Research Article

Melek Büyükeren*, Hasan Tolga Çelik, Şule Yiğit, Oytun Portakal, Banu Sancak and Murat Yurdakök

The role of the delta neutrophil index in determining the etiology of neonatal sepsis

Neonatal sepsis etiolojisinin belirlenmesinde delta nötrofil indeksinin rolü

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Abstract

Objectives: To demonstrate immature granulocyte (IG) count and delta neutrophil index (DNI) values (novel potential predictive marker for neonatal sepsis) for neonates.

Methods: This prospective controlled clinical study was consisted of 208 patients (77 in the study group and 131 in the control group) who were delivered between January 2016 and January 2018 at the Hacettepe University Neonatal Intensive Care Unit in Ankara, Turkey. In this study, we evaluated value of DNI in diagnosing neonatal sepsis by comparing the DNI values in culture positive septic neonates with healthy neonates.

Results: In our study, the median interquartile range (IQR = 25–75%) DNI was 0.1% (0.0–0.3%) in the control group and 1.5% (1.0–2.45%) in the sepsis group ($p < 0.05$). In our ROC curve analysis, the cut-off value for the DNI as a sepsis marker was 0.65%, with 96.2% specificity and 97.4% sensitivity. Those patients with gram-negative isolates had significantly higher DNI and IG counts when compared to those patients with gram-positive bacteria ($p < 0.05$).

***Corresponding author: Melek Büyükeren MD**, Department of Pediatrics, Division of Neonatology, Hacettepe University Faculty of Medicine Sıhhiye, Altındağ, 06100 Ankara, Turkey, Phone: +90 3123051390. E-mail: melekbuyukeren@hotmail.com
Hasan Tolga Çelik, Şule Yiğit and Murat Yurdakök: Department of Pediatrics, Division of Neonatology, Hacettepe University Faculty of Medicine, Ankara, Turkey, E-mail: htcelik@gmail.com (H.T. Çelik), suleyigit6@gmail.com (Ş. Yiğit), muratyurdakok@yahoo.com (M. Yurdakök)

Oytun Portakal: Department of Biochemistry, Hacettepe University Faculty of Medicine, Ankara, Turkey, E-mail: oytun@hacettepe.edu.tr

Banu Sancak: Department of Clinical Microbiology, Hacettepe University Faculty of Medicine, Ankara, Turkey, E-mail: banusancak@yahoo.com

Conclusions: Our findings indicated that the DNI counts are significant diagnostic biomarkers for neonatal sepsis. They may also have utility in determining the sepsis etiology (differentiating between gram-positive and gram-negative agents).

Keywords: delta neutrophil index; early granulocyte count; neonatal sepsis; newborn; sepsis.

Öz

Amaç: Yenidoğanlarda olgunlaşmamış granülosit (IG) sayısı ve delta nötrofil indeks (DNI) değerlerini (yenidoğan sepsisi için yeni potansiyel prediktif belirteç) göstermek.

Gereç ve Yöntem: Bu prospektif kontrollü klinik çalışmada, Ocak 2016–Ocak 2018 tarihleri arasında Hacettepe Üniversitesi Yenidoğan Yoğun Bakım Ünitesinde yatan 208 hasta (çalışma grubunda 77 ve kontrol grubunda 131) değerlendirildi. Bu çalışmada, kültür kanıtlı sepsis tanısı alan yenidoğanların DNI değerlerini, sağlıklı yenidoğanlarla karşılaştırarak; neonatal sepsis tanısı için DNI ölçümünü değerlendirdik.

Bulgular: Çalışmamızda, DNI değerleri; ortanca (% 25–75 persentil) kontrol grubunda % 0.1 (% 0.0–0.3) ve sepsis grubunda % 1.5 (% 1.0–2.45) idi ($p < 0.05$). ROC eğrisi analizinde, sepsis markeri olarak DNI için cut-off değeri % 0.65 (% 96.2 özgüllük ve % 97.4 duyarlılık) idi. Gram-negatif bakteriler ile sepsis olan hastalar, gram-pozitif bakteriler ile sepsis olan hastalara kıyasla önemli ölçüde daha yüksek DNI ve IG sayılarına sahipti ($p < 0.05$).

Sonuç: Bulgularımız DNI değerinin yenidoğan sepsisi için önemli bir tanısal biyobelirteç olabileceğini göstermiştir. Ayrıca sepsis etiolojisinin belirlenmesinde de faydalı olabilir (Gram-pozitif ve gram-negatif etkenler arasında ayrım yapılabilir).

Anahtar kelimeler: Delta nötrofil indeksi; erken granülozit sayısı; sepsis; yenidoğan; yenidoğan sepsisi.

Introduction

Neonatal sepsis is one of the major causes of mortality and morbidity in newborns [1, 2] and, the incidence of confirmed sepsis in developing countries is 16 per 1,000 live births [3]. Due to the mortality risk, it is important to diagnose sepsis early and initiate the appropriate treatment. The gold standard method for the diagnosis of sepsis is the isolation microorganism of the blood culture [4]. However, due to the physiological features of neonates and the limitations in the laboratory techniques, blood cultures require 48–72 h to detect bacterial growth, and they may even yield false negative results. Therefore, biomarkers can be used to obtain rapid results supporting a sepsis diagnosis, with new biomarkers constantly in development [5]. The most commonly used biomarkers are the C-reactive protein (CRP) level, procalcitonin level, total leukocyte count, and immature to total (I/T) neutrophil ratio [6, 7]. However, there is still no ideal laboratory test with high sensitivity and specificity; therefore, research is ongoing to identify inexpensive and more easily measured laboratory biomarkers. In recent clinical studies, it has been proposed that the immature granulocyte (IG) ratio automatically obtained from complete blood count devices (delta neutrophil index, DNI) can be used as a new sepsis biomarker.

In this study, we examined the use of the DNI in diagnosing neonatal sepsis by comparing the DNI values of healthy neonates (without sepsis) to those of neonates with blood culture-confirmed sepsis. In addition, we aimed to find a DNI cut-off value for use in the diagnosis of sepsis.

Materials and methods

This prospective observational clinical study was conducted between January 2016 and January 2018 at Hacettepe University Neonatal Intensive Care Unit in Ankara, Turkey. Approval was obtained prior to the study from Hacettepe University Clinical Research Ethics Committee (GO-17/824-29).

Patients

The research population included those patients being monitored in the neonatal intensive care unit who were diagnosed with sepsis based on bacterial growth in a blood culture. Newborns with at least one of the four groups (clinical, hemodynamic, tissue perfusion, and inflammatory variables) listed in Table 1 and whose findings were not identified with any other disease other than sepsis were included in the study [8, 9].

Table 1: Criteria for sepsis diagnosis (modified from Ref. [8]).

Clinical variables
Temperature instability (fever $>38.0^{\circ}\text{C}$, hypothermia $<36.0^{\circ}\text{C}$)
Heart rate > 2 SD above normal for age (≥ 180 beats/min, ≤ 100 beats/min)
Respiratory rate >60 breaths/min plus grunting/recession or desaturations
Lethargy/altered mental status
Glucose intolerance (plasma glucose >10 mmol/L)
Feeding intolerance
Hemodynamic variables
Arterial hypotension (blood pressure 2 SD below normal for gestational age)
Tissue perfusion variables
Capillary refill >3 s
Plasma lactate >3 mmol/L
Inflammatory variables
Leukocytosis (WBC count $>15,000/\text{mm}^3$)
Leukopenia (WBC count $<4,000/\text{mm}^3$)
Immature neutrophils (band forms) $>10\%$
Immature: Total neutrophil ratio >0.2
Thrombocytopenia ($<100,000/\text{mm}^3$)
CRP >0.8 mg/dL
Procalcitonin >2.0 ng/mL
CRP, C-reactive protein; WBC, white blood cell count.

The control group consisted of those patients admitted to the neonatal intensive care unit with no suspicion or diagnosis of sepsis and for whom a complete blood count was requested for any reason. No separate blood sampling was done for this study. In the control group, there were no signs of sepsis (Table 1) and antibiotic use until the sample was taken and three days later. Patients in the control group were selected from NICU were the similar to the study group in terms of gender, gestational age and birth weight.

A total of 281 patients were initially included in the study: 103 in the research group and 178 in the control group. However, 73 patients with incomplete consent forms and incomplete laboratory results were excluded. Therefore, the study was completed with a total of 208 patients: 77 in the study group and 131 in the control group. The patient flow chart is shown in Figure 1.

If the patients with the microorganisms isolated in the blood cultures, but not clinically compatible with sepsis, blood culture results were evaluated as contamination and these patients were not included in the study.

Biochemical analysis

Blood sampling: A complete blood count, peripheral blood smear, CRP level, procalcitonin level, and blood culture were obtained immediately before the antibiotic treatment was initiated for the patients suspected of having sepsis.

For the complete blood count, the blood samples were placed into tubes containing ethylenediaminetetraacetic acid (K2-EDTA) and sent to the laboratory within 1 h. Peripheral cell counting, differential, IG counting and blood smear were performed by Unicel DxH 800 (Beckman Coulter, Inc., Brea, CA, USA) automated cell counter. IG of

each patient was obtained from the system [10]. For blood smear Wright's stain was used.

I/T neutrophil ratio and immature and total neutrophil counts were determined by microscopic examination at 100× magnification [11].

To determine serum CRP and procalcitonin were taken into the ST tubes, and transport to the laboratory in 1 h. Serum procalcitonin levels were measured by a homogeny immunoassay method, TRACE (Time Resolved Amplified Cryptate Emission, Kryptor; Brahms, Germany) method, with a high limit of detection (0 ng/mL) and a linear range of 0.02–100 ng/mL. Serum CRP levels were measured by rate nephelometry (Beckman Coulter Inc., CA, USA) method with a linearity of 5–300 mg/dL.

In the study group, the DNI values unchecked after the antibiotic therapy.

Unicel DxH 800: The Unicel DxH 800 uses the electrical impedance, radiofrequency conductivity, and the volume, conductivity, and multiangle light scattering to count and distinguish between the leukocyte subpopulations [10]. The DNI percentage of each patient was obtained from the Unicel DxH 800. After that, the IG count is automatically calculated White blood cellxDNI percentage.

Statistical analysis

The statistical analyses of this study were done using IBM SPSS Statistics for Windows version 22 (IBM Corp., Armonk, NY, USA). The variables with normal distributions were evaluated using an independent samples *t*-test, and the variables with non-normal distributions were evaluated using the nonparametric Mann-Whitney *U* test. The categorical variables were analyzed using Fisher's exact and Pearson's chi-squared tests. *p*-values <0.05 were considered to be statistically significant. The counted variables were expressed as the mean (±standard deviation), and the measured values were expressed as the median (minimum–maximum, 25th–75th percentile).

Results

The demographic and neonatal characteristics of the 77 patients in the study group and 131 patients in the control group are shown in Table 2.

There were no statistical differences between the study group and the control group in terms of the gender ($p = 0.621$), weeks of gestation ($p = 0.953$), birth weight ($p = 0.568$), use of assisted conception techniques ($p = 0.247$), indirect hyperbilirubinemia ($p = 0.271$), or intracranial hemorrhage frequency ($p = 0.104$). However, the 5-min Apgar scores were significantly lower in the study group than in the control group ($p < 0.001$). Moreover, the study group showed significantly higher rates of resuscitation ($p < 0.001$), respiratory distress syndrome ($p = 0.011$), necrotizing enterocolitis ($p = 0.021$), patent ductus arteriosus ($p = 0.001$), bronchopulmonary dysplasia ($p = 0.003$), mortality ($p = 0.003$), blood sampling day ($p < 0.001$), and longer hospital stay ($p < 0.001$). Caesarean deliveries were significantly more common in the control group than in the research group ($p < 0.001$).

The complete blood counts and acute phase reactants of the patients are given in Table 3.

The hemoglobin and hematocrit values were significantly lower in the study group than in the control group ($p < 0.001$ for both), and there was no statistically significant difference between the groups regarding white blood cell count ($p = 0.150$). The study group had a significantly lower mean platelet count when compared to the control group ($p = 0.003$). However, the study group had statistically higher values for the absolute neutrophil count (ANC)

Figure 1: Flow chart of the study and control group.

Table 2: Demographic features of patients included in the study.

	Study group n = 77	Control group n = 131	p-value
Gender, n (%) (Female/Male)	39/38 (50.6/49.4)	71/60 (54.2/45.8)	0.621
Gestational age (week) ^a	34.3 ± 5.4	34.8 ± 3.5	0.953
Birth weight (gr) ^b	2,480 (1,110–3,235)	2,310 (1,700–3,160)	0.568
5th minute Apgar scores ^b	8 (7–9)	10 (10–10)	<0.001
Mode of delivery, n (%) (NSVD/CS)	22/55 (28.6/71.4)	3/128 (2.3/97.7)	<0.001
Resuscitation, n (%)	28 (36.4)	9 (6.9)	<0.001
Assisted reproductive technology, n (%)	9 (11.7)	8 (6.1)	0.247
RDS, n (%)	24 (31.2)	20 (15.3)	0.011
IHB, n (%)	21 (27.3)	27 (20.6)	0.271
NEC, n (%)	15 (19.5)	10 (7.6)	0.021
PDA, n (%)	15 (19.5)	5 (3.8)	0.001
BPD, n (%)	19 (24.7)	11 (8.4)	0.003
ICH, n (%)	7 (9.1)	4 (3.1)	0.104
Age at sepsis diagnosis (days) ^b	15 (7–33)		
Age at blood collection (days) ^b	15 (7–33)	9 (5–18)	<0.001
Duration of hospitalization (day) ^b	28 (15–72)	11 (5–20)	<0.001

VD, normal vaginal delivery; CS, cesarean delivery; RDS, Respiratory distress syndrome; IHB, Indirect hyperbilirubinemia; NEC, Necrotizing enterocolitis; PDA, Patent ductus arteriosus; BPD, Bronchopulmonary dysplasia; ICH, Intracranial hemorrhage.

^aMean ± standard deviation.

^bMedian (25th–75th percentile).

($p = 0.015$), I/T neutrophil ratio ($p < 0.001$), DNI percentage ($p < 0.001$), IG count ($p < 0.001$), and neutrophil to lymphocyte ratio (NLR) ($p < 0.001$). The CRP and procalcitonin values could not be compared because they were not evaluated in the control group.

In our study, the median interquartile range (IQR = 25–75%) DNI was 0.1% (0.0–0.3%) in the control group and 1.5% (1.0–2.45%) in the sepsis group ($p < 0.05$). We determined the DNI cut-off values according to the control and study groups using a receiver operating characteristic (ROC) curve analysis (Figure 2). In our study, a DNI cut-off

value of 0.65% had 96.2% specificity and 97.4% sensitivity as a sepsis biomarker.

The microorganisms isolated in the blood cultures of the 77 patients in the study group and their gram staining characteristics are shown in Table 4.

The blood cultures yielded gram-positive bacteria in 51 patients and gram-negative bacteria in 24 patients in the study group. Fungi were isolated in two patient blood cultures (2.6%).

Table 5 shows the comparison of the sepsis biomarkers between the patients with gram-positive and gram-

Table 3: The complete blood counts and acute phase reactants of the patients.

	Study group n = 77	Control group n = 131	p-value
Hemoglobin ^a (g/dL)	13.0 ± 2.9	14.8 ± 1.7	<0.001
Hematocrit ^a (%)	39.1 ± 8.7	46.3 ± 5.3	<0.001
Platelet count ^a (cells/ μ L)	1,74,000 (88,500–3,07,500)	2,40,000 (1,97,000–2,76,000)	0.003
WBC count ^b (cells/ μ L)	11,900 (7,150–18,350)	10,200 (8,600–13,000)	0.150
ANC ^b (cells/ μ L)	7,200 (3,700–11,500)	5,400 (3,900–7,300)	0.015
I/T ratio ^b	0.2 (0.18–0.25)	0.06 (0–0.11)	<0.001
DNI ^b (%)	1.5 (1.0–2.5)	0.1 (0–0.3)	<0.001
IG count ^b (cells/ μ L)	196 (88–355)	13 (0–35)	<0.001
CRP ^b (mg/dL)	3.02 (1.23–10.5)		
Procalcitonin ^b (ng/mL)	5.63 (1.05–16.53)		
NLR ^b	2.86 (1.38–6.45)	1.55 (1.04–2.00)	<0.001

WBC, White blood cell; ANC, Absolute neutrophil count; DNI, Delta neutrophil index; IG, Immature granulocyte, NLR, Neutrophil/lymphocyte ratio.

^aMean ± standard deviation.

^bMedian (25th–75th percentile).

Figure 2: Receiver operating characteristic curve for delta neutrophil index as a diagnostic marker of neonatal sepsis.

negative bacterial isolates in their blood cultures. The septic patients, whose blood cultures yielded *Candida* spp., were not included in the statistics because of their low number.

Those patients with gram-negative bacterial growth in their blood cultures had a significantly higher I/T neutrophil ratio, DNI percentage, IG count, and NLR value when

Table 5: Comparison of the sepsis biomarkers between the patients with gram-positive and gram-negative bacterial isolates in their blood cultures.

	Gram-positive, n = 51	Gram-negative, n = 24	p-value
Platelet count (cells/ μ L)	2,11,000 (1,32,000–3,37,000)	1,23,000 (26,250–1,78,250)	<0.001
WBC count (cells/ μ L)	12,000 (7,900–18,400)	10,350 (5,625–14,750)	0.175
ANC (cells/ μ L)	7,700 (3,600–12,000)	5,800 (3,900–12,125)	0.069
I/T ratio	0.2 (0.17–0.22)	0.24 (0.2–0.3)	<0.001
DNI (%)	1.2 (1.0–1.8)	2.7 (1.7–3.7)	<0.001
IG count (cells/ μ L)	175 (87–301)	318 (108–431)	<0.001
CRP (mg/dL)	2.31 (1.09–8.04)	4.30 (1.29–14.55)	0.103
Procalcitonin (ng/mL)	5.09 (0.75–16.53)	8.0 (1.97–35.32)	0.068
NLR	2.33 (1.27–7.19)	3.48 (1.76–6.35)	<0.001

WBC, White blood cell; ANC, Absolute neutrophil count; DNI, Delta neutrophil index; IG, Immature granulocyte; CRP, C-reactive protein; NLR, Neutrophil/lymphocyte ratio. Median (25th–75th percentile).

compared to those with the gram-positive isolates ($p < 0.001$ for all). There were no significant differences between the groups in terms of the white blood cell count ($p = 0.175$), ANC ($p = 0.069$), CRP level ($p = 0.103$), or procalcitonin level ($p = 0.068$).

Discussion

Sepsis is a major cause of mortality and morbidity that is frequently encountered in neonatal intensive care units [2]. While a positive blood culture remains the gold standard for diagnosis, isolating the agent in neonates is difficult for various reasons, and it requires time [4]. Therefore, sepsis biomarkers with high diagnostic sensitivity and specificity are needed.

One of the most commonly used parameters in neonatal units is I/T neutrophil ratio, a higher I/T neutrophil ratio indicates presence of a greater number of IGs (myelocyte, promyelocyte, and metamyelocyte) in the peripheral circulation. An I/T neutrophil ratio over 0.2 is accepted as an indicator of sepsis [11]; however, there may be observer-dependent variability in this parameter because, it is based on an observer evaluation of blood cells in a peripheral smear [7]. This has led to the recent development of automated hematology analyzers that are able to count IGs [12]. There is a growing body of evidence demonstrating the relationship between the automated IG count and infection [13–15].

Table 4: The microorganisms isolated in blood cultures.

Gram-positive bacteria n=51	n (%)	Gram-negative bacteria n = 24	n (%)
<i>Staphylococcus epidermidis</i>	15 (19.5)	<i>Klebsiella pneumoniae</i>	15 (19.5)
<i>Staphylococcus capitis</i>	11 (14.2)	<i>Serratia marcescens</i>	3 (3.9)
<i>Staphylococcus aureus</i>	6 (7.8)	<i>Enterobacter cloacae</i>	3 (3.9)
<i>Staphylococcus hemolyticus</i>	4 (5.2)	<i>Acinetobacter baumannii</i>	1 (1.3)
<i>Staphylococcus hominis</i>	3 (3.9)	<i>Sphingomonas paucimobilis</i>	1 (1.3)
<i>Paenibacillus</i> spp.	3 (3.9)	<i>Pseudomonas</i> spp.	1 (1.3)
<i>Enterococcus faecium</i>	3 (3.9)		
<i>Streptococcus agalactiae</i>	2 (2.6)		
<i>Bacillus licheniformis</i>	2 (2.6)		
<i>Streptococcus mitis/oralis</i>	1 (1.3)		
<i>Streptococcus parasanguinis</i>	1 (1.3)		

Nahm et al. [14] reported no statistical difference between the number of immature neutrophils counted by an automated hematology analyzer and that calculated by a hematologist based on a manual count of 200 cells in a peripheral smear. Therefore, we planned the present study to determine the sensitivity and specificity of the DNI calculated automatically by a hematology analyzer during a complete blood count in the diagnosis of sepsis. In our study, the median ([IQR] = 25–75%) I/T neutrophil ratio was 0.2 (0.18–0.25) in the study group, which was significantly higher than that in the control group [0.06 (0–0.11)] ($p < 0.001$). Similar to our findings, Zaki et al. [16] reported a higher I/T neutrophil ratio in the infected group than 0.12 $\pm$ 0.12 the control group (0.30 $\pm$ 0.17 vs. 0.12 $\pm$ 0.12).

Cimenti et al. [7] determined a DNI cut-off value as 1.3%, comparing DNI values of 21 septic newborns with 112 control newborns. Lee et al. [17] compared the DNI values of 24 newborns with sepsis and 48 babies without sepsis and found statistically significant differences. The mean DNI at the time of diagnosis was 6.5 $\pm$ 2.4% in the septic patients who died, 3.7 $\pm$ 1.8% in the septic patients who survived, and 1.1 $\pm$ 0.7% in the healthy controls. Çelik et al. [18] found that cut-off level of DNI was 4.6 with 85% sensitivity and 80% specificity in the study in which they examined cases with both proven and clinically neonatal sepsis. In our study, only cases with proven sepsis were included in the study. In our study, the median (IQR = 25–75%) DNI was 0.1% (0.0–0.3%) in the control group and 1.5% (1.0–2.45%) in the sepsis group ($p < 0.001$). In our ROC curve analysis, the cut-off value for the DNI as a sepsis marker was 0.65%, with 96.2% specificity and 97.4% sensitivity. According to many previous studies, the number of newborn infants is higher. Senthilnayagam et al. [19] reported that a study of 200 patients with 29 newborns, 0.5% for IG could be used for bacteraemia detection with 86.3% sensitivity and 92.2% sensitivity. Fernandes and Hamaguchi [20] found that the optimal timing of detect the immature granulocyte count was within 60 min after the blood was taken, when the IG value in healthy adults was below 0.52%. In other studies, there is insufficient data on the timeline of the blood samples were analyzed. In our study, blood samples taken at the time of sepsis diagnosis were analyzed within 60 min. In a study, involving more than 2,400 specimens, designed to determine the reference range of the immature granulocyte ratio according to age, the upper limit for IG in both children under 10 years and in infants was suggested as 0.3% [21]. There is a need for more prospective clinical trials involving larger numbers of cases of sepsis and healthy controls from different age groups.

We determined that the median IG count calculated using the DNI value was higher in the sepsis group than in the control group in the present study (196 vs. 13 μ l, respectively) ($p < 0.001$). Cimenti et al. [7] reported a cut-off value of 240/ μ l for the IG count in their study.

In our study, the median (IQR = 25–75%) NLR was 2.86 (1.38–6.45) in the sepsis group and 1.55 (1.04–2.00) in the control group. Alkan Özdemir et al. [22] reported 73% sensitivity and 78% specificity using an NLR cut-off value of 1.77, and Omran et al. [23] calculated a cut-off value of 2.7. In our study, the median NLR in the sepsis group was higher than the cut-off values reported in the other studies, and it was statistically significantly higher than in the control group ($p < 0.001$).

In this study, the most common causative agents isolated in the blood cultures of the septic patients were *Klebsiella pneumonia* (15 patients) and *Staphylococcus epidermidis* (15 patients). Gram-positive organisms were isolated in 51 patients (66.2%) and gram-negative organisms in 24 patients (31.2%). Those patients with gram-negative isolates had significantly higher DNI and IG counts when compared to those patients with gram-positive agents ($p < 0.001$ for both). We attributed this finding to the fact that gram-negative bacteria cause a more severe response to infection. Similar to the results of our study, in the study of Celik et al. [18], newborns with gram negative sepsis had higher DNI values than gram positives.

Chacha et al. [24] reported higher CRP values in the patients with gram-negative isolates. However, in our study, there were no statistical differences between the gram-positive and gram-negative groups in terms of the white blood cell count, ANC, CRP level, or procalcitonin levels. Lai et al. [25] analyzed 1,010 CRP values in neonates diagnosed as sepsis, and found that the patients with lower CRP levels had fewer gram-negative isolates. In another study by Fendler et al. [26] including 78 newborns with gram-negative and gram-positive isolates, the procalcitonin levels were found higher in the group with the gram-negative agents. We attributed the lack of a significant difference in our study to the smaller patient number.

Since only neonates were included in our study, no generalization or prediction was made regarding the value of DNI value in the diagnosis of sepsis in other pediatric age groups.

Our findings indicated that the DNI and IG count are significant diagnostic biomarkers for neonatal sepsis, and they may also have utility in determining the sepsis etiology (differentiating between gram-positive and gram-negative agents). As a parameter that can be automatically calculated by hematology analyzers along with the

complete blood count, the DNI stands out as an affordable biomarker that is easily obtained without additional blood sampling, making it more convenient and advantageous than other laboratory tests, such as inflammation markers. Our study shows that even in cases where there is no possibility to analyze CRP, Procalcitonin, IL-6, DNI values obtained automatically can be used as a laboratory marker to help diagnose neonatal sepsis alone.

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