vs 1500±231gm(p>0.05)], mean gestational age [31.5±2.08 weeksVs32.6±2.32 weeks(p>0.05)], AGA Vs SGA [3:1 Vs3:1].

In a study by Russel et al. that enrolled septic In preterm neonates with relative neutropenia i.e ANC <5000 /cmm there were significantly fewer deaths in the neonate screening G-CSF when compared to control group[5].In our study also mortality was significantly higher in the control group as compared to G-CSF group(35%Vs15%,p<0.05).13 out of 20 patients in control group and 17 out of20 in G-CSF group survived .G-CSF lead to rapid increase in ANC in all babies who received G-CSF. With G-CSF treatment ,ANC was>5000/cmm in 60% patients by day 5 and is 80% patients by day 7 of treatment as compared to 5% and 35% in control group on day 5, day 7 respectively .Baseline ANC was significantly higher in control group than G-CSF(3761±402/cmm Vs 3037±709/cmm, p<0.05) but on day 3, ANC had risen to significantly higher level in G-CSF group as compared to placebo group (4756±1089/cmm Vs 4212±354/cmm,p<0.05) and rise was sustained till day 5 (5008±1028/cmm Vs 4055±522/cmm, p,0.01).ANC reached its peak value by day 7 in G-CSF group. 17 out of 20 patients (85%) responded to G-CSF administration i.e.G-CSF led to an increased in ANC to 7500/cmm in 85% patients. The remaining of patients died and has ANC <5000/cmm till death. In term Baseline mean ANC was 2720/cmm. Mean increase in ANC was 1300/cmm but ANC did not reach 5000/cmm. Mortality was higher in patients in which ANC did not rise to significant levels. In 6 out of 7 case who died in control group, ANC was still <5000/cmm at the time of death. In these neonates mean baseline ANC was 3626±67/cmm and increase in ANC from baseline was478±33/cmm. Therefore, failure of ANC to rise was associated with mortality. Failure to mount a neutrophil response during infection and a relative neutropenia (ANC<5000/cmm) has been associated with high mortality among preterm neonates.[6,7,8] In our study mortality was significantly higher in the control group as compared to G-CSF group(35%Vs15%,p<0.05).13 out of 20 patients in control group and 17 out of20 in G-CSF group survived. In the study by Miura et al,44 preterm neonates weight 500gm to 2000gm with gestational age <37 weeks were randomized to treatment group(n=22) to receive 10microgm/kg of iv G-CSF once daily for 3 days and the placebo group(n=20).At 24 and 48 hrs ,ANC was significantly higher in the G-CSF recipients than the placebo group(mean9522/cmm Vs 4526/cmm at 24 hrs .p<0.06 and 16843/cmm Vs 4703/cmm at 48 hours, p<0.00042) although baseline ANC was comparable between two groups.

In the study, mean duration of relieving clinical symptoms of sepsis like feed intolerance, hypoglycaemia were significantly lower in G-CSF group as compared to control group (feed intolerance (4.71±3.30 days Vs 8.0±4.2 days, p<0.05,Hypoglycemia 4.0±1 days Vs7.0±1.78 days p<01).In study by Russel et alneonates with birth weight 500-1500gm,ANC <5000/cmm and clinical evidence of sepsis ,were randomly assigned to receive either G-CSF (10 microgm/kg) for maximum of 14 days intravenously (n=13) or placebo n=20) in addition to standard treatment.Babies

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treated with G-CSF spent fewer days in intensive care(average 8 days vs 12 days),spent fewer on ventilator (average 5 days vs 12 days,p<0.23).Although Their results did not reach statistical significance ,but there was trend towardsbenefits in their short term end points.[5] In astudy conducted by Kocherlakota et al ,14 septic neutropenic neonates were given G-CSF 10 microgm/kg/day and their outcome was compared with 11 concurrently treated case matched controlled septic neonates.13 out of 15 (92%) patients in G-CSF group and 5 out of 11 (55%) in control group survived to 28 days after onset of signs of sepsis .[11]This study supports index study.

CONCLUSION:

Foetus and neonates have a limited neutrophil storage pool which limits their capacity increasing the for release of neutrophil into the peripheral circulation in response to GI infections.It can be concluded from this study that G-CSF use in septic neutropenic neonates increases ANC and results in decreasing mortality.Only ANC notrecovering to >5000/cmm by day 7 had a significant correlation with mortality.

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