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Original

Is there clinical value in counting nucleated red blood cells and platelet indices in primary immunodeficiency disease?

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SUMMARY: Ekici-Günay N, Çakır I, Çelik T. Is there clinical value in counting nucleated red blood cells and platelet indices in primary immunodeficiency disease? Turk J Pediatr 2017; 59: 657-663.

Infections are the most common presentation of primary immunodeficiency diseases (PIDs). The increase of nucleated red blood cell (NRBC) count is interpreted as a systemic inflammatory response. Platelets play an important role in the pathogenesis of inflammatory disease. The relationship of platelet indices (PIs) and disease activity have been demonstrated in various inflammatory diseases. The aims of this study was to evaluate and compare NRBC and platelet/lymphocyte ratio (PLR), PIs [mean platelet volume (MPV), platelet distribution width (PDW), platelet large cell ratio (PLCR)] with a possible indirect inflammatory marker in children with PIDs. Data were recorded retrospectively from 66 PIDs patients, <16 years of age. The relationships between peripheral NRBC, C-reactive protein (CRP) and PIs were analyzed. NRBC was positively correlated with CRP (p<0.037), white blood cells (WBC) (p<0.020), PLR (p<0.044), PDW (p<0.037) and PLCR (p<0.001) and it was negatively correlated with platelet distribution width (PDW) (p< 0.036) in PIDs patients. A cutoff level of 0.80% NRBC, ≥15.55% PDW, ≥8.65 MPV and ≥43.67 PLR showed the best performance to predict PIDs, with 81% sensitivity, 27% specificity; 61% sensitivity, 37% specificity; 70% sensitivity, 43% specificity; 54% sensitivity, 40% specificity, respectively. Our results suggested that these indices may be used as auxillary diagnostic markers of PIDs with positive NRBC, showing more meaningful results than those known as the traditional infection markers for PIDs prediction. Elevated NRBC and MPV and low PDW are associated with infections and could be helpfull in the early diagnostic susception of PIDs. They can be used as rapidly accessible parameters for awareness of PIDs. These markers are easy to use in daily practice and without extra costs.

Key words: platelet indices, primary immunodeficiency, infection, nucleated red blood cells, platelet-to-lymphocyte ratio.

Immune deficiency syndromes that cause severe bacterial and/or viral infections lead to serious morbidity or death in infants or children. Early identification of this syndrome is critical to improve treatment outcomes.[1]

The incidence of primary immunodeficiency diseases (PIDs) are not rare:1/2000-10000 of live births are reported as PID.[2,3] Primary immunodeficiency diseases can present at any age from birth to adulthood. The presenting signs and symptoms are non-specific, so it may be not easy to recognize immunodeficiency syndromes in infants and children.[4]

The issue is that pediatric clinicians lack evocation with these rare genetic disorders and lack guidance regarding the appropriate use of routine hematologic index investigations.[5]

Other diseases are anticipated with a similar

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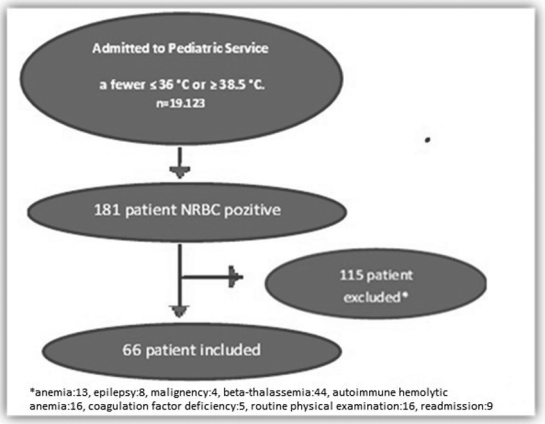

Fig. 1. Flowchart of patients.

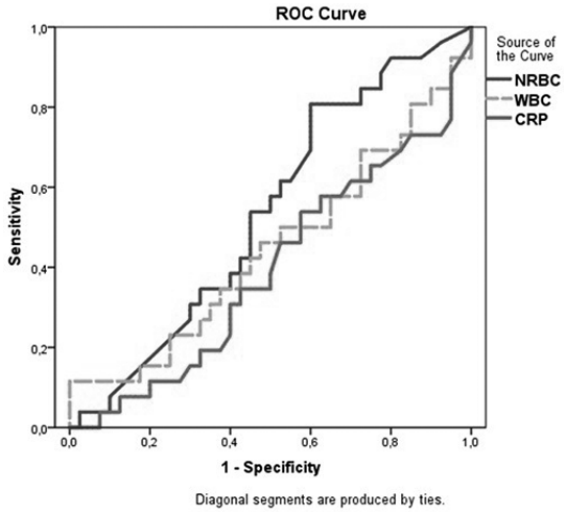

Fig. 2. Receiver operating characteristic curves of C-reactive protein (CRP), white blood cell (WBC) count, nucleated red blood cell (NRBC) for primary immunodeficiency diseases.

clinical picture and are primarily associated with recurrent infections, including allergies and metabolic disorders, so it is essential for the pediatric clinicians to determine which patient should be suspected for PIDs.

The NRBCs can be present in numerous conditions; even in thalassemic syndromes, myeloproliferative diseases (specifically myelofibrosis), bone marrow metastases of solid tumors, extramedullary hematopoiesis and all of the conditions of hematopoietic stress (eg, septicemia, massive hemorrhage, and severe hypoxia).[6] Studies showed that production of the inflammatory response, characterized by cytokine release, is associated with increased NRBC levels.[7,8].

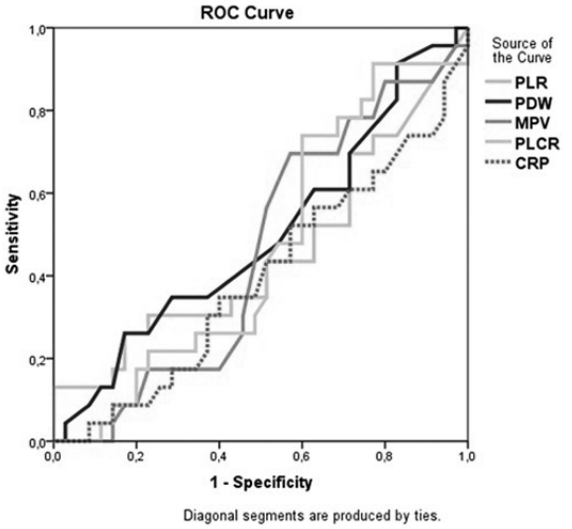

Fig. 3. Receiver operating characteristic curves of PLR, PDW, MPV, PLCR and CRP for primary immunodeficiency diseases.

Platelet indices (PI) as MPV, PDW, PLR and PLCR are a group of derived platelet parameters obtained as a part of the automatic complete blood count. Emerging evidence suggests that PIs may have diagnostic and prognostic value in certain diseases.[9]

The mean platelet volume provides an estimate of platelet dimension of and is a marker of platelet activation[10]. In addition to that, other markers of platelet morphology, such as PDW and PLCR may play important roles in vascular inflammatory process.[11]

In various studies, NRBC, MPV, PDW and PLR have been identified as markers of inflammation.[12-13]

The provision of rapid real-time NRBC counts has implications for earlier diagnosis, more rapid treatment and therefore improved prognosis in this group of patients[14,15]. Plateletes indices are a prognostic marker of many diseases, but it has not yet been established in primary immunodeficiency syndroms (PIDs). The aim of this study is to evaluate and compare NRBC parameter and PIs as inflamation index/markers in PIDs. To the best of our knowledge, the morphologic parameters that act as indicators of subclinical platelet activation, including the PDW, PLCR and PLR have not been investigated in PIDs, yet. Therefore, the aim of this study was to investigate indicators of platelet morphology, including the MPV,

PLCR, PDW, PLR and platelet counts in PID patients. Another objective of this study was to determine the diagnostic marker of these parameters.

Material and Methods

Data respectively analyzed from previously diagnosed primary immunodeficiency patients less than 16 years of age, admitted to pediatric emergency service with the infection precaution. Inclusion criteria for this study was based on a temperature less than 36°C or greater than 38.5°C and to be followed as PIDs. All of the patients were divided into two groups according to the status of NRBC detected or not detected. Finally, previously diagnosed PIDs patients with positive NRBC levels were selected. Secondary immunodeficiency patients were excluded by a physical examination and a careful history questioning nutritional disorders, defects in innate immunity, autoinflammatory disorders, splenectomy, hematological malignancy, immunosuppressive or immunomodulatory therapy and human immunodeficiency virus infection. The PID patients subtype distribution is shown in Table I. An age-sex match control group was created from those who were admitted to the routine general checkup clinic and reported as healthy. All of the children were treated between January 2014 and May 2014 in the Emergency Care Department of Pediatric Clinics of Training and Research Hospital (Kayseri, Turkey).

Before initiation of treatment, blood was sampled for laboratory analyses, including a complete blood count with differential (CBC), NRBC, WBC, lymphocyte (LY), monocyte (Mo), neutrophils (Ne), eosinophils (Eo), basophils (Ba), platelet indices including PDW, PLCR and MPV and then PLR was calculated. These examinations were done upon admission to the hospital before giving any drugs intravenously.

Peripheral venous blood samples were collected by antecubital venipuncture into vacutainer tubes (Becton Dickinson, Rutherford, NJ, USA) containing tripotassium ethylenediaminetetriacetic acid (EDTA). In order to minimize changings in platelet size analyses were done within one hour after the blood samples were drawn. Complete blood count with differential analysis was performed using the Mindray BC-6800 automated analyzer in line with the manufacturer's recommendations (Shenzhen *Mindray* Bio-*Medical* Electronics Nanshan Shenzhen, China).[16] The serum C-reactive protein (CRP) concentration was analyzed in an immunoturbidimetric assay analyzer (Dade Behring/*Siemens BN II, Germany*). Internal quality control was checked for each of the assays.

Statistical analyses

IBM SPSS Statistics version 21.0 (IBM Co, Armonk, NY, USA) was used to compare variables. Independent t-test was used for continuous variables with normal distribution, in addition to descriptive statistics expressed as mean±standard deviation. Spearman's correlation analysis was carried out to determine the relationship between two variables. The discriminative ability of each biomarker for immun deficiency syndromes was evaluated by plotting receiver operating characteristic (ROC) curves. Multiple logistic regression analyses were performed to find independent predictive factors for positive NRBC patients. ROC analysis was performed to determine the best cut-off value to predict the outcome. According to ROC analyses of NRBC and PIs, optimum cut-off values were decided using the maximum value of Youden's index (sensitivity+specifity-1) for each potential marker. All differences were considered significant at a value of $p < 0.05$.

Table I. The Primary Immunodeficiency Patients Distribution (Total n=66).

Type of immunodeficiency	n	%
Primary antibody deficiencies	49	74.2
Other well-defined immuno deficiency syndromes	5	7.6
Severe combined immunodeficiency	4	6.0
Other immunodeficiency	3	4.5
Complement deficiency	3	4.5
Phagocytic system disorders	2	3

The Institutional Scientific Review Board of Kayseri Training and Research Hospital approved this retrospective study. Prior to the study, institutional scientific research ethics committee permission was obtained to review the clinical records of pediatric patients (No: 20/October/2016-57). Personal identifiers were entirely removed and the records were analyzed anonymously. Informed consent was obtained from the parents of all the patients using the data.

Results

19,123 pediatric patients were admitted to the Pediatric Service with infection (a fever of ≤36°C or ≥38.5°C) were screened. The 181 children with febrile/hypotermic infections were found NRBC positive. 66 of them were identified with diagnosis of primary immunodeficiency syndromes (37 males and 29 females) (p< 0.685) and included in our study (Fig. 1). The majority of patients were followed for a primary antibody deficiency diagnosis (74.2%). Male predominance was upfront among PID cases (59%). Details of different PIDs diagnoses are showed in Table I. An age-matched non-PIDs group was formed. There was no difference between patients and control group's ages; 5.4±4 and 7.2±5 years, respectively (p< 0.689).

Descriptive analyses of hemotologic parameters are showed in Table II. Patients group's NRBC, CRP, WBC, PLT, PDW, MPV, PLCR and PLR levels and their correlations with each other were studied. In PIDs children group, PI components were correlated with %NRBC: PDW was significantly negatively (p< 0.003) and the others as PLR (p< 0.044), WBC (p< 0.037), CRP (p< 0.019) and PLCR (p< 0.036) were positively correlated. Monocyte percentage was also negatively correlated with NRBC levels (p< 0.022) (Table III, IV).

CRP levels was correlated with PLR (*P*<0.050). Monocyte percentages were found to be negatively correlated with CRP as well as

Table II. Descriptive Analyses of Hematologic Parameters of the Patient's Group.

Parameters	Mean±SD	Parameter	Mean±SD
NRBC (%)	36.52±120.7	PLT(x10^3 /μL)	408.05±233.9
CRP (mg/L)	20.85±23.64	MPV(fL)	9.04±1.05
WBC (x10^3 /μL)	14±7.1	PDW(%)	16.72±5.10
LY (%)	42.6±19.3	PLCR(%)	21.19±7.91
Ne (%)	6.7±5.33	PLR	34.33±25.84

NRBC: Nucleated red blood cell, CRP:C-reactive protein, WBC:white blood cells,LY:lymphocytes, Ne:neutrophils, PLT:platelet, MPV:mean platelet volume, PDW: platelet distribution width, PLCR:platelet large cell ratio, PLR:platelet/lymphocyte ratio.

Table III. Spearman's Correlation of The Nucleated Red Blood Cell Concentration With Other Hematologic Parameters (n=66).

Parameters	r	p
CRP (mg/L)	0.288	0.019*
WBC (x10^3/μL)	0.257	0.037*
PLT (x10^3 /μL)	0.146	0.278
PDW (fL)	-0.277	0.003**
MPV (fL)	0.356	0.050
PLCR (%)	0.458	0.036*
PLR	0.249	0.044*
Ne (%)	0.270	0.029*
Mo (%)	-0.282	0.022*
Ba (%)	0.397	0.001**

CRP: C-reactive protein, WBC: white blood cells, PLT: platelet, PDW: platelet distribution width, MPV: mean platelet volume, PLCR: platelet large cell ratio, PLR: platelet/lymphocyte ratio, Ne: neutrophils, Mo: monocyte, Ba: basophile. * Correlation is significant at the 0.05 level (2-tailed). **Correlation is significant at the 0.01 level (2-tailed).

Table IV. Spearman's Correlation of PLR with Other Hematologic Parameters (n=66).

Parameters	r	p
NRBC(%)	0.249	0.044**
MPV(fL)	0.280	0.831
PDW(%)	0.258	0.043*
PLCR(%)	0.000	0.998

NRBC: Nucleated red blood cell, MPV: mean platelet volume, PDW: platelet distribution width, PLCR: platelet large cell ratio.
*Correlation is significant at the 0.05 level (2-tailed). **Correlation is significant at the 0.01 level (2-tailed).

with NRBC ($p< 0.02$) (Table V).

Significant correlation was found between NRBC with PLR and PDW levels ($p<0.044$, $p< 0.003$ respectively).

Based on these findings, characteristic ROC curves were drawn for NRBC, PLR, PDW, MPV, PLCR and CRP, which were considered as inflammatory predictors for PIDs (Table VI), (Figs. 2 and 3).

The area under ROC curve analysis for CRP and WBC were lower than PDW, PLCR, MPV, PLT and PLR for PIDS. Areas under the ROC curve for NRBC and PDW were 0.537 and 0.498, respectively. A cut off level of ≥0.80% NRBC and of ≥15.55 % PDW showed the best performance to predict PIDs, with 81% sensitivity and 27% specificity; 61% sensitivity and 37% specificity respectively. The cutting edge value for MPV was 8.65 fL (70 %, 43%) and the cut off value for PLR was 43.66 (54% sensitivity, 40 % specificity).

Our study revealed that 0.94% of children, admitted to the internal Anatolian region hospital with febrile infection, had positive NRBC and 36% of patients with NRBC positive infection had PIDs.

Discussion

Early detection of PIDs results in improved clinical outcomes.[17] However, failure to recognize PIDs remains a major challenge for pediatric clinicians worldwide. High suspicion index is still the main stay for diagnosing PID.

In the pathogenesis of various inflammatory or infectious disease, platelets play important roles.[18] In relation to the disease activity, MPV and platelet counts have been studied as inflammatory markers.[19] NRBC and PIs has also been studied in various infectious disorders such as hepatitis B, acute appendicitis and sepsis and found as a positive inflammatory marker.[20-22]

In the present study, we found decreased PDW and increased levels of MPV in NRBC positive pediatric patients with PIDs so we suggest that these parameters act as positive acute phase reactants in PIDs. We also showed that PLR levels had a positive correlation with CRP levels in PIDs. We pointed out in this study that NRBC, PDW, MPV, PLCR and PLR were significantly higher than conventional infection markers, including the CRP and WBC level. We suggested that, NRBC may be an additional potential indicator for PIDs. The combined use of NRBC with PDW and other markers such as CRP, should be considered in the early diagnosis of PIDs.

Platelets play a crucial role in the hemostasis and inflammatory response. Trombocyte activation observed in the active period of the disease not only regulate coagulation, but also enhances mucosal inflammation. Plateletes

Table V. Spearman's Correlations of CRP with Other Parameters (n=66).

Parameters	r	p
WBC ($x10^3$ /μL)	0.447	0.004**
Ne (%)	0.447	0.000**
LY (%)	-0.363	0.005**
PLR	0.172	0.050*

WBC: White blood cells, Ne: neutrophils, LY: lymphocyte, PLR: platelet to lymphocyte ratio
*Correlation is significant at the 0.05 level (2-tailed). **Correlation is significant at the 0.01 level (2-tailed).

Table VI. Receiver Operating Characteristic Analysis of NRBC, PLR, PIs and Inflammatory Predictor That Predict Primary Immunodeficiency Diseases.

Variable	AUC	Standard error	P	95% CI
NRBC(%)	0.537	18.19	< 0.001	6.85- 66.2
WBC (x$10^3/\mu$L)	0.446	0.87	< 0.001	12.25- 15.76
CRP (mg/L)	0.395	2.90	< 0.001	15.04- 26.67
PLR	0.467	97.73	< 0.005	0.01- 521.82
PDW(%)	0.498	0.64	< 0.001	15.43- 18.02
MPV(fL)	0,459	0,13	<0.001	8,78- 9,32
PLCR	0.463	1.03	< 0.001	19.10- 23.26

AUC: area under curve, CI: confidence interval

initiate and support inflammatory processes by secretion of numerous biologically active substances such as β-thromboglobulin, CD40L, fibrinogen, IL-1β, platelet factor-4.[23]

MPV may be increased in mild inflammation due to the emergence of the large platelets in the peripheral circulation and conversely, may be decreased in severe inflammation because of the consumption of large platelets in the inflammatory area. Large platelets are relatively more active than small ones; they produce more glycoprotein Ib and glycoprotein IIb/IIIa receptors, release more thromboxane A2 and rapidly aggregate.[24] The increase of MPV and PLCR in conditions with increased platelet turnover is probably mediated by several cytokines (interleukins 6, 11 and thrombopoietin) that affect megakaryocyte ploidy and result in the production of larger and more reactive platelets.[25]

NRBC levels < 1.5% detected by the automated count may be present in patients with a pathologic bone marrow process or without increased erythropoiesis. In addition, while analysing NRBC positive patients without hematologic diseases, blood cytokine profile suggests that NRBC may be considered a parameter that sums inflammatory injuries.[26]

Although peripheral platelet counts do not correlate with NRBC, we have suggested that the differences in platelet index may have a greater impact on PID suspicion in this study.

Our study has some limitations. First, the number of patients was small. New studies are needed evaulating these parameters in larger sample size. Second, the children who were reported to be healthy in the patient records for admission were selected for the control group, but no advanced genetic and molecular researches were performed for PIDs.

Our study revealed that 0.94% of children, admitted to the internal Anatolian region of Turkiye hospital with febrile infection, had positive NRBC and 36% of patients with NRBC positive infection had PIDs. Therefore, evaluation of NRBC presence may be considered for the diagnosis of PIDs as an auxillary predictive hematologic parameter. Furthermore, it is essential to establish multi-center national-based screening of life-threatening PID diseases.

Positive family history of PID, previous sibling death from infection, parental consanguinity and where genetic testing access is unavailable, basic laboratory tests as NRBC presence and alterations of PIs may lead to PID suspicion.

When PIDs are suspected, advanced genetic studies can be costly and difficult to access. In this context, we recommend that easy and inexpensive laboratory parameters such as NRBC and PI be one of the markers for PID diagnosis, which should be included in accepted diagnostic guidelines.

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