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AGE-RELATED EVALUATION OF NEUTROPHIL-TO-LYMPHOCYTE RATIO IN WOMEN WITH POLYCYSTIC OVARY SYNDROME: A RETROSPECTIVE STUDY

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Abstract

Objective: To evaluate the effect of age on the neutrophil-lymphocyte ratio (NLR), a marker of systemic inflammation, and other hematological parameters in women diagnosed with polycystic ovary syndrome (PCOS).

Methods: This retrospective study examined 280 women aged 18-43 who had been diagnosed with PCOS according to the Rotterdam criteria between May 2022 and May 2025. The patients were divided into four groups according to their age: Group 1 (18-24 years, n=100); Group 2 (25-30 years, n=130); Group 3 (31-35 years, n=37); and Group 4 (36-43 years, n=13). The Kruskal-Wallis test was used to compare neutrophil, lymphocyte, and NLR levels between groups. Spearman correlation analysis was used to evaluate the relationship between age and inflammatory parameters. The independent effect of age on NLR was examined using multivariate linear regression analysis.

Results: No significant difference in NLR levels was found between age groups (p=0.160). However, the neutrophil count was significantly lower in Group 3 than in the other groups (p=0.013). A weak negative correlation was found between age and neutrophil count (r=-0.142, p=0.024), but no significant correlation was found between age and NLR (r=0.084, p=0.162). Multivariate regression analysis revealed that age had no independent effect on NLR (β=-0.088, p=0.156), whereas BMI positively affected NLR (β=+0.176, p=0.005).

Conclusion: In this cohort of women with PCOS, NLR did not show a significant association with age, whereas it was associated with BMI. These findings emphasise the importance of considering metabolic factors when evaluating the inflammatory process in PCOS.

Keywords: *Polycystic ovary syndrome, neutrophil-lymphocyte ratio, age, inflammation, body mass index.*

Introduction

Polycystic ovary syndrome (PCOS) is the most common endocrine disorder in women of reproductive age, with a prevalence ranging from 5% to 20% depending on the diagnostic criteria used.[1] PCOS is a heterogeneous syndrome characterised by oligo-anovulation, hyperandrogenism and polycystic ovarian morphology.[2] In addition to these symptoms, metabolic abnormalities such as insulin resistance, obesity, dyslipidaemia and diabetes mellitus are frequently observed in patients with PCOS.[3]

Recent studies have shown that low-grade chronic inflammation plays an important role in the pathophysiology of PCOS.[4] This inflammatory state is thought to contribute to the development of reproductive and metabolic complications associated with the syndrome. The neutrophil-to-lymphocyte ratio (NLR) is considered a readily accessible, inexpensive and reliable biomarker of systemic inflammation.[5] Numerous studies have demonstrated that NLR levels in women with PCOS are significantly higher than in healthy controls and that this increase is independent of obesity.[5, 6] Therefore, NLR stands out as a potential marker for assessing inflammation in PCOS.

The phenotypic characteristics of PCOS can change with age. While hyperandrogenism and menstrual irregularities are more prevalent in younger women, obesity and metabolic disorders become more prominent in older women.[7] Decreased androgen production and changes in ovarian morphology with age may also affect inflammatory markers.[8] However, the effect of age on inflammatory markers in PCOS remains unclear.

This study aims to investigate the potential role of age in inflammatory processes associated with PCOS by comparing clinical and haematological parameters in women diagnosed with PCOS according to age group.

Methods

This retrospective, cross-sectional study was conducted in the Department of Obstetrics and Gynaecology, following approval from the Non-Interventional Clinical Research Ethics Committee of the Yozgat Bozok University Faculty of Medicine (2025-GOKAEK-2515_2025.09.03_575). The study was conducted in accordance with the ethical principles of the Declaration of Helsinki. As this study was conducted in a tertiary training and research hospital, written informed consent regarding the use of anonymized clinical data for scientific research is routinely obtained from all patients at the time of application.

Between May 2022 and May 2025, 280 women aged 18-43 who visited the outpatient clinic and were diagnosed with PCOS according to the Rotterdam criteria were included in the study. A diagnosis of PCOS is made if at least two of the following three criteria are present: (1) oligoovulation or anovulation; (2) clinical and/or biochemical evidence of hyperandrogenism; and (3) a polycystic appearance of the ovaries on ultrasound. Data on the patients' age, height, weight and complete blood count (including neutrophils and lymphocytes) were obtained by searching the hospital automation system and patient files. Body mass index (BMI) was calculated using the formula weight (kg)/height (m^2). The NLR was obtained by dividing the absolute neutrophil count by the absolute lymphocyte count.

Oligo/anovulation was defined as a menstrual cycle length >35 days or fewer than eight menstrual cycles per year. Clinical hyperandrogenism was assessed by the presence of hirsutism, defined as a modified Ferriman–Gallwey score ≥8, and/or acne. Biochemical hyperandrogenism was defined as elevated serum total testosterone levels above the laboratory reference range. Polycystic ovarian morphology was defined as the presence of ≥12 follicles measuring 2–9 mm in diameter and/or an ovarian volume >10 mL in at least one ovary on transvaginal ultrasound. Ultrasound examinations were performed using a high-resolution transvaginal probe by experienced gynecologists in the outpatient clinic.

Venous blood samples were collected from all patients between 08:00 and 10:00 in the morning after an 8-12-hour fast from the antecubital vein. Haematological parameters (e.g. neutrophils, lymphocytes) were measured using an automated haematology analyser.

Patients with an active infection, chronic inflammatory disease, diabetes, hypertension, thyroid disease or malignancy; those who had undergone surgery within the last three months; those taking chronic medication; smokers; pregnant or breastfeeding women; and patients with incomplete data were excluded from the study.

The patients were divided into four groups according to their age: Group 1 (18-24 years old; n = 100); Group 2 (25-30 years old; n = 130); Group 3 (31-35 years old; n = 37); and Group 4 (36-43 years old; n = 13). As sample size calculations were not performed, all patients who met the inclusion criteria during the specified time period were included in the study.

Statistical analysis

Statistical analyses were performed using IBM SPSS Statistics version 25 (IBM Corp., Armonk, NY, USA). The normality of continuous variables was assessed using the Shapiro-Wilk test, as well as histograms and Q-Q plots. As the data did not show a normal distribution, the Kruskal-Wallis test was used to compare the four age groups. The Bonferroni-corrected Dunn post hoc test was applied to variables showing significant differences. The relationship between age and neutrophils, lymphocytes and NLR was evaluated using Spearman's correlation test. Multivariate linear regression analysis was performed to determine the independent effect of age on NLR. The dependent variable in the model was defined as NLR, and the independent variables were age, BMI, neutrophils and lymphocytes. Multicollinearity was controlled for using VIF and tolerance values. The effect size for the Kruskal-Wallis tests was calculated using chi-squared (ε^2). The level of statistical significance was set at $p<0.05$.

Results

A total of 280 women with PCOS were included in the study. Patients were distributed into the following age groups: Group 1 (18-24 years): 100 patients; Group 2 (25-30 years): 130 patients; Group 3 (31-35 years): 37 patients; and Group 4 (36-43 years): 13 patients.

Table 1 presents the comparison of the four age groups in terms of clinical and haematological parameters. There was a statistically significant difference in height values between groups (p=0.004, ε^2=0.004). No significant differences were found between groups in terms of weight or BMI (p=0.255 and p=0.457, respectively). There were statistically significant differences in neutrophil levels between groups (p=0.030, ε^2=0.067). No significant differences were found in terms of lymphocyte levels or NLR between groups (p=0.085 and p=0.155, respectively).

The results of the correlation analysis between age and inflammatory parameters are shown in Table 2. A weak but

statistically significant negative correlation was found between age and neutrophils (r=-0.142, p=0.024). No statistically significant correlation was found between age and lymphocytes (r=+0.091, p=0.137) or between age and NLR (r=-0.087, p=0.162).

Table 1. Comparison of Clinical and Hematological Parameters According to Age Groups in Women with PCOS

Parameters	Group 1 (18-24) n=100	Group 2 (25-30) n=130	Group 3 (31-35) n=37	Group 4 (36-43) n=13	*p*-value†	Effect Size (ε^2)
Height (cm)	135.26	141.60	148.38	147.35	0.828	0.004
Weight (kg)	143.68	131.52	158.58	154.38	0.255	0.018
BMI (kg/m²)	133.13	148.85	132.54	136.35	0.457	0.011
Neutrophils (10^3/µL)	151.79	141.87	105.34	140.04	**0.030***	**0.067**
Lymphocytes (10^3/µL)	146.81	130.10	144.72	184.00	0.085	0.052
NLR	144.37	146.62	117.30	115.62	0.155	0.038

† **Kruskal-Wallis test;**
PCOS: Polycystic Ovary Syndrome;
BMI: Body Mass Index;
NLR: Neutrophil-to-Lymphocyte Ratio;
* p<0.05 statistically significant

Table 2. Correlation Analysis Between Age and Inflammatory Parameters

Parameter	Spearman's r	*p*-value
Age - Neutrophil	-0.142	0.024*
Age - Lymphocyte	+0.091	0.137
Age - NLR	-0.087	0.162

Age was analyzed as a continuous variable.
NLR: Neutrophil-to-Lymphocyte Ratio;
* p<0.05 is statistically significant.

The results of the multivariate linear regression analysis, which was performed to determine the factors affecting NLR, are presented in Table 3. No statistically significant effect of age on NLR was found (β=-0.084, p=0.156). BMI was found to have a statistically significant positive effect on NLR (β=+0.176, p=0.005). Neutrophil (β =+0.492, p<0.001) and lymphocyte (β=−0.438, p<0.001) values were found to have a statistically significant effect on NLR. No multicollinearity problem was detected in the model (all VIF values<2).
The results of the Bonferroni-corrected Dunn post hoc test, which was conducted to identify the groups responsible for the significant difference in neutrophil levels observed, are presented in Table 4. Statistically significant differences were found between Groups 1 and 3 (Z=-2.71, p=0.007) and between Groups 2 and 3 (Z=-2.38, p=0.017). No significant differences were found in other group comparisons.

Table 3. Multivariate Linear Regression Analysis on NLR

Variable	β (Standardized)	Standard Error	t	*p*-value	VIF
Age	-0.084	0.017	-1.42	0.156	1.12
BMI	+0.176	0.023	2.85	**0.005**	1.21
Neutrophil	+0.492	0.032	7.98	**<0.001**	1.58
Lymphocyte	-0.438	0.028	-6.77	**<0.001**	1.45

Model R^2=0.42;
F=38.6, p<0.001;
BMI: Body Mass Index.

Table 4. Post-hoc Dunn Test Results for Neutrophils

Compared Groups	Z Value	*p* (Bonferroni)
Group 1 - Group 3	-2.71	**0.007**
Group 2 - Group 3	-2.38	**0.017**
Group 3 - Group 4	-1.55	0.121
Group 1 - Group 2	0.88	0.379
Group 1 - Group 4	0.93	0.351
Group 2 - Group 4	0.41	0.682

Group 1 (18-24 years old; n=100); Group 2 (25-30 years old; n=130); Group 3 (31-35 years old; n=37); and Group 4 (36-43 years old; n=13)

Discussion

This study examined how age affects inflammatory markers in women diagnosed with PCOS. The findings revealed no statistically significant difference in NLR levels across the different age groups. However, the neutrophil count was significantly lower in the 31-35 age group than in the other age groups. While a weak negative correlation was observed between age and neutrophil count in the correlation analysis, no significant correlation was detected between age and NLR. Multivariate regression analysis revealed that age had no independent impact on NLR, whereas BMI had a positive impact. Overall, these findings suggest that age may not be a major determinant of NLR within the reproductive age range studied; however, the unequal distribution across age groups-especially the small oldest group-may have limited the ability to detect subtle age-related differences.
In our study, no significant difference in NLR levels was found between age groups. This finding suggests that the chronic low-grade inflammation observed in PCOS does not increase with age, but rather follows a stable course throughout the reproductive years. Numerous studies in the literature show that NLR is elevated in women with PCOS.[6,9-11] However, most of these studies compared PCOS and control groups without considering the age factor. Our study offers a new perspective on this issue by comparing PCOS patients in different age groups. The absence of a significant effect of age on NLR suggests that the inflammatory process

in PCOS is related to pathophysiological mechanisms specific to the syndrome rather than aging.

Another notable finding of our study was that the neutrophil count in the 31-35 age group was significantly lower than in other age groups. This finding aligns with the weak negative correlation observed between age and neutrophil count. This finding supports the hypothesis of phenotypic transformation with age in polycystic ovary syndrome (PCOS). Hsu et al. reported that the PCOS phenotype, characterised predominantly by hyperandrogenism in younger individuals, transforms into a phenotype where metabolic components become more prominent with advancing age.[7] The 31-35 age range can be considered a critical period when this phenotypic transition becomes more apparent clinically.

The relative decrease in androgen levels observed during this period, alongside changes in metabolic parameters, may influence neutrophil activation and circulating neutrophil counts. Furthermore, the increased initiation of hormonal treatments or changes in lifestyle-related factors in this age group may also contribute to the relatively transient decrease in neutrophil counts. However, prospective and longitudinal studies evaluating hormonal profiles, treatment status and metabolic parameters together are needed to clearly establish these mechanisms.

In our regression analysis, no independent effect of age on NLR was detected; however, body mass index was shown to have a positive and significant effect on NLR. This suggests that obesity may be a significant factor in the inflammatory process observed in polycystic ovary syndrome. However, it is noteworthy that there are conflicting results in the literature on this subject. Some authors have reported that the increase in NLR levels in PCOS patients is independent of obesity.[6] A different study has shown that, even among normal-weight adolescents with PCOS, NLR is higher than in healthy controls; however, no direct relationship was found between NLR and BMI.[12]

In our study, the positive and independent effect of BMI on NLR may be related to the BMI distribution in our sample group, the frequency of metabolic syndrome components or other accompanying inflammatory processes. Furthermore, the more severe obesity and insulin resistance observed in the adult PCOS population may explain the discrepancy in results compared to those observed in adolescents.[13] In conclusion, these findings suggest that inflammation in PCOS is shaped not only by the syndrome's own pathophysiological mechanisms, but also by obesity, insulin resistance and other metabolic factors. Therefore, when interpreting inflammatory markers such as NLR, metabolic parameters should be evaluated alongside BMI.

The clinical significance and prognostic value of NLR

Although our study found that NLR is independent of age and associated with BMI, other studies have shown that NLR has significant clinical and prognostic value in PCOS that goes beyond merely indicating inflammation. For example, Dey et al. reported that elevated NLR is associated with poor cardiovascular health and an increased risk of metabolic syndrome.[14] Similarly, Pergialiotis et al. found that NLR showed a positive correlation with androgens such as free testosterone and androstenedione, and a negative correlation with SHBG and HDL.[15] These findings suggest that NLR is associated with both hormonal and metabolic disorders. Cho et al. observed that NLR was significantly higher in PCOS patients with insulin resistance, demonstrating a positive correlation with HOMA-IR; however, this association weakened when confounding factors such as BMI were considered.[16] This finding supports the independent effect of BMI on NLR observed in our study. In conclusion, NLR may be a cheap, accessible and useful biomarker for predicting not only inflammation, but also metabolic complications, insulin resistance and cardiovascular risk in PCOS. However, the complexity of this relationship and the influence of factors such as BMI suggest that caution should be exercised when using NLR in a clinical setting.

Limitations

Our study has several limitations. Firstly, due to its retrospective design, it is not possible to establish a causal relationship. Second, the cross-sectional data collection method does not permit the longitudinal assessment of age-related changes. Secondly, critical hormonal parameters (testosterone, SHBG, LH, FSH and estradiol) and insulin resistance indicators (HOMA-IR and fasting insulin levels), which play a key role in the pathophysiology of PCOS, were not evaluated in our study. This prevents us from clarifying the relationship between changes in neutrophil levels and hormonal profiles. Thirdly, information regarding the patients' treatment status, medication use (e.g. metformin and oral contraceptives) and disease duration was lacking. These factors may affect inflammatory markers and confound our results. Fourthly, the number of patients across the age groups is imbalanced, particularly in the 36-43 age group (n=13), which limits the generalisability of the findings for this group. Fifth, the absence of a control group prevents comparison of NLR levels in women with PCOS with those in a healthy population. Sixthly, PCOS phenotypes (hyperandrogenic, metabolic and ovulatory dysfunction) were not differentiated and it was not possible to evaluate the inflammatory profiles of the different phenotypes. Seventhly, lifestyle factors such as diet, physical activity and smoking, as well as socioeconomic status and psychological factors such as anxiety and depression, were not recorded. Finally, the single-centre design and relatively small sample size limit the generalisability of the findings. Future studies should be multicentre and prospective, with larger sample groups, and include hormonal profiles and metabolic parameters to better understand age-related inflammatory changes in PCOS.

Conclusion

In conclusion, this study found no significant difference in parameters such as NLR and BMI between age groups in women with PCOS, but neutrophil count differed in the 31-35 age group. This finding suggests that age may have complex effects on inflammatory processes associated with PCOS that cannot be explained by a single marker. The lack of variation in NLR across age groups in this cohort may indicate that low-grade inflammation in PCOS persists across the reproductive years; however, this interpretation is limited by the retrospective design and the small sample size of the oldest age group.

Conflict of Interest

The authors explicitly state that they have no conflict of interest with this article.

Ethics and permissions

The study was approved by the Local Ethics Committee of Yozgat Bozok University (2025-GOKAEK-2515_2025.09.03_575). All authors involved in the study fully complied with the principles of the Declaration of Helsinki.

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Author Contributions
Ç.A.: Study idea/Hypothesis; Ç.A., M.B.: Design; Ç.A., M.B.: Data Collection; Ç.A.: Analysis; Ç.A. Literature review; Ç.A., M.B.: Writing; Ç.A., M.B.: Critical review.

Footnote
Preliminary results of this study were presented as a poster abstract at the 1st International Women's Health Congress, Istanbul, September 18–20, 2025.

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