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Evaluation of (Neutrophil-to-lymphocyte Ratio (NLR)) as an Inflammatory Immune Marker in Patients with Acute Infections and Comparison- with Healthy Subjects

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Abstract

General Background: Inflammation is a fundamental biological response in infectious diseases involving alterations in circulating immune cells, particularly neutrophils and lymphocytes.**Specific Background:** The neutrophil-to-lymphocyte ratio (NLR), derived from routine complete blood count testing, reflects the balance between innate and adaptive immunity and is increasingly utilized in clinical evaluation. **Knowledge Gap:** Despite its clinical use, further validation of NLR as a reliable inflammatory immune marker in acute infections compared with healthy individuals remains necessary. **Aims:** This study aimed to evaluate NLR as an inflammatory immune marker in patients with acute infections and compare findings with healthy controls. **Results:** The study included 30 patients and 20 controls. Patients demonstrated significantly higher absolute neutrophil count (8.49 ± 1.58 vs. 3.83 ± 0.88), markedly elevated NLR (10.62 ± 3.26 vs. 1.67 ± 0.45), and reduced absolute lymphocyte count (0.86 ± 0.27 vs. 2.37 ± 0.45) ($p < 0.001$). ROC analysis showed excellent diagnostic performance, with NLR and neutrophil count achieving an AUC of 1.000, and lymphocyte count an AUC of 0.995. Correlation analysis revealed positive association between neutrophils and NLR and negative association between lymphocytes and NLR. **Novelty:** This study provides integrated statistical and diagnostic validation of NLR using hematological indices and ROC analysis within a defined cohort. **Implications:** NLR represents a simple, low-cost, and accessible biomarker for assessing inflammatory and immune responses in acute infections and may support clinical evaluation and disease monitoring.

Highlights:
- Marked Elevation of Neutrophils Alongside Reduced Lymphocytes Identified in Infected Individuals
- Diagnostic Analysis Demonstrated Near-Perfect Classification Accuracy Between Groups
- Correlation Patterns Confirmed Ratio Structure Driven by Opposing Hematological Components

Keywords: Neutrophil Lymphocyte Ratio, Acute Infection, Inflammatory Marker, Complete Blood Count, Biomarker

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Introduction

Inflammation is one of the body's primary defense mechanisms during infection, as it involves changes in immune cells circulating throughout the body, particularly neutrophils and lymphocyte. Therefore, the immune system is essential for protection against harmful factors [1]. Many studies have confirmed that the complex interaction between cells such as neutrophil, lymphocyte and monocyte contribute to the development of inflammation, and that its degree is primarily determined by the number and percentage of cells [2]. During systemic inflammation and stress conditions, neutrophil count tend to increase whereas lymphocyte counts often decrease, Neutrophils are key component of innate immune system, playing a major role in the acute inflammatory response, while lymphocytes are essential for long-term immune protection, i.e., adaptive immunity [3]. The neutrophil-to-lymphocyte ratio (NLR) is obtained from a routine CBC test, which reflects the balance between adaptive and innate immune responses and has been approved as a useful biological therapy during systemic infections [4]. Elevated (NLR) values are associated with disease onset in various cases and press reports, as demonstrated by numerous studies [5],[6]. Therefore, the study purpose was to evaluation NLR as an inflammatory immunomarker in patients with acute infections and compare it to healthy individuals. this marker has received increasing attention in clinical practice and biomedical research due to its simplicity, availability, and low cost.

Samples collection

The research was collected indirectly from various private laboratories in Basra Governorate, Iraq, for the period from 1/8/2025 to 1/2/2026 (i.e., for a period of sex months). Blood was collected for thirty people with acute inflammation, including (17) males and (13) females, while twenty healthy people were prepared, including (12) males and (8) females.

Material and Methods

The (CBC) performed by venous Place the blood sample into EDTA test tubes and then fed into an automated hematology analyzer (Sysmex – from the Japanese company Sysmex corporation). This analyzer automatically mixes the samples, draws a precise amount of blood and accurately analyzes the results. Using both absolute values of both neutrophils and lymphocytes, the NLR was calculated and statistically analyzed [7].

Statistical analysis

Statistically analyzed by using IBM SPSS Statistics for Windows, Version 28.0 . All data were tested by using the Pearson Chi-square (χ^2) test univariate analysis of variance, and Diagnostic accuracy was evaluated using (ROC) curve analysis,

Results

50 people who underwent a CBC test, the number of those infected was limited (30) patients (17) males, (13) females, but the number of those who were healthy was (20) person (12) males and (8) females.

Table (1) : Distribution of Sex According to Study Groups (Patients vs. Control).

Sex	Patients (n = 30)	Control (n = 20)	Total (N = 50)	Chi-square test
Male	17 (56.7%)	12 (60.0%)	29 (58.0%)	$\chi^2(1) = 0.055$ $p = 0.815$ NS
Female	13 (43.3%)	8 (40.0%)	21 (42.0%)	
Total	30 (100%)	20 (100%)	50 (100%)	

Figure (1): Distribution of Sex According to Study Groups (Patients vs. Control).

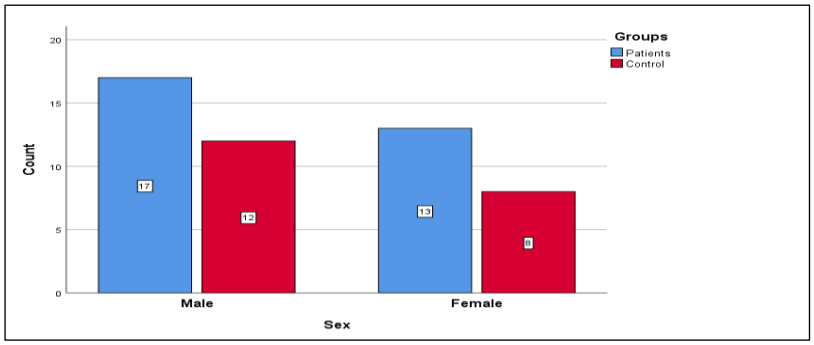

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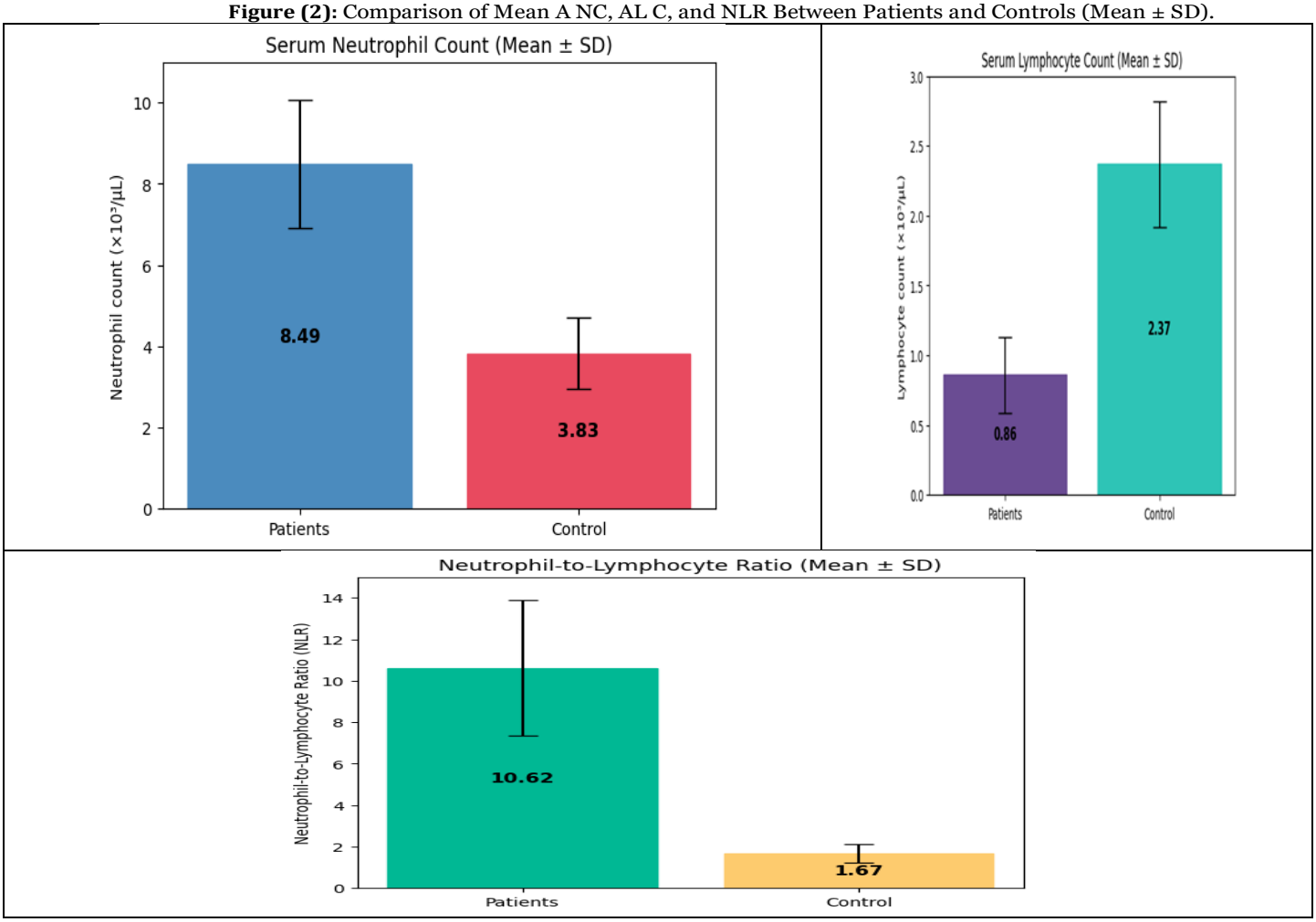
Table (2): Comparison of Hematological Indices and NLR Between Study Groups.

Parameter	Patients (n = 30) Mean ± SD	Control (n = 20) Mean ± SD	Mean Difference	t-value	p-value	95% CI of Difference
Neutrophil count (×10³/µL)	8.49 ± 1.58	3.83 ± 0.88	4.66	11.99	<0.001	3.87 – 5.44
Lymphocyte count (×10³/µL)	0.86 ± 0.27	2.37 ± 0.45	-1.51	-14.86	<0.001	-1.72 – -1.31
Neutrophil-to-Lymphocyte Ratio (NLR)	10.62 ± 3.26	1.67 ± 0.45	8.95	12.17	<0.001	7.47 – 10.43

Figure (2): Comparison of Mean A NC, AL C, and NLR Between Patients and Controls (Mean ± SD).



within the range considered outstanding (>0.90), indicating very high classification accuracy. Overall, the magnitude of AUC values and the perfect or near-perfect sensitivity and specificity strongly support the utility of ANC and NLR as powerful inflammatory biomarkers capable of accurately distinguishing patients from healthy controls in this cohort.

Table (3): (ROC) Analysis of absolute Neutrophil Count, absolute Lymphocyte Count, and (NLR).

Parameter	AUC	Std. Error	p-value	95% CI	Optimal Cutoff	Sensitivity (%)	Specificity (%)

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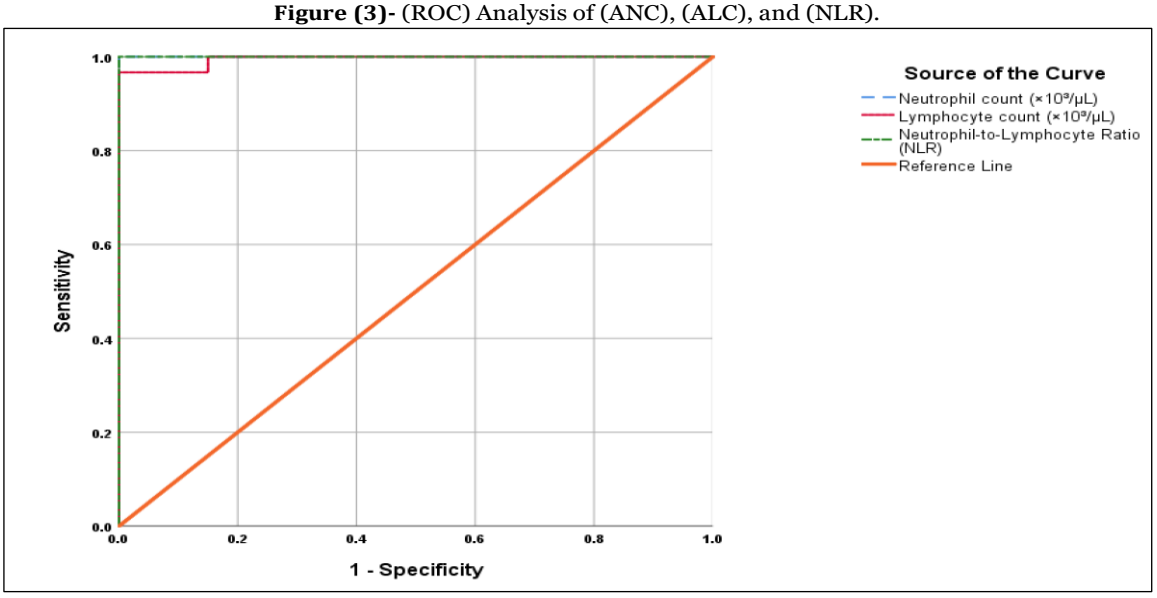
Parameter	AUC	Std. Error	p-value	95% CI	Optimal Cutoff	Sensitivity (%)	Specificity (%)
Neutrophil count (×10³/µL)	1.000	0.000	<0.001	1.000–1.000	≥ 6.35	100.0	100.0
Lym c (×10³/µL)	0.995	0.006	<0.001	0.983–1.000	≤ 1.26	96.7	100.0
Neutrophil-to-Lymphocyte Ratio (NLR)	1.000	0.000	<0.001	1.000–1.000	≥ 3.50	100.0	100.0

Figure (3)- (ROC) Analysis of (ANC), (ALC), and (NLR).



Overall, the correlation structure confirms that NLR behaves consistently as a composite inflammatory index, strongly driven by neutrophil elevation and lymphocyte suppression, while remaining independent of age in both groups.

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Table (4): Pearson Correlation Analysis Between Age, ANC, ALC , and NLR in Patients and Control Groups.

Groups	Variable	Neu. c (×10³/µL)	Lym. ct (×10³/µL)	NLR
Patients Group (n = 30)	Age	0.073 (p = 0.703)	-0.021 (p = 0.911)	0.074 (p = 0.697)
	Neu-c (×10³/µL)		0.084 (p = 0.660)	0.506** (p = 0.004)
	Lym c (×10³/µL)	-		-0.725** (p < 0.001)
Control Group (n = 20)	Age	0.033 (p = 0.889)	-0.054 (p = 0.821)	0.084 (p = 0.726)
	Neu. c (×10³/µL)	-	0.086 (p = 0.718)	0.690** (p = 0.001)
	Lym. c (×10³/µL)	-	-	-0.644** (p = 0.002)

Figure 4. Correlation Between ANC and NLR in Patients and Controls.

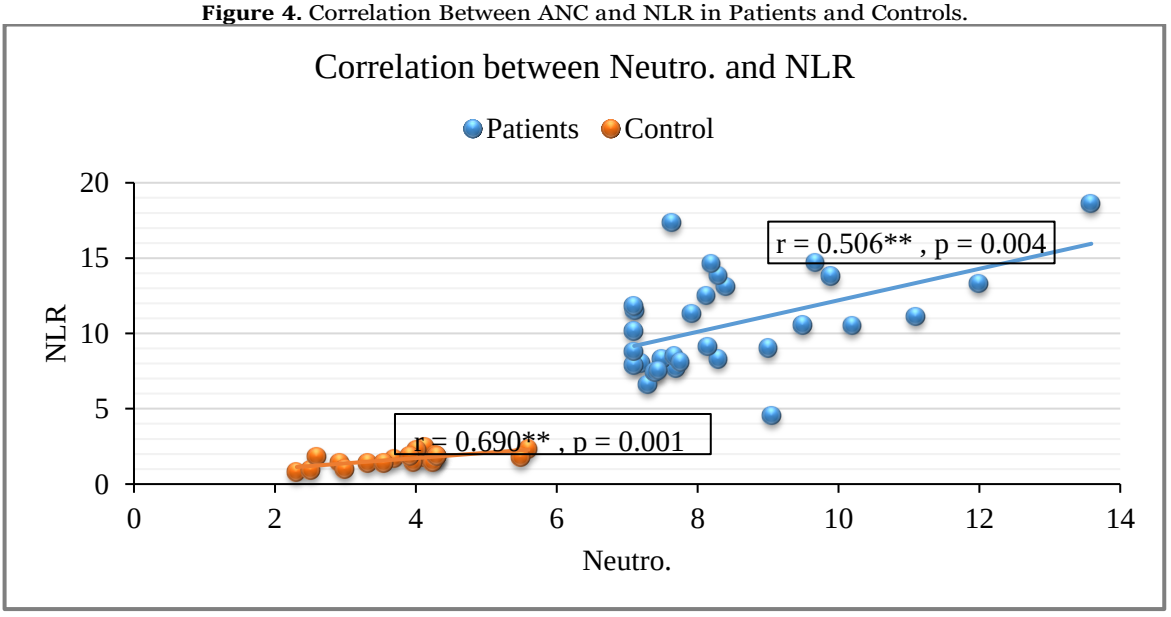

Figure (4) observed a positive -significant between absolute neutrophil count and NLR in both patients (r = 0.506, p = 0.004) and controls (r = 0.690, p = 0.001), indicating that higher neutrophil levels are directly NLR values increase

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Figure (5): Correlation Between ALC and NLR in Patients and Controls.

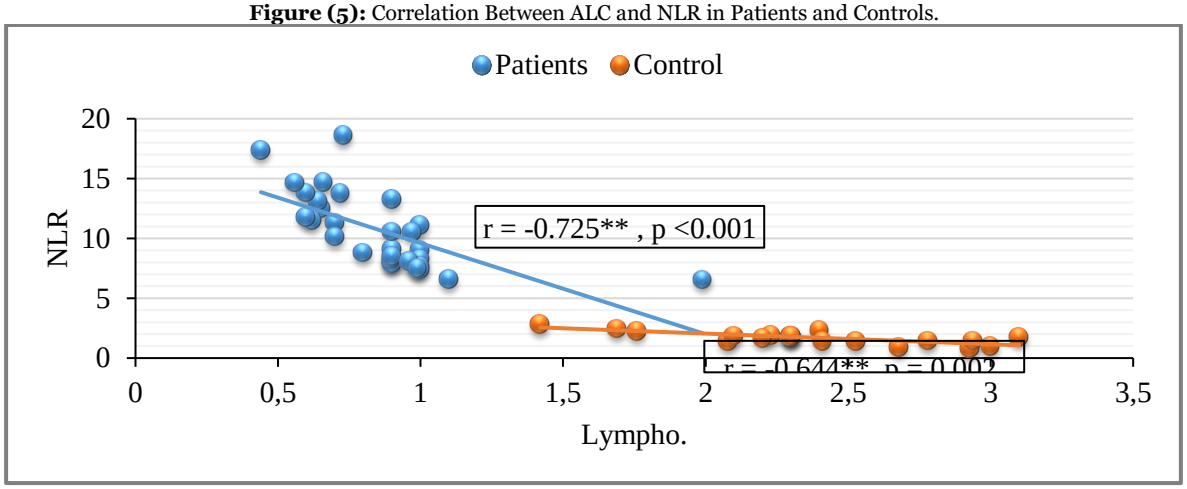

Figure (5) shows a strong negative correlation was identified between

ALC and NLR in patients (r = −0.725, p < 0.001) and controls (r = −0.644, p = 0.002), reflecting the inverse contribution of lymphocyte levels to the calculated NLR index.

Discussion

The classification and quantification of cells were performed through routine blood tests , as measuring NLR is a readily available and cost-effective inflammatory marker, as demonstrated by numerous recent studies [8],[9]. Table (1) presents the distribution of sex across the patient and control groups. Among patients, males constituted 56.7% (n = 17), while females represented 43.3% (n = 13). The same trend was followed in the control group whereby males represented 60.0% (n = 12) and females 40.0% (n = 8). On balance, 58.0% of the total population of the study was made of males. The chi-.square test showed that there There was no statistically significant relationship between the study group and gender. (kh2(1) = 0.055, p = 0.815). The fact that there were no major differences also shows that the two groups were similar in terms of sex distribution [10]. This homogeneity in the baseline demography features enhances the internal validity of the research since this minimizes the chances of sex being a confounding factor which affects the future clinical or biochemical comparisons. Figure (1): indicates similar balance in numbers of both Females and males plus controls. Male was a little bit more predominant in both groups, between groups no significant difference (kh2 = 0.055, p = 0.815), which proves the homogeneity of demographics. Table (2) indicates a significant hematologic difference between patients and healthy controls. The average number of neutrophils in the study patients (8.49 +- 1.58 x103/uL) was significantly greater than the neutrophil count in the controls (3.83 +- 0.88 x103/uL), with an extremely significant difference (t = 11.99, p < 0.001). The average of 4.66 * 103/uL with a small standard deviation of 3.875-5.44, shows a significant rise by neutrophils in patients. This trend is an indicator of an increased inflammatory or immune activation state. On the other hand, the lymphocyte count was low in patients (0.86 +- 0.27 x103/uL) in relation to the controls (2.37 +- 0.45 x103/uL), with a mean difference of 1.51 x103/uL (t = 14.86, p < 0.001). A negative confidence interval ([?]1.72 to 1.31) proves constant lymphocytic suppression of the patient group. This decrease can be an indication of immune dysregulation or loss of lymphocytes because of stress (11). Most significantly, the mean of the (NLR) of patients (10.62 +- 3.26) compared to the controls (1.67 +- 0.45) was significantly higher as well (mean difference of 8.95) with t = 12.17 and p = 0.001). Since NLR is the amalgamation of neutrophilia and lymphopenia, its severe increase is a strong sign of inflammatory imbalance in the system. The size of the separation between the groups indicates that NLR can be an effective discriminatory inflammatory biomarker in this group of the population under study. Figure (2) illustrates significant differences in inflammatory hematological parameters between patients and controls. The mean Absolute neutrophil count was markedly higher in patients (8.49 ± 1.58 ×10³/μL) compared with controls (3.83 ± 0.88 ×10³/μL), reflecting pronounced neutrophilia in the patient group.

Conversely, Absolute lymphocyte count was substantially reduced in patients (0.86 ± 0.27 ×10³/μL) relative to controls (2.37 ± 0.45 ×10³/μL), indicating relative lymphopenia. As a consequence of these combined changes, the (NLR) was dramatically elevated in patients (10.62 ± 3.26) compared with controls (1.67 ± 0.45). . Overall, the graphical representation clearly demonstrates a pronounced inflammatory imbalance in the patient group, characterized by increased neutrophil levels, decreased Absolute lymphocyte counts, and a markedly elevated NLR, supporting the statistical findings reported in the corresponding table (p < 0.001 for all comparisons).Table (3) summarizes the diagnostic performance of ANC ,ALC , and using ROC curve analysis. Both neutrophil count and NLR achieved a perfect area under the curve (AUC = 1.000, p < 0.001), indicating complete discrimination between patients and controls. The 95% confidence limits were set to 1.000-1.000 which indicates complete segregation of the values of the groups with no quantifiable overlap. This amount of precision indicates an extremely high inflammatory selectivity among the study group. Absolute lymphocyte count also demonstrated near-perfect diagnostic performance (AUC = 0.995, 95% CI: 0.983–1.000, p < 0.001). Although slightly lower than the other two markers, the AUC remains [12]. Table (4) presents Pearson correlation coefficients among age, absolute neutrophil count, absolute lymphocyte count, and NLR within both study groups. In the patient group (n = 30), age showed no significant correlation with absolute neutrophil count (r = 0.073, p = 0.703), absolute lymphocyte count (r = -0.021, p =

0.911), or NLR (r = 0.074, p = 0.697), suggesting that inflammatory alterations were independent of age in this cohort [13]. A moderate positive correlation was observed between absolute neutrophil count and NLR (r = 0.506, p = 0.004), indicating that elevated neutrophils substantially contribute to the rise in NLR values. More prominently, was identified negative correlation between absolute lymphocyte count and NLR (r = -0.725, p < 0.001), reflecting the mathematical and biological inverse relationship between lymphocyte levels and the calculated ratio. Similarly, in the control group (n = 20), age was not significantly associated with any hematological parameter. The t (ANC) demonstrated a strong positive correlation with the NLR (r=0.690, p=0.001), identifying it as a significant determinant of the inflammatory ratio within the study population., and absolute lymphocyte count showed a was identified negative correlation with NLR (r = -0.644, p = 0.002) [14]. The strength of these binding seems to be slightly higher in neutrophil controls, and slightly higher in patients lymphocytes controls. The tendency is probably attributed to the inherent structural dependence of NLR on its elements where the lymphocyte variability has a particularly strong effect on the patient group [15].

Conclusion

To conclude, this study found that the NLR is an excellent inflammatory immune marker for acute infections. Patients affected by acute infections had a statistically higher absolute neutrophil count (ANC) and NLR, and a lower absolute lymphocyte count (ALC) than healthy patients. Such changes indicate an obvious inflammatory imbalance evidenced by the coexistence of neutrophilia and lymphopenia leading to the increase of NLR seen in patients with infection. In addition, ROC curve analysis confirmed NLR and ANC as excellent diagnostic tools, with sensitivity and specificity values close to their maximum, suggesting both very high sensitivity and specificity to separate between infected patients and healthy controls. The positive correlation of NLR with neutrophilia, the negative correlation between NLR and lymphopenia, and the correlation analysis that further validated NLR as a composite indicator of systemic inflammatory response. These findings imply that neutrophil-to-lymphocyte ratio (NLR) based on frequently used complete blood counts may be a simple, cheap, and readily available biomarker in identifying and following inflammatory response whenever they may occur in patients presenting with acute infections. Adding NLR to the standard clinical evaluation of these patients may facilitate infection severity risk stratification, allowing early medical intervention from the clinician. Nevertheless, the relatively small sample size and limited geographic sampling were limitations of the study; therefore, larger multicenter populations, the determination of the prognostic value of NLR in different infectious diseases, and its association with other inflammatory biomarkers to enhance diagnostic accuracy and clinical decision-support aids in the management of infectious disease are important future research directions.

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