

The Significance of Inflammatory Markers in the Diagnosis of Cervical Cancer

Ph.D. thesis
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1. Introduction

Cervical cancer is the fourth most common cancer among women worldwide in terms of both incidence and mortality, despite the fact that local treatments for cervical intraepithelial neoplasia (CIN) are highly effective. Moreover it is considered as a preventable disease as its etiology and evolution is largely understood, HPV infections are responsible for more than 99% of cervical cancer cases. Besides, it is considered as the “disease of inequalities,” given the fact that in countries with low human development indices (HDI), the incidence was twice as high, while the mortality was five times higher compared to countries with a very high HDI. Currently, the most commonly used method involves the following: cytology (Pap test) and, in cases of high risk of malignancy, conization, which also serves as a treatment. HPV infection can be detected through HPV DNA testing, and the signaling of cell cycle dysregulation caused by HPV is indicated by the p16 marker.

Overall, according to the data mentioned earlier, it would be necessary to introduce more accessible screening methods for risk classification. This is especially important given the fact that, in general, the progression of cancer can cause inflammation that is detectable systemically.

Inflammatory biomarkers are already used as prognostic markers for other malignant tumors, including the platelet-to-lymphocyte ratio (PLR), the lymphocyte-to-monocyte ratio (LMR), and the neutrophil-to-lymphocyte ratio (NLR). These ratios represent the balance between chronic inflammation and the immune response. Their advantage is that they require only a single peripheral blood sample, making them a simple, cost-effective, and easy-to-perform test.

2. Objectives

The overall aim of this dissertation was to investigate the potential diagnostic, predictive, and prognostic value of routinely available systemic inflammatory biomarkers in cervical cancer and its precursor lesions.

The specific objectives were:

- To examine whether the neutrophil-to-lymphocyte ratio (NLR), platelet-to- lymphocyte ratio (PLR), and lymphocyte-to-monocyte ratio (LMR) can be associated with p16 positivity and ultimately with cervical cancer prediction.
- To evaluate the association of NLR, PLR, and LMR with cervical cancer progression and adverse clinicopathological characteristics.
- To assess the potential prognostic value of NLR, PLR, and LMR in patients with cervical cancer.
- To investigate the diagnostic and predictive value of NLR in patients undergoing cervical cytology and subsequent conization.
- To determine an optimal NLR cut-off value for identifying patients at increased likelihood of underlying invasive cervical cancer.
- To explore the potential role of NLR in risk stratification and the development of more individualized cervical cancer screening and diagnostic strategies.

3. Methods

As part of the SCOPE study (Simmelweis Conization and Inflammation Outcomes with Predictive Evaluation), we conducted two retrospective observational analyses. Medical records from 2021 to 2024 were reviewed, focusing on demographic information, HPV status, preoperative complete blood count, and the final histopathological result after LEEP conization. The cytology results were also required for the second study. To analyze the data, we used non-parametric tests (Kruskal–Wallis and Mann–Whitney U tests for group comparisons) and ROC analysis. The study included patients aged 18 years or older who underwent LEEP conization at the Department of Obstetrics and Gynecology at Semmelweis University; they had complete sets of histological and hematological data, including the results of immunohistochemical staining for NLR, PLR, MLR, and p16. Exclusion criteria applied to patients who had a history of cervical cancer, who had previously undergone cervical surgery, who had been diagnosed with an autoimmune disease, or who were currently receiving or had previously received immunosuppressive therapy. In addition, participants with incomplete follow-up data or missing relevant clinical, hematological, or immunohistochemical data were excluded. Thus, of the 417 patients selected, 395 met the inclusion criteria in Study 1, and 374 met the inclusion criteria in Study 2. It should be noted that, based on the results of cervical cancer screening, patients were classified into four risk categories which does not correspond to the pathological histological grade classification. In accordance with the classification in cytology and conisation, Grade 1 included negative results, Grade 2

included LSIL and ASC-US, Grade 3 included HSIL, ASC-H, AGC, and AIS, and Grade 4 included invasive cancerous lesions.

4. Results

4.1 Neutrophil/Lymphocyte Ratio (NLR) as a Predictive Marker for p16 Positivity and Cervical Cancer Progression: Insights from the SCOPE Study

4.1.1. Laboratory Parameters and p16 Positivity

Laboratory Parameters	Mann–Whitney U	<i>z</i>	<i>p</i>
NLR	3687.5	−2.555	0.011 *
PLR	4308.0	−0.904	0.366
LMR	4064.5	−1.459	0.145

Table 1. Relationship between laboratory parameters and p16 positivity.

*It Shows the Mann–Whitney U test results for the association between p16 positivity in conization and hematological parameters, including neutrophil/lymphocyte ratio (NLR), platelet/lymphocyte ratio (PLR), and lymphocyte/monocyte ratio (LMR). * $p < 0.05$.*

As Table 1. demonstrates, a significant association was found between p16 positivity and the NLR ($p = 0.011$). Nevertheless no significant association was found between p16 positivity and the PLR ($p = 0.366$) or the LMR ($p = 0.145$).

4.1.2. Laboratory Markers and HPV DNA Status

A significant correlation was found only for the NLR, specifically when the NLR value exceeded 1.31 ($p = 0.04$) (Figure 1.).

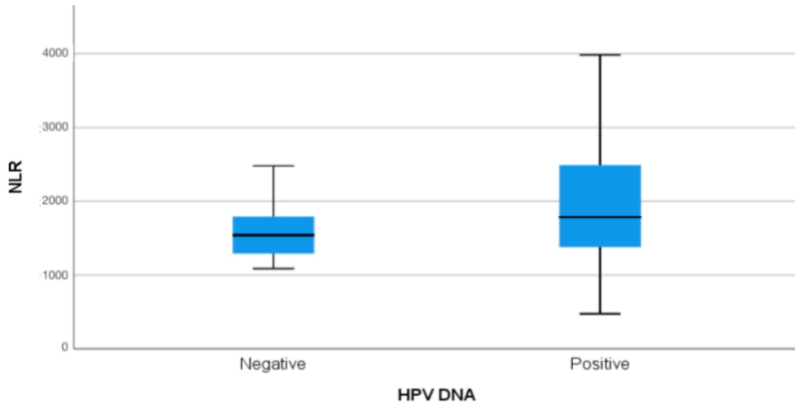


Figure 1. Boxplot showing the distribution of neutrophil/lymphocyte ratio (NLR) values by HPV DNA status.

HPV DNA-positive individuals exhibit higher median NLR values compared to HPV DNA-negative individuals. This illustrates the significant association between elevated NLR values and HPV DNA positivity

4.1.3. Diagnostic Performance Test

As shown by the data presented in Figure 2, the ROC analysis conducted as part of the evaluation of diagnostic performance. The NLR showed the highest discriminatory power (AUC = 0.601), while the AUC values for the PLR and LMR were 0.540 and 0.568, respectively. For the NLR, the optimal cutoff value determined using the Youden index was ≥ 1.530 , at which sensitivity was 71.0% and specificity was 51.0%. An increase in NLR was significantly associated with the outcome of interest ($p = 0.026$; OR = 1.596; 95% CI: 1.059–2.406).

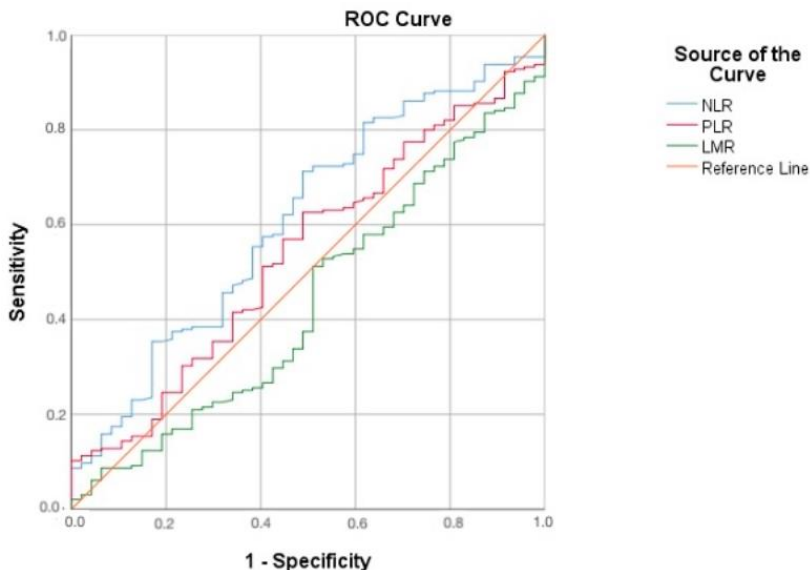


Figure 2. The Receiver Operating Characteristic (ROC) curve compares the diagnostic performance of the neutrophil/lymphocyte ratio (NLR), platelet/lymphocyte ratio (PLR), and lymphocyte/monocyte ratio (LMR) for identifying p16 positivity. Sensitivity (true positive rate) is plotted on the y-axis, while 1-specificity (false positive rate) is on the x-axis.

4.2. The Neutrophil-to-Lymphocyte Ratio (NLR) as a Potential Predictor in Conization Outcomes for Cervical Cancer

4.2.1. Relationship Between Laboratory Parameters and Cytological Results

Even though the evaluation of cytological categories revealed that neutrophil-to-lymphocyte ratio (NLR) values did not differ significantly across diagnostic groups, the trend in the distribution of NLR values is distinct (*Figure 3*). Moreover, patients with Grade II lesions (LSIL, ASC-US) demonstrated a

median NLR of 1.63, those classified as Grade III (HSIL, ASC-H, AGC, AIS) had a median value of 1.88, while individuals diagnosed with Grade IV cancer showed a median NLR of 2.31 (Table 2).

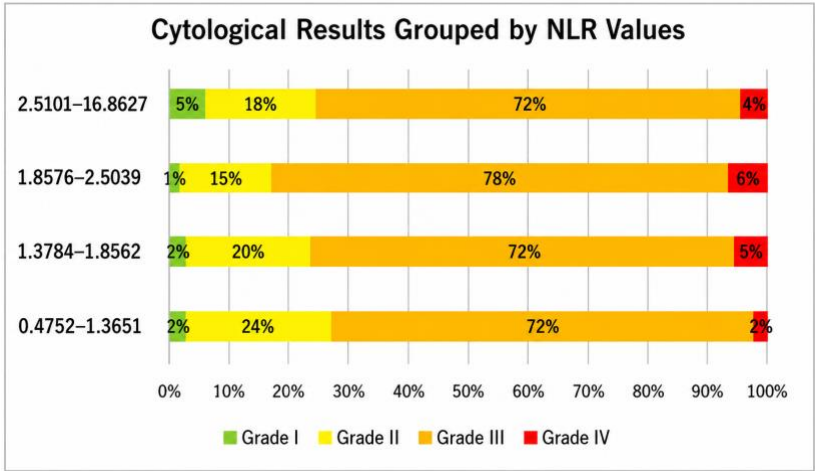


Figure 3. Distribution of cytological results by NLR categories. Stacked bar plots illustrate the proportion of cytology grades stratified by NLR categories. The x-axis represents the percentage of patients, while the color-coded bars indicate negative (green), mild (yellow), severe (orange), and cancerous (red) findings. Higher NLR values are associated with more severe cytological abnormalities.

The examination of cytological categories revealed a gradual increase in NLR values as the lesions became more severe, a finding supported by the graphical representation (Figure 4). However, this trend could not be confirmed by statistical analysis, as the Kruskal–Wallis test did not reveal a significant difference between the individual cytological grades ($p = 0.123$). Furthermore, the Spearman’s correlation coefficient also indicated an extremely weak association between NLR and

cytological severity ($\rho=0.047$; $p = 0.364$), suggesting that the visually observable upward trend does not reflect a true biological relationship.

Table 2. NLR values by cytological outcome.

	Median	N	Range	Minimum	Maximum
Grade I	2.51132	10	4.560	0.811	5.371
Grade II	1.62500	73	9.713	0.738	10.451
Grade III	1.88246	274	10.429	0.475	10.904
Grade IV	2.31022	17	4.575	1.216	5.791
Total	1.85055	374	10.429	0.475	10.904

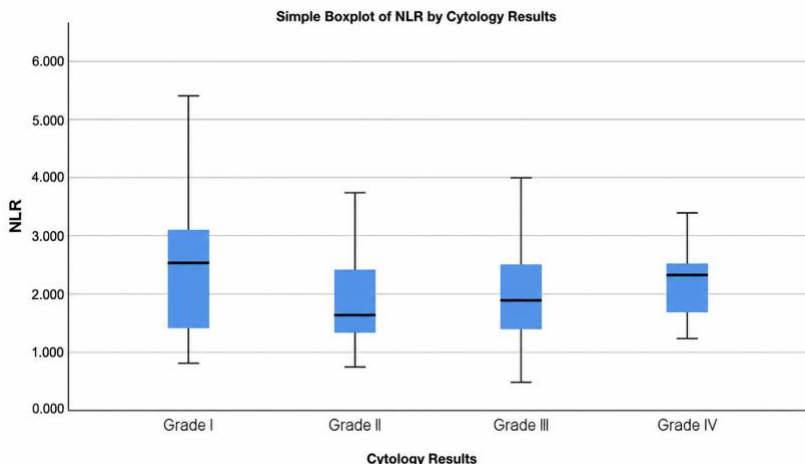


Figure 4. *Boxplot of NLR Values by Cytological Outcome.* This boxplot illustrates the distribution of neutrophil-to-lymphocyte ratio (NLR) values across different grades of cytological outcome categories. The median and interquartile range are displayed for each group, showing a trend of increasing NLR values with worsening cytological results.

4.2.1. Relationship Between Laboratory Parameters and Conisation Results

It should be mentioned that no significant difference was found between grades I, II, and III, suggesting that an elevated NLR is primarily associated with malignant conization results. The data showed that the mean NLR value increased proportionally as conization results became more severe (Figure 5). Specifically: the median NLR value for patients with Grade II (LSIL, ASC-US) conization results was 1.63; for Grade III (HSIL, ASC-H, AGC, AIS) results, the median NLR was 1.85; while the average NLR value for patients diagnosed with Grade IV, neoplastic results was 2.5 (Table 3).

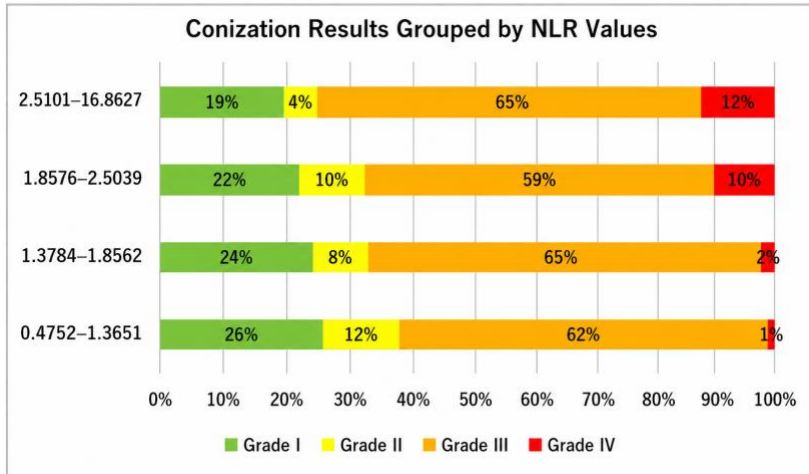


Figure 5. Distribution of cytological results by NLR categories.

Stacked bar plots illustrate the proportion of cytology grades stratified by NLR categories. The x-axis represents the percentage of patients, while the color-coded bars indicate negative (green), mild (yellow), severe (orange), and cancerous (red) findings. Higher NLR values are associated with more severe cytological abnormalities.

Table 3. NLR values by cytological outcome.

	Median	N	Range	Minimum	Maximum
Grade I	1.73973	84	4.566	0.804	5.371
Grade II	1.63434	32	2.695	0.660	3.355
Grade III	1.84531	234	16.387	0.475	16.863
Grade IV	2.50000	23	9.688	1.216	10.904
Total	1.84713	373	16.387	0.475	16.863

Pairwise NLR comparisons between conization result groups and further analyses showed that an NLR value above 2.86 was more likely to be associated with a neoplastic conization result ($p = 0.045$) (*Figure 6*). *Figure 6* also illustrates how NLR values are distributed within each histological grade. Grade IV showed not only a higher median but also a wider interquartile range compared to grades I–III. This wider distribution may reflect greater inflammatory variability among patients with confirmed malignancy and further supports the potential role of NLR in identifying high-grade lesions.

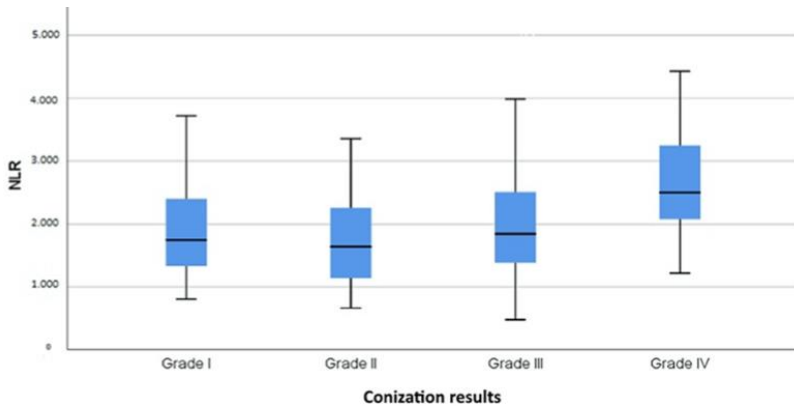


Figure 6. Boxplot of NLR values by conization outcomes.

A receiver operating characteristic (ROC) analysis was performed to assess the diagnostic value of the NLR in predicting cancer (*Figure 13*). The area under the ROC curve (AUC) was 0.734, indicating moderate discriminatory power. The asymptotic 95% confidence interval ranged from 0.643 to 0.825, with a standard error of 0.047 ($p < 0.001$), confirming statistical significance.

At the optimal NLR cutoff determined by the Youden index (≥ 1.865), sensitivity was 87.0%, while specificity was 53.8%. In addition, using the Closest Top Left method, the best NLR cutoff value was found to be ≥ 2.041 , resulting in a sensitivity of 78.3% and a specificity of 61.0%.

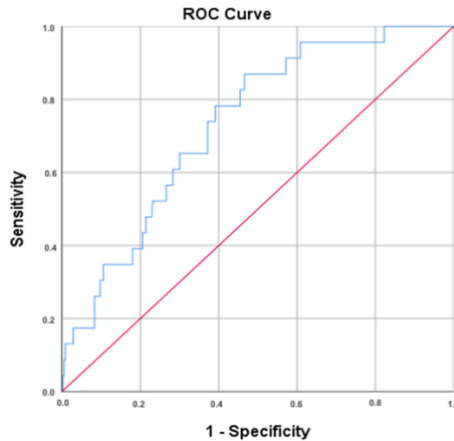


Figure 13. Receiver Operating Characteristic (ROC) Curve for NLR in Predicting cervical cancer.

The ROC curve illustrates how effectively the neutrophil-to-lymphocyte ratio (NLR) can identify cervical cancer, comparing its sensitivity (true-positive rate) on the vertical axis with 1-specificity (false-positive rate) on the horizontal axis. An AUC value of 0.734 reflects a moderate level of diagnostic performance.

5. Conclusions

To summarise the findings, it can be concluded that elevated inflammatory markers show an association with the occurrence of cervical cancer.

Apart from MLR and PLR, NLR stood out as it showed significant differences in terms of p16 expression, HPV DNA positivity, cytological grades, and conisation samples derived from cases of invasive carcinoma. Moreover, we were able to identify a significant NLR cutoff of value (≥ 1.865) for predicting the presence of cervical cancer. With NLR, we have been able to identified a method that has a high negative predictive value, substantial sensitivity, and moderate specificity.

Overall, our results have proved that the use of NLR may offer a new avenue for identifying patients at risk of cervical cancer. It has opened up the possibility of an easily accessible, low-cost, supplementary diagnostic tool that could complement current screening protocols, particularly in low-income countries.

6. Bibliography of the candidate's publications

Publications related to the thesis:

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ΣIF: 13