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Original Article

White blood cell count and ratio changes in newborns after granulocyte colony-stimulating factor treatments

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Abstract

Background Granulocyte-colony stimulating factor (G-CSF) is frequently used to treat neonatal neutropenia. There is a paucity of data in the literature on when immature to total neutrophil ratio (I/T ratio) can be accurately used as a sepsis marker after G-CSF therapy, as well as when I/T ratio returns to normal values expected in newborns who did not receive G-CSF.

Objective To investigate changes in white blood cells counts and ratios in neonates with neutropenia before and after G-CSF therapy.

Methods This retrospective study included newborns admitted to the NICU of Hacettepe University Ihsan Dogramaci Hospital, Ankara, Turkey, between 2005 and 2017 who received G-CSF therapy for neutropenia. Subjects underwent complete blood counts on the day before receiving G-CSF therapy (day 0) as well as days 1, 2, and 3 after treatment; I/T ratios were recorded from peripheral smears.

Results Twenty-eight neonates were included in the study. Subjects' median gestational age (interquartile range 25-75%) was 32.6 (29.7-37.6) weeks, and median birth weight was 1,630 (1,040-2,980) g. On day 3, there were significant increases in white blood cell counts compared to day 0. There were statistically significant elevations in the I/T ratios between day 0 and day 1 and between day 0 and day 2. On day 3, the I/T ratio decreased, but was not significantly different between day 0 and day 3.

Conclusion The changes in I/T ratio observed after G-CSF treatments in our study suggest that the I/T ratio can be used as a reliable sepsis marker starting 72 hours after G-CSF administration. However, I/T ratio is significantly affected within 72 hours of G-CSF administration, and therefore, is unreliable as a sepsis marker during that period. **[Paediatr Indones. 2021;61:240-6 ; DOI: 10.14238/pi61.5.2021.240-6].**

Keywords: *newborn; granulocyte colony-stimulating factor; neutropenia; immature/total ratio*

Neutropenia affects 6–8% of infants in neonatal intensive care units (NICUs).[1] However, the incidence of neonatal neutropenia is higher in infants who are small for gestational age (SGA), born to mothers with hypertension and/or preeclampsia, and those with sepsis.[2,3] Neutropenia is defined as having an absolute neutrophil count (ANC) below the 5th percentile according to the reference range, or $<1,000\text{-}1,500/\mu L$.[4,5] The etiology of neutropenia includes inadequate production, excessive margination, or increased destruction of neutrophils.[6]

Neonates exhibit insufficient endogenous production of G-CSF, a physiological factor that stimulates granulocyte-specific colony development and increases polymorphonuclear leukocyte (PMNL) count. G-CSF is also involved in neutrophil phagocytosis, chemotaxis, and cytotoxicity.[6,7] An exogenous G-CSF source can be used to increase neutrophil counts in neutropenic newborns.[8] The most common indication

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for G-CSF use in newborns is sepsis accompanied by neutropenia.[9]

Hematological changes following G-CSF therapy have been evaluated in numerous studies in children; however, G-CSF doses, durations, and treatment response times vary among these studies.[10-12] Nevertheless, very few studies have investigated the hematological changes after G-CSF therapy in newborns. Moreover, studies on the accurate use of I/T PMNL ratio (I/T ratio<0.2) to diagnose neonatal sepsis have been limited.[4,12,13] There is also a paucity of literature on how the I/T ratio changes after G-CSF therapy, as well as when it returns to normal in newborn neutropenia patients.

In this retrospective study, we aimed to investigate the changes in leukocyte counts and ratios in neonates diagnosed with neutropenia (absolute neutrophil count/ANC<1,000/mm3) who received G-CSF therapy in the NICU. We evaluated the effect of G-CSF administration on neutrophil counts and I/T ratio, in addition to the duration of time for the I/T ratio to return the normal values (I/T ratio<0.2).

Methods

This retrospective study included infants hospitalized in the NICU of Hacettepe University İhsan Doğramacı Children's Hospital, Ankara, between 2005 and 2017 who received G-CSF therapy (10 μg/kg/dose) for neutropenia. Subjects' demographic data, neonatal findings, neutropenia etiology, timing and duration of G-CSF therapy, as well as hematological laboratory parameters were evaluated. The ANC was calculated based on white blood cell counts from a complete blood count and the PMNL fraction in a peripheral smear (ANC = total white blood cell count x PMNL percentage).

The G-CSF therapy was administered to patients with ANC < 1,000/mm^3 based on complete blood count and peripheral smear results obtained during follow-ups in the NICU. G-CSF was administered once daily at a dose of 10 μg/kg. Patients with persistent neutropenia (ANC<1,000) according to the daily complete blood counts and peripheral smears were
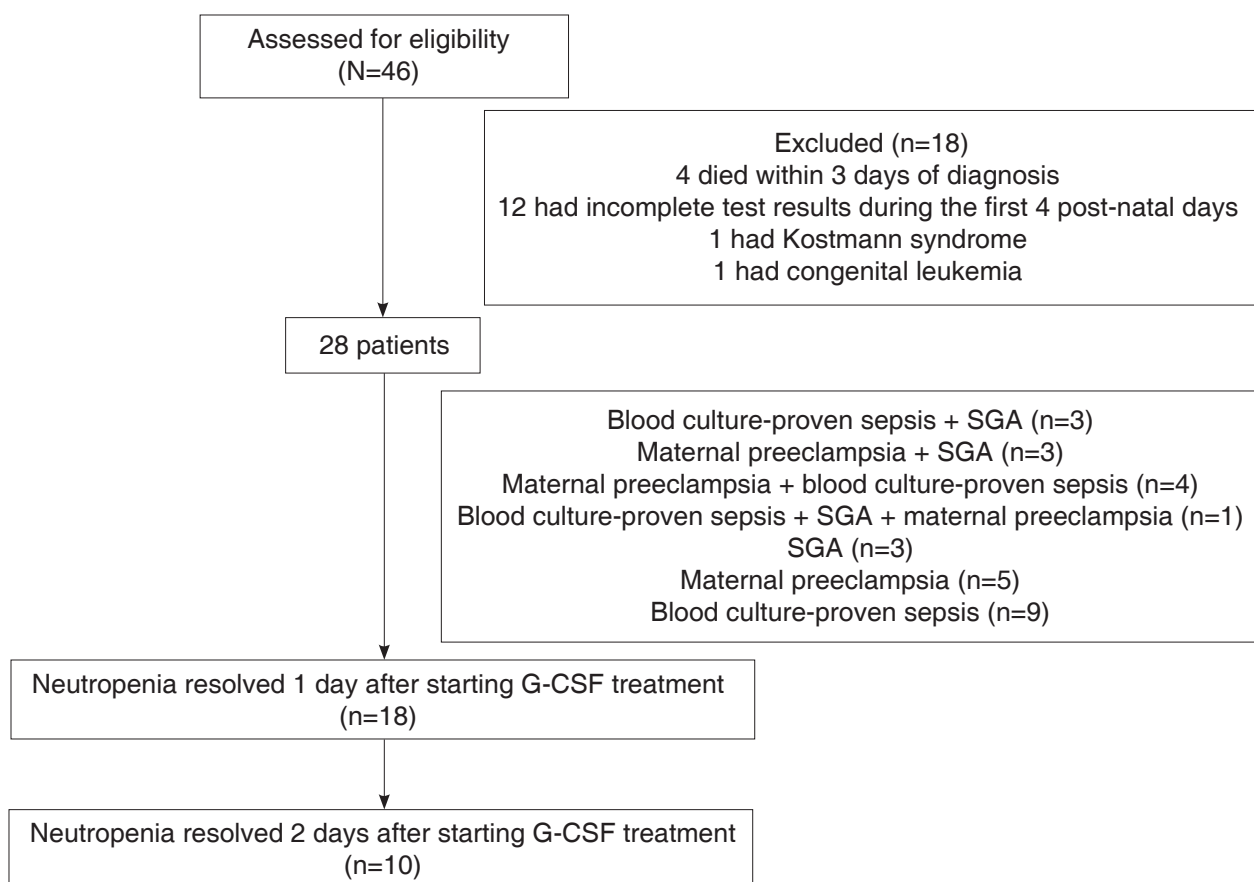

Figure 1. Study flow chart

given a second dose of 10 μg/kg on day 2. The study flow chart is shown in **Figure 1**.

Immature-to-total neutrophil ratio was calculated by the following formula: immature neutrophil count (%) / total neutrophil count (mature+immature) (%). If the I/T ratio was higher than 0.2, sepsis is considered.[14] Newborns with at least one of the signs or symptoms from each of the four categories are diagnosed with sepsis. The categories are clinical (temperature instability >37.9°C or <36°C, heart rate of >180 beats/min,, respiratory rate >60 breaths/min., lethargy/ altered mental status, glucose intolerance, feeding intolerance), hemodynamic (arterial hypotension), tissue perfusion (decreased capillary refill, increased plasma lactate) or inflammatory variables (leukocytosis, leukopenia, immature neutrophils, increased I/T ratio, thrombocytopenia, increased C-reactive protein, procalcitonin) and for whom no other reason explained the findings other than sepsis.[15,16]

Statistical analyses were performed using *IBM SPSS Statistics for Windows* (*version 22; IBM Corp.*, Armonk, NY, USA). Variables with normal distributions were evaluated using independent T-test, while those with non-normal distributions were calculated using the non-parametric Mann-Whitney U test. A P value of < 0.05 was considered to be statistically significant. Additionally, a related-samples Friedman's two-way analysis of variance by ranks test was used to evaluate changes in complete blood count parameters between the days. Numerical values are shown in mean [standard deviation (SD)] and median [interquartile range 25-75% (IQR)]. The Clinical Research Ethics Committee of Hacettepe University approved this study.

Results

A total of 46 patients who received G-CSF therapy were identified from medical records. Of these, 4 patients died within 3 days of G-CSF administration, 12 patients had incomplete data, 1 patient had Kostmann syndrome, and 1 patient had congenital leukemia. These 18 patients were excluded, therefore, a total of 28 patients were included in the study. Subjects' demographic and neonatal characteristics are presented in **Table 1**. Of the 28 subjects, 12 were male, 21 were premature (<37 weeks of gestation), and 13 had

mothers with preeclampsia. Median gestational age was 32.6 (IQR 29.7-37.6) weeks and median birth weight was 1,630 (IQR 1,040-2,980) g. Ten of the newborns were SGA. Subjects' median post-natal day of G-CSF initiation was day 5 (IQR 2-13) and median number of G-CSF doses was 1.0 (IQR 1.0-2.0). Seventeen subjects had culture-proven bacterial sepsis. The median length of the hospital stay was 25 (IQR 15-36) days and the survival rate was 67.9% (19 patients).

The most common causes of neutropenia in the NICU were sepsis, SGA birth weight, and maternal preeclampsia.[2] In our study, 13/28 infants were born to mothers with preeclampsia and 10/28 infants were SGA. Seventeen out of 28 patients were concurrently diagnosed with proven sepsis and neutropenia. Ten patients in our study required a second dose of G-CSF. Of these, 6/28 were diagnosed with culture-proven sepsis. There was no statistically significant difference between patients with and without sepsis in terms of a second G-CSF dose requirement (P=1.000).

Table 1. Demographic data and neonatal characteristics of subjects

Characteristics	(N=28)
Gender, M/F, n (%)	12/16 (42.9/57.1)
Maternal preeclampsia, n (%)	13 (46.2)
Mode of delivery, NSVD/CS, n (%)	7/21 (25.0/75.0)
Gestational age, weeks	
Mean (SD)	33.3 (4.3)
Median (IQR)	32.6 (29.7-37.6)
Birth weight (g)	
Mean (SD)	1,908 (922)
Median (IQR)	1,630 (1,040–2,980)
Apgar score (5th min)	
Mean (SD)	7.9 (1.6)
Median (IQR)	8 (7–9)
Resuscitation at birth, n (%)	16 (57.1)
SGA, n (%)	10 (35.7)
Onset of G-CSF treatment, day of life	
Mean (SD)	7.5 (7.2)
Median (IQR)	5 (2–13)
Number of doses	
Mean (SD)	1.4 (0.5)
Median (IQR)	1.0 (1.0–2.0)
Sepsis, n (%)	17 (60.7)
Duration of hospitalization, days	
Mean (SD)	32 (29)
Median (IQR)	25 (15–36)
Discharge/death, n (%)	19/9 (67.9/32.1)

NSVD=normal spontaneous vaginal delivery, CS=cesarean section

During follow-up, 3/28 patients developed acute respiratory distress syndrome, 1/28 developed bronchopulmonary dysplasia, 1/28 developed necrotizing enterocolitis, and 2/28 developed pneumonia. Nine out of 28 patients received phototherapy for indirect hyperbilirubinemia.

Subjects' complete blood counts on day 0 (immediately before receiving the G-CSF therapy) and days 1, 2, and 3 days after treatment are shown in **Table 2**. On day 3, there were significant decreases in hemoglobin, hematocrit, and lymphocyte percentage values (P=0.043, P=0.005, and P<0.001, respectively) and significant increases in white blood cell counts, ANCs, and lymphocyte counts (P<0.001, P<0.001, and P<0.001, respectively).

There were statistically significant elevations in I/T ratios between day 0 and day 1 (P<0.001) and between day 0 and day 2 (P<0.001), however, I/T ratio was not significantly different between day 0 and day 3 (P=1.000). I/T ratio increased and remained elevated for 24-48 hours after treatment.

At day 0, ANC values were significantly lower in the sepsis group (n = 17) than in the non-sepsis group (n = 11) (P=0.005). In addition, at day 0, I/T ratio was significantly higher in the sepsis group than in the non-sepsis group (P= 0.008). There were no significant differences between the groups in terms of ANC and I/T ratios at other times (**Table 3**).

Patients were divided into three subgroups of risk factors (sepsis, SGA, and maternal preeclampsia): one risk factor (n=17), two risk factors (n=10) and three risk factors (n=1). Statistical analysis was not performed on the three-risk factor group because there was only one patient. There was no statistically significant difference between the groups with one and two risk factors in terms of ANC and I / T ratio at all time points (**Table 4**).

Table 2. Complete blood and peripheral smear counts in median (IQR)

Parameter	Day 0	Day 1	Day 2	Day 3	P value
Hemoglobin, g/dL	13.0 (9.9-14.5)	13.1 (10.5-13.9)	11.7 (9.7-13.6)	11.1 (9.8-12.5)	0.043
Hematocrit, %	37.9 (30.9-43.9)	37.8 (33.6-41.3)	33.2 (30.6-38.7)	32.9 (30.8-35.5)	0.005
White blood cell count, /mm^3	2,450 (2,100-3,250)	5,300 (4,450-8,600)	9,350 (7,400-15,350)	9,200 (6,850-14,050)	0.001
Platelet count, /mm^3	122,000 (69,000-188,000)	115,000 (57,000-163,000)	129,000 (62,000-179,000)	147,000 (102,000-218,000)	0.180
I/T ratio	0.00 (0.00-0.07)	0.19 (0.11-0.36)	0.22 (0.13-0.33)	0.06 (0.02-0.14)	<0.001*
Lymphocyte ratio, %	62 (53-81)	58 (39-76)	40 (31-50)	35 (30-46)	<0.001
Lymphocyte count, /mm^3	1,500 (1,112-2,620)	3,388 (1,543-4,771)	3,880 (2,334-6,013)	3,131 (2,424-6,161)	<0.001
ANC, /mm3	470 (192-770)	1,801 (859-3,905)	5,332 (3,161-7,570)	4,907 (3,429-7,813)	<0.001

All data was expressed as median (25-75% interquartile range); P value for day 0 *vs.* day 3 (except * for day 0 *vs.* day 1)

Table 3. The ANC and I/T ratios

Parameter	Sepsis		P value
	Positive (n=17)	Negative (n=11)	
ANC, /mm^3 day 0	204 (10-440)	672 (354-843)	0.005
ANC, /mm^3 day 1	2058 (1145-4566)	1708 (540-3100)	0.168
ANC, /mm^3 day 2	5564 (3161-7892)	5100(3111-6120)	0.712
ANC, /mm^3 day 3	5022 (3429-7486)	4508 (3355-9724)	0.382
I/T ratio day 0	0.20 (0.12-0.25)	0.00(0.00-0.00)	0.008
I/T ratio day 1	0.23 (0.11-0.36)	0.14 (0.10-0.38)	0.515
I/T ratio day 2	0.24 (0.11-0.34)	0.14 (0.13-0.33)	0.555
I/T ratio day 3	0.08 (0.00-0.15)	0.06 (0.03-0.12)	0.989

All data was expressed as median (25-75% interquartile range).

The difference in I/T ratio between day 1 and day 2 was not significant (P=1.000), but there was a significant decrease on day 3 compared to days 1 and 2 (P<0.001 and P=0.002, respectively). The changes in the I/T ratio according to days of G-CSF therapy are shown in **Figure 2.**

Discussion

Although neutropenia is a common problem in the NICU, there is no consensus on optimal treatments. G-CSF therapy is among the treatments most commonly administered to newborns with neutropenia;[7] however, conflicting results have been reported regarding its use. A previous review reported no adverse effects, but noted that G-CSF efficacy for sepsis treatment or prophylaxis in preterm infants was uncertain.[17]

Table 4. ANC, IT ratios, and risk factors

Parameter	Risk factors (sepsis, SGA, preeclampsia)		P value
	Patients with one risk factor (n=17)	Patients with two risk factors (n=10)	
ANC, /mm3 day 0	500 (222-792)	300 (126-756)	0.224
ANC, /mm3 day 1	2,288 (972-4566)	1,708 (760-3312)	0.195
ANC, /mm3 day 2	5,564 (3161-7892)	4,752(2652-7540)	0.961
ANC, /mm3 day 3	4,212 (3350-8401)	5,334 (3458-8372)	0.443
I/T ratio day 0	0.00 (0.00-0.06)	0.00(0.00-0.00)	0.563
I/T ratio day 1	0.14 (0.08-0.30)	0.24 (0.14-0.40)	0.166
I/T ratio day 2	0.17 (0.13-0.31)	0.24 (0.15-0.37)	0.476
I/T ratio day 3	0.06 (0.15-0.12)	0.06 (0.00-0.17)	0.434

All data were expressed as median (25-75% interquartile range)
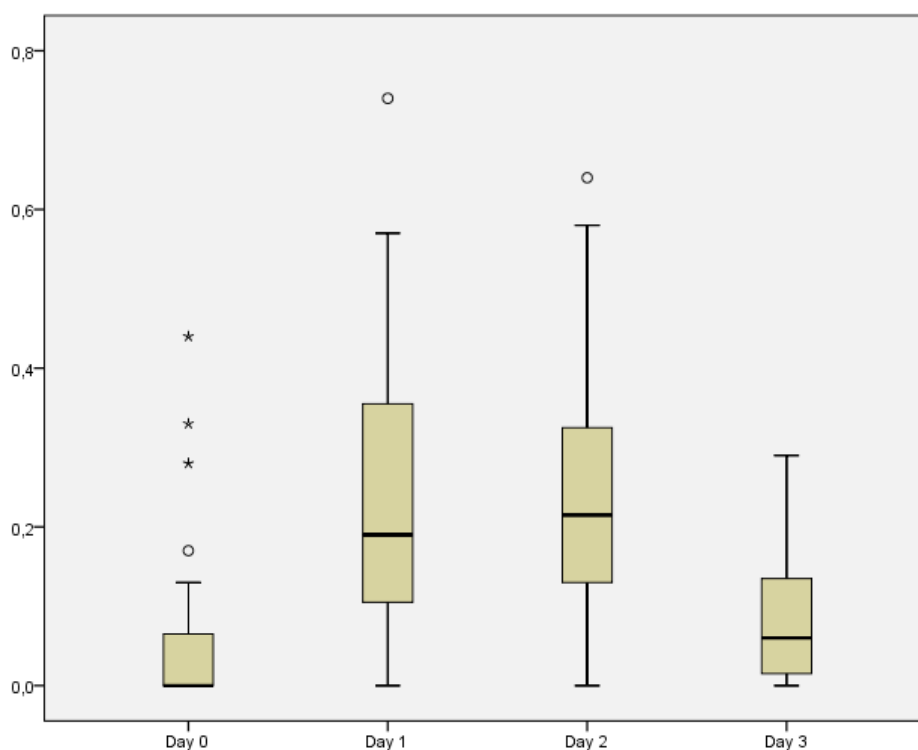

Figure 2. Distribution of I/T ratio based on G-CSF treatment day

According to a Cochrane review of 257 infants with suspected systemic bacterial infections, there was insufficient evidence for G-CSF either as treatment of established systemic infection or as prophylaxis to prevent systemic infection in high risk neonates. No toxicity from G-CSF use was reported in any study included in that review. They concluded that G-CSF treatment may reduce mortality when systemic infection is accompanied by severe neutropenia and this should be investigated further in adequately powered trials which recruit sufficient infants infected with organisms associated with a significant mortality risk.[18] In a more recent study, G-CSF therapy decreased the hematological recovery time, however, it was associated with higher secondary sepsis and mortality rates in neutropenic infants.[8] As a result, G-CSF is not routinely recommended for newborns with sepsis or suspected sepsis. According to the Cochrane review, G-CSF is only recommended for neutropenic newborns with proven sepsis.[18] In our study, G-CSF was used only in neutropenic newborns with proven sepsis or in newborns with severe neutropenia mostly due to maternal preeclampsia. We believe that the significant decreases in hemoglobin and hematocrit values after G-CSF administration in our study were secondary to the frequent blood specimen collections for laboratory tests, prematurity, and other existing neonatal problems. Although sepsis,[3,13] SGA birth weight,[2] and maternal preeclampsia[19] are risk factors for thrombocytopenia in newborns, we observed no significant differences in platelet values in our study (P=0.180). A study reported that a G-CSF treatment group exhibited no change in platelet values after G-CSF administration when compared to baseline.[10]

In our study, there was a statistically significant decrease in lymphocyte percentage, and it was not associated with a decrease in total lymphocyte count. It was likely due to significant and rapid increases in white blood cell counts and ANC values observed after G-CSF administration. A previous study reported no difference between absolute lymphocyte counts determined 1 day before and 28 days after G-CSF treatment.[10]

In our subjects, neutropenia resolved a median of 1 day after G-CSF therapy. Aktaş *et al.*[12] studied 56 neonates with sepsis, with and without G-CSF treatment [mean gestational age 28.9 (2) *vs.* 29.7 (2) weeks, respectively]. Neutropenia resolved in a mean of 3 (SD 0.5) days in infants who received G-CSF therapy. Neutropenia resolution time was shorter in our study. A study observed a three-fold increase in ANC values within 24 hours after G-CSF administration in the treatment of neutropenic infants born to normotensive mothers and preeclamptic hypertensive mothers.[20] We also observed a rapid increase in ANC in our study (median ANC values increased more than 3 times in 24 hours).

There are conflicting results in the literature regarding the evaluation of I/T ratio in infants treated with G-CSF. A study observed no differences in I/T ratio between groups with and without G-CSF treatment, on days 1, 2, and 3.[12] In a previous double-blind, randomized controlled trial involving 40 non-neutropenic newborns with sepsis and gestational age 33-36 weeks, I/T ratio remained steady throughout treatment in the G-CSF group, but it significantly decreased in the group that did not receive G-CSF.[21] However, the existing literature is insufficient in terms of when I/T ratio returns to normal and when it can be reliably used again as a marker of sepsis. Additional randomized controlled trials with larger patient populations are needed to reach a more definitive conclusion.

In conclusion, despite the small sample size of our study, changes in I/T ratio observed after G-CSF treatment suggest that I/T ratio can be confidently used again as a sepsis marker starting 72 hours after G-CSF administration. However, I/T ratio within the first 72 hours of G-CSF administration may not be safely used as a sepsis marker.

Conflict of Interest

None declared.

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