Original Investigation

Delta Neutrophil Index and Red Blood Cell Distribution Width as New Markers to Predict Endometriosis

Kayacık Günday and Yılmazer. DNI, endometriosis

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Abstract

Objective: The study aimed to evaluate the efficacy of delta neutrophil index (DNI) in predicting endometriosis.

Material and Methods: A retrospective case-control study was performed in a tertiary care center. DNI, red cell distribution width (RDW), and other blood parameters obtained from the complete blood count of 267 patients, consisting of 122 endometriosis patients with proven pathology reports of stages 3-4, and a control group of 145 people who underwent laparoscopy for simple ovarian cyst and/or diagnostic purposes and had normal findings in the pathology, were compared between the two groups. ROC and logistic regression analyses were performed.

Results: DNI and RDW were significantly higher in endometriosis patients than in the control group (p=0.034, 0.003, respectively). For parameters calculated from other complete blood counts (leukocyte, neutrophil, lymphocyte, monocytes, platelet, NLR), there was no difference between the two groups (P>0.05). For DNI, at a cut-off value of 0.025, AUC was 0.572 and it was statistically significant (p=0.042; 95%CI=0.503- 0.642, sensitivity: 45.9%, specificity: 67.6 %, Youden's Index = 0.135). For RDW, AUC=0.601 for cut-off value of 13.65 was statistically significant (p= 0.004, 95% CI= 0.553- 0.669, sensitivity= 50.8%, specificity= 67.6 %, Youden's Index= 0.184). The logistic regression model established with the combined marker obtained by multiplying the DNI and RDW was statistically significant (p<0.001, Nagelkerke R^2=0.72, 95% CI=2.58- 47.26, B: 2.40, NPV=78.6 %, PPV=37.7 %).

Conclusion: DNI, a new inflammatory marker, and RDW, known to be associated with inflammation, seem to be useful for clinically diagnosing endometriosis without the need for surgery.

Keywords: Endometriosis, delta neutrophil index, red blood cell distribution width, inflammation, biomarker

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Introduction

Endometriosis is an estrogen-dependent disease defined as the implantation and growth of endometrial cells outside the uterine cavity and affects approximately 10% of young women of reproductive age (1). It is a challenging disease for both patients and physicians as it is difficult to diagnose and treat and causes a decrease in quality of life. Although dysmenorrhea and dyspareunia are the most common symptoms, they can also cause bladder and/or intestinal pathologies. Clinical diagnosis is difficult as these symptoms are not specific to the disease. Even though imaging techniques such as ultrasonography and magnetic resonance imaging (MRI) are beneficial, especially in the diagnosis of deep infiltrating endometriosis and ovarian endometrioma (OMA), (2) laparoscopy is still the gold standard method for its definitive diagnosis, which provides the histopathological diagnosis. However, both surgeries for endometriosis with deep infiltrating into the pelvic organs and visual diagnosis during laparoscopy require significant surgical experience (3). Invasive surgical methods do not help in minimal and mild endometriosis (4). In addition, since it is an invasive procedure, most patients do not want to have surgery, and this causes a delay in diagnosis of up to 8 years (5). Although the most commonly used biomarker for the preoperative diagnosis of endometriosis is CA 125, which is synthesized by the coelomic epithelium, it is not specific for endometriosis and has low sensitivity and specificity for the diagnosis of endometriosis compared to laparoscopy(6). At this point, finding a biomarker that would ensure the accuracy of the preoperative diagnosis of endometriosis and OMA has become an essential need in managing endometriosis and an ongoing research topic (6).

In endometriosis, the suggestion of cytokines play a role in the ectopic implantation of endometrial cells (7), the high levels of proinflammatory cytokines reported in the pelvic fluids of women with endometriosis compared to the control group, the changes in circulating white blood cell (WBC) counts, the increase in serum proteins such as C-reactive protein (CRP) (8), and the demonstration of neutrophilia and lymphocytopenia are evidence to consider endometriosis as a local inflammatory disease with systemic subclinical manifestations(9). Inflammation in endometriosis is associated with immune clearance, modification of endometrial cell proliferation, prevention of invasion, and angiogenesis (10). Subsequent studies on the mechanism of inflammation in endometriosis patients focused on inflammatory cells, and endometriosis has been indicated to be a risk factor for developing the severe pelvic inflammatory disease (11).

Delta neutrophil index (DNI) is defined as the immature granulocytes (IG) fraction, which reflects the ratio of circulating IG to the total neutrophil count and can be detected by automatic hematology analyzers thanks to the latest technological developments (12). The term IG describes the myelocytes, promyelocytes, and metamyelocytes (neutrophil precursors) found in the bone marrow after the neonatal period. It has been revealed that these immature neutrophil forms enter the circulation during infection (12). In recent years, DNI has been suggested to be predictive and prognostic in infectious conditions such as acute appendicitis, bacterial peritonitis, and sepsis (12-14). Although red cell distribution width (RDW) has been defined as a biomarker associated with anemia, it has recently been accepted as a marker related to inflammation (15). Inflammation disrupts iron metabolism, shortens the lifespan of erythrocytes, and the erythropoietin response causes an increase in RDW levels (16).

Even though increased inflammatory response in patients with endometriosis has been evaluated for various markers in the literature, the relationship between DNI, a new inflammatory marker, and endometriosis has not been studied. The present study aimed to investigate the efficacy of DNI, which can be determined easily with complete blood count parameters, in diagnosing stage 3-4 endometriosis, which still does not have an ideal and reliable marker and unfortunately requires invasive procedures such as laparoscopy.

Material and Methods

The presented retrospective clinical study was performed between September 2019 and March 2022 at University, Faculty of Medicine, Department of Obstetrics and Gynecology. The study was approved by Health Sciences University Clinical Research Ethics Committee Medical Ethics Committee (2022/ 507).

A total of 353 patients' medical records were reviewed retrospectively, and clinical, demographic, laboratory and surgical data were obtained. The patient group consisted of 122 endometriosis patients who were operated on for endometriosis and/or endometrioma and who had endometriosis proven by pathology reports. The control group was formed by 145 age-matched patients who underwent laparoscopy or laparotomy due to unexplained infertility, chronic pelvic pain, bilateral tubal ligation, and simple ovarian cyst, who had no macroscopic endometriotic lesions, no history of endometriosis, and normal findings in pathology evaluation. All patients were caucasian non-pregnant women aged 18-45 years. Patients with systemic and infectious-inflammatory diseases, endocrine disorders, autoimmune diseases, tuberculosis, malignant disease, menopause, obesity, hepatic and renal diseases, and hematopoietic system diseases were excluded. Therefore, 41 patients with missing complete blood count parameters, 38 patients older than 45 years of age, two patients younger than 18 years of age, two patients with menopause, and three patients with pelvic inflammatory disease were not included in the study.

The histopathological diagnoses of all patients and blood analyses obtained during preparation for the operation were recorded (complete blood count: Sysmex XE-2100 hematology analyzer; Kobe, Japan, Ca 125: electrochemiluminescence immunoassay; Cobas 8000 e602).

For this study, the primary outcome was whether there was a difference in DNI between the endometriosis and control groups, and the secondary outcome was to investigate the predictive value of DNI for endometriosis.

Statistical analysis

The distribution of continuous variables was presented as mean and standard deviation (SD) values, while categorical variables as ratios and percentages of the total. Comparison of continuous variables between groups was performed with Student's t-test or Mann-Whitney U-test, depending on the normality of the distribution. Receiver operating characteristic (ROC) analysis determined the appropriate cut-off point for individual indicators and calculated sensitivity and specificity. The optimal significant cut-off value was calculated with the Youden’s Index. LR was determined as sensitivity/ (1- specificity). Logistic regression analysis was used to predict the effect of the combined biomarker on endometriosis, which was calculated by multiplying the RDW level with the DNI at a 95% confidence interval.

Results

The study consisted of 122 patients who were diagnosed with endometriosis histopathologically and 145 control groups without endometriosis determined during surgery and/or histopathological evaluation (Total number: 267). The patients in the endometriosis group were patients with deep pelvic endometriosis, tubal diffuse endometriosis, and stage 3-4 (moderate-severe) endometriosis due to OMA (17). No patient findings suggested mild endometriosis in the patient records. There was no difference in mean age between the two groups (mean± SD: patient: 34.84 ± 6.75; control: 34.09 ± 6.94; p= 0.379). DNI, RDW and CA 125 were significantly higher in the endometriosis group than in the control group (DNI: patient: 0.0278±0.0197; control: 0.220±0.0092; p= 0,034/ RDW: patient: 14.443±2.515; control: 13.594±2.0164; p= 0.003/ CA 125: patient: 82.19±178.51; control: 25.81±35.62; p<0.001). No differences were observed between the two groups in other complete blood count parameters (leukocytes, neutrophils, lymphocytes, monocytes, platelets, and NLR) (p>

0.05) (Table 1). DNI, RDW, and CA 125 were significantly positively correlated with the diagnosis of endometriosis ($p<0.05$; $r= 0.13, 0.19, 0.44$, respectively). In ROC analysis, for DNI, the cut-off value was 0.025 and AUC was 0.572, being statistically significant ($p= 0.042$; 95% CI= 0.503- 0.642, sensitivity: 45.9%, specificity: 67.6 %, Youden's Index= 0.135). For RDW, the cut-off value was 13.65 and AUC was 0.601, being also statistically significant ($p= 0.004$, 95% CI= 0.553- 0.669, sensitivity= 50.8%, specificity= 67.6%, Youden's Index= 0.184). In the patient records, the number of patients whose CA 125 value could be reached was 141 (endometriosis n= 85; control group n= 56), and similar to the literature(6), CA 125 was significantly higher in the endometriosis group ($p<0.05$). When ROC analysis was performed for CA 125, for the cut-off value of 28.54, AUC was 0.759, being statistically significant ($p<0.001$). In our ROC analysis with the combined marker (DNI and RDW), the specificity was close to the analysis for CA 125 alone (78.6% vs. 76%) (Figure 1) (Table 2). For CA 125, although the AUC value was higher than both RDW and DNI, the number of patients for whom we could reach CA 125 was much less (n:267 vs. 141). The combined marker obtained by multiplying DNI and RDW significantly predicted the diagnosis of endometriosis ($p<0.001$, Nagelkerke $R^2= 0.72$, 95% CI= 2.58- 47.26, B: 2.40, NPV= 78.6%, PPV= 37.7%) (Table 3). The significant cut-off value for the combined marker was 0.38 ($p= 0.003$; AUC= 0.606; 95% CI: 0.537-0.674; Youden's Index: 0.20; sensitivity= 44.3%; specificity= 76%) (Figure 2).

Discussion

In our study, the combined marker of two serum markers (DNI and RDW) had a better AUC (0.606) performance for moderate-to-severe endometriosis and a better specificity (68, 68, and 76, respectively). CA 125 alone had greater both AUC (0.760) and sensitivity (65%), but its specificity was similar to that of the combined marker (79%). The fact that the number of patients with a CA 125 value could be reached was lower and that CA 125 had low sensitivity in the diagnosis of endometriosis in previous studies (6) highlights DNI as a new marker combined with RDW in our study. In addition, CA 125 is a molecule that changes according to the menstrual cycle phase (18). Kitawaki et al. demonstrated that CA 125 level was below 20 IU/mL in 10.6% of OMA patients and 15.6% of middle-stage endometriosis patients (19). Thus, CA 125 alone does not appear to be sufficient as a marker for endometriosis. To date, no single marker with high sensitivity and specificity has been determined for endometriosis. Instead, it has been suggested that a combination of markers may more accurately predict endometriosis(6). We also combined DNI with RDW, and the result was statistically significant for endometriosis ($p= 0.003$).

Although the sensitivity and specificity for DNI were not at the desired level, the result was significant for the cut-off value of 0.025 (AUC= 0.572; P= 0.042). Surprisingly, the cut-off value for RDW was 13.65 (AUC= 0.601; P= 0.004). The fact that both markers are obtained very simply from complete blood count data seems very useful. In our study, all patients were recorded as stage 3-4 because of ovarian involvement (OMA) and widespread pelvic-peritoneal-tubal endometriosis in all patients who received surgical treatment (17). There are three clinical forms of the disease in clinical practice: superficial peritoneal endometriosis, deep infiltrating endometriosis, and OMA(20). However, their histopathological and immunohistochemical features are similar (21). Although there were no mild endometriosis patients in our study, this situation suggests that DNI and RDW would be useful for predicting endometriosis at all stages due to similar pathogenesis. The insidious, chronic and progressive nature of endometriosis causes a delay of up to 8 years in diagnosing and treating the disease (5). Patients with severe dysmenorrhea may have small lesions in the pelvic cavity, while other patients with moderate to severe endometriosis may be asymptomatic. In addition, diagnostic laparoscopy does not eliminate all possible complications (22). This situation may lead to the risk of infertility in young patients in the following years (23). The

gold standard for the definitive diagnosis of advanced endometriosis is laparoscopy. However, laparoscopy in the early stage may be insufficient for the diagnosis (4). In addition, for OMA, although imaging methods are helpful (2), there is too much variation in the number of organized blood products within the endometrioma and in the measurement of OMA diameter, which complicates the differential diagnosis of the cystic structure (24). Therefore, complete blood count parameters remain remarkable as a new noninvasive marker for the diagnosis of endometriosis, sensitive at all stages and locations of the disease and unaffected by the time of collection.

NLR is the most commonly studied inflammation marker among complete blood count parameters. In Jing et al.'s study of 662 patients with endometriosis and 83 patients with pathologically benign ovarian tumors, lymphocytes, CA 125, and NLR were significantly higher in endometriosis patients. For distinguishing endometriosis from other benign ovarian tumors, the combination of NLR and CA125 (81.3%) showed greater sensitivity than CA 125 alone (80.6%) (25). The sensitivity of NLR alone (32.9%) in this study was lower than the sensitivity (46%) determined for DNI in our study. Kim et al. reported that the severity of endometriosis was not associated with either NLR or CA 125 levels (26). The results of our study were also consistent with these studies. Therefore, NLR does not appear to be an ideal marker. Since peritoneal markers vary greatly according to hormonal effect and amount of peritoneal fluid and are more invasive, markers in serum are more useful in showing the disease's activity. Furthermore, although a large number of molecules have been studied in the bloodstream, including a wide variety of cytokines, hormones, growth factors, adhesion molecules, and antibody levels (6), the analysis of these molecules has difficulties in routine clinical practice, such as precise threshold calculation and high cost. However, DNI and RDW, which were significant in our study, are calculated automatically in whole blood analysis.

Neutrophils play a role in innate immunity and have been found to have more functions than antimicrobial responses in various tissues under pathological conditions (27). There is growing evidence that neutrophils have a role in endometriosis patients (28). Systemic inflammation leads to the destruction of circulating mature neutrophils and the loss of active neutrophils. To compensate for this situation, the number of immature neutrophils (metamyelocytes, myelocytes, and promyelocytes) in the circulation increases and a left shift occurs where the immature/total granulocyte ratio increases, which is an indicator of sepsis and inflammation (29). Therefore, DNI has been studied as a marker for many inflammatory and infectious diseases. Besides being reported as a diagnostic tool that better predicts mortality during sepsis than CRP (30), it has been indicated to predict perforation in patients with appendicitis (31). DNI has also been studied in obstetric patients. In women with severe preeclampsia, serum DNI value was increased compared to women with normal pregnancy or mild preeclampsia (32). In another study, DNI was a predictive marker for histological chorioamnionitis in patients with preterm premature rupture of membranes (33). In other studies, a higher DNI has been reported as a prognostic marker of conditions such as cardiac arrest and pulmonary embolism, and based on these studies, DNI values were considered to reflect both the severity of the infection and the severity of diseases associated with systemic and sterile inflammation in the absence of infection (34, 35). Also, DNI is time and cost-effective, as it is simply analyzed with a complete blood count (36). Our study found DNI to be significantly higher in endometriosis patients since it is known that endometriosis is associated with inflammatory response, and DNI increases inflammation.

In the present study, RDW was significantly higher in the endometriosis group compared to the control group, and its specificity was the same as DNI in predicting endometriosis ($p<0.05$, 68%). Recently, RDW has been recognized as an inflammation-related marker. Inflammation is also a key feature of endothelial dysfunction, and this effect results in an

increased RDW, indicating abnormal erythrocyte survival (15). Besides the disruption of iron metabolism during inflammation and the effect of cytokines released during inflammation, the disruption of the erythropoietin response leads to anisocytosis and an abnormal RDW. Some evidence indicates the potential role of iron metabolism disorders in the pathogenesis of endometriosis. Iron accumulated in the peritoneal cavity of women with endometriosis causes free radical production, inflammation, and cell damage (37). As a result of all these, it is plausible that RDW is affected in endometriosis, an inflammatory condition (38). In addition, Lippi et al. demonstrated that RDW significantly correlates with CRP and erythrocyte sedimentation rate (39). In a study consisting of 98 patients, RDW was significantly higher in the endometriosis (n: 50) group compared to the control group (n: 48), and RDW was found to be associated with the severity of endometriosis (40). In our study, RDW was significantly higher in the endometriosis group, and the number of patients was much higher (n: 267). Qin et al. determined a positive correlation between endometriosis score and RDW; however, surprisingly, there was no significant association between CA 125 and NLR. As the study population included only women with moderate to severe endometriosis, as in our study, they could not exclude the possibility that NLR is associated with the severity of early-stage endometriosis. However, NLR was not a good marker for assessing the severity of endometriosis in patients with moderate to severe endometriosis (41). In another study, a comparison between patients with stage 3 (n: 96) and stage 4 (n: 87) endometriosis showed that mean levels of CA-125 and RDW were significantly higher in stage 4 patients than in stage 3 patients (42).

Although OMA is a condition in which advanced endometriosis can be diagnosed preoperatively, most advanced endometriosis patients may be asymptomatic. Also, it has been suggested that in patients with stage 3/4 endometriosis, removing only the OMA and leaving possible pelvic and intestinal endometriotic foci in place would be an inadequate treatment (43). In this context, a marker that will enable the preoperative identification of stage 3/4 endometriosis patients can provide an idea about the necessity of extensive pelvic surgery in advance.

Study Limitations

This study had some limitations. First, the data used were obtained from a single center, and since it was a retrospective study, causality cannot be determined. DNI was calculated for each patient from a one-off blood sample only. Therefore, we did not know the changes over time. In our clinic, automatic IG count parameters could be reached after 2018, and the number of patients remained relatively limited. We could not include the patients' body mass indexes since they were not recorded in the patient files. Besides, all patients consisted of moderate-to-advanced endometriosis patients. The first-time investigation of DNI in endometriosis is the strength of this study.

Conclusion

Inflammation-mediated mechanisms play a critical role in the etiology of endometriosis. Therefore, DNI, which is prognostic in many inflammatory and systemic diseases, can be used as a new low-cost and rapid marker in endometriosis. Elucidating how and why DNI is associated with the endometriosis may provide increased understanding of pathophysiology. In this sense, well-designed prospective studies are needed better to understand the role of DNI.

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Table 1. The comparison of inflammatory markers and baseline characteristics between endometriosis and control groups

	Endometriosis patients, (n=122)	**Control group, (n=145)**	**p**
DNI (IG: µl)	0.0278±0.0197	0.0220±0.0092	[a]**0.034**
RDW	14.443±2.515	13.594±2.0164	[b]**0.003**
Combined marker	0.41±0.32	0.23±0.14	[a]**0.003**
CA 125 (IU/ mL)	82.19±178.51	25.81±35.62	[a]**<0.001**
NLR	3.58±4.042	2.84±1.75	[a]0.634
WBC (10^3/µl)	7.77±2.018	7.77±1.976	[b]0.997
Lymphocyte (10^3/µl)	1.98±0.68	2.02±0.63	[b]0.612
Neutrophil (10^3/µl)	5.05±2.19	5.06±1.78	[a]0.553
Platelets (10^3/µl)	268.71±66.17	265.92±69.13	[b]0.737
MPV (fL)	10.57±0.96	10.49±0.98	[b]0.461
Age (years)	34.84±6.75	34.09±6.94	a0.379
Having child	% 47.6	% 41.5	c0.347
Irregular menstruation	% 56.7	% 43.3	c0.169

DNI: Delta neutrophil index; RDW: Red blood cell distribution width; WBC: White blood cell; NLR: Neutrophil-to-lymphocyte ratio; MPV: Mean platelet volume, [a]Mann-Whitney U-Test, [b]Student T- Test, [c]Pearson Chi-Square

Table 2. Comparison of the ROC Analyses of of four markers (DNI, RDW, combination of DNI and RDW, CA125) for prediction of stage 3-4 endometriosis

Markers	AUC	Sensitivity, (%)	Specificity, (%)	Cut-off	(95% CI)		Youdan Index	p
					Lower bound	Upper bound		
DNI	0.572	45.9	67.6	0.025	0.503	0.642	0.13	**0.042**
RDW	0.601	50.8	67.6	13.65	0.553	0.669	0.18	**0.004**
DNI and RDW	0.606	44.3	76.0	0.38	0.537	0.674	0.20	**0.003**
CA125	0.760	64.7	78.6	28.54	0.678	0.841	0.43	**<0.001**

ROC: Receiver operating characteristic, AUC: Area-under-curve, P<0.05 is significant

Table 3. Logistic regression analysis showing the predictive effect of combined markers on endometriosis (omnibus tests of model coefficients: p=0.001; Nagelkerke R2: 0.72)

Variables	B	OR	95% CI		Sensitivity, %	Specificity, %	PPV, %	NPV, %	p
			Lower	Upper					
Combined marker	2.4	11.04	2.58	47.26	44.3	76	37.7	78.6	0.001

PPV: Positive predictive value, NPV: Negative predictive value for combined marker (DNI and RDW) CI: Confidence interval, p<0.05 is significant

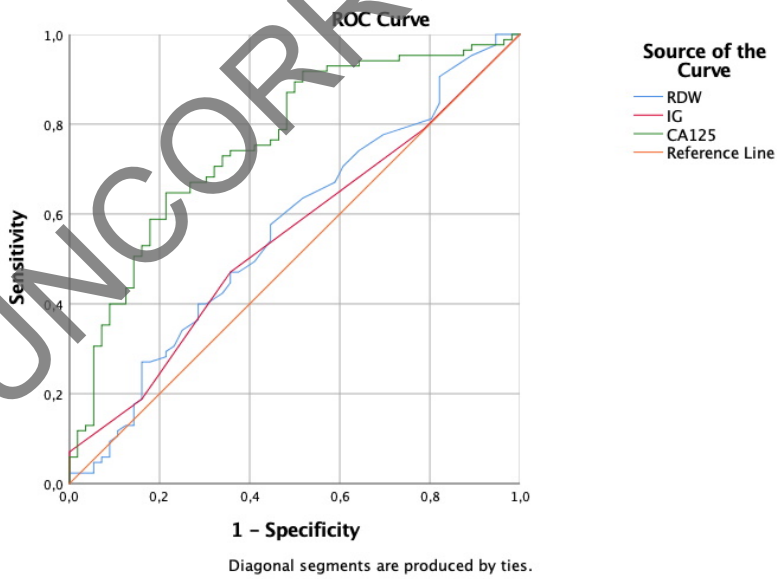

Figure 1. ROC Analyses of DNI, RDW, and CA 125 for prediction of stage 3-4 endometriosis

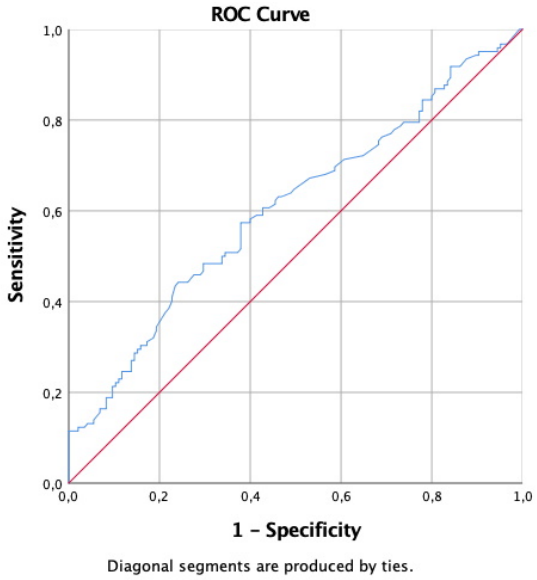

Figure 2. ROC Analyses of the combination of DNI and RDW for prediction of stage 3-4 endometriosis