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Original Article

Diagnostic performance of hematological inflammatory biomarkers for assessing endometriosis severity and pelvic pain severity

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Abstract

Endometriosis is a chronic inflammatory disease frequently associated with pelvic pain and infertility. Since systemic inflammation may reflect disease activity, hematological inflammatory biomarkers obtained from routine blood tests have been proposed as non-invasive indicators of endometriosis severity. The aim of this study was to evaluate the association of the neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), systemic immune-inflammation index (SII), and monocyte-to-lymphocyte ratio (MLR) with endometriosis severity and pelvic pain severity, as well as to assess their diagnostic performance. A cross-sectional study was conducted at Dr. Zainoel Abidin Hospital, Banda Aceh, Indonesia, among women with histopathologically confirmed endometriosis. Endometriosis severity was classified intraoperatively according to the revised American Society for Reproductive Medicine (rASRM) staging system. Pelvic pain severity was assessed preoperatively using the Numeric Rating Scale (NRS). Hematological inflammatory biomarkers were calculated from preoperative complete blood count results. The associations and diagnostic performance of the biomarkers in relation to endometriosis severity and pelvic pain severity were evaluated using correlation analysis and receiver operating characteristic (ROC) curve analysis. Our data indicated that NLR (r=0.460; p<0.001), SII (r=0.439; p=0.001), and MLR (r=0.391; p=0.003) had significant positive correlations with endometriosis severity, whereas PLR demonstrated a borderline correlation (r=0.261; p=0.050). ROC analysis showed good diagnostic performance for NLR (AUC=0.784; p=0.005), SII (AUC=0.777; p=0.006), and MLR (AUC=0.783; p=0.005), while PLR was not statistically significant (AUC=0.679; p=0.078). NLR (r=0.295; p=0.026) and SII (r=0.275; p=0.038) showed weak but significant positive correlations with pelvic pain severity, whereas PLR and MLR had no significant association. None of the biomarkers demonstrated significant diagnostic performance for pelvic pain severity. NLR, SII, and MLR may serve as accessible and inexpensive adjunctive indicators of endometriosis severity, particularly in resource-limited settings; however, their utility for assessing pelvic pain severity appears limited.

Keywords: Endometriosis, endometriosis severity, pelvic pain, neutrophil-to-lymphocyte ratio, monocyte-to-lymphocyte ratio

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Introduction

Endometriosis is a chronic gynecological disorder characterized by the presence of endometrial-like tissue outside the uterine cavity and commonly presents with chronic pelvic pain and infertility. The disease affects approximately 6–10% of women of reproductive age and remains a major cause of morbidity and impaired quality of life worldwide [1]. The pathophysiology of endometriosis involves a complex chronic inflammatory process characterized by immune activation and the release of inflammatory mediators that contribute to the development and progression of ectopic endometrial lesions [2]. Increasing evidence suggests that systemic inflammatory responses play an important role in disease severity and symptom manifestation, leading to interest in identifying reliable inflammatory biomarkers for disease assessment [2-4].

Hematological inflammatory biomarkers, including the neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), systemic immune-inflammation index (SII), and monocyte-to-lymphocyte ratio (MLR), have emerged as potential indicators of systemic inflammation [5]. Previous studies have demonstrated significant associations between these biomarkers and the severity of endometriosis based on the revised American Society for Reproductive Medicine (rASRM) classification [3,4]. Since laparoscopic visualization and histologic confirmation remain the gold standard for the diagnosis and staging of endometriosis [6], the identification of accessible and non-invasive biomarkers may facilitate preoperative assessment and disease stratification.

Pelvic pain is one of the most common and debilitating manifestations of endometriosis and is strongly associated with reduced quality of life [7]. Recent studies have reported positive correlations between hematological inflammatory biomarkers and pelvic pain severity, possibly reflecting the inflammatory activity underlying pain generation in endometriosis [8,9]. However, evidence regarding the relationship of these biomarkers with both endometriosis severity and pelvic pain severity remains limited and inconsistent, particularly for newer composite indices such as SII and MLR [3,8,10]. Therefore, the aim of this study was to evaluate the association and diagnostic performance of NLR, PLR, SII, and MLR with endometriosis severity and pelvic pain severity in patients with endometriosis.

Methods

Study design and setting

A cross-sectional analytic study was conducted at the Obstetrics and Gynecology outpatient clinic and inpatient ward of Dr. Zainoel Abidin Hospital, Banda Aceh, Indonesia, between January 2025 and December 2025. Patients suspected of having endometriosis and scheduled for laparoscopy or laparotomy were invited to participate. Patients underwent clinical history taking, general physical examination, gynecological examination, and ultrasonography. Preoperative complete blood count results were used to calculate NLR, PLR, SII, and MLR. Pelvic pain severity was assessed using the Numeric Rating Scale (NRS). During the laparoscopy or laparotomy, endometriosis severity was determined using rASRM classification. The diagnosis of endometriosis was confirmed by histopathological examination, and only histopathologically confirmed cases were included in the final analysis.

Patients and criteria

Patients were included if they had suspected endometriosis or endometriotic cysts, were willing to undergo hematological assessment for the calculation of NLR, PLR, SII, and MLR, had no history of laparotomy or cesarean section, and had not received hormonal therapy within the previous three months. Patients with suspected pelvic inflammatory disease or peritoneal tuberculosis based on clinical, laboratory, and ultrasonographic findings, as well as those with suspected malignancy, were excluded from the study.

Sample size and sampling

The minimum sample size was calculated using the Lemeshow formula for a single population proportion, with a 95% confidence level, an estimated endometriosis prevalence of 2%, and a 5%

margin of error. This calculation yielded a minimum sample size of 30 participants. After accounting for an anticipated 20% dropout or incomplete data rate, the minimum required sample size was increased to 36 participants. The consecutive sampling method was employed. In the final analysis, 57 patients with histopathologically confirmed endometriosis were included.

Data collection

Patient demographic and clinical data were recorded at baseline, including age, education level, marital status, parity, and gynecological symptoms (dysmenorrhea and dyspareunia). Pelvic pain intensity was assessed before surgery using NRS. Venous blood samples were collected as part of routine clinical evaluation and analyzed using complete blood count examinations for calculation of hematological inflammatory biomarkers. All participants subsequently underwent laparoscopy or laparotomy, during which intraoperative findings were documented for endometriosis severity assessment. Surgical specimens obtained during the procedure were submitted for histopathological examination to confirm the diagnosis of endometriosis.

Study variables

The dependent variables of this study were endometriosis severity and pelvic pain severity, while the independent variables were hematological inflammatory biomarkers: NLR, PLR, SII, and MLR. Endometriosis severity was determined intraoperatively according to the rASRM classification from stage I to IV [11]. Pelvic pain severity was assessed using NRS, with scores ranging from 0 to 10, where higher scores indicated greater pain intensity. For descriptive and discriminative analyses, endometriosis severity was categorized as mild (stage I–II) or moderate-to-severe (stage III–IV) and pain severity was categorized as mild-to-moderate (NRS 1–6) or severe (NRS 7–10).

Hematological inflammatory biomarkers were calculated from preoperative complete blood count results. NLR was calculated as the ratio of neutrophil count to lymphocyte count, PLR as the ratio of platelet count to lymphocyte count, MLR as the ratio of monocyte count to lymphocyte count, and SII as the product of neutrophil and platelet counts divided by lymphocyte count.

Statistical analysis

Descriptive statistics were used to summarize patient characteristics and clinical features. Categorical variables were presented as frequencies and percentages, while continuous variables were presented as median and range (minimum–maximum). Correlations between hematological inflammatory biomarkers (NLR, PLR, SII, and MLR) and endometriosis severity, as well as pelvic pain severity, were analyzed using Spearman's rank correlation test. The discriminative ability of each biomarker was evaluated using receiver operating characteristic (ROC) curve analysis, expressed as area under the curve (AUC) with 95% confidence intervals (95%CIs), for both endometriosis severity and pelvic pain severity. Optimal cut-off values were determined using the Youden Index for biomarkers that demonstrated statistically significant discriminatory ability in ROC analysis. Diagnostic performance at the optimal cut-off points was reported in terms of sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy. A $p<0.05$ was considered statistically significant. Data were analyzed using the Statistical Package for the Social Sciences (SPSS) version 26.0 (IBM Corp., Armonk, NY, USA).

Results

Characteristics of the patients

A total of 57 women with histopathologically confirmed endometriosis were included in this study and their baseline characteristics and clinical features are presented in **Table 1**. More than half of the patients were aged 20–35 years (56.1%), followed by those aged >35 years (38.6%) and <20 years (5.3%). Most patients had completed senior high school education (63.2%). Dysmenorrhea was reported by 96.5% of patients, whereas dyspareunia was reported in 10.5%. Most patients had moderate-to-severe endometriosis based on the rASRM staging (82.5%), while only 17.5% had mild severity. Pelvic pain severity assessed using the NRS showed that 66.7% of patients had mild-to-moderate pain (NRS 1–6), while 33.3% had severe pain (NRS 7–10). The median values

of hematological inflammatory biomarkers were NLR 2.23 (0.90–5.92), PLR 157.42 (44.62–426.72), SII 910.00 (246.75–3654.54), and MLR 0.22 (0.08–0.62) (**Table 1**).

Table 1. Characteristics and clinical features of patients with endometriosis included in the study (n=57)

Characteristic	Frequency (%)
Age	
<20 years	3 (5.3)
20–35 years	32 (56.1)
>35 years	22 (38.6)
Education level	
Junior high school	1 (1.8)
Senior high school	36 (63.2)
Diploma	7 (12.3)
Bachelor's degree	13 (22.8)
Marital status	
Unmarried	4 (7.0)
Married	53 (93.0)
Parity	
Nulliparous	38 (66.7)
Primiparous	7 (12.3)
Multiparous	12 (21.1)
Dysmenorrhea	
Yes	55 (96.5)
No	2 (3.5)
Dyspareunia	
Yes	6 (10.5)
No	51 (89.5)
Endometriosis severity (based on rASRM classification)	
Mild (stage I–II)	10 (17.5)
Moderate-to-severe (stage III–IV)	47 (82.5)
Median score (min–max)	4 (2–4)
Pelvic pain severity	
Mild-to-moderate (NRS 1–6)	38 (66.7)
Severe (NRS 7–10)	19 (33.3)
Median score (min–max)	5 (0–10)
Hematological biomarkers, median (min–max)	
Neutrophil-to-lymphocyte ratio (NLR)	2.23 (0.90–5.92)
Platelet-to-lymphocyte ratio (PLR)	157.42 (44.62–426.72)
Systemic immune-inflammation index (SII)	910.00 (246.75–3654.54)
Monocyte-to-lymphocyte ratio (MLR)	0.22 (0.08–0.62)

NRS: Numeric Rating Scale; rASRM: revised American Society for Reproductive Medicine

Correlations of inflammatory biomarkers with endometriosis severity and pelvic pain severity

Correlations between hematological inflammatory biomarkers and endometriosis severity are presented in **Table 2**. NLR showed a significant positive correlation with endometriosis severity (r=0.460; p<0.001). SII also demonstrated a significant positive correlation (r=0.439; p=0.001) as well as MLR (r=0.391; p=0.003). PLR showed a borderline correlation with endometriosis severity (r=0.261; p=0.050) (**Table 2**).

Table 2. Correlations between hematological inflammatory biomarkers and endometriosis and pelvic pain severity (n=57)

Outcome	Biomarker	Correlation coefficient (r)	p-value
Endometriosis severity	Neutrophil-to-lymphocyte ratio (NLR)	0.460	<0.001
	Platelet-to-lymphocyte ratio (PLR)	0.261	0.050
	Systemic immune-inflammation index (SII)	0.439	0.001
	Monocyte-to-lymphocyte ratio (MLR)	0.391	0.003
Pelvic pain severity	Neutrophil-to-lymphocyte ratio (NLR)	0.295	0.026
	Platelet-to-lymphocyte ratio (PLR)	0.143	0.289
	Systemic immune-inflammation index (SII)	0.275	0.038
	Monocyte-to-lymphocyte ratio (MLR)	0.161	0.231

Correlation analysis between hematological inflammatory biomarkers and pelvic pain severity is presented in **Table 2**. NLR and SII showed significant positive correlation with pelvic pain severity, r=0.295; p=0.026 and r=0.275; p=0.038, respectively. In contrast, PLR and MLR did not show significant correlations with pelvic pain severity (**Table 2**).

Discriminative ability of inflammatory biomarkers for endometriosis severity

ROC analysis of hematological inflammatory biomarkers for endometriosis severity is presented in **Table 3** and **Figure 1**. NLR showed good diagnostic performance with an AUC of 0.784 (95%CI: 0.601–0.967; p=0.005) (**Figure 1A**). SII demonstrated a comparable discriminative ability with an AUC of 0.777 (95%CI: 0.623–0.931; p=0.006) (**Figure 1B**). MLR also showed good performance with an AUC of 0.783 (95%CI: 0.601–0.965; p=0.005) (**Figure 1C**). In contrast, PLR demonstrated lower diagnostic performance compared to other biomarkers and did not reach statistical significance with an AUC of 0.679 (95%CI: 0.502–0.855) (**Table 3**).

Table 3. Receiver operating characteristic (ROC) analysis of hematological inflammatory biomarkers for endometriosis and pelvic pain severity

Outcome and biomarkers	AUC	Standard error	95% confidence interval	p-value
Endometriosis severity				
Neutrophil-to-lymphocyte ratio (NLR)	0.784	0.093	0.601–0.967	0.005*
Platelet-to-lymphocyte ratio (PLR)	0.679	0.090	0.502–0.855	0.078
Systemic immune-inflammation index (SII)	0.777	0.079	0.623–0.931	0.006*
Monocyte-to-lymphocyte ratio (MLR)	0.783	0.093	0.601–0.965	0.005*
Pelvic pain severity				
Neutrophil-to-lymphocyte ratio (NLR)	0.580	0.079	0.426–0.734	0.326
Platelet-to-lymphocyte ratio (PLR)	0.544	0.085	0.379–0.710	0.588
Systemic immune-inflammation index (SII)	0.580	0.081	0.421–0.740	0.326
Monocyte-to-lymphocyte ratio (MLR)	0.558	0.079	0.403–0.713	0.477

AUC: area under the receiver operating characteristic curve
* Statistically significant at p<0.05

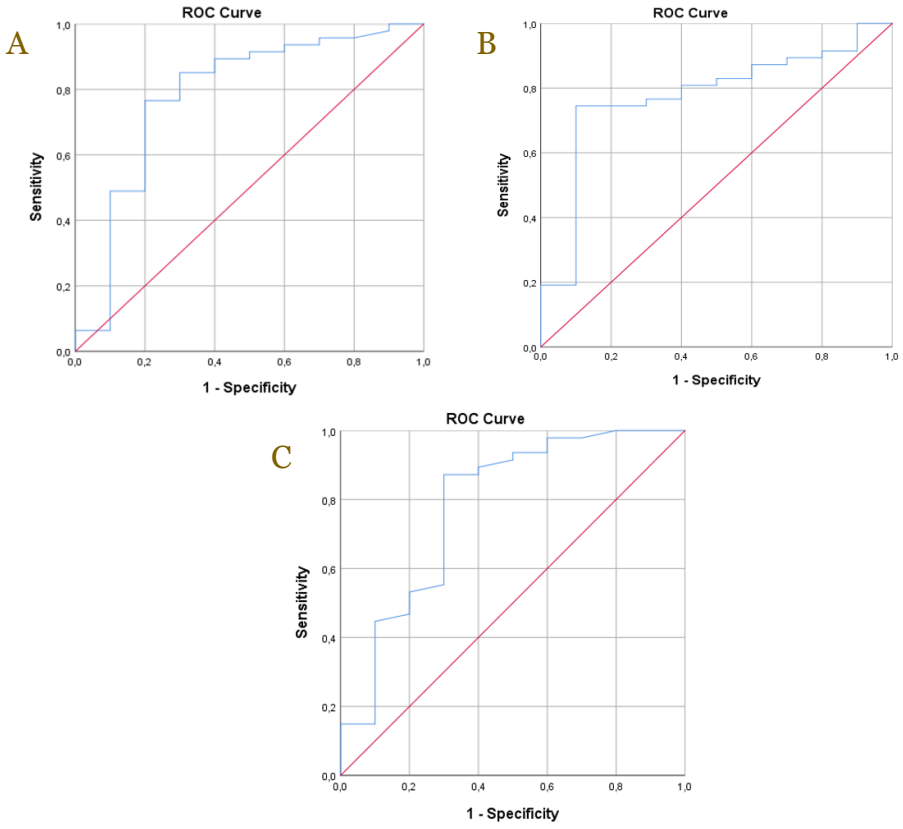

Figure 1. Receiver operating characteristic (ROC) curves of hematological inflammatory biomarkers for identifying moderate-to-severe endometriosis. (A) Neutrophil-to-lymphocyte ratio (NLR); (B) systemic immune-inflammation index (SII); and (C) monocyte-to-lymphocyte ratio (MLR).

ROC analysis of hematological inflammatory biomarkers for pelvic pain severity is presented in **Table 3**. All biomarkers demonstrated limited discriminatory ability for pelvic pain severity and did not reach statistical significance.

Diagnostic performance of inflammatory biomarkers for endometriosis severity

The optimal cut-off values and diagnostic performance of hematological inflammatory biomarkers for identifying moderate-to-severe endometriosis are presented in **Table 4**. NLR showed an optimal cut-off value of 1.723, with a sensitivity of 76.6% and specificity of 80.0%. SII had an optimal cut-off value of 687.33, with a sensitivity of 74.5% and the highest specificity among the biomarkers at 90.0%. MLR showed an optimal cut-off value of 0.1768 and had the highest sensitivity at 87.2%, although its specificity was lower at 70.0%. These findings indicate that NLR, SII, and MLR may have diagnostic utility as accessible inflammatory markers for identifying moderate-to-severe endometriosis, with SII offering the best specificity and MLR offering the best sensitivity (**Table 4**).

Table 4. Optimal cut-off values and diagnostic performance of hematological inflammatory biomarkers for identifying moderate-to-severe endometriosis

Biomarker	Cut-off	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)
NLR	1.723	76.6	80.0	94.7	42.1	77.2
SII	687.33	74.5	90.0	97.2	42.9	77.1
MLR	0.1768	87.2	70.0	93.2	53.8	84.2

MLR: monocyte-to-lymphocyte ratio; NLR: neutrophil-to-lymphocyte ratio; NPV: negative predictive value; PPV: positive predictive value; SII: systemic immune-inflammation index.

Discussion

This study aimed to evaluate the association and diagnostic performance of hematological inflammatory biomarkers, including NLR, PLR, SII, and MLR, in relation to endometriosis severity and pelvic pain severity. The findings demonstrated that NLR, SII, and MLR were significantly correlated with endometriosis severity and showed good discriminatory performance for identifying moderate-to-severe disease. Among these biomarkers, MLR showed the highest sensitivity, whereas SII demonstrated the highest specificity. In contrast, PLR showed only a borderline association with endometriosis severity and did not achieve significant diagnostic performance. Regarding pelvic pain severity, NLR and SII showed weak but significant positive correlations, whereas PLR and MLR were not significantly associated. However, none of the biomarkers demonstrated significant discriminatory ability for distinguishing severe from mild-to-moderate pelvic pain.

The clinical characteristics of the study population were generally consistent with the known epidemiology of endometriosis. Most participants were women of reproductive age, and a large proportion were nulliparous, findings that have been commonly reported among patients with endometriosis [12,13]. Notably, 82.5% of patients had moderate-to-severe disease, likely reflecting referral bias inherent to tertiary-care centers, where patients often present after prolonged symptoms and previous evaluations [14,15]. The predominance of advanced-stage disease may also have influenced the diagnostic performance of the evaluated biomarkers, particularly their relatively low negative predictive values.

The significant associations observed between NLR, SII, and MLR with endometriosis severity support the central role of systemic inflammation in disease progression. Endometriosis is increasingly recognized as a chronic inflammatory disorder characterized by dysregulated immune responses, persistent inflammatory signaling, and progressive tissue remodeling [16-18]. More severe disease is associated with increased production of inflammatory mediators, including interleukin-8, prostaglandin E2, and vascular endothelial growth factor, which promote neutrophil recruitment, angiogenesis, and lesion survival [8,19]. Consequently, increased neutrophil counts together with relative lymphocyte suppression contribute to elevated NLR values in patients with more severe disease. These findings are supported by previous studies demonstrating significantly higher NLR values among women with endometriosis, particularly those with moderate-to-severe disease [3,8]. Similarly, SII demonstrated a

significant association with endometriosis severity and good discriminatory performance. Because SII incorporates neutrophil, platelet, and lymphocyte counts into a single index, it may provide a more comprehensive representation of the inflammatory milieu associated with disease progression [20,21].

MLR also demonstrated a significant association with endometriosis severity and showed discriminatory performance comparable to NLR and SII. This finding may be explained by the important role of monocytes and macrophages in endometriosis pathogenesis. Circulating monocytes are recruited into the peritoneal cavity through chemokines such as monocyte chemoattractant protein-1, where they differentiate into macrophages that contribute to angiogenesis, extracellular matrix remodeling, and immune dysregulation [22]. As disease severity increases, enhanced monocyte recruitment combined with reduced adaptive immune activity may lead to higher MLR values. Previous studies have similarly reported elevated MLR levels in women with endometriosis and suggested that MLR may serve as a useful inflammatory biomarker for disease assessment [9,23].

Unlike the other biomarkers, PLR demonstrated only a borderline correlation with endometriosis severity and failed to achieve statistically significant diagnostic performance. Several mechanisms may explain this observation. Platelet counts can be influenced by various physiological and pathological conditions independent of endometriosis-related inflammation. Iron deficiency anemia, which is common among women with endometriosis because of chronic menstrual blood loss, may induce reactive thrombocytosis and consequently alter PLR values [13,24]. Furthermore, platelets appear to be more closely associated with fibrosis and adhesion formation than with overall disease severity. Previous studies have suggested that PLR correlates more strongly with pelvic adhesions than with rASRM severity classification, indicating that PLR may reflect specific pathological components of endometriosis rather than the overall severity of disease progression [8,25]. This may account for the weaker association observed in the present study.

Regarding pelvic pain severity, NLR and SII demonstrated significant but weak positive correlations with pain scores, suggesting that systemic inflammation contributes to symptom burden in women with endometriosis. Chronic inflammation promotes the production of prostaglandins, cytokines, and other mediators capable of sensitizing peripheral nociceptors and amplifying pain perception [20,26]. However, the relatively modest correlation coefficients observed in this study indicate that inflammatory activity alone cannot fully explain the complexity of pelvic pain. Endometriosis-associated pain is increasingly recognized as a multidimensional phenomenon involving not only peripheral inflammation but also central sensitization, hormonal influences, lesion location, and psychological factors [26,27]. These mechanisms likely contribute to the considerable variability in pain experience among patients with similar levels of disease severity.

An important finding of this study is that none of the evaluated biomarkers demonstrated significant discriminatory ability for pelvic pain severity despite the significant correlations observed for NLR and SII. Although these findings may initially appear contradictory, correlation analysis and ROC analysis evaluate different aspects of association. Correlation analysis assesses whether biomarker levels increase in parallel with pain scores, whereas ROC analysis evaluates the ability of biomarkers to accurately classify patients into distinct pain severity categories. The lack of significant ROC performance suggests that hematological inflammatory biomarkers alone are insufficient to reliably distinguish patients with severe pain from those with mild-to-moderate pain. This observation is consistent with previous studies reporting inconsistent relationships between inflammatory biomarkers and pelvic pain, highlighting the multifactorial nature of pain perception in endometriosis [26-28]. Therefore, these biomarkers may serve as adjunctive indicators of inflammatory activity but should not be considered standalone tools for pain assessment.

This study has some limitations that need to be acknowledged. The relatively small sample size and single-center design may limit the generalizability of the findings. In addition, hematological inflammatory biomarkers are nonspecific markers that can be influenced by factors unrelated to endometriosis, while pelvic pain assessment remains inherently subjective. Finally, other established biomarkers such as CA-125, interleukin 6, and vascular endothelial

growth factor were not evaluated. Future multicenter studies with larger sample sizes and additional biomarkers are needed to validate these findings.

Conclusion

Three hematological inflammatory biomarkers (NLR, SII, and MLR) were significantly associated with endometriosis severity and demonstrated good discriminatory ability for identifying moderate-to-severe disease. Among these biomarkers, MLR showed the highest sensitivity, whereas SII demonstrated the highest specificity. In contrast, PLR showed limited utility, with only a borderline association with endometriosis severity and no significant discriminatory performance. Although NLR and SII were significantly associated with pelvic pain severity, all evaluated biomarkers demonstrated limited ability to discriminate between severe and mild-to-moderate pelvic pain. These findings suggest that hematological inflammatory biomarkers, particularly NLR, SII, and MLR, may serve as accessible and inexpensive adjunctive markers for assessing endometriosis severity, while their role in evaluating pelvic pain severity appears limited.

Ethics approval

The study protocol was reviewed and approved by the Ethics Committee of the Dr. Zainoel Abidin General Hospital, Banda Aceh, Indonesia (Approval No.340/ETIK-RSUDZA/2025). All patients provided written informed consent prior to enrollment.

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Competing interests

All the authors declare that there are no conflicts of interest.

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Underlying data

Derived data supporting the findings of this study are available from the corresponding author on request.

Declaration of artificial intelligence use

Artificial intelligence (AI) tools, ChatGPT and QuillBot, were employed to improve grammar, sentence structure, and readability. The authors critically reviewed and revised all AI-generated outputs to ensure accuracy, coherence, and alignment with the study's objectives. The final decisions, interpretations, and manuscript content reflect the authors' independent judgment and intellectual contributions.

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