Diagnostic Value of Hemogram Parameters as a Biomarker in Infection and Sepsis

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Abstract

Red Cell Distribution Width (RDW), Platelet Distribution Width (PDW), Mean Platelet Volume (MPV), and Neutrophil-Lymphocyte Count Ratio (NLCR) are part of hemogram parameters in Complete Blood Count which inexpensive and easy to obtain, but their clinical usefulness in sepsis management is controversial. We conducted a cross-sectional study where RDW, PDW, MPV, and NLCR rates were evaluated on 64 sepsis patients, 30 patients with infection, and 71 control at Central Laboratory of Dr. Saiful Anwar Hospital. Statistically significant difference was found between 3 groups in all parameters. Both RDW and NLCR showed an area under curve (AUC) values over 0.90 in differentiating healthy group to a patient with infection. For sepsis, RDW (AUC 0.91, sensitivity 86.7%, specificity 80.3%) and NLCR (AUC 0.979, sensitivity 93.3% specificity 97.2%) showed a similar accuracy. Median PDW was higher in the sepsis patient ($p<0.001$) and patient with infection ($p=0.078$). Median MPV was significantly different in the infection group ($p<0.05$), but not significant in patients with sepsis ($p=0.464$). Our study shows RDW and NLCR have good diagnostic values; therefore it could be promising markers in predicting infection and sepsis.

Keywords — *Red Cell Distribution Width, Platelet Distribution Width, Mean Platelet Volume, Neutrophil-Lymphocyte Count Ratio, sepsis*

I. INTRODUCTION

Sepsis is a medical emergency, which is one of the leading causes of death and critical illness; were estimated, millions of people each year worldwide. Furthermore, causes of death in 1 in 4 and often more [1]-[4]. Increased incidence over the past 30 years may be caused by the growth of the elderly population, antibiotic resistance, the use of corticosteroids, and invasive surgery [3]-[5].

The concept of sepsis, according to the latest definition (Sepsis-3), is based on the discovery of infection and dysregulation of body response, which characterized by organ dysfunction as an acute increase in total Sequential Organ Failure Assessment (SOFA) score [2]. In this context, sepsis raises several diagnostic problems and prognosis, so further studies are needed to identify criteria that are useful for establishing a fast and accurate diagnosis and effective therapy [4].

Thereby, clinicians are dealing with challenges in recognizing the infection and assessing the disease severity. In clinical practice, suspicion of infection is based on the emergence of signs and symptoms from the host response. However, those manifestations do not always occur, especially in patients with comorbidities, such as the elderly population or patients with immune system disorders. Physicians use infection biomarkers as a complementary beside clinical assessments and laboratory results, which contribute to rule in or rule out infection. Furthermore, it helps to make triaging decisions about the need for admission to the intensive care unit (ICU) or ward and antibiotic administration [6].

Red Cell Distribution Width (RDW), Platelet Distribution Width (PDW), Mean Platelet Volume (MPV) are routinely measured by automated hematology analyzers using either electrical impedance or optical fluorescence method, which do not incur an additional cost. RDW is an evaluation of erythrocyte size variability and used extensively in the differential diagnosis of anemia [4], [7]. High RDW values can be found in conditions of increased erythrocyte destruction and nutritional deficiencies. Such as iron, vitamin B12, and folic acid deficiency anemia, or blood transfusion [8].

PDW is an indicator of the release of activated platelets and describing platelet size variation [9]. And MPV refers to the average size of platelets. In addition to its role in homeostasis, platelets also interact as inflammatory cells. In response to inflammatory stimulation, platelets can become active. Activated platelets tend to be larger, by changing from discoid to a spherical shape and the formation of pseudopodia. So that PDW and MPV reflect the level of platelet activation and production [4], [10], [11]. The vertical diameter of platelets is important in measuring platelet volume, which is measured using electric field deformation based on impedance technology [12].

In 2001, Zahorec *et al* firstly introduced Neutrophil Lymphocyte Count Ratio (NLCR) as an inflammatory and stress parameter in critical illness,

which was obtained easily, quickly, and cheap [13]. A study by Loonen *et al* stated that NLCR as the most promising biomarker in determining whether patients with bacteremia or not in the emergency department setting [14]. NLCR is suggested as an indicator of sepsis in the early phase and also has a prognostic value in the advanced sepsis phase [15]. Based on the description above, although hemogram parameters are inexpensive and easy to obtain, their clinical use as a potential marker in overcome morbidity and mortality due to sepsis is still controversial and data limited. It needs further investigation about the diagnostic value of RDW, PDW, MPV, and NLCR in infection and sepsis detection.

II. REASEARCH METHOD

This study was conducted as a single-center, prospective observational with a cross-sectional study design carried out in the period from July to December 2018 at the Central Laboratory of Dr. Saiful Anwar General Hospital Malang, which is a Teaching Hospital. 165 adult patients (> 18 years) consecutively enrolled in this study, which fulfills inclusion-exclusion criteria. All participants who agree to be included in this study will sign an informed consent and the study was approved by the Hospital Ethics Committee.

The inclusion criteria were patients suspected infection and sepsis, as judged by the physician, who carries out laboratory examinations at Dr. Saiful Anwar General Hospital. Exclusion criteria in this study were: i) Age <18 years; ii) Patients with hematological diseases, such as hematological malignancies, bone marrow metastases by malignant infiltration, recovery after bone marrow hyperplasia or acute bleeding; iii) Severe and immunocompromised chronic disease conditions; iv) Pregnancy. The presence of infection is determined based on the following criteria: culture / microscopic pathogens from the focus of infection, positive urine dip tests in patients with urinary tract infections, linking pathogens with serology, or Polymerase Chain Reaction (PCR), or pneumonia that help with chest X-Rays.

While sepsis is established based on the Third International Consensus, condition of organ dysfunction that life-threatening, which caused by dysregulation of the host response to infection, by calculating SOFA scores ≥ 2 points and septic shock is hypotension in sepsis that requires vasopressors to maintain mean arterial pressure ≥ 65 mmHg and adequate fluid resuscitation, and serum lactate > 2 mmol/L [2].

The control group was healthy adults who underwent a general check-up, and there was no diagnosis of infectious diseases, with normal leukocyte counts on laboratory examinations. RDW, PDW, MPV, and neutrophil and lymphocyte counts were obtained from peripheral venous blood samples of patients with EDTA tubes that were examined by Sysmex XN-1000.

Statistical analysis using the SPSS program for Windows version 25.0. The data normality test uses the Kolmogorov-Smirnov test, and data proportion present with frequency (%), while the non-parametric numeric data is described by the median (inter-quartile range / IQR). The Kruskal-Wallis test was used as a comparative test. The diagnostic value was calculated using the Receiver Operating Characteristic (ROC) curve, then the Area Under Curve (AUC) is obtained and the value of p <0.05. Determination of optimum cut-off based on the Youden Index.

III. RESULT

The total population of study subjects was 165 patients (median age: 42 years old [IQR: 29-57], with an age range of 19-85 years old, consist of 82 (49.7%) males and 83 (50.3%) females, which was divided into 3 groups, 60 sepsis patients, 34 patients with infection, and 71 patient as a control group. The characteristics of the study subjects are listed in Table 1. The comparative test uses the Kruskal-Wallis test between the 3 groups and the results show that there were significant differences so that it was continued with the post hoc test, namely the Mann-Whitney U test which can be seen in Table 2.

We analyzed diagnostic value in patients with sepsis using the ROC curve for each parameter, showed in Fig. 1. AUC NLCR results (0.971 (95% Confidence Interval (CI): 0.92-1; p<0.001), higher than RDW (0.910 (95% CI: 0.85-0.96; p<0.001), PDW (0.607 (95% CI) : 0.48-0.73; p=0.078), and MPV (0.347 (95% CI: (0.23-0.45); p=0.011). The ROC curve for each parameter in diagnosing sepsis is shown in Fig. 2, with an AUC NLCR 0.979 (95% CI: 0.95-1; p <0.001), RDW 0.910 (95% CI: 0.86-0.95; p < 0.001), PDW 0.672 (95% CI: 0.57-0.77; p <0.001) and MPV 0.463 (95% CI: 0.36-0.56; p = 0.464). We selected diagnostic accuracy and optimum cut-off values for each parameter based on the Youden Index, listed in Table 3.

Table 1. Characteristics of Research Subject

	Healthy (n=71) f(%) or Median (IQR)	Infection (n=34) f(%) or Median (IQR)	Sepsis (n=60) f(%) or Median (IQR)
Gender :			
Male	31 (43.7%)	11 (32.4%)	40 (66.7%)
Female	40 (56.3%)	23 (67.6%)	20 (33.3%)
Age (year)	29 (27-34)	46 (35.75-57.75)	57 (49-69)
RDW (%)	13 (12.3-13.2)	14.8 (14.0-15.7)	15.1 (13.8-16.9)
PDW (fL)	10 (9.4-10.8)	10.7 (9.5-11.5)	10.9 (9.7-13.3)
MPV (fL)	10.3 (9.6-11.9)	9.9 (9.2-10.4)	10.3 (9.3-11.3)
NLCR	1.90 (1.5-2.5)	11.1 (6.5-25.2)	18.8 (8.7-24)

IQR : interquartile range; f : frequency
*Chi Square test analysis
**Kruskal-Wallis test analysis

Table 2. Post-Hoc test with *Mann-Whitney U* for Each Parameter

P-value	Healthy vs Infection	Healthy vs Sepsis	Infection vs Sepsis
RDW	0.000	0.000	0.595
PDW	0.078	0.001	0.145
MPV	0.011	0.464	0.067
NLCR	0.000	0.000	0.281

Table 3. Performance of each Parameter in Diagnosing Infection and Sepsis

Parameter	*Optimum Cut-off*	Sensitivity (%)	Specificity (%)	LR (+)	LR (-)	Youden Index (J)
Healthy vs Infection						
RDW (%)	13.35	88.2	78.9	4.18	0.15	0.671
PDW (fL)	10.65	52.9	73.2	1.97	0.64	0.262
MPV (fL)	14.35	2.9	98.6	2.07	0.98	0.015
NLCR	3.83	94.1	97.2	33.61	0.06	0.913
Healthy vs Sepsis						
RDW (%)	13.45	86.7	80.3	4.4	0.17	0.669
PDW (fL)	11.6	43.3	95.8	10.31	0.59	0.391
MPV (fL)	10.85	45	66.2	1.33	0.83	0.112
NLCR	3.74	93.3	97.2	33.32	0.07	0.905

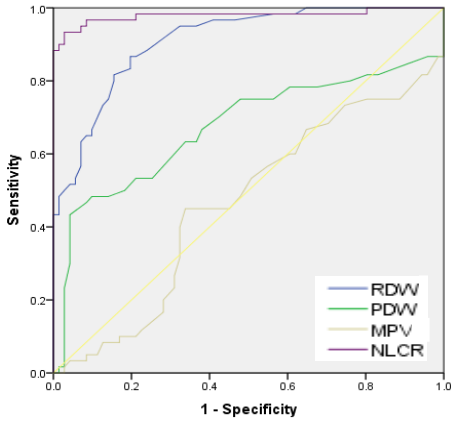

Fig. 1. ROC Curve of RDW, PDW, MPV, NLCR in Patient with Infection

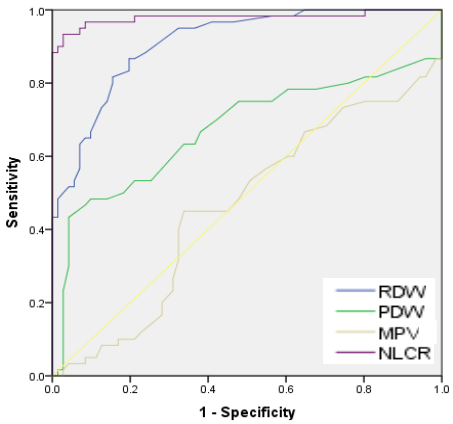

Fig. 2. ROC Curve of RDW, PDW, MPV, NLCR in Patient with Sepsis

IV. DISCUSSION

Sepsis is now defined as life-threatening organ dysfunction caused by dysregulation the body's systemic immunological response to an infectious process [2]. An accurate early diagnosis of sepsis has an important role related to therapy's effectiveness and improves patient outcomes [16]. Nevertheless, Sepsis-3 did not redefine infection. In consequence, it is needed to raise recommendations for infection and/or inflammation biomarkers, that could help to recognize infection and sepsis among infection suspected patients [6].

Male was more often developed to sepsis, as many as 40 people, and 20 women in the sepsis group, which significant difference statistically. The epidemiological studies report consistently about the higher incidence of sepsis in men [17], [18]. Nasir *et al* in 2015 examined the relationship between the incidence and mortality of sepsis to gender. It was concluded that men had a 70% greater mortality rate in sepsis compared to women, related to respiratory tract infection rates and IL-6 levels [19].

The average study subjects in the sepsis group were 57 years old (range of 49-69 years old), older than the infection and control groups, but did not make any difference statistically. The incidence of sepsis increases in older adults. There is a strong relationship between age and increases the risk of death at the beginning of hospitalization described by medical researchers [20].

In this study, NLCR had the highest accuracy (AUC 0.971; optimum cut-off 3.83) and sepsis (AUC 0.979; optimum cut-off 3.74). Previous studies, on 140 patients suspected of being infected in the Emergency Department, obtained AUC 0.770 (95% CI: 0.662-0.879) [14]. NLCR also correlated significantly with patients with SIRS (Systemic Inflammatory Response Syndrome) [21].

This study's range of NLCR values in the control group was 0.1-4.3%. It is known that NLCR value will be below five in normal condition and increase under conditions of severe infection or SIRS [21]. This is concordant with SIRS pathophysiology, which is marked by an increase in the number of circulating leukocytes, and the number of neutrophils which increases as first-line antimicrobials. On the other hand, lymphopenia arises as a result of lymphocyte margination and redistribution in the lymphatic system, increasing the acceleration of apoptosis. In addition to predictive of sepsis patients, it has been evaluated also NLCR roles for survival rates of patients with cancer: lung, prostate, pancreas, esophagus, colorectal, hepatocellular carcinoma; cardiovascular disease; and inflammatory bowel disease [4].

RDW also showed good diagnostic value in identifying infection (AUC 0.910; optimum cut-off 13.35%), and diagnosis of sepsis (AUC 0.910; optimum cut-off 13.45%). In sepsis conditions, the pro-inflammatory status has an important role in

causing erythropoiesis insufficiency which effects changes in the structure and function of erythrocytes. Cytokines such as TNF-α, IFN-ɣ, IL-1β, and IL-6 can influence the production and survival of erythrocytes, which results in variations in the volume of erythrocytes and RDW [22]. As such, RDW is also a non-specific marker of inflammation, in many other diseases, such as heart failure, stroke, peripheral arterial disease, or chronic lung disease [4].

The median PDW in sepsis and infection patients was higher than in the control group, which was found to be statistically significant in sepsis patients (10.9 fL (9.7-13.3) vs 10 fL (9.4-10.8); AUC 0.607; p<0.001), but did not differ significantly in infectious patients (10.9 fL (9.7-13.3) vs 10 fL (9.4-10.8); p = 0.078). A previous study in 2016 by Zhang H. B *et al*, investigated the diagnostic value of RDW, PDW and NLCR on 120 patients with positive and negative blood cultures, with AUC respectively: 0.621 (95% CI: 0.520-0.722; p=0.023), 0.636 (95% CI: 0.537-0.741; p = 0.010), 0.718 (95% CI: 0.625-0.811; p<0.001) [9].

There was a difference between the median MPV in the control group and patients with infection (10.3 fL (9.6-11.9) vs 9.9 fL (9.2-10.4); p = 0.011; AUC 0.347). Whereas MPV in sepsis patients was not different significantly compared to the control group (10.3 fL (9.6-11.9) vs 10.3 fL (9.3-11.3); p = 0.464). In a healthy population, the MPV value is inverse to the platelet count. MPV describes the size of platelets circulating in peripheral blood. Elevated MPV during the sepsis process is caused by an increase in platelet destruction which requires an increase in the production of young platelets which has greater size [3].

A retrospective study by Aydemir *et al*, concluded that reducing the number of platelets and MPV was found in the first 3-5 days of the onset of sepsis [23]. While the previous study stated that MPV acts as a positive acute-phase reactant, but some studies also found the role of MPV as an acute phase was negative [24], [25]. Diquattro *et al* also stated, under conditions of platelet count <20x10^9/l, Volume Index Platelets, especially MPV, can cause significant discrepancies [26].

There was no significant difference in all parameters in differentiating patients with infection and sepsis. According to a study in Turkey by Ates *et al*, there were significant differences between MPV in SIRS patients (cut-off 8.123, sensitivity 69.6%, specificity 62.5%) and sepsis patient (cut-off 8.915, sensitivity 71%, specificity 63.9%) compared to the healthy control group, but did not have a significant difference between the median MPV in 69 patients with SIRS and 69 sepsis patients (9.45 vs 10.07 fL, p=0.261). And it was stated, although significant results were obtained in MPV, still has not fulfilled the standards to be used as a screening test, because it needed to eliminate predisposing factors to MPV [12], such as diabetes, cardiovascular, smoking, hypertension, hypercholesterolemia, obesity and metabolic syndrome [27].

This study has an important advantage, that we use the latest Sepsis-3 definition in the classification of patients. On the other hand, it has become limited for us to compare with previous studies that used the old sepsis definition. And this study is single-center research, with results that might be not the same in other centers. Another weakness of this study is parameters that are only performed once and without seeing the onset of sepsis.

V. CONCLUSION

Based on this research, we conclude that RDW and NLCR had excellent diagnostic values and could be a helpful tools in predicting infection and sepsis. PDW also had good diagnostic value in sepsis detection. The diagnostic value of MPV for sepsis and infection is weak. RDW, PDW, MPV, and NLCR cannot distinguish between patients with infections and sepsis. Further research is needed with a cohort and multi-center design. And we also recommend considering possible predisposing factors of each parameter that might influence the result.

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