Neutrophil-to-Lymphocyte Ratio as a Marker for Eradication Failure of *Helicobacter pylori*

Rizk Sayad Rizk Sarhan[a*], Raafat R Mohammed[b]

[a]Department of Internal Medicine, Faculty of Medicine, Benha University, Benha, Egypt.
[b]Department of Clinical Pathology, Benha Hospital Laboratory, Benha, Egypt.

Abstract

Background: *Helicobacter pylori* (HP) infection results in multiple complications, and its chronic form was defined as the most prominent risk factor for gastric cancer.

Objectives: evaluation of the association between HP infection and neutrophil/lymphocyte ratio (NLR) and its ability to identify patients with complicated or failed infection eradication. The study aimed to assess the performance of NLR for distinguishing patients with complicated or failed infection eradication

Patients and methods: 135 patients with complaints suggestive of HP infection underwent endoscopic examination and biopsy taking, and HP diagnostic tests, and blood samples were obtained for complete blood count to calculate the NLR and platelet/lymphocyte ratio (PLR). Patients with positive HP diagnosis and no history of treatment and patients with HP infection who received treatment were grouped as S1 and S2 groups, and the C-group included patients with negative diagnosis and endoscopy.

Results: The NLR and PLR were significantly ($P < 0.001$) higher in patients than controls. Further, NLR, HP-IgG titer, and PLR are the highly significant predictors for positive endoscopy ($P < 0.001$, 0.001, and 0.002, respectively), and high NLR, total leucocytic count, and PLR as the highly significant predictors for infection eradication failure ($P < 0.001$, $P < 0.001$, and 0.001, respectively).

Conclusion: Hematological inflammatory markers are practical and easily obtainable parameters related to HP infection. The NLR is pertinent to developing HP infection-induced complications and may be a useful identifier for cases with failed eradication.

Keywords: *Helicobacter pylori* (HP) infection; Neutrophil/Lymphocyte ratio; Platelet/Lymphocyte ratio; Endoscopic findings; Infection-eradication failure.

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***Correspondence**: rizk.r.sarhan72023@gmail.com

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Introduction

Helicobacter pylori (HP) infection is still among the most prevalent infections with variable worldwide spread **(Cho et al., 2021)**. Despite the high prevalence of HP infection, most of the infected adults are asymptomatic **(Nelson et al., 2021)**.

Chronic HP infection was defined as the most prominent risk factor for precancerous lesions and gastric cancer (GC) **(Gao et al., 2025)**. However, only 1-3% of chronically infected individuals will develop GC **(Morris et al., 2025)**.

Further, HP infection results in gastrointestinal and extra-gastrointestinal complications **(Abdel-Razeq et al., 2024; Galan et al., 2024).** Arteriosclerosis, dyslipidemia, diabetes, obesity, hypertension, and cardiovascular disease are the common extra-gastrointestinal complications of HP infection **(Šačić et al., 2024)**. Thus, bacterial eradication is mandatory to prevent these complications, especially with the increased resistance of HP bacteria to antibiotics, which has made management more challenging **(Zhang et al., 2025)**.

Hemogram inflammatory markers, namely, the neutrophil-to-lymphocyte ratio (NLR), the platelet-to-lymphocyte ratio (PLR), red-cell distribution width, and mean platelet volume, are associated with multiple disease states **(Hassanzadeh et al., 2025)**. NLR was associated with the development and severity of the metabolic syndrome, independent of age and race/ethnicity **(Sarrafan-Chaharsoughi et al., 2024; Qian et al., 2025)**.

Further, NLR, PLR, and C-reactive protein (CRP) were used to represent systemic inflammation **(Huang et al., 2025)**; high NLR was identified as an independent predictor of unfavorable outcomes of non-HIV nontuberculous mycobacterial disease patients **(Chai et al., 2025)**. Also, combined use of NLR testing with serum levels of procalcitonin, CRP, and total leucocytic count (TLC) was found to improve the diagnostic sensitivity of pediatric bloodstream infections, reducing the risk of missed diagnoses, thereby enhancing the early diagnosis **(Li et al., 2025)**.

The current study aimed to evaluate the association between HP infection and NLR for patients with a positive diagnosis of HP infection. Also, the study tried to assess the ability of NLR to identify patients who had complicated HP infection or failed complete eradication on the previously received treatments.

Patients and Methods

Design: Prospective interventional comparative clinical trial.

Setting: Department of Internal Medicine, Faculty of Medicine, Benha University, in conjunction with Benha University Hospital Lab.

All patients presenting with complaints like (Abdominal pain, nausea, vomiting, bitter water in the mouth, bad breath, early satiety, anorexia) suggestive of HP infection, previously treated or not, were included for evaluation for the presence of inclusion and exclusion criteria. Patients' demographic and disease-related data were obtained.

Inclusion criteria: Patients with a possibility of having *H. pylori* infection who were free of the exclusion criteria and accepted to participate in the study were included.

Exclusion criteria: The presence of complaints that influence the accuracy of HP diagnostic tests, such as disturbed bowel movement and upper gastrointestinal bleeding, previously known hematological diseases, systemic diseases, maintained on drugs that may alter the hematological parameters, and parasitic and other infectious diseases.

Diagnostic protocol

A. Gastroduodenoscopy

Endoscopic examination was performed under mild sedation using intramuscular injection of midazolam 20 minutes before the procedure. Diagnosis relied on obtaining endoscopic biopsies from the antrum and corpus of the greater curvature **(Lan et al., 2012)** for microscopic examination with Giemsa staining to identify *H. pylori*. Both the esophagus and duodenum were explored endoscopically for the presence of esophagitis or duodenal ulcers (DU).

B. *H. pylori* infection diagnostic test

Before testing for *H. pylori* infection, patients were asked to stop taking antibiotics for at least four weeks, proton pump inhibitors (PPI) and bismuth preparations for at least two weeks, and antacids and histamine H2-receptor antagonists for at least one day. Patients were subjected to the following diagnostic tools before enrolment:

1. **Stool analysis** to exclude the presence of parasitic and/or bacterial infections.
2. **Urea breath test (UBT)** depends on the urease activity of *H. pylori*; the ^{13}C-labeled urea ingested by the patient is hydrolyzed to labeled CO_2 in the stomach, which is absorbed in the blood and exhaled by breathing to be measured by a UBT kit. UBT is an accurate noninvasive test for the diagnosis of *H. pylori* infection with sensitivity and specificity rates of 96% and 93% **(Ferwana et al., 2015)**. UBT was performed using the Heliforce, a breath test kit (Beijing Richen-force Science and Technology Co., Ltd., China), which is a quick, about 30-minute, non-radioactive test with sensitivity and specificity of 96% and 98%, respectively, as documented by the manufacturer.
3. **Monoclonal stool antigen test (SAT)** is an accurate noninvasive method for the initial diagnosis of *H. pylori* infection with good sensitivity and specificity, 94% and 97%, respectively **(Gisbert et al., 2006)**. Stool samples were obtained in a sterile clean container and preserved at a low temperature to maintain the antigenicity and tested over a short period or stored at -80 °C till being assayed **(Shimoyama, 2013)**. SAT depends on the detection of *H. pylori* antigen in stool samples and was performed using Pylori-Strip (Sterilab Services Co., UK), which is a 10-min test and has sensitivity, specificity, positive, and negative predictive values of 96%, 98.2%, and 92.3% and 99.1%, respectively, and an accuracy rate of 97.8% as documented by the manufacturer.

C. Blood tests

5-ml peripheral blood samples were aseptically obtained and divided into two parts:

a. One ml of blood was collected in a tube containing tri-potassium ethylenediaminetetraacetate (K3-EDTA) for a complete blood count, including differential leucocytic counts using the fully automated hematology analyzer (Mythic™ 22, Orphée, SWISS Hematology Solution).
b. Four ml of blood were put in a plain tube, allowed to colt in a warm water bath at 37ºC for 5 minutes, and centrifuged at 5000 rpm for 2 min to separate the serum. The resultant serum sample was collected in a dry Eppendorf tube and kept frozen until

ELISA estimation for *H. pylori* IgG using the IgG plus Abcam ELISA Kit (Cat. No. ab178645, Abcam Inc., San Francisco, USA; Detection range: 1.39 - 150 IU/mL; Intra-assay C.V.: 1.76-3.34, Inter-assay C.V.: 8.94-13.44) according to the manufacturer guidelines using the quantitative sandwich enzyme immunoassay technique. The analysis results were read by an ELISA reader (Dynatech, MR 7000) using a 96-well microplate.

Sample size calculation

The suggested null hypothesis of the study is the ability of NLR to differentiate patients with failed eradication of *H. pylori* infection as manifested by the persistence of complaints, positive *H. pylori* infection diagnostic tests, and positive endoscopy. Accordingly, to achieve a study power of 80% with an α-error of 5%, the sample size as calculated by G*Power (Version 3.1.9.2) was 45 patients per group to allow ascertaining or refusal of the null hypothesis **(Faul et al., 2007)**.

Grouping

Patients with a positive diagnosis of *H. pylori* infection were grouped as HP+ with no history of *H. pylori* infection treatment (S1 Group) or HP+ with a previous history of *H. pylori* infection treatment (S2 Group). Patients with a negative diagnosis of *H. pylori* infection and negative endoscopy were categorized as the Control group (C Group).

Study outcomes

The diagnostic performance of NLR for patients with failed eradication of *H. pylori* infection and to differentiate patients with complicated *H. pylori* infection as judged by endoscopic findings among HP+ patients.

Ethical registration

The Institutional Ethics Committee, Faculty of Medicine, Benha University, approved the study protocol by the approval number RC14-2-2025 and patients signed the written consent were enrolled in the study. Sample size was exempted by the Ethics committee because the study design is observational.

Statistical analysis

The Kolmogorov-Smirnov test was applied to assess the data normality. The data are presented as mean, standard deviation (SD), numbers, and percentages. The unpaired t-test and Chi-square test (X^2 test) were used to evaluate the significance of the inter-group differences. Pearson's correlation analysis was applied to evaluate correlations between studied variables. The multivariate regression and the receiver characteristic curve (ROC) analyses were used to define the identifiers for the development of *H. pylori* infection-induced endoscopic findings and patients with *H. pylori* infection eradication failure as yes or no. The IBM® SPSS® Statistics (Version 22, 2015; Armonk, USA) was used to convey the statistical analyses at a p-value of <0.05.

Results

The preliminary evaluation included 151 patients; 16 patients were excluded for having a parasitic infection (n = 8), irritable bowel (n = 4), hematemesis (n = 3), and coagulopathy (n =1). The remaining patients were divided into three groups **(Fig. 1)**. The enrolment criteria for patients of the three groups and the frequencies of complaints showed insignificant intergroup differences, as shown in **(Table.1)**.

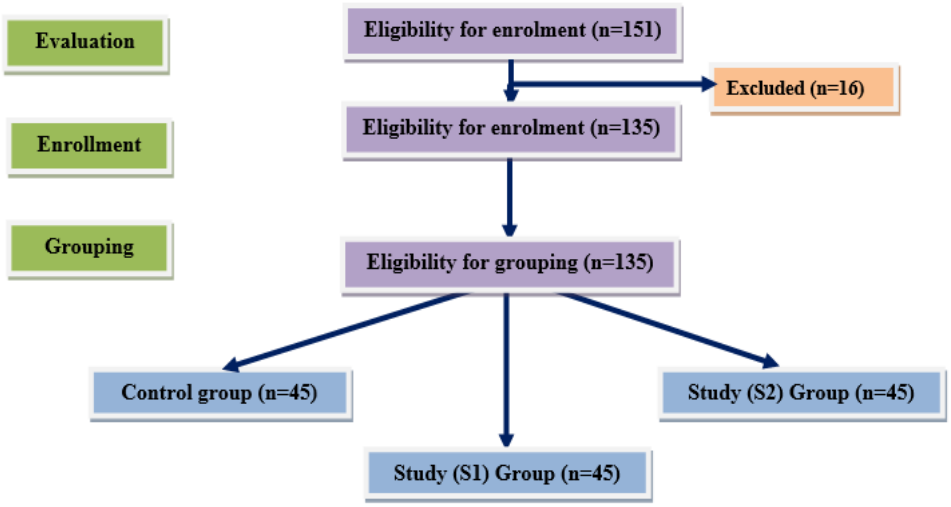

Fig.1. Study flow chart

Table 1. Patients' enrolment data

Item / Group		Control (n=45)	HP+/Treatment- (S1; n=45)	HP+/Treatment+ (S2; n=45)	Significance of the difference between		
					C vs. S1	C vs. S2	S1 vs. S2
Age (years)		**46.6±10.5**	48.6±8.5	50.6±11.1	0.323	0.339	0.083
Gender*	**Males**	**21 (46.7%)**	28 (62.2%)	26 (57.7%)	0.138	0.291	0.666
	Females	**24 (53.3%)**	17 (37.8%)	19 (42.2%)			
Body mass index data	**Weight (kg)**	**82.4±9.3**	80.6±10	81.4±11.9	0.379	0.658	0.731
	Height (cm)	**167.8±3.6**	169±2.8	168.6±4.2	0.081	0.335	0.596
	BMI (kg/m^2)	**29.3±3.73**	28.2±3.66	28.7±4.51	0.162	0.492	0.566
Complaints*	**Abd pain**	**31 (68.9%)**	25 (55.6%)	30 (66.7%)	0.292	0.502	0.080
	Nausea	**5 (11.1%)**	9 (20%)	12 (26.7%)			
	Vomiting	**0**	3 (6.7%)	7 (15.6%)			
	Bitter water in the mouth	**2 (4.4%)**	3 (6.7%)	5 (11.1%)			
	Bad breath	**3 (6.7%)**	3 (6.7%)	5 (11.1%)			
	Early satiety	**1 (2.2%)**	4 (8.9%)	6 (13.3%)			
	Anorexia	**3 (6.7%)**	4 (8.9%)	5 (11.1%)			

Unpaired t-test and Chi-square test*

Positive endoscopic findings were detected in 52 (57.8%) patients of the S1 and S2 groups, and mucosal erythema and swelling were the most frequent endoscopic findings with a significantly (P = 0.033) higher frequency among patients of the S2 group, (Table 2).

The UBT was positive in 66 patients (73.3%) with a significantly higher frequency among patients of the S2 group compared to patients of the S1 group (P = 0.017). The SAT was positive in 74 patients (82.2%) with significantly (P = 0.027) higher frequency in the S2 than in the S1 group. A positive HP IgG test was detected in samples of 68 patients (75.6%) with an insignificant (P = 0.141) lower frequency among the S1 group **(Table. 2)**.

Table 2. The findings of the HP diagnostic tests

Item / Group			HP+/Treatment- (S1; n=45)	HP+/Treatment+ (S2; n=45)	P
Endoscopy	Mucosal lesions	Negative	24 (53.3%)	14 (31.1%)	0.033
		Positive	21 (46.7%)	31 (68.9%)	
	Endoscopic findings in cases had mucosal lesions	Erythema	18 (85.7%)	28 (90.3%)	0.882
		Mucosal swelling	10 (47.6%)	19 (61.3%)	
		Erosions and ulcers	5 (23.8%)	7 (22.6%)	
		Bleeding spots	7 (33.3%)	9 (29%)	
		Diffuse redness and disappearance of RAC	3 (14.3%)	6 (19.4%)	
		Visible vascular pattern and rugal atrophy	1 (4.8%)	5 (16.1%)	
		Whitish elevated lesion	1 (4.8%)	3 (9.7%)	
Urea breath test	Positive		28 (62.2%)	38 (84.4%)	0.017
	Negative		16 (37.8%)	7 (15.6%)	
Stool antigen test	Positive		33 (73.3%)	41 (91.1%)	0.027
	Negative		12 (26.7%)	4 (8.9%)	
HP IgG	Positive		31 (68.9%)	37 (82.2%)	0.141
	Negative		14 (31.1%)	8 (17.8%)	

RAC: regular arrangement of collecting venules; Chi-square test*

The TLC was significantly higher among patients of the S2 group (P < 0.001) and S1 group (P = 0.039) than in the C group, with significantly (P < 0.001) higher TLC in samples of the S1 group. Neutrophil count was significantly higher in S2 group (P < 0.001) and in S1 group (P = 0.031) than in samples of the C group. Lymphocyte count was significantly higher in samples of the C group patients than in samples of the S1 (P < 0.001) and S2 (P = 0.005) groups; consequently, the NLR was significantly (P < 0.001) higher in samples of the S1 and S2 groups than in the C group, with a significantly (P < 0.001) higher ratio in samples of the S1 than in samples of the C

groups. Further, the PLR was markedly lower in the control samples than in the samples of the S1 (P = 0.005) and the S2 (P = 0.0002) groups, with an insignificantly (P = 0.095) higher ratio in samples of the S2 than the S1 groups **(Table.3, Fig. 2).**

Table 3.The complete blood count data of the studied patients

Variables			Control	S1	S2	Significance of difference		
Item		Group				S1 vs. C	S2 vs. C	S1 vs. S2
Hemoglobin concentration (g/dl)			12.7±0.48	12.76±0.39	12.5±0.79	0.517	0.150	0.051
Leucocytic count (10^3/µl)	Total		6375±852.9	6688±524.2	8601±820.8	0.039	<0.001	<0.001
	Differential	Neutrophil	3907.8±664.5	4167.4±431.2	5940.8±723	0.031	<0.001	<0.001
		Lymphocytes	1749.4±303.8	1562.9±304.6	1454.4±306.6	0.005	<0.001	0.095
		Others	717.8±477	957.7±277.3	1205.8±465.8	0.0045	<0.001	0.0028
Platelet count (10^3/µl)			251.9±14.3	250.2±15.3	246.2±14.3	0.587	0.065	0.204
Ratio	Neutrophil/lymphocyte		2.24±0.14	2.75±0.5	4.24±0.9	<0.001	<0.001	<0.001
	Platelet/lymphocyte		0.148±0.03	0.165±0.026	0.177±0.04	0.005	0.0002	0.095

Unpaired t-test

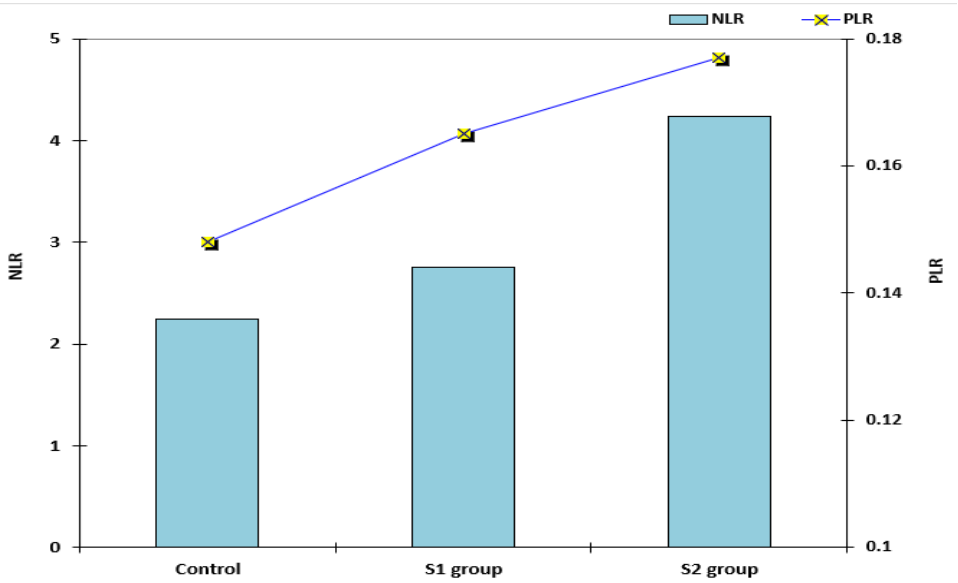

Fig. 2. The mean value of the NLR and PLR in the studied groups

The results of the HP diagnostic tests, total and differential leucocytic counts, and the NLRs and PLRs were positively and significantly correlated with the positive findings on endoscopy and the presence of failure of HP infection eradication **(Table. 4)**.

Table 4. Correlation analysis of HP diagnostic tests, TLC, NLR and PLR with the results of endoscopy and HP infection eradication

Variables	Positive endoscopy		Failed eradiation	
	"r"	P	"r"	P
Urea breath test	0.410	<0.001	0.334	0.025
Stool antigen test	0.341	<0.001	0.243	0.107
H. pylori IgG	0.397	<0.001	0.362	0.014

Total leucocytic count	0.435	<0.001	0.399	0.007
Neutrophil/Lymphocyte Ratio	0.574	<0.001	0.773	<0.001
Platelet/Lymphocyte Ratio	0.459	<0.001	0.581	<0.001

Multivariate regression analysis defined high NLR as significant predictor for positive endoscopy (β = 0.381, P < 0.001), followed by the high *H. pylori* IgG titer (β = 0.236, P = 0.001), and high PLR (β = 0.232, P = 0.002). ROC curve analysis assured the results of the regression analysis with the widest AUC for NLR (AUC = 0.844 ± 0.043, P < 0.001, 95% CI: 0.759-0.929) followed by the AUC for PLR (AUC = 0.784 ± 0.052, P < 0.001, 95% CI: 0.683-0.886) and lastly the AUC for the *H. pylori* IgG (AUC = 0.729 ± 0.043, P < 0.001, 95% CI: 0.644-0.813), while the other tests were excluded as predictors for positive endoscopy **(Table.5, Fig. 3).**

Table 5. Multivariate regression and ROC Curve analyses for *H. pylori* diagnostic test and lab parameters as predictors for the results of endoscopy

Analyses	**Regression analysis**		**ROC curve analysis**			
Variables	**β**	**P**	**AUC**	**SE**	**P**	**95% CI**
Urea breath test	0.111	0.284	Excluded			
Stool antigen test	0.072	0.583	Excluded			
***H. pylori* IgG**	0.236	0.001	0.729	0.043	<0.001	0.644-0.813
Total leucocytic count	0.137	0.145	Excluded			
Neutrophil/Lymphocyte Ratio	0.381	<0.001	0.844	0.043	<0.001	0.759-0.929
Platelet/Lymphocyte Ratio	0.232	0.002	0.784	0.052	<0.001	0.683-0.886

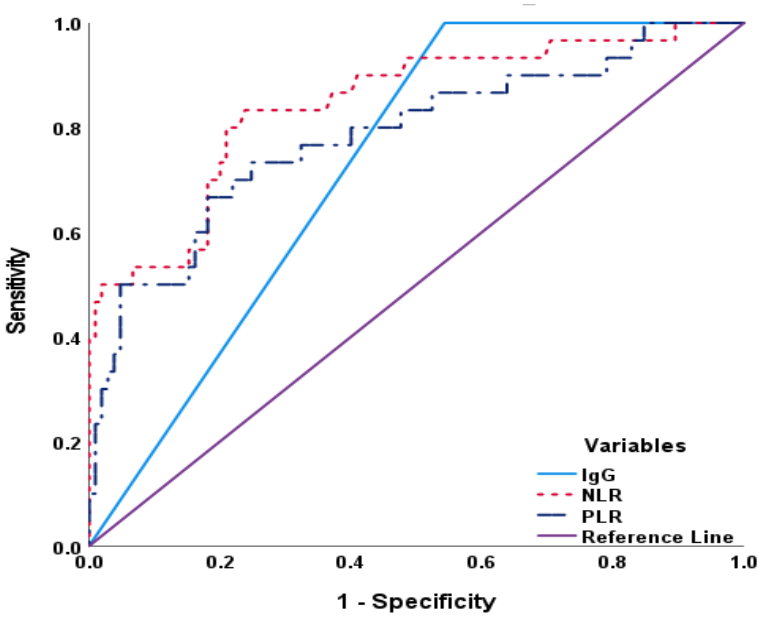

Fig. 3. ROC curve analyses for *H. pylori* diagnostic test and lab parameters as predictors for the results of endoscopy

Multivariate regression analysis defined high NLR as significant predictor for *H. pylori* infection eradication failure (β = 0.508, P < 0.001), followed by high TLC (β = 0.322, P < 0.001), high PLR (β = 0.295, P = 0.001) and lastly high *H. pylori* IgG titer (β = 0.205, P = 0.011), while excluding the other variables as predictors for eradication failure. ROC curve analysis ensured the high predictability of high NLR for eradication failure with the largest AUC (AUC = 0.943 ± 0.038, P < 0.001, 95% CI: 0.870-1.000) followed by high PLR (AUC = 0.847 ± 0.058, P < 0.001, 95% CI: 0.734-0.959) and high TLC (AUC = 0.783 ± 0.069, P = 0.002, 95% CI: 0.646-0.919), while excluding high *H. pylori* IgG **(Table.6, Fig. 4).**

Table 6.Multivariate Regression and ROC curve analyses for *H. pylori* diagnostic tests and lab parameters as identifiers for cases that had *H. pylori* eradication failure

Analyses Variables	Regression analysis		ROC curve analysis			
	β	P	AUC	SE	P	95% CI
Urea breath test	0.003	0.969	Excluded			
Stool antigen test	0.014	0.892	Excluded			
***H. pylori* IgG**	0.205	0.011	0.643	0.081	0.111	0.484-0.802
Total leucocytic count	0.322	<0.001	0.783	0.069	0.002	0.646-0.919
Neutrophil/Lymphocyte Ratio	0.508	<0.001	0.943	0.038	<0.001	0.870-1.000
Platelet/Lymphocyte Ratio	0.295	0.001	0.847	0.058	<0.001	0.734-0.959

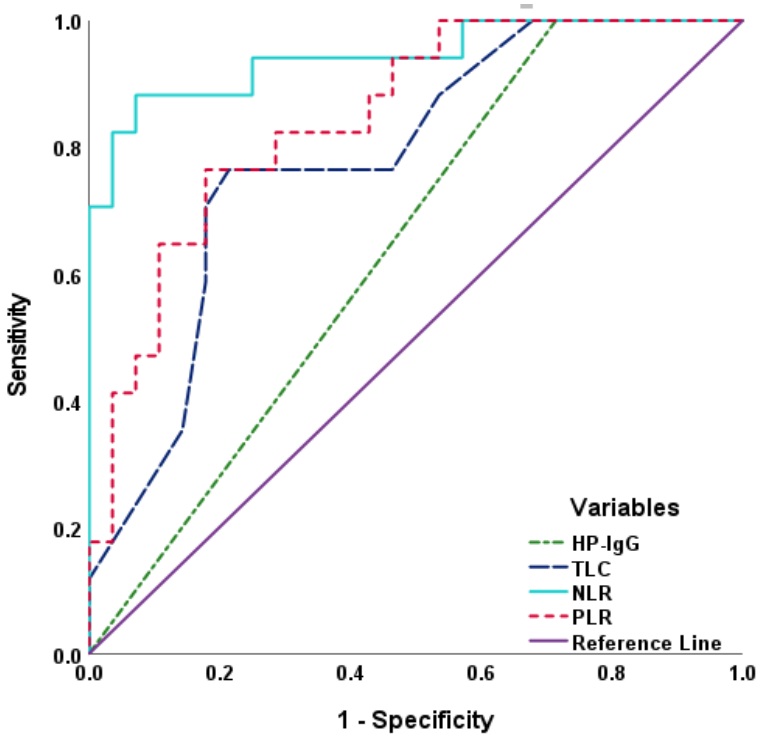

Fig. 4. ROC curve analyses for *H. pylori* diagnostic tests and lab parameters as predictors for the *H. pylori* eradication failure

Discussion

The reported relation between disturbed levels of hematological inflammatory cells and the presence of *H. pylori* infection complications, as judged by endoscopy, and with the presence of eradication failure irrespective of the presence of complications. Furthermore, Regression analyses defined high NLR, PLR, and TLC as preliminary markers for identifying patients who had complicated *H. pylori* infection and as identifiers for patients with eradication failure where the *H. pylori* infection diagnostic tests failed to identify. It is to be noted that high NLRs were the most significant predictors for either the presence of complications or eradication failure.

These findings align with those of **Bulbuloglu et al. (2020)**, who found high pain scores and NLR might predict scores on the Operative Link for Gastritis Assessment system and estimate the severity of gastritis in endoscopy patients. Also, **Sengul et al. (2022)** reported significant differences in PLR and NLR between patients with and without *H. pylori* infection, while other parameters in the hemogram could not help to assess the intensity and severity of inflammation. Thereafter, **Sağlam and Civan (2023)** detected a significant increase in neutrophil count and PLR values in *H. pylori*-positive patients, and **Doğan and Kekilli (2023)** also detected significantly higher NLR in *H. pylori-positive* patients than in *H. pylori-negative* patients and in *H. pylori-positive* patients with DU than in *H. pylori-positive* patients who were free of DU and concluded that NLR may be used as a non-invasive test for documenting the presence of *H. pylori* infection and *H. pylori*-related DU. Additionally, **Coşgun and Aras (2023)** reported that NLR was significantly higher in patients with peptic ulcers than in those with non-ulcer dyspepsia and in patients with peptic and gastric ulcers compared to those with peptic ulcers with DU.

Using multivariate analysis, **Yasuda et al. (2024)** showed that among evaluated parameters, only a high peripheral blood NLR was significantly associated with eradication failure. They also documented that the persistently elevated NLRs after eradication indicated a high risk for newly developed GC and a significantly higher mortality rate, with a strong association between high NLR and a history of GC and hypertension with GC development.

Multiple recent studies have assured the efficacy of NLR as a predictor of complications and as a prognostic indicator for various disorders. The National Health and Nutrition Examination survey concluded that among U.S. adults, NLR was associated with the development and severity of the metabolic syndrome, independent of age and race/ethnicity **(Sarrafan-Chaharsoughi et al., 2024)**. Then, **Chai et al. (2025)** found that high NLR was identified as an independent predictor of unfavorable outcomes of nontuberculous mycobacterial infection and can predict mortality with high AUC.

Despite the high diagnostic performance of *H. pylori* diagnostic tests for the detection of HP-positive patients, it failed to identify patients with positive endoscopic findings or with failed eradication of *H. pylori* infection, except for high H. pylori IgG titer that showed high discriminative ability for patients with positive endoscopic findings but weak discriminative ability for patients with eradication failure of *H. pylori* infection. These findings support the earlier studies, where **Liu et al. (2018)** found *H. pylori* eradication rates based on UBT results were relatively high with comparable outcomes of the triple and quadruple therapy, and **Miernyk et al. (2018)** reported a 94% concordance regarding the prevalence

of *H. pylori* using UBT or anti-H. pylori IgG.

Conclusion

Hematological inflammatory markers are practical and easily obtainable parameters related to the inflammatory phases of *H. pylori* infection. The NLR is pertinent to developing *H. pylori* infection-induced complications and may be a useful identifier for cases that had *H. pylori* infection eradication failure and as a prognostic parameter during follow-up.

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