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Can immature platelet fraction be an early predictor for congenital pneumonia?

Olgunlaşmamış trombosit fraksiyonu konjenital pnömoni için erken belirleyici olabilir mi?

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The known about this topic

Immature platelet fraction, a new hematologic parameter, has been used to gather clinical information on the prognosis of thrombocytopenia, as well as to measure the level of inflammatory activity in adult patients. Timely diagnosis and treatment of congenital pneumonia are crucial in neonates. Although other platelet indices have been subject to some research for their usefulness in neonatal infection, studies on immature platelet fraction are limited.

Contribution of the study

To best of our knowledge, this is the first study to suggest that immature platelet fraction may have an early predictive role in the diagnosis of congenital pneumonia in neonates with respiratory distress.

Abstract

Aim: Timely diagnosis and treatment of congenital pneumonia are crucial. A new hematologic parameter, immature platelet fraction, has been used to gather clinical information on the prognosis of thrombocytopenia, as well as to measure inflammatory activity in adult patients. This study aimed to compare immature platelet fraction and sepsis biomarkers in late-preterm infants diagnosed as having congenital pneumonia and to evaluate its predictive value for congenital pneumonia.

Material and Methods: Late-preterms were categorized based on infectious vs. non-infectious etiology of respiratory distress. Two sets of blood samples for markers were taken at 12–24 (sample-1) and 48–72 hours (sample-2) after birth. Immature platelet fraction was measured using a Sysmex XN-3000 analyzer.

Results: From a total of 30 non-thrombocytopenic late-preterms, 16 were included in the congenital pneumonia group and 14 comprised the transient tachypnea group. The groups were comparable in terms of gestational age, birth weight, and cesarean section rate. The proportion of prolonged membrane rupture was significantly higher in the

Öz

Amaç: Konjenital pnömoninin zamanında tanı ve tedavisi önemlidir. Yeni bir hematolojik parametre olan olgunlaşmamış trombosit fraksiyonu, yetişkin hastalarda trombositopeninin seyri hakkında klinik bilgi edinmek yanında inflamatuar aktiviteyi ölçmek için de kullanılmıştır. Bu çalışmada, konjenital pnömoni tanılı geç preterm bebeklerde olgunlaşmamış trombosit fraksiyonunun bilinen sepsis biyobelirteçleri ile karşılaştırılması ve konjenital pnömoni için belirleyici değerinin araştırması amaçlanmıştır.

Gereç ve Yöntemler: Çalışmaya alınan geç preterm bebekler, enfeksiyöz ve enfeksiyöz olmayan solunum sıkıntısına göre kategorize edildi. Doğumdan sonraki 12–24. (örnek-1) ve 48–72. saatlerde (örnek-2) olgunlaşmamış trombosit fraksiyonu ve biyobelirteçler için kan örnekleri alındı. Olgunlaşmamış trombosit fraksiyonu, Sysmex XN-3000 ile ölçüldü. Karşılaştırma için tam kan parametreleri, C-reaktif protein ve prokalsitonin gibi sepsis biyobelirteçleri kullanıldı.

Bulgular: Çalışmaya konjenital pnömoni grubundan 16, geçici takipne grubundan 14 olmak üzere toplamda 30 tane trombositopenik olmayan geç preterm bebek alındı. Gruplar arasında gebelik haftası, doğum ağırlığı ve sezaryen oranları açısından fark yoktu. Konjenital pnömoni grubunda

Cont. ➡

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congenital pneumonia group. Values of immature platelet fraction-1, immature platelet fraction-2, and procalcitonin-2 were significantly higher in the congenital pneumonia group than in the transient tachypnea group. No significant differences were found between the groups in other biomarkers. It was determined that an immature platelet fraction-1 cut-off value of 2.9% could predict congenital pneumonia with a sensitivity of 65%, a specificity of 71.4%, a positive predictive value of 70.5%, and negative predictive value of 63.7% (area under the curve=0.724; p=0.028).

Conclusion: Immature platelet fraction may have an early predictive role in the diagnosis of congenital pneumonia.

Keywords: Congenital pneumonia, early predictor, immature platelet fraction

uzamış membran rüptürü oranı anlamlı olarak yüksekti. Geçici takipne grubuna göre konjenital pnömoni grubunda olgunlaşmamış trombosit fraksiyonu-1, olgunlaşmamış trombosit fraksiyonu-2 ve prokalsitonin-2 değerleri anlamlı olarak daha yüksekti. Diğer biyobelirteçlerde gruplar arasında anlamlı fark bulunmadı. Olgunlaşmamış trombosit fraksiyonu-1'in %2,9 kesme değerinde %65 duyarlılık, %71,4 özgüllük, %70,5 pozitif tahmini değer ve %63,7 negatif tahmini değer ile konjenital pnömoniyi öngörebildiği belirlendi (eğrinin altındaki alan, 0,724; p=0,028).

Çıkarımlar: Sonuçlarımız olgunlaşmamış trombosit fraksiyonunun konjenital pnömoni tanısında erken belirleyici role sahip olabileceğini ortaya koymaktadır.

Anahtar sözcükler: Erken belirleyici, konjenital pnömoni, olgunlaşmamış trombosit fraksiyonu

Introduction

Pneumonia during the neonatal period is an important respiratory infectious disease with its potential of high mortality and morbidity (1, 2). Despite recent advances in management strategies, the nonspecific clinical signs of neonates and paucity of early objective diagnostic evaluations contribute to a delay in the initiation of treatment (3).

When the timing of infection manifestations is considered, pneumonia presenting in the first week of life is described as early-onset pneumonia. Congenital pneumonia (CP), which occurs within the first day of life and is associated with prolonged premature rupture of membranes, chorioamnionitis, and perinatal maternal infections, is accepted as a variant of early-onset pneumonia (2, 3). Pneumonia also commonly occurs in patients with early-onset sepsis (EOS) of the neonate (4).

Timely diagnosis and prompt treatment of CP is a key factor for the prevention of persistent pulmonary hypertension and septic shock (3–5). The conventional screening parameters for neonatal infections include laboratory tests, such as complete blood count (CBC), absolute neutrophil count (ANC), immature/total neutrophil (I/T) ratio, and C-reactive protein (CRP) (6, 7). However, given the nonspecific character of these parameters, distinguishing CP from non-infectious causes of respiratory distress such as transient tachypnea of the newborn (TTN) or respiratory distress syndrome (RDS) becomes a major clinical challenge (3–5). Although blood cultures are the most helpful diagnostic tests, an etiologic agent may not be identified in many cases (3).

Recent diagnostic advances in the field of hematology allow the use of novel CBC parameters such as immature platelet fraction (IPF), which has been used to gather clinical information on the prognosis of thrombocytopenia, as well as to measure the level of inflammatory activity (8–10). Studies involving critically ill adults have suggested that IPF can be used to diagnose sepsis, and it may even represent a more accurate inflammatory biomarker as compared with CRP and procalcitonin (PCT) (11–13). However, although other platelet indices have been subject to some research for their usefulness in neonatal infections, studies on IPF are limited (14–17).

Late-preterm infants represent a growing population of preterms, and although the risk of early sepsis and respiratory morbidity is higher in this population, they are often perceived by physicians as physiologically similar to full-term infants (18, 19).

In this study, we aimed to compare the IPF and common sepsis biomarkers in late-preterm infants diagnosed as having CP, and to evaluate the predictive value of IPF for CP.

Material and Methods

This prospective study was planned between February and August 2018 in the neonatal intensive care unit (NICU), Kocaeli Derince Training and Research Hospital. The study protocol was approved by the local ethics committee of Kocaeli University (KIA 2018/20) and conducted in accordance with the principles of the Declaration of Helsinki. Late-preterms admitted to the NICU were enrolled in the study after obtaining written informed consent from their parents. Detailed information on prenatal history was recorded at admission.

Late-preterms were categorized based on the etiology of respiratory distress as either of infectious (CP-group) or non-infectious origin (TTN-group). Infants with other respiratory disorders, particularly those with RDS receiving surfactant therapy, as well as those with thrombocytopenia, were excluded.

For the study purposes, late-preterm infants were defined as those born from 34 through 366/7 weeks of gestation and a platelet count of less than 150 000/mm^3 was considered to indicate thrombocytopenia (18, 20). Infants with a history of maternal infection were evaluated for sepsis using the following laboratory criteria (apart from platelet count): total leukocyte count (WBC) <5000/mm^3, ANC<1000/mm^3, I/T ratio ≥0.2 and CRP >5 mg/L or PCT >0.5 ng/mL (6, 7). All chest X-rays were re-evaluated by experienced radiologists.

Late-preterms were diagnosed as having CP based on the presence of the following: (1) tachypnea, retraction, cyanosis, groaning symptoms in the first day of life, as well as the appearance of radiologic features of pneumonia (e.g. reticulonodular pattern and bilateral alveolar densities with air-bronchograms) in chest X-rays; (2) hypoxemia (Pa <50 mm Hg), acidosis (pH <7.25) and $PaCO_2$ >60 mm Hg in blood gases; (3) at least one risk factor for prenatal infection (maternal fever >38°C, chorioamnionitis, urinary tract infection, prolonged rupture of membranes >18 hours) or elevated acute phase reactants or positive blood culture for a proven pathogen. Additionally, deterioration of respiratory symptoms within 12–24 hours and the need for mechanical ventilation were used as supportive findings for diagnosis (2, 3, 21, 22).

The diagnosis of TTN was based on the following criteria: (1) respiratory rate higher than 60 per-minute within six hours after delivery, grunting sounds with breathing, flaring of the nostrils, retractions; (2) tachypnea lasting for at least 12 hours; (3) chest X-ray indicating at least one of the following: increased aeration, flattening of the ribs, fluid accumulation in interlobar fissures and costophrenic angle, vascular congestion and depression of diaphragmatic domes or increased anteroposterior diameter or both; (4) exclusion of either known respiratory (meconium aspiration, RDS, pneumonia, congenital heart diseases) or non-respiratory disorders (neurological, metabolic, hematologic disorders) likely to cause tachypnea because the symptoms of TTN may sometimes persist as long as 72 hours or longer (23, 24).

Blood samples

Blood samples were collected at two time-points (12–24 and 48–72 hours after birth) simultaneously in ethylenediaminetetraacetic acid (EDTA) tubes (for CBC and IPF), as well as in plain tubes (for CRP and PCT). Peripheral smears were prepared for determining I/T ratio, and blood cultures were obtained in Bactec culture bottles before antibiotic administration. Samples obtained at 12–24 hours and 48–72 hours after birth were referred to as sample 1 and sample 2, respectively (e.g. IPF-1, IPF-2). The routine hematologic investigations performed in EDTA tubes on a multi-channel automated cell counter included CBC indices such as WBC, ANC, platelet count and indices; platelet distribution width (PDW), mean platelet volume (MPV). Also, the platelet mass index (PMI) was calculated using the following formula: PMI= platelet count x MPV/103 (14).

Measurement of IPF

The collected blood samples of 2–3 mL in EDTA tubes for IPF were kept at room temperature for 4–6 hours before being sent to the hematology laboratory for analyses. Identification was performed using flow cytometry with a nucleic acid-specific fluorescent dye in the platelet channel of an automated hematology analyzer (Sysmex XN-3000; Sysmex Corporation, Japan). This dye penetrates through the cell membrane and stains RNA in immature platelets. The automated system provides a graphical image of different populations based on size, RNA quantity, and estimates indices. IPF is characterized by platelets with larger size and high fluorescence intensity. It is estimated as the ratio of immature platelet to total platelet population and expressed as a percentage (%) (25).

Statistical Analysis

The IBM SPSS 20.0 (IBM Corp., Armonk, NY,USA) and (MedCalc Statistical Software version 19.1.3 (MedCalc Software bv, Ostend, Belgium; https://www.medcalc.org;2019) statistical programs were used to analyze data. Normality was tested using the Kolmogorov-Smirnov test. Variables are expressed as mean±standard deviation (SD), median (25^{th}–75^{th} percentiles), and counts (percentages). Comparisons of variables between the groups were performed using the Student's t, Mann-Whitney U, Monte Carlo, and Fisher's exact Chi-square tests. For comparisons between study time-points, the paired-samples t and Wilcoxon t-test were used. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of IPF were calculated. The IPF cut-off value was attained from a receiver operating characteristic curve (ROC). A p-value <0.05 was considered statistically significant.

Results

A total of 30 late-preterm infants (18 females and 12 males) were included in the study, with a median gestational age and mean birth weight of 35.5 (range, 34.5–37) weeks and 2570±586.3 g, respectively. The mean platelet count of sample 1 was 272 000 (211 000–310 500)/mm^3 and the mean platelet count of sample 2 was 262 000 (228 000–310 000)/mm^3. The mean IPF-1 was 3.88±2.11% and IPF-2 was 3.91±2.30%. No significant differences were found between mean sample 1 and sample 2 in terms of platelet count and IPF (p>0.05).

The groups were comparable in terms of gestational age, birth weight, sex, and cesarean section rate (p>0.05).

The proportion of patients with a history of prolonged rupture of membranes was significantly higher in the CP group (p=0.001), but rates of maternal urinary tract infection and antenatal prophylactic cefazolin use were similar (p>0.05).

Empirical antibiotic treatment with ampicillin and gentamicin was initiated at admission in both groups. The median duration of antibiotic use and hospitalization was longer in the CP group (p=0.042 and p=0.044, respectively). Growth was not detected in first blood cultures in either group. The characteristics of the late-preterm groups are shown in Table 1.

Table 1. The characteristics of late-preterm groups

	TTN group (n=16)	CP group (n=14)	p
Gestational week, median (25th–75th)	**36** (35.1–37)	**35.3** (34.5–36.2)	0.189[a]
Birth weight (g), mean±SD	**2626**±426.5	**2526**±700.2	0.141[b]
Male/Female, n (%)	**7/9** (44/56)	**5/9** (36/64)	0.407[c]
Caesarean section, n (%)	**12** (75)	**11** (78.6)	0.635[c]
Maternal urinary tract infection, n (%)	**12** (75)	**14** (78.6)	0.921[c]
Prolonged rupture of membrane, n (%)	**2** (12.5)	**14** (100)	**0.001**[c]
Use of antenatal prophylactic cefazolin, n (%)	**15** (93.8)	**13** (92.9)	0.852[c]
Duration of antibiotic use in NICU (days), median (25th–75th)	**3** (2–4)	**7** (5–10)	**0.042**[a]
Duration of NICU hospitalization (days), median (25th–75th)	**6** (5–7)	**11** (8–15)	**0.044**[a]

TTN: Transient tachypnea of the newborn; CP: Congenital pneumonia; NICU: Neonatal intensive care unit; a: Mann-Whitney U test; b: Student's t-test; c: Chi-square test

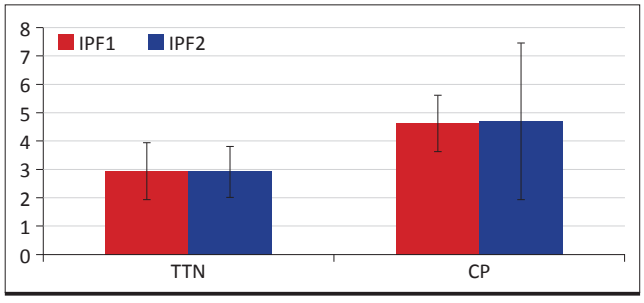

Figure 1. The comparison of IPF-1 and IPF-2 between the study groups

Table 2 shows sample 1 and sample 2 results for sepsis biomarkers, platelet indices, and IPFs in both study groups. In the CP group, WBC-1 was significantly lower and PCT-2 was significantly higher than in the TTN group (p=0.048 and p=0.016, respectively).

A significant decrease in the TTN group and a significant increase in the CP group were observed in PCT values (p=0.035 and p=0.046, respectively).

IPF-1 and IPF-2 were significantly higher in the CP group vs. the TTN group (4.62±2.53% vs. 2.93±0.75% and 4.70±2.76% vs. 2.91±0.89%, p=0.032 and p=0.038, respectively) (Table 2 and Fig. 1).

No differences were noted in other biomarkers and platelet indices between the groups (p>0.05) (Table 2).

For the predictive value of IPF for CP, the area under the curve (AUC) value of IPF-1 and IPF-2 was 0.724 (95% CI: [0.510–0.882]; p=0.028) and 0.701 (95% CI: [0.487–0.866]; p=0.060), respectively. An IPF-1 cut-off value of 2.9% could predict CP in late-preterm infants with a sensitivity of 65%, a specificity of 71.4%, PPV of 70.5%, and NPV of 63.7% (Fig. 2).

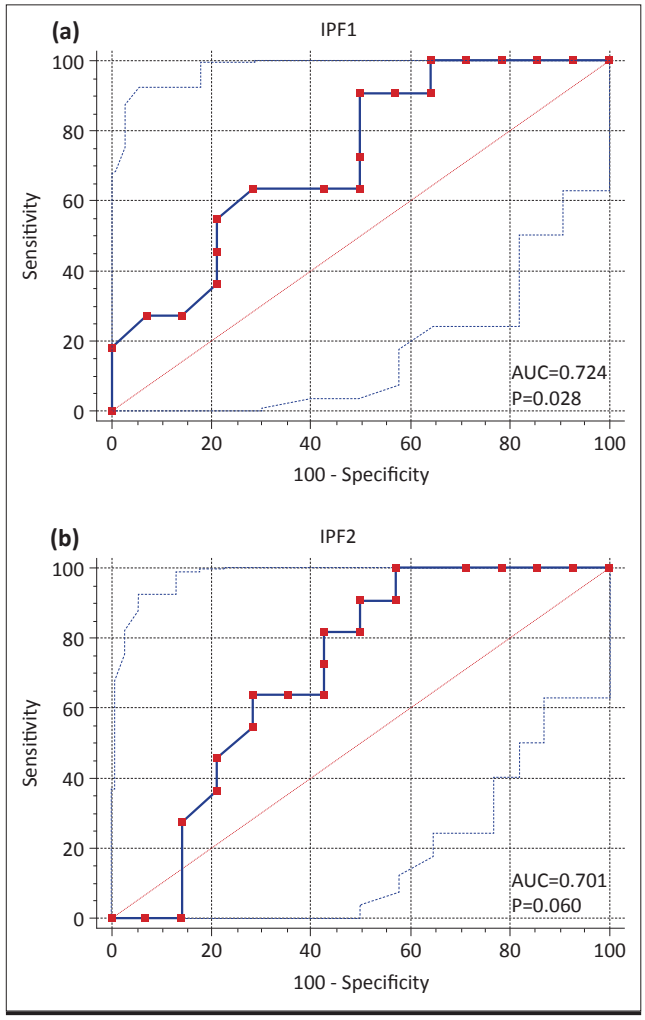

Figure 2. (a) Receiver-operating characteristic curve and area under the curve of IPF-1. The difference between areas was significant (95% confidence interval, CI; 0.510–0.882) (p=0.028). (b) Receiver-operating characteristic curve and area under the curve of IPF-2. The difference between areas was not significant (95% confidence interval, CI; 0.487–0.886) (p=0.060)

Table 2. The results of sepsis biomarkers, platelet indices and IPFs in late-preterm groups

	TTN group (n=16)	CP group (n=14)	p
WBC-1* (/mm³)	20 572±7508	15 161±5 097	**0.048**[b]
WBC-2& (/mm³)	17 542±6797	13 700±4 193	0.144[b]
p[d]	0.006	0.374	
ANC-1*	9164±4325	6687±2975	0.112[b]
ANC-2&	7755±2441	6552±2434	0.314[b]
p[d]	0.108	0.731	
I/T ratio-1*	0.05±0.03	0.06±0.04	0.256[b]
I/T ratio-2&	0.07±0.04	0.09±0.04	0.190[b]
p[d]	0.089	0.094	
Platelet-1* (/mm³)	285 000 (207 000–313 000)	260 500 (211 000–299 000)	0.403[a]
Platelet-2& (/mm³)	280 000 (217 000–320 000)	269 000 (229 000–299 500)	0.845[a]
p[e]	0.476	0.754	
PDW-1* (%)	15.02±2.33	15.09±1.76	0.928[b]
PDW-2& (%)	15.19±1.69	15.38±1.71	0.800[b]
p[d]	0.555	0.659	
MPV-1* (fL)	8.35±0.48	8.29±0.40	0.730[b]
MPV-2& (fL)	8.42±0.54	8.34±0.50	0.740[b]
p[d]	0.109	0.282	
PMI-1* (fL/nL)	2.28±0.68	2.20±0.32	0.343[b]
PMI-2& (fL/nL)	2.32±0.60	2.22±0.41	0.750[b]
p[d]	0.232	0.687	
IPF-1* (%)	2.93±0.75	4.62±2.53	**0.032**[b]
IPF-2& (%)	2.91±0.89	4.70±2.76	**0.038**[b]
p[d]	0.936	0.897	
PCT-1* (ng/mL)	5.14 (1.04–7.80)	5.40 (0.16–11.53)	0.809[a]
PCT-2& (ng/mL)	0.71 (0.17–3.06)	9.43 (0.65–16.52)	**0.016**[a]
p[e]	**0.035**	**0.046**	
CRP-1* (mg/L)	0.1 (0.1–2.5)	0.1 (0.1–4.0)	0.999[a]
CRP-2& (mg/L)	0.1 (0.1–4.5)	1.1 (0.1–6.5)	0.235[a]
p[e]	0.715	0.359	

TTN: Transient tachypnea of the newborn; CP: Congenital pneumonia; WBC: White blood cell; ANC: Absolute neutrophil count; I/T: Immature/total neutrophil ratio; PDW: Platelet distribution width; MPV: Mean platelet volume; PMI: Platelet mass index; IPF: Immature platelet fraction; PCT: Procalcitonin; CRP: C-reactive protein; *: Sample1, sample taken at 12–24 hours after birth; &: Sample2, sample taken at 48–72 hours after birth; a: Mann-Whitney U test; b: Student's t-test; d: Paired-samples t-test; e: Wilcoxon t-test

Discussion

In this study, late-preterm infants with CP had higher IPF values before PCT elevation, suggesting a predictive role of IPF at 12–24 hours after birth for a diagnosis of CP. The cut-off value was 2.9% with acceptable sensitivity, specificity, and PPV.

A novel hematologic parameter, IPF, which mainly provides clinical information on the state of thrombopoiesis, has also been recently tested for its role in the diagnosis of sepsis in adult patients. However, studies of IPF in newborns are scarce (8–13, 15–17).

Although the risk of death due to pneumonia is highest during the neonatal period in children, CP presents significant challenges for physicians both in terms of diagnosis and treatment (1, 2). Important risk factors for CP include maternal colonization with group B streptococcus and chorioamnionitis. Pneumonia results from aspiration of infected amniotic fluid during the intrauterine period or birth process. The disease generally manifests itself within the first hours of birth and may be part of early sepsis or confined to the lungs. Clinical signs are often nonspecific and are also shared by a range of non-infectious re-

spiratory conditions. A chest X-ray should be obtained in all infants with respiratory distress. The most common finding suggestive of pneumonia includes dense bilateral alveolar infiltrates with air-bronchograms (2–5).

Compared with term infants, late-preterms have an increased risk of sepsis and respiratory morbidity (17, 18). It is unclear, however, if sepsis evaluations are promoted by often suspected clinical signs of sepsis or by premature birth. The reported incidence of culture-proven sepsis is low in late-preterm infants. In one study, only 0.4% of cases were found to have a confirmed episode of EOS among a group of late-preterms admitted to the NICU for sepsis evaluation (26). Although bacteriologic cultures are widely used and represent the most helpful diagnostic test, several factors may interfere with the ability to cultivate a likely pathogen from suspected sites, such as pretreatment with antibiotics limit the *in-vitro* but not *in-vivo* growth, contaminants that overgrow the pathogen, and pathogens that do not replicate in culture systems (3). Therefore, the CP diagnosis of participants was mainly based on maternal history, and clinical and laboratory findings in the present study.

The indices of CBC are widely used for the diagnosis of neonatal infections. Multicenter studies reported that WBC <5000/mm^3, ANC <1000/mm^3, and an I/T ratio ≥0.2 were associated with culture-proven sepsis (6). In our study, there were no significant differences between the groups in terms of the above-mentioned parameters, except for lower WBC in the CP group. However, WBC values in both groups were within the normal range (6000–30 000/mm^3) for the first 24 hours of life (27). Therefore, this difference may be considered negligible.

In a cohort study, newborns with sepsis were found to have MPV elevation and thrombocytopenia (15, 28). Elevated MPV indicates a release of larger, younger platelets into circulation, and PDW reflects heterogeneity in platelet size, and both parameters usually change in the same direction. Also, variation in MPV has an impact on PMI (14). Thrombocytopenia and PMI generally exhibit a significant inverse relationship, and PMI has been reported to reliably predict intracranial hemorrhage in newborns with sepsis (29). In the current study, although no differences were observed in platelets and platelet indices between the groups, a positive correlation was detected between immature platelet indices, IPF-2, and MPV-2 (r=0.73, p=0.049) in the CP group (data not shown).

The use of conventional sepsis biomarkers to support the diagnosis of suspected infections (including pneumonia) remains a controversial issue due to their limited diagnostic value (3). Although CRP has high sensitivity, its specificity is relatively low in the early stages of infection. Despite the relatively high specificity of PCT, which has been reported to be useful in the identification of bacteremia among children and neonates with pneumonia, its sensitivity is low and cost remains an issue (13). In recent studies involving adult patients, IPF has been reported to provide higher sensitivity and specificity for the diagnosis of infection (11–13). In our study, both measured IPF values were significantly higher in patients with CP as compared with TTN, and the second PCT value was significantly elevated among patients with CP. Although IPF measured in the first 12–24 hours of life was associated with acceptable sensitivity, specificity, and PPV, with a cut-off value of 2.9%, the small sample size of our study should be taken into consideration when interpreting our findings. However, our observations seem to suggest that IPF can represent a promising, early biomarker for preterm infants with respiratory distress, and IPF values ≥2.9 within the first 24 hours after birth may be indicative of pneumonia.

The IPF represents an immature stage in process of thrombopoiesis and reflects the fraction of platelets with small amounts of RNA and rough endoplasmic reticulum (25). Several plausible explanations have been put forward for the association of IPF with sepsis. For instance, a higher IPF may be a part of an ongoing inflammatory response to sepsis, in which platelets play important roles. It has been demonstrated that platelets express toll-like receptors (TLR) that regulate innate immune response to pathogens. Activation of these receptors leads to the release of interleukin (IL)-1β and the formation of neutrophil extracellular traps, which trap invading pathogens at infection sites. Therefore, when the TLR-mediated mechanism is triggered by infections, higher IPF values, as well as high plasma thrombopoietin, reflect recruitment of newly formed platelets (30, 31). In a study of Di Mario et al. (32), IPF was reported to be significantly higher in patients with positive blood cultures than in those with negative blood cultures (4.86% vs. 1.79%, respectively), suggesting that IPF could be used for screening of bacterial infections.

Despite the scarcity of data in newborns, studies examining the role of IPF as a sepsis marker in adults suggest that this parameter could also provide clinical information regarding inflammatory activity. In a study of critically-ill adults, De Blasi et al. (11) reported that with a specificity of 90% and a sensitivity of 56.2%, IPF values above 4.7% could predict the development of sepsis up to 3 days before clinical manifestations. To investigate the performance of IPF in sepsis, Enz Hubert et al. (12) evaluated patients with severe or non-complicated sepsis and

observed significantly higher IPF levels in patients with severe sepsis (6.2±3.0% vs. 3.6±2.6%). Neither platelets nor CRP was able to differentiate the groups, yet they revealed that IPF was correlated with sepsis severity scores and offered the highest diagnostic accuracy for the presence of sepsis. Again, Liu et al. (13) showed that the value of reticulated platelets (RP) in diagnosing infection was higher than conventional inflammation markers and that its combined use with CRP/PCT could further increase early diagnostic rate of infectious diseases. In their study, for an RP cut-off value of 5.5%, sensitivity and specificity were 84.2% and 72.2%, respectively, and patients with RP% ≥5.5%, and ≥9.72% were more likely to have infections and serious infections, respectively.

Our results were similar to these previous studies, although there were differences in IPF values. We observed significantly higher IPF among the CP group as compared with the TTN group at 12–24 hours and 48–72 hours after birth (4.62±2.53% vs. 2.93±0.75% and 4.70±2.76% vs. 2.91±0.89%, respectively), but there were no significant differences in platelets, platelet indices, and CRP between the groups. Additionally, PCT elevation was seen later in the CP group. We suggest that at a cut-off value of 2.9%, with IPF measured 12–24 hours after birth, could predict CP with a sensitivity, specificity and PPV of 65%, 71.4%, and 70.5%, respectively.

In Liu et al.'s (13) study stated above, the authors also analyzed dynamic changes in RP in 10 infected patients, observing a return of RP to normal levels 2 to 7 days before normalization of body temperature. These findings suggest that IPF can also be used to guide the clinical use of antibiotics. In our study, although there were no statistical differences between IPFs, a slight increase in the CP group, and a slight decrease in the TTN group were observed within three days (Table 2). However, because of the limited sample size, we were not able to track a potential decline in IPF levels in patients with CP. Serial IPF measurements would be more suitable for assessing the ongoing inflammatory process, as well as for following up on the outcome of treatments administered.

Our observations are at odds with findings reported in the limited number of previous studies evaluating IPF and other biomarkers in infants with sepsis and thrombocytopenia. In Cremer et al.'s study (16), neonates with elevated pro-inflammatory cytokines were compared with controls. In general, infected neonates had significantly lower platelet counts, although displaying similar IPF. Brown et al. (17) examined RP and thrombopoietin in 20 neonates with sepsis. Although thrombopoietin concentrations were increased in the early infection phase and inversely correlated with platelets, they observed reduced absolute RPs. However, in the study by Enz Hubert et al. (12), as well as in our study, where there was a suggestion that IPF might be associated with sepsis, platelet counts were normal. It may be more plausible to evaluate IPF as a predictor biomarker during the time window when platelet counts are still within the normal range because IPF is thought to increase before platelet destruction due to sepsis (11, 12, 30, 31).

Recently, the neonatal reference values of IPF were determined for monitoring and treating thrombocytopenia. In two studies, the reported IPF values were 4% and 4.1±1.8% on the first day of life among non-thrombocytopenic neonates with 35.8±4.3 and 37 weeks' gestation, respectively (16, 33). Compared with these data, IPF values obtained on the first day in our study were similar or relatively higher in the CP group and lower in the TTN group. Possible explanations for IPF differences observed across the studies include differences in the site of blood sampling, study sample size, the timing of measurements, laboratory equipment, and sex distribution (13).

The present study has certain limitations. Due to ethical concerns, no blood samples could be obtained from healthy neonates for comparison. Also, the sample size was small and the blood cultures of participants were sterile. Therefore, the evaluation of IPF's inflammatory role was based on the infectious or non-infectious etiology of respiratory distress. Larger patient series with culture-proven sepsis would allow a better assessment of IPF. Also, larger series would provide more detailed information on the relationship between immature platelet indices.

In conclusion, to the best of our knowledge, this is the first study to suggest that IPF measured 12–24 hours after birth may have an early predictive role for the diagnosis of CP in neonates with respiratory distress. Similar to platelet counts, IPF measurements can be performed routinely as a part of the CBC in premature infants to monitor the inflammatory activity using only a small volume of blood. However, further studies with larger sample sizes are warranted to confirm the diagnostic value of IPF in neonatal infections.

Ethics Committee Approval: The study was conducted in accordance with the principles of the Declaration of Helsinki. Approval was obtained from the local ethics committee of Kocaeli University Faculty of Medicine (KIA 2018/20).

Informed Consent: Written informed consent was obtained from the parents of participants.

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