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The Significance of Inflammatory Markers in the Diagnosis of Cervical Cancer

Ph.D. Thesis

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LIST OF ABBREVIATIONS

(-)LR	Negative Likelihood Ratios
(+)LR	Positive Likelihood Ratio
ACS	American Cancer Society
AGC	Atypical Glandular Cells
AIS	Adenocarcinoma in Situ
ANOVA	Analysis of Variance
ASC	Atypical Squamous Cells
ASC-H	Atypical Squamous Cells—cannot exclude High-grade lesion
ASC-US	Atypical Squamous Cells of Undetermined Significance
AUC	Area Under the Curve
BMI	Body Mass Index
CI	Confidence Interval
CIN	Cervical Intraepithelial Neoplasia
CIS	Carcinoma In Situ
CKC	Cold Knife Conisation
CRP	C-Reactive Protein
CT	Computed Tomography

CTLA-4	Cytotoxic T-Lymphocyte-Associated protein 4
df	degrees of freedom
DNA	Deoxyribonucleic Acid
e.g.	exempli gratia (for example)
EMT	Epithelial-Mesenchymal Transition
Exp(B)	Exponentiated B
FIGO	International Federation of Gynecology and Obstetrics
FoxP3+	Forkhead box P3+
G1 phase	first Gap/Growth phase
G2 phase	second Gap/Growth phase
HDI	Human Development Index
HIV	Human Immunodeficiency Virus
HPV	Human Papillomavirus
HR-HPV	High-Risk Human Papillomavirus
HSIL	High-Grade Squamous Intraepithelial Lesion
hTERT	Human Telomerase Reverse Transcriptase
IFN- γ	Interferon- γ
IL-6	Interleukin-6
IL-10	Interleukin-10

INK4a	Inhibitor of Cyclin-dependent Kinase 4
IQR	Interquartile Range
JAK/STAT3	Janus Kinase / Signal Transducer and Activator of Transcription 3
LEEP/LETZ	Loop Electrosurgical Excision Procedure/ Loop Excision of the Transformation Zone
LMIC	Low- and Middle-Income countries
LMR	Lymphocyte-to-Monocyte Ratio
LSIL	Low-grade Squamous Intraepithelial Lesion
M phase	Mitosis phase
Max.	Maximum
MDSCs	Myeloid-Derived Suppressor Cells
Min.	Minimum
MMP-9	Matrix Metalloproteinase-9
MRI	Magnetic Resonance Imaging
N	Number of valid cases
NCR/URR	Non-Coding Region/Upstream Regulatory Region
NETs	Neutrophil Extracellular Traps
NK	Natural Killer
NLR	Neutrophil-to-Lymphocyte Ratio

NPV	Negative Predictive Values
OR	Odds Ratio
ORF	Open Reading Frame
Ori	Origin of replication
pAE	Early Polyadenylation site
pAL	Late Polyadenylation site
Pap test	Papanicolaou Test
PD-1	Programmed cell Death protein 1
PD-L1	Programmed Death-ligand 1
PDGF	Platelet-Derived Growth Factor
PLR	Platelet-to-Lymphocyte Ratio
PPV	Positive Predictive Values
pRb	Retinoblastoma protein
PSA	Prostate-Specific Antigen
Rb	Retinoblastoma
ROC	Receiver Operating Characteristic
S phase	Synthesis phase
S.E.	Standard Error

SCOPE	Semmelweis University Colonization and Inflammation Outcomes with Predictive Evaluation
SE RKEB	Institutional Review Board of Semmelweis University
Sig.(p)	Significance level (p-value)
SII	Systemic Immune-Inflammation Index
SIRI	Systemic Inflammatory Response Index
TAMs	Tumor-Associated Macrophages
TANs	Tumor-Associated Neutrophils
TGF- β	Transforming Growth Factor-beta
TIM-3	T-cell Immunoglobulin and Mucin-domain containing-3
TME	Tumor Microenvironment
TNF- α	Tumor Necrosis Factor-alpha
Treg cells	regulatory T cells
VEGF	Vascular Endothelial Growth Factor
WHO	World Health Organization

1. INTRODUCTION

1.1. Cervical Cancer

1.1.1. Epidemiology

Cervical cancer (carcinoma cervicis uteri) still appears among the leading gynecologic cancers worldwide. Current data indicate that it is the fourth most common cancer in terms of incidence and mortality among women worldwide despite being considered as a preventable disease as its etiology and evolution is largely understood (1, 2). 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 (3). As previously indicated, it is considered as the “disease of inequalities,” given the fact that significant geographic and socioeconomic differences are present in its incidence and mortality rates. This is best illustrated by the 2022 statistics, which show that 94% of recorded deaths were associated with low- and middle-income countries (LMIC) (4). High incidence rates are observed in Eastern, Middle, Southern and Western Africa, Melanesia, Micronesia, South-central and South-eastern Asia and Central America and the Caribbean. According to a 2019 survey, in some African countries (e.g., eSwatini, Malawi, Zambia), the estimated annual age-standardized incidence rate exceeds 40 per 100, 000 women, which is many times higher than the rates in Egypt, Finland or Malta (<5 per 100 000 women) (*Figure 1*) (5).

A study from 2023 found that only about 44% of patients with cervical cancer are diagnosed at an early stage, at which point the 5-year relative survival rate is estimated to be 92%. Inconsistent screening leads to a higher stage at diagnosis, which reduces survival and increases the risk of cancer recurrence. It has been shown that the 5-year relative survival rate drops to 60% when cervical cancer is diagnosed after local or regional lymph node involvement, while in cases of distant metastases, the 5-year relative survival rate at the time of diagnosis is only 19% (6).

The elimination of cervical cancer has now become a global public health goal. The World Health Organization (WHO) has set the 90-70-90 goal, aiming to be achieved by 2030 in LMICs as well. The three proposed pillars are: 90% of girls receive the HPV vaccine; 70% of women undergo a high-performance test by age 35, and again at age 45; and at least 90% of women diagnosed with precancerous lesions or invasive cervical cancer

receive adequate treatment (7). The social significance of the disease is further underscored by the young average age of death, at a time when women are often still raising children (8). It is deeply disappointing that the high mortality rate is closely linked to late diagnosis, the limited capacity of the healthcare system, social and health behavior factors, and the lack of organized screening programs. Consequently, the late treatment of cervical cancer places a particularly heavy financial burden on LMICs, precisely where access to screening and preventive health services is limited and inadequate. Thus, this emerging vicious cycle must not be allowed to escalate; rather, science should be encouraged to develop screening protocols that are more accessible (9).

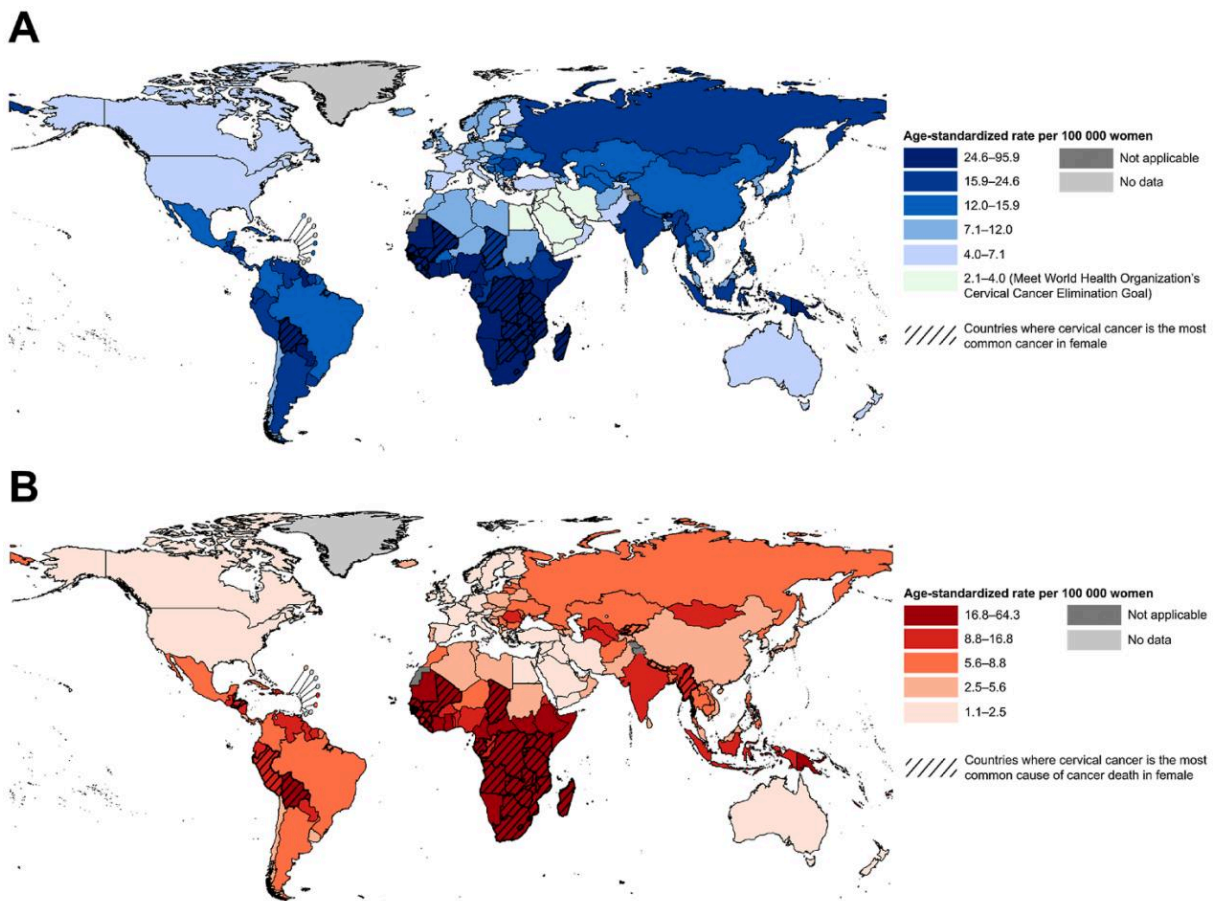


Figure 1. Estimated age-standardized (A) incidence and (B) mortality rates (per 100,000 women) of cervical cancer in 2022 (10).

1.1.2. Anatomy

Anatomically and histologically, the uterine cervix can be divided into two regions: the ectocervix, which is covered by a multilayered, non-keratinizing squamous epithelium, and the endocervix, which is lined by a single layer mucus-producing columnar epithelium. The boundary between these two different types of epithelium forms the squamocolumnar junction, representing the most vulnerable area of the cervical surface and thus the most prevalent region of cervical tumorigenesis. The two main types are as follows: squamous cell carcinoma, which develops from the ectocervix in 90% of cases, and adenocarcinoma, which develops from the endocervix. Both subtypes arise from precursor lesions. Precancerous lesions are referred to as cervical intraepithelial neoplasia (CIN) and are classified into three grades based on the extent of epithelial involvement. CIN 1 represents mild dysplasia, which often regresses. CIN 2 and CIN 3 lesions represent a more advanced state of abnormality and carcinoma in situ (CIS) is the ultimate phase before progression into invasive cancer, thus these carry a significantly higher risk of transformation into invasive carcinoma. The evolution of cervical cancer typically takes several years, or even a decade, which enables screening tests for early detection (6).

1.1.3. Etiology

The transformation zone is a dynamic structure that can change depending on age, hormonal influences, and environmental factors. The process of neoplastic changes developing here is influenced by numerous cofactors, including smoking, which exerts a local immunosuppressive effect; HIV infection and other immunodeficiency states; long-term use of oral contraceptives; and factors related to sexual behavior (young age at the onset of sexual activity, multiple sexual partners, and high pregnancy rates) (6).

This area is particularly susceptible to HPV infection, as the metaplastic cells found here exhibit increased proliferative activity, which promotes viral persistence and the accumulation of genetic damage. The vast majority of precancerous lesions, and subsequently cervical cancer itself, originate from this region (11). The development of cervical cancer is a multiphase, progressive process during which normal epithelium gradually transforms into invasive carcinoma through precancerous stages (12, 13).

1.1.4. Human Papillomavirus Infection and the Pathogenesis of Cervical Cancer

Worldwide, hundreds of thousands of new cases of HPV infection are reported each year—it is likely that every sexually active person will become infected at some point in their life—but the vast majority of infections are transient, remain mild, and the immune system clears the virus within 1–2 years. However, in persistent cases, HPV infection can lead to malignant transformations, which are responsible for more than 99% of cervical cancer cases and, consequently, for a significant number of deaths. To develop targeted screening protocols, it is important to recognize that population-based HPV prevalence studies show that the highest prevalence of high-risk HPV occurs among adults under 25 years of age, while deaths from cervical cancer peak among middle-aged women between 40 and 50 years of age. Studies have shown that HPV-related cervical disease largely resolves spontaneously in women under 25, but at this age, infection with multiple HPV types is less likely to resolve on its own (6).

Human papillomaviruses are small, non-enveloped, double-stranded DNA viruses, of which more than 200 genotypes are known. Of these, approximately 17 types are classified as high-risk (oncogenic), with HPV types 16 and 18 most commonly involved in the development of cervical intraepithelial neoplasia and its progression to invasive carcinoma (12). It has been shown that these two types account for more than 75% of cervical cancer cases (12). HPV is transmitted through skin-to-skin contact, including sexual intercourse, hand contact with the genital area, and oral sex. Thus, the infection can affect the skin, the throat, the genital area, and the anus. Infection occurs at the level of proliferating basal epithelial cells, typically through microtraumas (6). The HPV virion binds to molecules on the surface of the host's epithelial cells and enters via receptor-mediated endocytosis. In the cell nucleus, the virus either immediately exploits the host cell's differentiation processes to replicate or persists within the cells for an extended period. Thus, it is important to note in diagnosis that the increasing DNA replication of the virus is closely linked to the differentiation of the host cell. Amplification of the HPV genome, production of capsid proteins, and the release of new virions all occur within surface cells. The virus is ultimately shed from the body through the desquamation of epithelial cells, generally without the classic phenomenon of cell lysis (14).

Three regions can be distinguished in the HPV genes: the E (early) region, the L (late) region associated with capsid proteins, and the NCR/URR region that regulates the viral genome (*Figure 2*). The following genes stand out in the first region: E1, which is responsible for viral DNA replication; E2, which regulates viral DNA transcription and replication; E4, which supports the viral life cycle and virion release; E5, which is responsible for growth signaling and evades the immune system; and E6 and E7, which disrupt the host cell cycle. Other early proteins have also been identified (E3, E5, and E8), but their levels of expression are not commonly observed within the entire Papillomaviridae family (15). Overexpression of the E6 and the E7—key oncoproteins—forms the molecular basis of HPV-associated malignant transformation, as they are capable of binding to the host cell's regulatory proteins, thereby disrupting physiological regulatory mechanisms and leading to uncontrolled cell proliferation, i.e., tumor formation (16).

The E6 protein induces the degradation of the tumor suppressor p53 through ubiquitination, thereby preventing the repair of damaged DNA or the induction of apoptosis. In addition, E6 also influences the activation of hTERT telomerase, which leads to an extended ability of cells to divide. The E7 protein, in turn, inhibits the function of another tumor suppressor, the retinoblastoma protein (pRb). Under normal conditions, pRb inhibits the E2F transcription factors during the G1 phase of the cell cycle, which would otherwise facilitate the transition to the next phase, the S phase (*Figure 3*). However, the virus's E7 protein inactivates pRb, resulting in increased E2F activity, which leads to uncontrolled cell cycle activation and increased genomic instability. Integration of the viral DNA into the host cell genome induces further genetic and epigenetic changes that further enhance the development of invasive tumors (15).

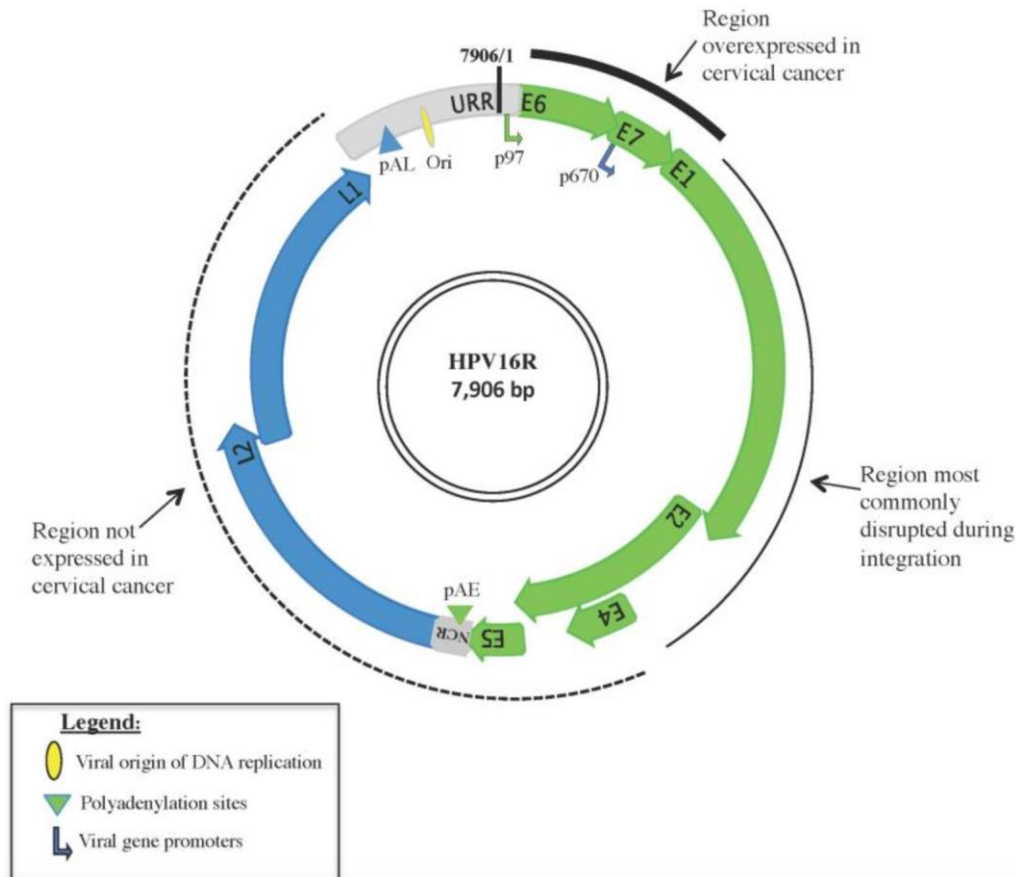


Figure 2. The Structure of the HPV16 Genome

The HPV16 genome displayed shows the general organization of the HPV open reading frames (ORFs) and regulatory regions. Early (E) genes and related transcriptional regulatory motifs are shown in green; late (L) genes and related transcriptional regulatory are shown in blue; and the 2 noncoding regions are shown in grey, the smaller region between E5 and L2 is termed the NCR (non-coding region), and the larger region between L1 and E6 is known as the URR (Upstream Regulatory Region). The URR contains regulatory elements including the viral DNA origin of replication (Ori) depicted by a yellow oval and the early gene promoter, p97 depicted by a green arrow. The blue arrow in the E7 ORF depicts late gene expression regulated by the late promoter, p670. Polyadenylation sites termed early (pAE) or late (pAL) are shown as green or blue triangles, respectively. Arrowheads at the tip of each ORF depict their overlap. The thick solid line represents genomic regions commonly overexpressed in cancer, the thin black line represents genomic regions frequently disrupted by integration, and the dashed line represents the genomic region rarely expressed in cancer (15).

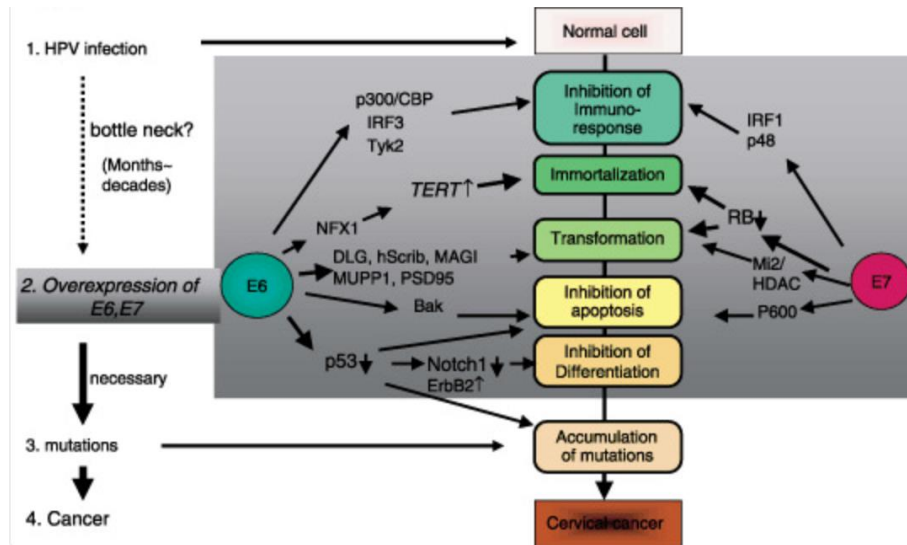


Figure 3. E6 and E7 cooperatively function in the development of cervical cancer.

Multistep carcinogenesis for human papillomavirus (HPV)-induced cervical cancer. The authors would like to emphasize that the bottleneck step to cancer is the overexpression of E6 and E7, which is usually achieved by accidental integration of a viral genome into a host chromosome. Once E6 and E7 genes are overexpressed, subsequent events (in the dark box) might be expected to occur within a short period of time because E6 and E7 can cooperatively induce chromosomal instability (17).

1.1.5. Main Options for Prevention, Screening, Diagnosis, and Treatment

Given that the development of cervical cancer is usually preceded by premalignant conditions that can persist even for years, it is particularly important to provide targeted education and to establish effective and complementary screening protocols (18).

The primary prevention of cervical cancer is HPV vaccination, which is estimated to be 90% effective. The vaccine is recommended for both women and men between the ages of 9 and 45. As of 2025, there are 5 approved HPV vaccines worldwide which have received WHO prequalification, and each of them prevents risk of infection with the high-risk HPV types 16 and 18 (19).

It is recommended to undergo an annual routine gynecological physical examination to assess the external and internal genitalia. Warning signs may include visible cervical abnormalities (e.g., erosion, fixed masses), fetid vaginal discharge, parametrial infiltration, lower-extremity oedema or unusual bleeding (20). In addition, it is advisable to perform a colposcopic examination, where the cervix is made visible using a speculum. To detect malignant conditions, a 3–5% acetic acid solution is applied to the cervix, where cells with a higher nucleic acid content may turn whitish. In cases where the white discoloration on the cervix is more dense, the likelihood of a more severe lesion is higher. The Schiller test is also recommended by applying Lugol's solution, the glycogen-rich squamous cells—which are indicative of malignancy—turn brown when exposed to iodine. Thus, the following findings on colposcopy may indicate malignancy: aceto-whiteness, atypical vascular pattern, coarse mosaic pattern, leukoplakia, ulceration, contact bleeding (necrotic and exophytic), and irregular areas (21).

However, the physical examination and colposcopy has low sensitivity; it may not yield a positive result in patients with cancer, so the Papanicolaou test (Pap test) and/or HPV test are essential for the evaluation and diagnosis of patients with cervical cancer and its precursor lesions (6, 22). According to the American Cancer Society (ACS), even women who are not at high risk should undergo primary HPV testing every 5 years, starting no later than age 25 and continuing until at least age 65. If this is not available, it is advisable to undergo cotesting (HPV testing in combination with cytology) every 5 years; if even that is not possible, then cytology alone every 3 years is strongly recommended (23). In accordance with the ASCCP's 2019 risk-based guidelines, HPV 16/18 positivity requires immediate colposcopy, and in cases of HSIL cytology, immediate excision is often

recommended. The ASCCP determines the next steps based on the risk of CIN3+, which may include colposcopy, observation, or excision. Furthermore, post-treatment follow-up is also determined by HPV-based screening (24). To supplement the evaluation of abnormal cytology findings and/or persistent, high-risk HPV infection, acetic acid is applied to the affected area and assessed with the naked eye or via colposcopy; however, even then, multiple endocervical biopsies may often be necessary. Abnormal colposcopic findings may include a white-to-pink color change upon application of acetic acid, abundant vascularity, atypical blood vessels, mosaicism, and punctate lesions (6, 25).

Another method for assessing the risk of HPV infection is p16/Ki-67 double staining. An advantage of this approach is that it allows for reproducible and objective evaluation, complementing the cytological examinations mentioned earlier, which rely to a greater extent on the examiner's experience (26). This is based on the previously mentioned loss of pRb function, which leads to processes that can also be utilized in diagnostics, as it triggers a negative feedback mechanism during which the cell compensatorily increases p16(INK4a) expression, thereby simultaneously resulting in a characteristic biomarker pattern. Under physiological conditions, p16—in response to increased E2F levels—stimulates the maintenance of pRb's active state by inhibiting the CDK4/6 enzymes, thereby arresting the cell cycle. Thus, elevated p16 levels detected by immunohistochemistry as a result of viral infection can serve as an indirect marker of HR-HPV-induced transformation, which is currently one of the most important supplementary diagnostic tools for precancerous and malignant cervical lesions. Overexpression of p16 strongly correlates with high-grade cervical intraepithelial neoplasia (CIN2 and CIN3) as well as invasive carcinoma (*Figure 4*). A diffuse, block-like p16-positive staining pattern is particularly characteristic of HR-HPV-positive lesions, whereas in low-grade lesions (CIN1) it is often absent or appears only focally (27, 28).

Furthermore, it is important to mention the Ki-67 nuclear protein, which is expressed exclusively in proliferating cells and serves as a marker for the active phases of the cell cycle (G1, S, G2, M). Thus, immunohistochemical measurements make it possible to estimate what proportion of the tissue is actively involved in proliferation. In normal cervical epithelium, Ki-67 expression is limited to the basal and parabasal cell layers, while it is not detectable in differentiated surface cells. Similarly, p16 expression is low

even under physiological conditions and is not typical in differentiated cells. However, in the case of HPV-induced transformation, an abnormal phenomenon can be observed: excessive p16 expression and proliferative activity (Ki-67 positivity) occur simultaneously within the same cell. This is a biologically paradoxical state, since p16 normally participates in cell cycle inhibition; thus, active cell division would not be expected in its presence. This dual positivity in cells therefore indicates a disorder in cell cycle regulation and, consequently, the presence of a transforming HPV infection.

Taking advantage of this, in modern screening and diagnostic algorithms, p16 is often used not as a standalone marker but as part of a combined p16/Ki-67 double staining assay (29, 30).

The Bethesda system includes the evaluation of HPV tests and immunocytochemical results. It is a unified system that has standardized the understanding of cervical cancer through computer-assisted interpretation, thereby simplifying the interpretation of treatment protocols worldwide. The system considers the following factors essential for making a diagnosis during cervical cancer screening: whether the sample contains a sufficient number of cells, whether the transformation zone is present, whether it contains precancerous or cancerous cells, and what category the epithelial cell changes fall into. The latter category includes LSIL (Low-grade Squamous Intraepithelial Lesion) and HSIL (High-grade Squamous Intraepithelial Lesion), which are generally associated with HPV infection; HSIL is a true precancerous condition that, without treatment, may persist as CIN2–3 or progress to cancer. The system also takes into account the category of atypical squamous cells (ASC). ASC-US (Atypical Squamous Cells of Undetermined Significance) often indicates nonspecific inflammation, which may be caused by HPV, while ASC-H (Atypical Squamous Cells—cannot exclude HSIL) may indicate a neoplastic lesion, including the presence of HSIL. In the Bethesda system, glandular lesions are also classified into separate categories. In the case of AGC (Atypical Glandular Cells), endocervical or endometrial neoplasia may be present. In contrast, the AIS (Adenocarcinoma in situ) and adenocarcinoma categories represent unambiguously malignant lesions (26, 31).

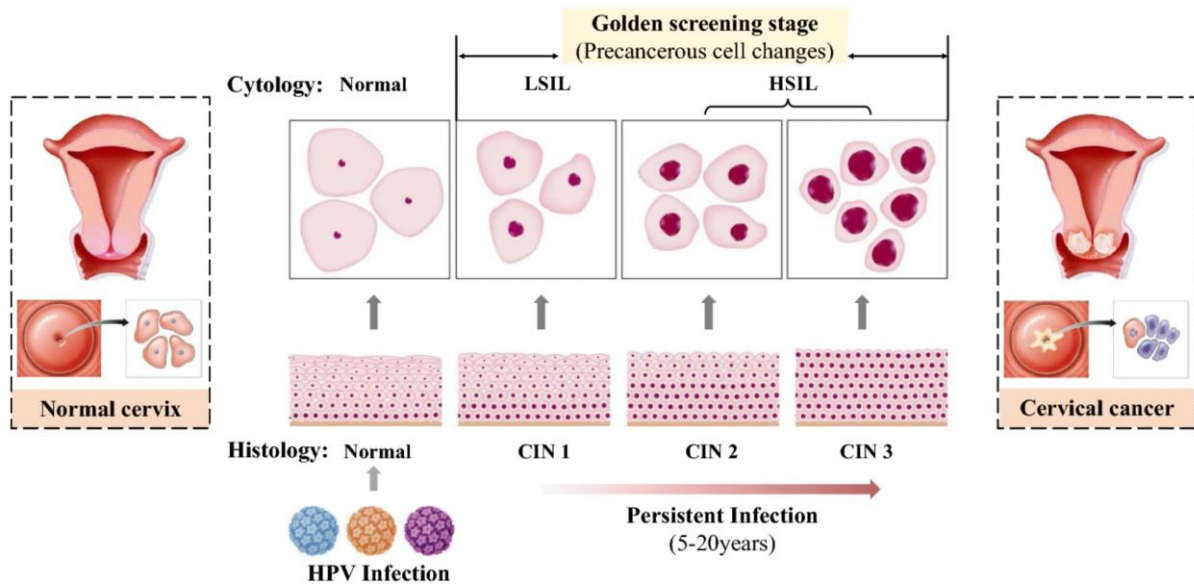


Figure 4. The natural evolution of HPV-infected cervical cancer (32)

In cases where cytology, HPV tests and colposcopy yield inconclusive or alarming results, the next step is most often to proceed with conisation. This allows us to determine with certainty the presence, type and depth of the invasion. However, the procedure is not only diagnostic but also constitutes a definitive oncological intervention at an early stage. The essence of the surgical procedure is the excision of the abnormal epithelial area of the cervix in one piece, in a cone-shaped resection. The lesion usually affects the transformation zone; therefore, the excision encompasses the entire epithelial surface (exocervix + endocervix), the subepithelial stroma, any invasive area and part of the cervical canal as well. There are several methods for this, depending on the choice of instrument: e.g. cold knife conisation (CKC), loop electrosurgical excision procedure/loop excision of the transformation zone (LEEP/LETZ) and laser conisation. The advantage of conisation is that the procedure is relatively quick (10-30 min), generally requires only outpatient care, and the removed tissue can be preserved and assessed pathologically. The orientable tissue sample also reveals whether there is any residual disease and whether further intervention is necessary. However, it should be noted that the disadvantages of conisation are that it depends on the quality of the incision made by the surgeon. Moreover, it is an inadequate procedure in the case of extensive tumours; that it does not assess parametrial invasion; that it does not reveal lymph node status; and that, if the sample is too small, it may underestimate the extent of invasion (33, 34).

Furthermore, it is standard practice to determine the stage according to the International Federation of Gynecology and Obstetrics (FIGO) classification, which requires basic tests available, such as pathological histology, X-ray, CT, MRI and a combination of basic laboratory tests (35). The FIGO staging system describes the extent of cervical cancer, including how deep the tumour has invaded, its approximate size, whether surrounding structures are affected, and whether there are lymph node metastases. In brief, in Stage I the tumour is located only within the cervix; in Stage II it has spread beyond the cervix but has not reached the pelvic wall or the lower third of the vagina. Stage III involves involvement of the pelvic wall, the lower third of the vagina, hydronephrosis or lymph nodes, whilst Stage IV indicates infiltration of the bladder or rectum and distant metastases. The classification is detailed further in Table 1 (36, 37).

Table 1. 2018 FIGO Staging System for Uterine Cervical Cancer (38)

Stage	Description
I	Carcinoma is strictly confined to the cervix (extension to the uterine corpus should be disregarded)
IA	Invasive carcinoma that can be diagnosed only with microscopy, with maximum depth of invasion <5 mm
IA1	Stromal invasion <3 mm in depth
IA2	Stromal invasion ≥3 mm and <5 mm in depth
IB	Invasive carcinoma confined to the uterine cervix, with measured deepest invasion ≥5 mm
IB1*	Tumor measures <2 cm in greatest dimension
IB2*	Tumor measures ≥2 cm and <4 cm in greatest dimension
IB3*	Tumor measures ≥4 cm in greatest dimension
II	Carcinoma invades beyond the uterus, but has not extended onto the lower third of the vagina or to the pelvic wall
IIA	Limited to the upper two-thirds of the vagina without parametrial involvement
IIA1	Tumor measures <4 cm in greatest dimension
IIA2	Tumor measures ≥4 cm in greatest dimension
IIB	With parametrial involvement but not up to the pelvic wall
III	Carcinoma involves the lower third of the vagina and/or extends to the pelvic wall and/or causes hydronephrosis or nonfunctioning kidney and/or involves pelvic and/or para-aortic lymph nodes
IIIA	Involves the lower third of the vagina, with no extension to the pelvic wall
IIIB	Extension to the pelvic wall and/or hydronephrosis or nonfunctioning kidney from tumor
IIIC*	Involvement of pelvic and/or para-aortic lymph nodes, irrespective of tumor size and extent [†]
IIIC1*	Pelvic lymph node metastasis only
IIIC2*	Para-aortic lymph node metastasis
IV	Carcinoma has extended beyond the true pelvis or has involved (biopsy-proven) the mucosa of the bladder or rectum
IVA	Spread to adjacent pelvic organs
IVB	Spread to distant organs

Note.— Imaging and pathologic analysis, where available, can be used to supplement clinical findings for all stages. FIGO = International Federation of Gynecology and Obstetrics. (Adapted, under a CC BY license, from reference 1.)

* Indicates stages that are new from the 2009 FIGO system.

[†] Stage IIIC should be annotated with *r* (radiology) or *p* (pathologic analysis) to indicate the method used to allocate this stage. Imaging modality or pathologic technique should also be documented.

Regarding treatment methods, excision is often used for precancerous conditions. If the extension of the lesion allows for total resection with free margins the conization and the loop electrosurgical excision procedure (LEEP) are often the surgical options of choice. The advantage of the widely used conization procedure is that the excised tissue can be examined by a pathologist, thereby providing the surgeon with precise confirmation that

the tumor has been completely removed. After the diagnosis of invasive cancer, considering the symptoms, histological findings, and imaging results, surgical resection is usually the only treatment required—ranging from repeat conization to pelvic exenteration. However, in more advanced stages, chemotherapy, radiation therapy, and immunotherapy may also be necessary (6, 39).

1.2. The Significance of Inflammatory Markers in Cancer

It is well known that the development of cancer is caused by the emergence of neoplastic characteristics in normal cells, leading to their development in a genetically unstable state. However, we now know that when we talk about cancer, it is not enough to simply refer to cells with defective genetic material that proliferate uncontrollably and chronically. These cells interact with one another, but they also affect the entire body. It has therefore become essential to study the tumor microenvironment as well, since it plays such a major role in tumor progression (40).

The development and progression of tumors can not be fully understood without considering the body's inflammatory and immunological processes. In the early stages of tumor development, “immune surveillance” can be observed, when the immune system is still capable of recognizing and eliminating transformed cells (41). This involves both the innate and adaptive immune systems. Natural killer (NK) cells, dendritic cells, and macrophages recognize cells expressing stress or tumor antigens and activate cytotoxic T lymphocytes. These CD8⁺ T cells are capable of directly destroying tumor cells by releasing perforin and granzyme and by activating apoptosis-inducing signaling pathways. During this phase, inflammatory cytokines—such as interferon-gamma (IFN- γ)—are produced, which enhance antigen presentation and the effectiveness of the immune response (42, 43).

As time progresses, the tumor develops mechanisms that allow it to avoid the immune system: this is known as the “immune escape” phase (42). To ensure their survival, tumor cells can reduce the expression of molecules necessary for antigen presentation—for instance MHC class I—thereby becoming “invisible” to cytotoxic T cells. In addition, they can increase the expression of molecules such as PD-L1, which binds to the PD-1 receptor on T cells and inhibits their activity. As a result, antitumor effector T cells

become exhausted (T-cell exhaustion), their proliferation and cytotoxic function decrease, and they ultimately become functionally inactive (44-46).

At the same time, the tumor microenvironment (TME) becomes increasingly immunosuppressive. The tumor actively recruits and functionally reprograms diverse cell populations to support its progression. Regulatory T cells (Treg cells) proliferate and inhibit the function of effector T cells through certain cytokine-mediated mechanisms (as an example the production of IL-10 and TGF- β) as well as through direct cell-cell interactions (47). Myeloid-derived suppressor cells (MDSCs) likewise exert a significant influence by inhibiting T-cell activation and proliferation via arginase and reactive oxygen species production. Macrophages also play a key role in this process (48). Tumor-associated macrophages (TAMs) commonly shift toward an M2 phenotype, which facilitates tissue repair, angiogenesis, and immunosuppression, in contrast to the classically activated, antitumor M1 macrophages. M2-polarized macrophages release growth factors—such as VEGF—along with proteases and inflammatory mediators that drive neovascularization, tumor infiltration, and the breakdown of the extracellular matrix (49).

One of the defining characteristics of the inflammatory environment maintained by the tumor is the increased production of cytokines and chemokines. Interleukin-6 (IL-6) plays a particularly important role; it activates the JAK/STAT3 signaling pathway, thereby promoting the proliferation, survival, and immune evasion of tumor cells. Tumor necrosis factor-alpha (TNF- α), along with other pro-inflammatory mediators, further contributes to sustaining chronic inflammation. These molecules not only exert their effects locally but also have systemic consequences, such as inducing an acute-phase reaction in the liver (50).

Paradoxically, however, the chronic inflammation that develops in this way does not promote tumor elimination but rather its progression. Inflammatory cells and mediators promote DNA damage, cell proliferation, and the inhibition of apoptosis. In addition, they support the epithelial-mesenchymal transition (EMT), which is key to increasing the invasiveness and metastatic potential of tumor cells (51). The inflammatory environment also contributes to the formation of what is known as the premetastatic niche, which prepares distant organs to receive tumor cells (52).

Collectively, the findings indicate that the relationship between tumors and inflammation is therefore a bidirectional and self-perpetuating process. The tumor triggers an inflammatory response and then harnesses this inflammatory environment to facilitate its own growth and spread. The antitumor immune response is gradually depleted and suppressed, while immunosuppressive and proinflammatory mechanisms become dominant. This transformation is of fundamental importance in tumor biology and determines both the clinical course of the disease and therapeutic responses. Systemic inflammation associated with tumors can therefore no longer be interpreted merely as an epiphenomenon, but rather as one of the fundamental organizing principles of tumor biology. The interaction between the tumor and the host is a dynamic, bidirectional process in which the tumor exerts immunomodulatory, proinflammatory, and immunosuppressive effects (53, 54).

1.2.1. Blood-detectable inflammatory markers

The inflammatory processes resulting from tumor progression lead to measurable changes not only in the peripheral blood but also throughout the entire body. Inflammation also alters hematopoiesis: myelopoiesis is enhanced, leading to an increase in the number of innate immune cells, while components of the adaptive immune response are suppressed. This phenomenon clearly illustrates the shift in balance toward the innate, inflammatory response, at the expense of effective antitumor immunity (30, 31).

An increase in neutrophil granulocytes is one of the most consistent hematological phenomena associated with tumor-associated inflammation. Neutrophils are not only effector cells but also active modulators of the tumor microenvironment. Tumor-associated neutrophils (TANs) exhibit substantial phenotypic diversity, with N1 cells mediating antitumor effects and N2 cells promoting tumor progression. N2 neutrophils enhance angiogenesis—through the production of VEGF and MMP-9—facilitate extracellular matrix degradation, and, by generating neutrophil extracellular traps (NETs), may contribute to the survival of circulating tumor cells and metastasis (55) (56). This functional polarization explains why an elevated neutrophil count is not merely an inflammatory marker but also an active tumor-promoting factor. In the tumor microenvironment, neutrophils are not merely effector cells but active modulators of tumor biology. They are capable of producing reactive oxygen and nitrogen species,

releasing matrix metalloproteinases, and producing angiogenesis-stimulating factors (such as VEGF). In addition, through neutrophil extracellular traps (NETs), they can promote the survival of circulating tumor cells and metastatic colonization. In contrast, lymphocytes—particularly CD8⁺ cytotoxic T cells—are key players in the antitumor immune response; a reduction in their numbers or functional exhaustion indicates a weakening of immune surveillance (57, 58).

The activity of the monocyte–macrophage axis also plays a crucial role in the relationship between tumors and inflammation. Circulating monocytes, upon the tumor microenvironment, differentiate into tumor-associated macrophages (TAMs), which predominantly adopt an M2 phenotype. These cells produce immunosuppressive cytokines (IL-10, TGF- β), stimulate angiogenesis (VEGF), and promote tumor invasiveness and metastatic potential.

Monocytosis is therefore one of the systemic indicators of a tumor’s “myeloid dominance” and often correlates with a poor prognosis. However, a drawback is that hematologic disorders significantly influence its levels (59, 60).

Thrombocytosis is a particularly important, multifactorial phenomenon associated with malignant inflammation. Thrombopoiesis is stimulated by IL-6 and other cytokines produced by the tumor, which increase thrombopoietin production in the liver. However, platelets are not merely passive cellular components but active mediators of tumor progression. By forming aggregates with tumor cells, they provide an “immune shield” in the circulation, reducing NK-cell-mediated cytotoxicity. In addition, they release pro-angiogenic factors (VEGF, PDGF) and adhesion molecules that promote adhesion to the endothelium and extravasation. Thus, thrombocytosis is not only an inflammatory marker but also a functional facilitator of metastasis. However, bleeding and other factors influence its levels (61).

In contrast, the lymphocyte compartment shows changes in the opposite direction. Lymphopenia is one of the most important systemic manifestations of tumor-induced immunosuppression, reflecting the functional exhaustion of the adaptive immune response. The number and activity of CD8⁺ cytotoxic T cells decrease, while the phenomenon of T-cell exhaustion (increased expression of PD-1, CTLA-4, and TIM-3) becomes dominant. Concurrently, expansion of regulatory T cells (FoxP3⁺ Tregs) is observed, which inhibit the effector immune response through the production of IL-10

and TGF- β . Lymphopenia is thus not merely a quantitative abnormality but a biomarker of the functional collapse of antitumor immunity (62, 63).

Other sensitive biochemical indicators of systemic inflammation is C-reactive protein (CRP), which is produced in the liver during an IL-6-mediated acute-phase response. Although CRP is a highly sensitive indicator of systemic inflammatory activity, its poor specificity substantially limits its diagnostic value. Elevated CRP levels can arise in numerous clinical contexts—such as infections, autoimmune disorders, tissue damage, or malignancies—making it challenging to determine whether the increase reflects tumor-related inflammation rather than another underlying condition (64, 65).

Fibrinogen and other components of the coagulation system represent also a common intersection between tumor biology and inflammation. Fibrinogen is not only a coagulation factor but also a component of the extracellular matrix that promotes the adhesion and migration of tumor cells and the formation of the metastatic niche. Tumor-induced hypercoagulability underlies the pathomechanism of Trousseau syndrome and also explains the high incidence of thromboembolic complications in patients with malignancies (66).

1.2.2. Combined Hematological Indices

In recent years, inflammatory biomarkers that can be calculated from simple, routinely available blood test values have received increasing attention in clinical practice and medical research. These include the neutrophil-to-lymphocyte ratio (NLR), the lymphocyte-to-monocyte ratio (LMR), and the platelet-to-lymphocyte ratio (PLR), as well as the more complex SIRI and SII indices.

These can be calculated as follows (67, 68).

$$NLR = \frac{\text{Neutrophils}}{\text{Lymphocytes}} \quad LMR = \frac{\text{Lymphocytes}}{\text{Monocytes}} \quad PLR = \frac{\text{Thrombocytes}}{\text{Lymphocytes}}$$

$$SIRI = \frac{\text{Neutrophils} \times \text{Monocytes}}{\text{Lymphocytes}} \quad SII = \frac{\text{Neutrophils} \times \text{Thrombocytes}}{\text{Lymphocytes}}$$

It should be noted that these parameters are not isolated diagnostic tools, but rather components of an integrated biological signaling system that reflect the various stages of the tumor immunoediting process (elimination–equilibrium–escape). Their clinical significance lies in the fact that, while classical tumor staging is based on anatomical and histological parameters, these markers provide functional immunological information. This is particularly important because tumor progression depends not only on local invasion but also on the effectiveness of the host's immune response. These markers are capable of simultaneously providing information on tumor activity, the host's immunocompetence, and the extent of systemic inflammation, while remaining cost-effective and routinely available. Their advantage is that they utilize the ratio or combination of different cell lines and reflect the balance between the innate and adaptive immune responses. Thus, they may be more sensitive in determining prognosis than individual parameters. At the same time, due to their limited specificity, numerous confounding factors (infection, medications, stress response, chronic inflammation), making a thorough medical history even more critical in these cases.

The NLR has been extensively studied over the past two decades in various fields of oncology. Numerous meta-analyses of breast, colorectal, head and neck, lung, gastric, pancreatic, and urological tumors have confirmed that a high NLR is significantly associated with poorer prognosis across various tumor types (69-75). This association has been replicated in multiple independent studies, establishing the NLR as a robust prognostic biomarker. In gynecologic cancers—such as cervical cancer, endometrial carcinoma, and ovarian cancer—a high NLR has consistently been described as an indicator of an unfavorable prognosis (76-79). In cervical cancer, for example, a high preoperative NLR is often associated with more advanced disease, larger tumor volume, lymphovascular invasion, and a poorer therapeutic response. In addition, a high NLR is associated with shorter survival and a higher risk of recurrence. Similar observations have been made in endometrial carcinoma, where the NLR correlates with tumor grade, myometrial invasion, and lymph node involvement (80, 81).

Monocytes respond more slowly; they differentiate into macrophages and participate in the later phases of inflammation, and they also play a role in shaping the tumor microenvironment. A low LMR is therefore often associated with a poorer prognosis in hematologic malignancies, chronic infections—such as tuberculosis—and cardiovascular

diseases. Its advantage is that it is more stable and less variable than the NLR; however, its disadvantage is that it responds more slowly to acute processes and is also nonspecific (81).

Platelets not only play a role in blood clotting, but also produce inflammatory mediators and may promote tumor angiogenesis. High PLR levels are therefore often associated with poorer outcomes, particularly in gastrointestinal cancers, as well as with an increased risk of thrombosis in cardiovascular diseases. An advantage is that platelet counts are relatively stable; a disadvantage, however, is that conditions such as iron-deficiency anemia or splenomegaly can significantly affect them (82).

Among the more complex markers, the SIRI—systemic inflammation response index—summarizes the combined effects of neutrophils, monocytes, and lymphocytes. Because it simultaneously takes into account two important cell lines of the innate immune response and the lymphocyte component of the adaptive immune response, it may be a more sensitive indicator of the body's inflammatory burden than simple ratios. A high value indicates a more aggressive inflammatory response and a poorer prognosis in conditions such as lung cancer, pancreatic cancer, and upper tract urothelial carcinoma, among others. Its advantage lies in its complexity and the resulting, presumably greater predictive power. Its disadvantage is that monocyte levels fluctuate greatly during infections, periods of stress, and when taking medications. As a result, the SIRI is more prone to distortion and has lower specificity for tumor states than the NLR (83).

The SII, or systemic immune-inflammation index, provides even more comprehensive information, as it uses a combination of platelets, neutrophils, and lymphocytes. This index is particularly sensitive to imbalances in inflammation and the immune system. A high SII value is associated with increased inflammation, a prothrombotic state, and a reduced adaptive immune response, all of which are linked to poorer survival in many cancers. Its advantage is that, according to studies, it is often more accurate than the NLR. Its downside is that it can be strongly influenced by fluctuations in platelet count and is not yet widely used in clinical practice (79, 84-86).

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 investigate the association between routinely derived inflammatory indices, including the neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), and lymphocyte-to-monocyte ratio (LMR), and p16 positivity.
- 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. MATERIALS AND METHODS

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

3.1.1. Patients and Characteristics

We conducted a retrospective observational study as part of the SCOPE Study (Semmelweis University Conization and Inflammation Outcomes with Predictive Evaluation), which was ethically approved by the Institutional Review Board of Semmelweis University, (SE RKEB: 195/2024). We reviewed the detailed medical documentations of 395 patients out of 417 initially recorded patients between 2021 and 2024, collecting a wide range of data on gynecological, clinical, laboratory and sociodemographic variables (included age, body weight, height, BMI, smoking status, alcohol consumption, known diabetes or hypertension, and the presence of immunological disorders affecting immune function). The patient's age was calculated by subtracting the year of birth from the year of conization. We also calculated the body mass index (BMI) using the standard formula based on measurements recorded during the preoperative clinical visit: body weight in kilograms divided by the square of height in meters (kg/m^2). Smoking status was also recorded during the clinical intake and classified into two categories: current smoker or nonsmoker. Preoperative laboratory parameters focused on biomarkers associated with systemic inflammation, particularly neutrophil, platelet, and monocyte counts, as well as lymphocyte count. From these values, we calculated the neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), and lymphocyte-to-monocyte ratio (LMR). All peripheral blood samples were collected within one month prior to the conization procedure to ensure their temporal relevance to the conization procedure. In order to ensure compliance with quality and accreditation standards, all laboratory tests were conducted in laboratories accredited by the Hungarian National Accreditation Authority. Only patients who were at least 18 years old and had undergone LEEP conization at the Department of Obstetrics and Gynecology at Semmelweis University were enrolled in the study.

During screening for cervical dysplasia, the results of cervical cancer screening and HPV status (with particular attention paid to the presence of high-risk HPV infection) were

evaluated, as well as the results of p16 immunohistochemical staining. The data also included the results of conization and the histopathological findings obtained during conization, which included p16 positivity and the presence of glandular involvement. Formalin-fixed, paraffin-embedded histological sections obtained from LEEP conization samples were subjected to p16 immunohistochemical staining.

Excluded were those who were receiving current or previous immunosuppressive therapy, who had a history of cervical cancer, or who had undergone prior cervical surgery that could have affected the histopathological or hematological results. Participants with autoimmune diseases affecting inflammatory markers were also excluded. Furthermore, we did not include those with incomplete follow-up data or relevant clinical information, or cases where the hematological or immunohistochemical data required for statistical analysis were incomplete (*Figure 5*).

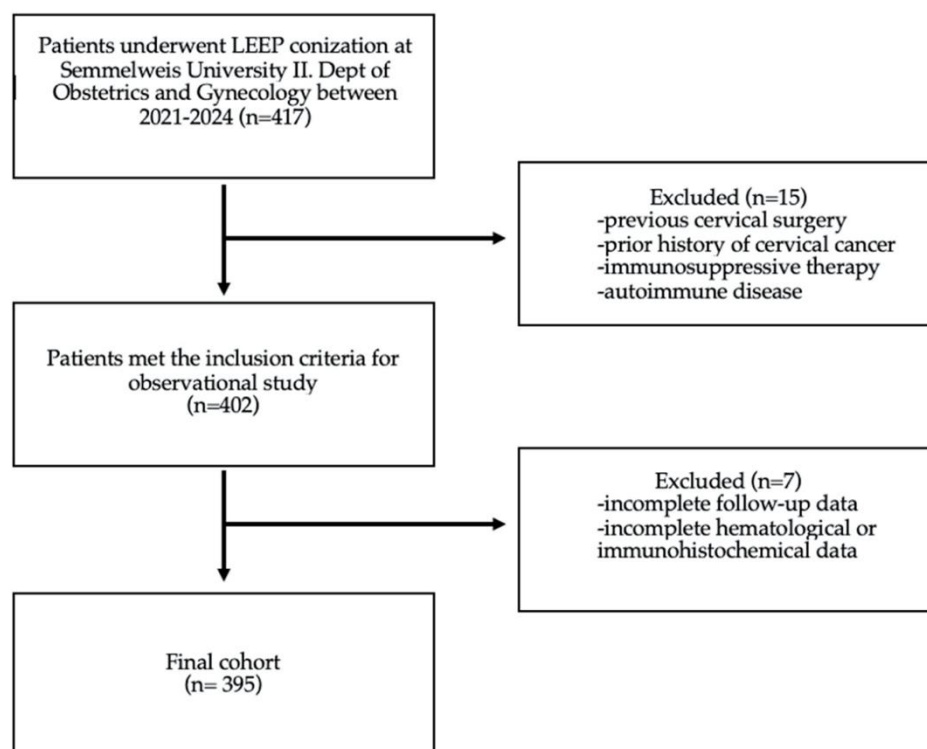


Figure 5. Flowchart of patient selection for the SCOPE Study I.

Source: Adapted from Keszthelyi et al (87)

3.1.2. Data Management and Statistical Analysis

Prior to the analysis of the dedicated and comprehensive database, strict checks were performed to ensure data quality, including an examination of outliers and inconsistencies; box plots were also used to identify extreme values that could skew the results. To ensure the suitability and reliability of the dataset for statistical analysis, missing data were systematically handled according to predefined criteria.

Statistical analyses were performed using IBM SPSS Statistics for Windows, Version 25.0 (2017 release, IBM Corp., Armonk, NY, USA), and descriptive statistics were calculated for all continuous variables, including the mean, standard deviation, median, minimum, and maximum values, to characterize the study population. The Mann–Whitney U test was used for comparisons between groups, with particular focus on examining differences in the neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), and lymphocyte-to-monocyte ratio (LMR) in relation to p16 positivity. Chi-square tests were used to analyze categorical variables. The level of significance was set at $p < 0.05$. Receiver operating characteristic (ROC) curves were constructed to further evaluate the predictive ability of p16 positivity. This analysis provided insight into the sensitivity and specificity of NLR, PLR, and LMR as potential biomarkers in the progression of cervical carcinoma. The results were used to determine optimal cutoff values for clinical applicability.

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

3.2.1. Patients and Characteristics

Following the same method as in the study described above, we conducted a retrospective study a retrospective observational study as part of the SCOPE Study (Simmelweis University Conization and Inflammation Outcomes with Predictive Evaluation), which was ethically approved by the Institutional Review Board of Semmelweis University, (SE RKEB: 195/2024). We reviewed the detailed medical documentation of 374 patients out of 417 initially recorded patients between 2021 and 2024, collecting a wide range of data on gynecological, clinical, laboratory and sociodemographic variables (included age, body weight, height, BMI, smoking status, alcohol consumption, known diabetes or hypertension, and the presence of immunological disorders affecting immune function).

The patient's age was determined by subtracting the year of birth from the year in which the conization was performed. Body mass index (BMI) was calculated using measurements obtained during the preoperative visit, applying the standard formula: body weight in kilograms divided by height in meters squared (kg/m^2). Smoking status was recorded at clinical intake and classified as either current smoker or nonsmoker.

A prerequisite for all patients was the availability of complete histopathological and hematological laboratory data. Preoperative laboratory assessments focused on biomarkers indicative of systemic inflammation, including neutrophil and lymphocyte counts. These parameters were used to derive the neutrophil-to-lymphocyte ratio (NLR). All peripheral blood and cytology samples were collected within one month prior to conization to ensure temporal relevance to the surgical procedure. To maintain compliance with quality and accreditation standards, all laboratory analyses were performed in facilities accredited by the Hungarian National Accreditation Authority. Only patients who were at least 18 years old and had undergone cervical cytology examination and LEEP conization at the Department of Obstetrics and Gynecology at Semmelweis University were enrolled in the study. During screening for cervical dysplasia, the results of cervical cancer screening and HPV status (with particular attention paid to the presence of high-risk HPV infection) were evaluated.

Excluded were those who were receiving current or previous immunosuppressive therapy, who had a history of cervical cancer, or who had undergone prior cervical surgery that could have affected the histopathological or hematological results. Participants with autoimmune diseases affecting inflammatory markers were also excluded. Furthermore, we did not include those with incomplete follow-up data or relevant clinical information, or cases where the hematological or immunohistochemical data required for statistical analysis were incomplete (*Figure 6*).

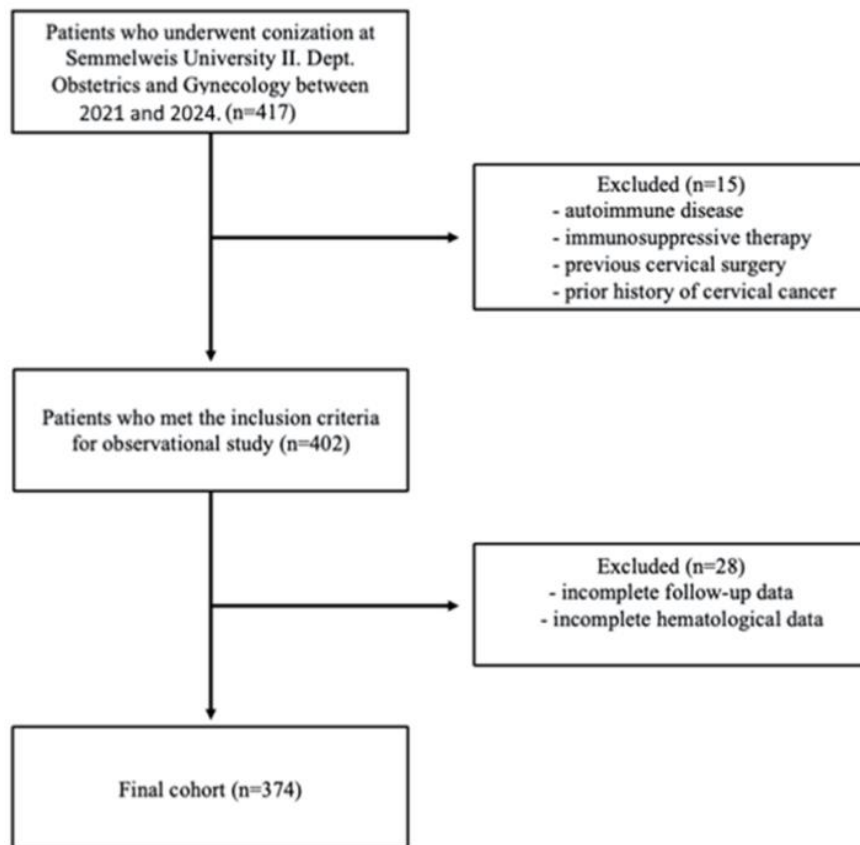


Figure 6. Flowchart of patient selection for the SCOPE Study II.

Source: Adapted from Keszthelyi et al (88)

It should be noted that, based on the results of cervical cancer screening, patients were classified into four risk categories. It is important to emphasize that this classification does not correspond to the pathological histological grade classification, but rather is a risk classification. Its use was justified by the fact that it allowed for the grouping of cytological and histological categories with similar clinical outcomes and treatment strategies. This approach allows us to reduce diagnostic heterogeneity and avoid the statistical underrepresentation of smaller subgroups, in line with the latest literature, which emphasizes the overlap of morphological features and risk-based categorization in the interpretation of cervical screening results (89). In accordance with the classification in cytology, 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. Similarly, the surgical data included the results of conization and the histological findings from the conization procedure, with conization specimens evaluated

according to the same classification: Grade 1 for negative results, Grade 2 for LSIL and ASC-US, Grade 3 for HSIL, ASC-H, AGC, and AIS, and Grade 4 for invasive carcinoma (Table 2).

Table 2. Risk-grade classification of cervical cancer

Source: Adapted from Keszthelyi et al (88)

Grade I.	Negative test results
Grade II.	LSIL (Low-grade Squamous Intraepithelial Lesion) AUSC-US (<i>Atypical Squamous Cells of Undetermined Significance</i>)
Grade III.	HSIL (High-grade Squamous Intraepithelial Lesion) ASC-H (Atypical Squamous Cells – cannot exclude HSIL) AGC (Atypical Glandular Cells) AIS (Adenocarcinoma in situ)
Grade IV.	CxCa (Cervix Carcinoma)

3.2.2. Data Management and Statistical Analysis

Following the previous research methodology, we also collected the data for this retrospective analysis in a specialized database created for the SCOPE study. Prior to conducting the analyses, we carefully checked the data, including the detection of inconsistencies and outliers, and used box plots to identify extreme values that could potentially skew the results. Statistical analyses were performed using IBM SPSS Statistics for Windows, Version 25.0 (2017 release, IBM Corp., Armonk, NY, USA). Here, descriptive statistics (mean, standard deviation, median, and range) were calculated for continuous variables, while frequencies and percentage distributions were reported for categorical data to characterize the study population. Comparisons of the neutrophil-to-lymphocyte ratio (NLR) between cytological and histopathological grades were performed using the Kruskal–Wallis test, and pairwise post-hoc comparisons were

conducted with Dunn–Bonferroni correction to control for errors arising from multiple testing. For each Kruskal–Wallis test, the effect size was estimated using eta-squared (η^2) to contextualize the p-values and assess the magnitude of the observed differences. Univariate and multivariate binary logistic regression analyses were performed to examine the association between NLR and histologically confirmed cervical malignancy. The binary logistic regression was performed using the “Enter” method in IBM SPSS Statistics (v25) “Enter” method, in which all selected predictors were simultaneously included in the model. After testing multiple predictors, only the neutrophil-to-lymphocyte ratio (NLR) remained statistically significant ($p = 0.008$), and thus it was included in the final model. Automatic stepwise selection procedures (e.g., Forward LR or Backward LR) were not used, in order to ensure that the inclusion of variables was based entirely on clinical and theoretical considerations. In the multivariate model, potential confounding factors (age, smoking status, HPV DNA positivity) were included using a forward stepwise (likelihood ratio) method. Odds ratios (OR) were reported along with 95% confidence intervals (CI) for all predictors retained in the final models. Missing data were handled using the listwise deletion method in all regression models, so only cases with complete data for all variables were included in the multivariate analyses. The number of valid cases (N) for each model was reported separately. The discriminatory power of the NLR was further examined using receiver operating characteristic (ROC) curve analysis. The area under the curve (AUC) was calculated to evaluate the model’s performance. The optimal NLR cutoff values were determined based on the Youden index and the “Closest Top Left” method. Based on these, sensitivity, specificity, positive and negative predictive values (PPV, NPV), positive and negative likelihood ratios (+LR, –LR), and overall accuracy were calculated. Model fit was assessed using Cox & Snell R^2 and Nagelkerke R^2 measures. The significance of the regression models was tested using an omnibus chi-square test and, where relevant, ANOVA. Missing data were handled using the listwise deletion method in all analyses, and the number of valid cases was specified separately for each statistical procedure. Finally, a post hoc statistical power analysis was performed using the G*Power 3.1.9.7 software. Given the observed effect size (OR = 1.372), $\alpha = 0.05$, and a desired statistical power of 80%, the required minimum sample size was 213. The total sample size ($n = 374$) exceeded this threshold, ensuring that the study had sufficient statistical power to detect the observed association. All

statistical tests were two-sided, and a p-value < 0.05 was considered statistically significant. This rigorous and transparent statistical approach supports the validity of the results and ensures their proper interpretation from both statistical and clinical perspectives. The methods used allow for a comprehensive examination of the predictive role of NLR in the diagnosis and progression of cervical carcinoma, as well as a reliable assessment of its clinical utility as a non-invasive biomarker.

4. RESULTS

4.1. Results in The Neutrophil/Lymphocyte Ratio (NLR) as a Predictive Marker for p16 Positivity

4.1.1. Patient Characteristics

The characteristics of the participants are summarized in Table 3. Among the 395 individuals included, the median age was 40 years (interquartile range (IQR): 23–78) and the median BMI was 22.85 (interquartile range: 14.6–46.4). Median values for the inflammatory markers were as follows: NLR was 1.85 (IQR: 0.47–16.86), PLR was 8.89 (IQR: 3.3–184.2), and LMR was 4.34 (IQR: 0.32–18.5). Overall, HPV positivity was detected in 94.3% of patients, and p16 positivity was reported in 198 patients (79.8%) following conization. Over 90% of the participants reported no history of alcohol consumption, diabetes, or hypertension, and no immunological disorders were present. Smoking was documented in 326 patients, representing 83% of the study population.

Table 3. Basic Characteristics of the sample. I.

Source: Adapted from Keszthelyi et al (87)

Characteristics (<i>n</i> = 395)	N (Range or %)
Total	395
Median Age (years)	40 (23–78)
BMI	22.85 (14.6–46.4)
Median NLR	1.85 (0.47–16.86)
Median PLR	8.89 (3.3–184.2)
Median LMR	4.34 (0.32–18.5)
HPV positivity	214 (94.3%)
p16 positivity after conization	198 (79.8%)
Smoking status	
Yes	63 (16%)
No	326 (83%)
N/A	6 (1%)
Alcohol consumption	
Yes	2 (0.5%)
No	386 (98%)
N/A	7 (2%)
Known Diabetes	
Yes	3 (1%)
No	392 (99%)
Known Hypertension	
Yes	37 (9%)
No	358 (91%)
Immunological disorders	
Yes	27 (7%)
No	368 (93%)

4.1.2. Relationship Between Laboratory Parameters and p16 Positivity

The association between p16 positivity in the conization sample and three hematological markers—neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), and lymphocyte-to-monocyte ratio (LMR)—was evaluated using the Mann–Whitney U test (*Table 4*). A significant association was observed for NLR, with p16-positive cases showing higher values ($U = 3687.5$, $p = 0.011$). In contrast, PLR ($p = 0.366$) and LMR ($p = 0.145$) did not demonstrate statistically meaningful associations with p16 status.

Table 4. Relationship between laboratory parameters and p16 positivity.

*The table 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). * $p < 0.05$.*

Source: Adapted from Keszthelyi et al (87)

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

4.1.3. Laboratory Markers and Correlation of HPV DNA Status

Analysis of the hematological markers showed clear differences between individuals with HPV DNA positivity and those without (*Tables 5 and 6*). Patients who tested positive for HPV DNA exhibited higher median neutrophil-to-lymphocyte ratios ($NLR = 1.79$) compared with HPV DNA-negative participants (median = 1.54). A similar pattern was observed for the platelet-to-lymphocyte ratio (PLR), which was also increased in the HPV DNA-positive group (median = 9.08 vs. 7.64). In contrast, the lymphocyte-to-monocyte ratio (LMR) was lower among HPV DNA-positive patients (median = 4.39) compared with those who were HPV DNA-negative (median = 5.38). A statistically significant association emerged only for NLR values above 1.31 ($p = 0.04$), indicating that elevated NLR is strongly linked to HPV DNA positivity. These findings suggest that systemic inflammatory activity potentially associated with HPV infection status.

Table 5. Laboratory markers and correlation of HPV DNA status.

Summary of laboratory markers (NLR, PLR, and LMR) in relation to HPV DNA status. The table displays the median, range, minimum, and maximum values for each marker across HPV DNA-negative and HPV DNA-positive groups. The results highlight higher median NLR and PLR values and lower median LMR values in the HPV DNA-positive group compared to the HPV DNA-negative group. Source: Adapted from Keszthelyi et al (87)

HPV DNA Status		NLR	PLR	LMR
Negative	Median	1.54	7.64	5.38
	N	12	12	11
	Range	1.39	5.88	2.94
	Min.	1.09	5.87	3.96
	Max.	2.48	11.75	6.90
Positive	Median	1.79	9.08	4.39
	N	213	212	212
	Range	16.39	155.71	17.90
	Min.	0.48	3.34	0.62
	Max.	16.86	159.05	18.52
Total	Median	1.76	8.93	4.46
	N	225	224	223
	Range	16.39	155.71	17.90
	Min.	0.48	3.34	0.62
	Max.	16.87	159.05	18.52

Table 6. Relationship between laboratory parameters and HPV positivity.

The table shows the Mann-Whitney U test results for the association between HPV positivity and hematological parameters, including neutrophil/lymphocyte ratio (NLR), platelet/lymphocyte ratio (PLR), and lymphocyte/monocyte ratio (LMR). Source: Adapted from Keszthelyi et al (87)

Laboratory Parameters	Mann–Whitney U	z	p
NLR	988.0	−1.322	0.186
PLR	1013.0	−1.186	0.366
LMR	775.0	−1.874	0.061

4.1.4. Diagnostic Performance

For the prediction of p16 positivity, receiver operating characteristic (ROC) analysis was performed to determine the diagnostic value of the NLR, PLR, and LMR. The area under the curve (AUC) for the NLR was 0.610, indicating moderate diagnostic accuracy (Figure 7).

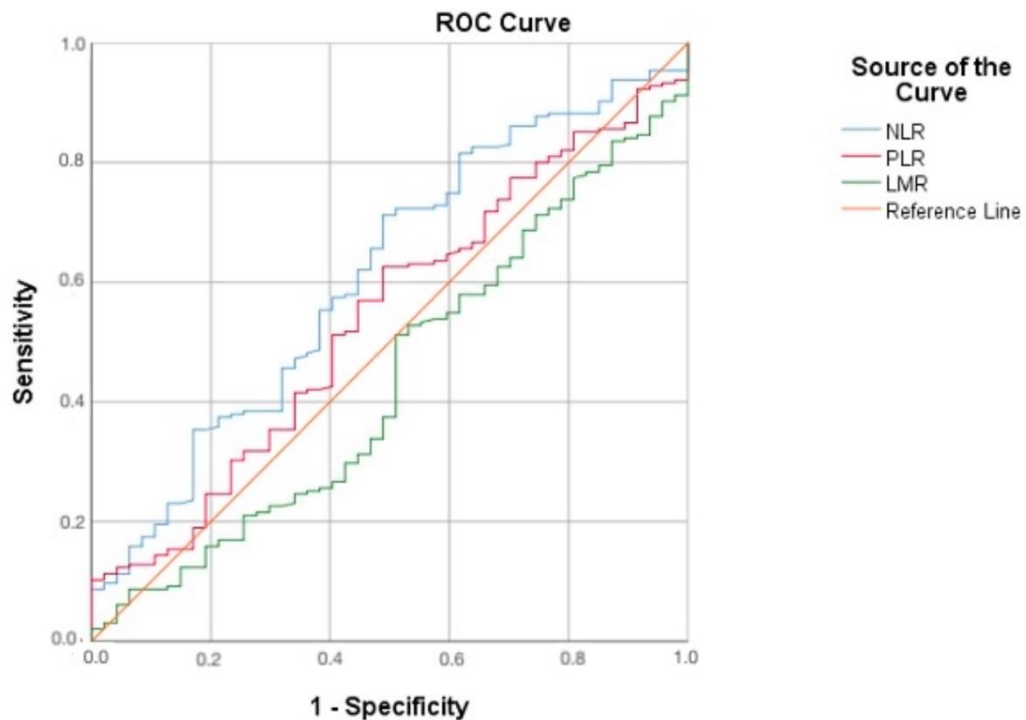


Figure 7. The ROC curve compares the diagnostic performance of the neutrophil/lymphocyte ratio (NLR), platelet/lymphocyte ratio (PLR), and lymphocyte/monocyte ratio (LMR) for identifying p16 positivity.

Sensitivity (true positive rate) is plotted on the y-axis, while 1-specificity (false positive rate) is on the x-axis. Source: Adapted from Keszthelyi et al (87)

The analysis determined that the optimal cutoff value for the neutrophil-to-lymphocyte ratio (NLR) was ≥ 1.530 . At this threshold, the maximum specificity was 51%, while the maximum sensitivity was 71% (Figure 8). For PLR, the area under the curve (AUC) was 0.540, indicating only minimal discriminatory capability. LMR showed similarly limited diagnostic performance, with an AUC of 0.568. Taken together, these findings demonstrate that neither PLR nor LMR provides sufficient standalone predictive value for identifying p16 positivity, especially when compared with the markedly stronger performance of NLR.

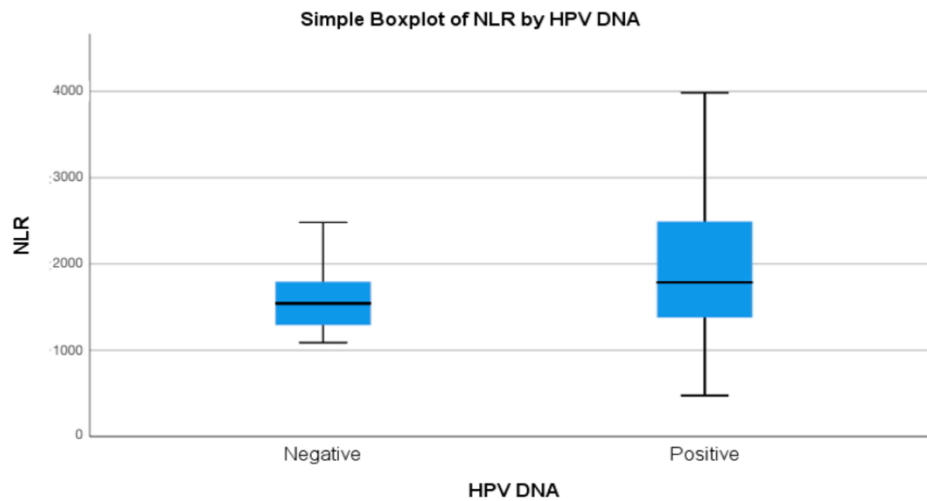


Figure 8. Boxplot showing the distribution of neutrophil/lymphocyte ratio (NLR) values by HPV DNA status.

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. Source: Adapted from Keszthelyi et al (87)

4.1.5. Influencing Factors of the Findings

No significant associations were identified between the hematological markers (NLR, PLR, and LMR) and health-related variables such as smoking, alcohol intake, diabetes, or immunological conditions. In contrast, age and hypertension showed measurable relationships with certain laboratory parameters. However, specific associations were observed with regard to age and hypertension (*Table 7*).

Table 7. Association between laboratory markers and influencing factors.

Statistical associations between laboratory markers (NLR, PLR, and LMR) and influencing factors such as age, smoking, alcohol consumption, hypertension, diabetes, and immunological disorders. * $p < 0.05$. Source: Adapted from Keszthelyi et al (87)

	NLR			PLR			LMR		
	Pearson Correlation		p	Pearson Correlation		p	Pearson Correlation		p
Age	0.074		0.145	0.038		0.458	-0.127		0.012*
	U	z	p	U	z	p	U	z	p
Smoking	9421.5	-0.930	0.353	9663.0	-0.557	0.578	9504.0	-0.680	0.497
Alcohol consumption	0.0	-1.728	0.084	3.0	-1.700	0.089	1.0	-1.718	0.086
Hypertension	5056.5	-2.304	0.021*	4901.0	-2.498	0.012*	5374.5	-1.500	0.134
Diabetes	314.5	-1.376	0.169	547.5	-0.170	0.865	229.0	-1.801	0.072
Immunological disorders	4841.5	-0.104	0.917	4586.5	-0.511	0.610	4007.5	-1.501	0.133

Given the observed association between hypertension and the NLR, a logistic regression analysis was performed to determine whether NLR independently predicts p16 positivity. The regression model demonstrated that NLR remained a statistically significant predictor of p16 expression ($B=0.468$, $p=0.026$, $\text{Exp}(B)=1.596$, 95% CI: 1.059–2.406).

This indicates that each one-unit increase in NLR corresponds to an approximately 59.6% rise in the probability of p16 positivity. In contrast, hypertension did not retain statistical significance in the model ($B=0.541$, $p=0.402$, $\text{Exp}(B)=1.717$, 95% CI: 0.485–6.076), suggesting that it does not independently modify the relationship between NLR and p16 status. The logistic regression model demonstrated limited explanatory power (Nagelkerke $R^2 = 0.055$), suggesting that although NLR was identified as a significant predictor, it accounted for only a small proportion of the variation in p16 positivity. Nevertheless, adding the predictor variables resulted in a statistically significant improvement in overall model performance ($\chi^2(2) = 8.671$, $p = 0.013$). Collectively, these findings indicate that while hypertension is associated with elevated NLR values, it does not act as a confounding factor. Thus, NLR remains an independent and meaningful predictor of p16 positivity within this cohort.

4.1.6. Validation Tests of HPV Positivity and p16 Positivity

To further verify the reliability of our histopathological observations, additional statistical analyses were carried out. A chi-square test demonstrated a robust association between p16 positivity and HPV DNA status ($\chi^2 = 13.768$, $p < 0.001$), reinforcing the validity of the histological assessments and confirming the strength of the analytical approach applied.

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

4.2.1. Patient Characteristics

The baseline characteristics of the study population are detailed in Table 8. After quality control and the exclusion of outliers, a total of 373 conization procedures were analyzed. The median age of the patients was 40 years, with an interquartile range of 33 to 47 years. The median body mass index (BMI) was 22.85, with a range of 14.6 to 46.4. Regarding HPV status, 61% of patients were HPV-positive and all of them was detected with high-risk HPV infection. The median neutrophil-to-lymphocyte ratio (NLR) was 1.85, with a range of 0.47 to 16.86.

Based on the results of cervical cancer screening, patients were classified into four groups: 10 patients (2.7%) were in Category I (negative), 73 patients (19.5%) were

classified into Category II (LSIL, ASC-US), 274 patients (73.3%) into Category III (HSIL, ASC-H, AGC, AIS), and 17 patients (4.5%) into Category IV (tumor) (*Table 8*). Histopathological evaluation of the conization specimens revealed a similar distribution pattern: 84 cases (22.5%) fell into Category I (negative), 32 cases (8.6%) into Category II (LSIL, ASC-US), 234 cases (62.3%) fell into Category III (HSIL, ASC-H, AGC, AIS), while 23 cases (6.2%) fell into Category IV (cancer) (*Table 8*).

Table 8. Basic Characteristics

Categorical parameters are presented as n. Continuous data are presented as median (range).

Source: Adapted from Keszthelyi et al (88)

Characteristics (n = 374)	N (Range or %)
Total	374
Median Age (years)	40 (23–78)
Median BMI	22.85 (14.6–46.4)
Median NLR	1.85 (0.47–16.86)
HPV positivity	228 (61%)
Hr HPV positivity	228 (61%)
Cytology results	374
Grade I	10 (2.7%)
Grade II	73 (19.5%)
Grade III	274(73.3%)
Grade IV	17 (4.5%)
Conization results	373
Grade I	84 (22.5%)
Grade II	32 (8.6)
Grade III	234 (62.7%)
Grade IV	23 (6.2%)

4.2.2. Relationship Between Laboratory Parameters and Cytological Results

The evaluation of cytological categories revealed that neutrophil-to-lymphocyte ratio (NLR) values did not differ significantly across diagnostic groups. The Kruskal–Wallis analysis ($\chi^2 = 5.785$, $p = 0.123$) indicated that NLR lacks sufficient discriminatory capacity to distinguish between varying degrees of pre-malignant cytological abnormalities (*Table 9*). Although pairwise Mann–Whitney U tests showed a significant difference only between cytological categories II and IV ($p = 0.046$), while comparisons between the other groups did not reach statistical significance. 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 10*). Even though it is impossible to precisely determine the NLR threshold above which cytological results would differ, the trend in the distribution of NLR values is distinct (*Figure 9*).

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 10*). 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. Even though higher median NLR values were associated with less favorable cytological findings, given the lack of statistical evidence, the NLR is not suitable for reliably distinguishing between cytological grades. These results suggest that the NLR does not show a meaningful association with the severity of cytological abnormalities and, therefore, cannot be considered a reliable biomarker for distinguishing between different cytological categories.

Table 9. Pairwise comparison of NLR values between cytological groups.

Source: Adapted from Keszthelyi et al (88)

Comparison Between Groups	Mann–Whitney U	z	Bonferroni Corrected p
Grade I versus Grade II	263.0	−1.427	0.933
Grade I versus Grade III	1077.5	−1.147	1
Grade II versus Grade III	8989.5	−1.328	1
Grade I versus Grade IV	78.0	−0.352	1
Grade II versus Grade IV	427.0	−1.995	0.279
Grade III versus Grade IV	1826.5	−1.493	0.815

As mentioned above, Table 9 presents a pairwise comparison of NLR values across cytological severity categories using the Kruskal–Wallis test followed by the Mann–Whitney U test and Dunn–Bonferroni post hoc analysis. Although a nominally significant difference was observed between cytological categories II and IV ($p = 0.046$), this difference was no longer statistically significant after applying the Bonferroni correction. We found no statistically significant differences among the other cytological categories. The U-statistic, z-score, and p-value indicate whether a statistically significant difference ($p < 0.05$) can be detected between the individual groups. The table presents the median neutrophil-to-lymphocyte ratio (NLR) values for the different cytological categories. It also presents the median, minimum, maximum, and range values for each group. Based on these results, it is also important to note that NLR values show an upward trend as cytological abnormalities worsen; however, this increase was not statistically significant.

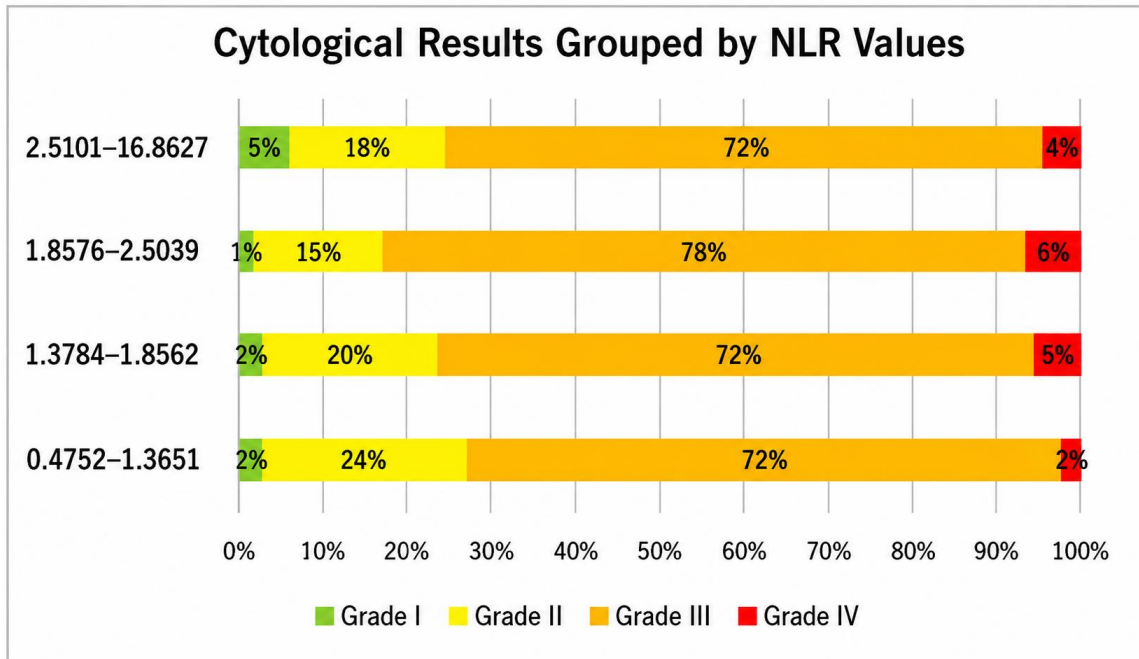


Figure 9. Distribution of cytological results by NLR categories.

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. Source: Adapted from Keszthelyi et al (88)

Table 10. NLR values by cytological outcome.

Source: Adapted from Keszthelyi et al (88)

	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

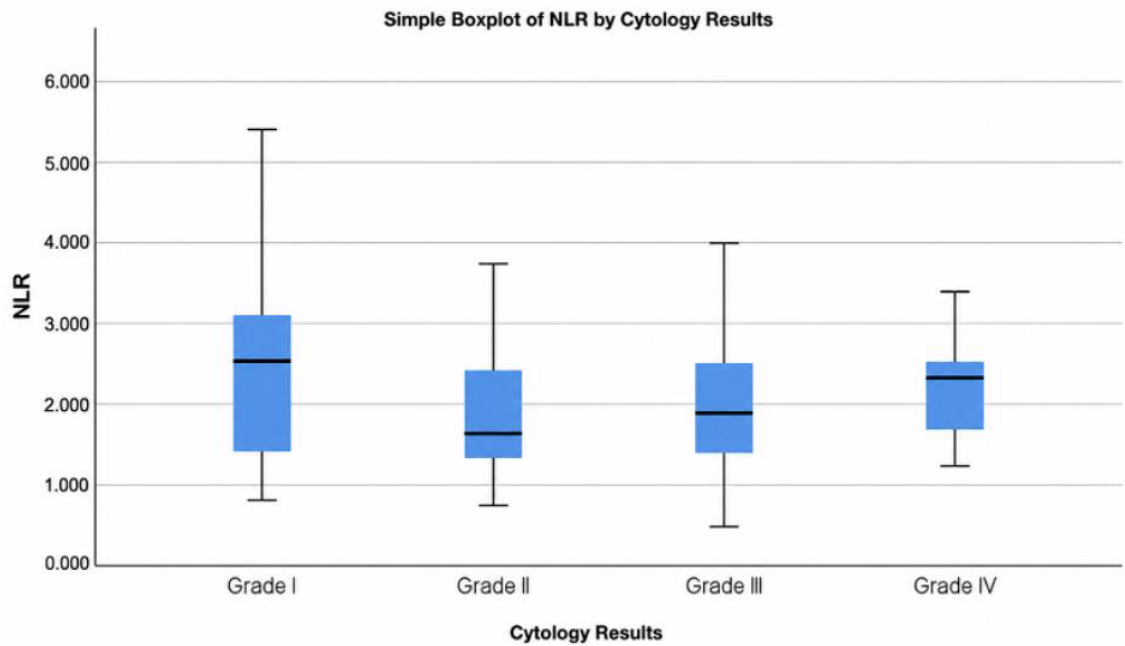


Figure 10. Boxplot of NLR Values by Cytological Outcome.

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. Source: Adapted from Keszthelyi et al (88)

4.2.3. Relationship Between Laboratory Parameters and Histology Results After Conization

In a manner analogous to the cytological evaluation, we examined the relationship between the results of the conization specimens and the neutrophil-to-lymphocyte ratio (NLR) using the Kruskal–Wallis test, which revealed a significant correlation ($p = 0.001$). Pairwise comparisons using the Mann–Whitney U test confirmed that NLR values were significantly higher in malignant lesions classified as Grade IV compared with Grade I ($p < 0.001$), Grade II ($p < 0.001$), and Grade III ($p = 0.001$) groups (*Table 11*). To clarify the interpretation of the statistical results, we also calculated effect sizes. The Kruskal–Wallis test indicated a negligible effect size ($\eta^2 = 0.0075$) when comparing the four grades in the cytology groups, whereas a small effect size ($\eta^2 = 0.0352$) was observed in the histology categories based on conization specimens.

Table 11. Pairwise comparison of NLR values between conization outcome groups. Pairwise comparisons of neutrophil-to-lymphocyte ratio terminated by conization, using Dunn–Bonferroni post hoc test was statistically significant ($\chi^2 = 15.99$, $p = 0.0011$), and post hoc analysis revealed that Grade IV differed significantly from both Grade I and Grade II (adjusted $p < 0.05$), suggesting a strong association between higher NLR values and invasive malignancy. Source: Adapted from Keszthelyi et al (88)

Comparison Between Groups	Mann–Whitney U	z	Bonferroni Corrected p
Grade I versus Grade II	1212.5	−0.812	1
Grade I versus Grade III	9344.5	−0.669	1
Grade II versus Grade III	3200.5	−1.332	1
Grade I versus Grade IV	486.0	−3.640	0.002
Grade II versus Grade IV	147.0	−3.771	0.001
Grade III versus Grade IV	1519.5	−3.444	0.003

Meanwhile, 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 11*). 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 12*). 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 12*). Figure 12 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.

Table 12. NLR values by conization outcomes.

This table presents the median, range minimum, and maximum NLR values for different conization outcomes. The results suggest an increasing trend in NLR values with worsening histopathological findings. Source: Adapted from Keszthelyi et al (88)

	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

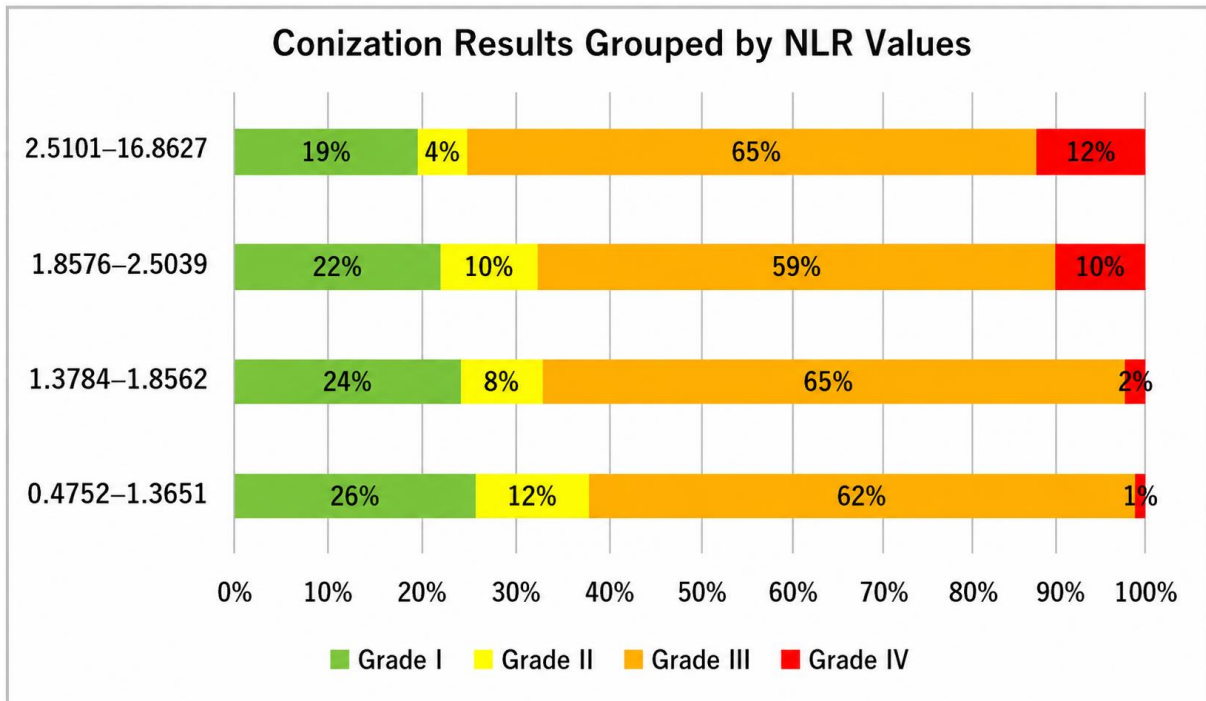


Figure 11. Distribution of Conization Results by NLR Categories Percentage distribution of conization results by histopathological grade is shown across different NLR levels.

The x-axis represents the percentage of patients, while the color-coded bars indicate Grade I (green), Grade II (yellow), Grade III (orange), and Grade IV (red) conization findings. Higher NLR values are associated with more severe conization outcomes.

Source: Adapted from Keszthelyi et al (88)

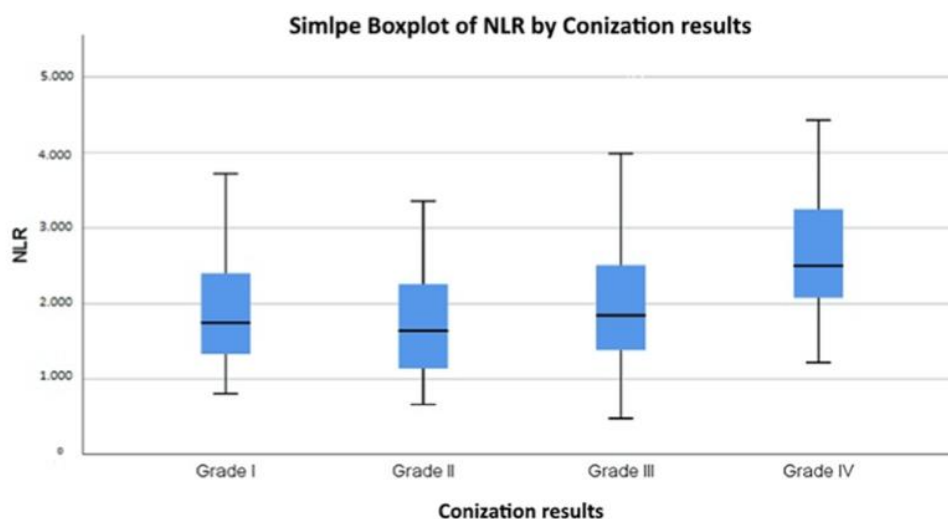


Figure 12. Boxplot of NLR values by conization outcomes.

Source: Adapted from Keszthelyi et al (88)

These findings support the idea that elevated NLR values may indicate more severe conization outcomes, which supports the possible role of systemic inflammation in the progression of cervical dysplasia. The correlation between the NLR and histological findings was found to be very weak and positive (Spearman's $\rho = 0.085$, $p = 0.102$), which indicates that as the severity of conization lesions increases, mean values of the NLR tendency also show an upward trend. The correlation between NLR and histological outcomes was very weak but positive (Spearman's $\rho = 0.085$; $p = 0.102$), suggesting that as the severity of conization abnormalities increases, mean NLR values also show an upward trend.

We used a box plot to illustrate the distribution of NLR values across the different conization outcome categories in each group, and we reported the median and interquartile range for each group. The box plot also shows that NLR values increase as histological findings worsen (*Figure 12*).

Moreover, we also took potential confounding factors into account and thus performed a multivariate binary logistic regression analysis using a stepwise (likelihood ratio) method. Of the variables examined (NLR, age, smoking status, HPV DNA positivity), only NLR and age were retained in the final model. Both variables remained statistically significant predictors of histologically confirmed cervical cancer. It should be noted that the odds ratio (OR) for NLR was 1.293 ($p = 0.033$) and that for age was 1.068 ($p = 0.025$), suggesting that an increase in these variables is associated with an increased risk of cancer (*Table 13*).

Table 13. Logistic regression analysis of NLR and age as predictors of malignancy in conization outcomes.

This table presents the results of a multivariate logistic regression analysis. B indicates the unstandardized regression coefficient; S.E., standard error; Wald, Wald chi-square test statistic; df, degrees of freedom; Sig.(p), significance level; Exp(B), odds ratio; 95% CI for Exp(B), 95% confidence interval for the odds ratio. Source: Adapted from Keszthelyi et al (88)

Predictor	B	S.E.	Wald	df	Sig.(p)	Exp(B)	95% CI for Exp(B)
NLR	0.257	0.12	4.565	1	0.033	1.293	[1.021–1.638]
Age	0.066	0.029	5	1	0.025	1.068	[1.008–1.132]

4.2.4. Logistic Regression Model

Logistic regression analyses demonstrated that the NLR is a statistically significant predictor of cancer diagnosis. In the univariable model, the odds ratio was 1.372 (95% CI: 1.085–1.735, $p = 0.008$), indicating that each one-unit increase in NLR corresponds to a 37.2% rise in the likelihood of cancer (*Table 14*). After adjusting for age, HPV status, and smoking, the NLR remained an independent predictor in the multivariable model (OR = 1.293, 95% CI: 1.021–1.638, $p = 0.033$), although the magnitude of the effect was modest. The logistic regression model assessing the influence of NLR on cancer risk was statistically significant overall ($\chi^2(1) = 8.012$, $p = 0.005$), confirming that NLR contributes meaningfully to the model's predictive capacity. Despite this, the Cox & Snell R^2 (0.021) and Nagelkerke R^2 (0.057) values indicate that NLR accounts for only a small portion of the variance in cancer status. The regression coefficients further support its predictive role: the NLR variable showed a significant positive association with cancer ($B = 0.316$, S.E. = 0.119, Wald = 7.013, $p = 0.008$), with an odds ratio of 1.372. ANOVA results also confirmed the model's significance ($F(1, 371) = 5.950$, $p = 0.015$), reinforcing the relevance of NLR in estimating cancer risk. Overall, these findings suggest that incorporating NLR into predictive models can aid in identifying patients at higher risk for cervical cancer, although its practical impact remains limited. Future research should explore interactions with additional biomarkers to better define its clinical utility.

Table 14. Logistic regression analysis of NLR as a predictor of cancer diagnosis.

Source: Adapted from Keszthelyi et al (88)

	B	S.E.	Wald	df	Sig.(p)	Exp(B)	95% CI for Exp(B)
NLR	0.316	0.119	7.013	1	0.008	1.372	[1.085–1.735]
Constant	−3.467	0.372	86.973	1	0.000	0.031	

4.2.5. Diagnostic Performance

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%.

In addition to the AUC and the sensitivity/specificity values obtained from the ROC analysis, further diagnostic performance indicators were calculated for both NLR cut-off points (1.865 and 2.041). These metrics included PPV, NPV, positive and negative likelihood ratios (+LR, -LR), and overall accuracy. Although the NPV values were consistently high ($>97\%$), the PPV values remained low ($<12\%$), suggesting that the NLR is more effective for ruling out disease than for confirming its presence (*Table 15*). The table presents the optimal NLR thresholds determined using the Youden index and the Closest Top-Left method, allowing comparison of how each approach balances sensitivity and specificity.

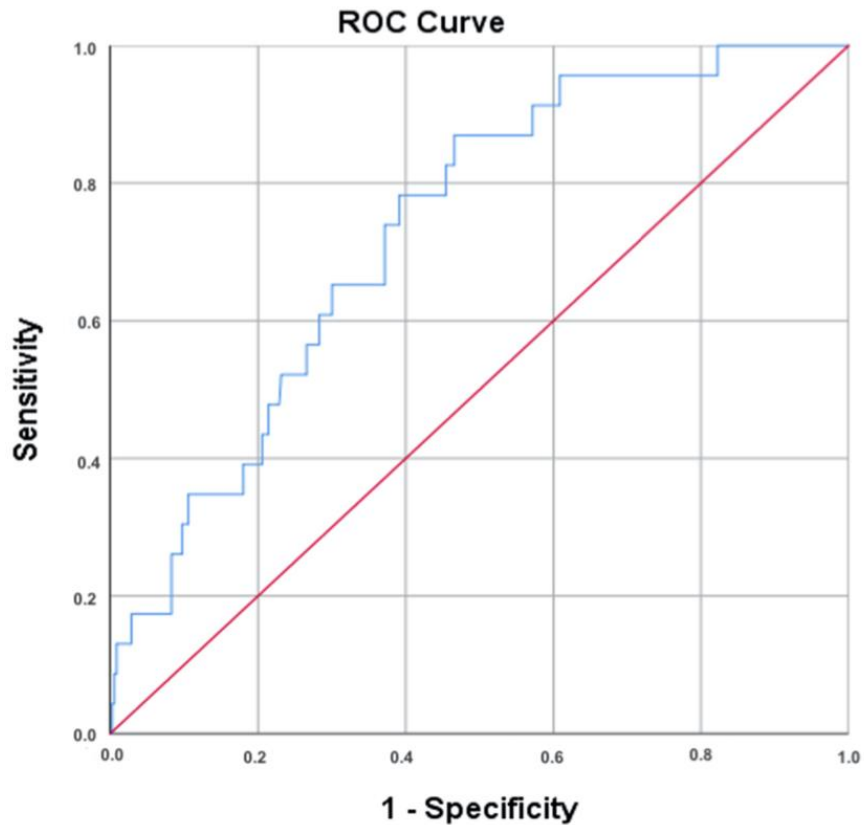


Figure 13. Receiver Operating Characteristic (ROC) Curve for NLR in Predicting cervical cancer.

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. Source: Adapted from Keszthelyi et al (88)

Table 15. Cut-off values via the Youden index and the Closest Top Left method.

Source: Adapted from Keszthelyi et al (88)

	NLR Cut-Off	Sensitivity	1-Specificity	Positive Predictive Value (PPV)	Negative Predictive Value (NPV)	Positive Likelihood Ratio (+LR)	Negative Likelihood Ratio (-LR)	Accuracy
Youden index	1.86510	0.870	0.462	0.109	0.984	1.867	0.244	0.555
Closest Top Left method	2.04119	0.783	0.390	0.116	0.977	1.999	0.357	0.619

5. DISCUSSION

The two complementary SCOPE studies provide consistent evidence that the neutrophil-to-lymphocyte ratio (NLR) can serve as a biomarker with meaningful diagnostic and prognostic significance in cases of HPV-associated cervical lesions.

Cervical cancer, regrettably, remains a significant public health challenge worldwide, with chronic and high-risk HPV infection continuing to be the primary etiological factor for cervical cancer (90). According to the GLOBOCAN 2022 database, 662,301 new cases of cervical cancer were registered in 2022, resulting in 348,874 deaths. Moreover, it is important to note that there are global disparities in the distribution of the disease; countries with low HDI had twice the incidence rate and five times the mortality rate compared to those with high HDI. A 2025 study, if the 2022 rates remain unchanged, the global burden of cervical cancer is expected to rise to 760,082 new cases (a 14.8% increase) and 411,035 deaths (a 17.8% increase) by 2030 (3). Probably the reason for this is that access to current screening methods is limited, so patients often arrive at clinics at a late stage. Thus, current measures have proven insufficient to meet the WHO's 90-70-90 targets set for 2030 (7). Consequently, there was a need to further expand personalized screening strategies in order to address these disparities and accelerate the elimination of cervical cancer.

This research provides important insights into the pathophysiological processes underlying HPV-associated cervical diseases and, by extension, into the potential diagnostic and prognostic role of inflammatory markers. It has been determined that 99.7% of cervical cancer cases are attributable to HPV infection (91). Numerous studies, such as the one conducted by Kalliala and colleagues, emphasize the role of HPV persistence and, consequently, its significance in risk assessment and the management of patient care (92). HPV-induced cervical cancer can be detected using the p16 immunohistochemical method, which can also be used to predict the progression of cervical cancer to CIN3 with 100% sensitivity and a high negative predictive value (93).

The first discovery was made by Virchow in 1963: that cancer is generally not a localized disease, but rather that its development and progression are usually accompanied by

systemic inflammatory and immunological processes (94). Based on our current understanding, up to 25% of chronic inflammatory conditions may be linked to the development of cancer, and the characteristics of cancerous tumors may contribute to the onset of inflammation (95). The resulting inflammation can be detected in the blood and may manifest as relative neutrophilia, monocytosis, thrombocytosis, or lymphocytopenia (96). Identifying this is very simple, inexpensive, and easy to repeat and reproduce, because it requires only a routine peripheral blood sample (97). To interpret them more comprehensively, ratio pairs can be used: PLR, LMR, NLR (98). Among these, the NLR is of particular importance in cases of other tumors, such as head and neck and gastrointestinal cancers: it has been shown that pre-treatment NLR values are inversely correlated with survival time (99, 100). Regarding gynecological cancers—such as endometrial cancer, ovarian cancer, and cervical cancer—previous studies have already demonstrated that the NLR is associated with cancer progression (76-79). However, the role of the NLR in terms of its potential diagnostic and prognostic value remains unclear at this time.

Our first SCOPE study confirmed the hypothesis that systemic inflammation, as indicated by an elevated NLR, promotes the persistence of high-risk HPV and subsequent neoplastic transformation. The NLR showed a significant association with both HPV DNA positivity (mean NLR in HPV-positive patients: 2.15; in HPV-negative patients: 1.61; $p = 0.04$) and p16 positivity ($p = 0.011$). In contrast, PLR and LMR showed no detectable significant correlation with the association between systemic inflammation and transcriptionally active, high-risk HPV infection. These findings are consistent with previous studies, such as those by Du and colleagues, which highlighted the prognostic value of an elevated NLR in early-stage cervical cancer (101). Similarly, Xu and colleagues observed significant differences in NLR values among the CIN1, CIN2, and CIN3 groups and found higher NLR values associated with HPV16 persistence (102). However, in our study, we found no statistically significant association between PLR and LMR. Thus, it can be concluded that, in HPV-associated cervical diseases, NLR is the most robust and consistent biomarker of systemic inflammation. These findings are supported by the observations in the 2024 meta-analysis by Han et al., in which only the NLR showed prognostic value in cervical cancer, there was no such value for the PLR or

the LMR (76). Studies by Origoni and colleagues showed also that the recurrence rate among patients with abnormal cervical lesions was lower—only 15.2% (<2.0) for those with a lower NLR— whereas with a higher NLR (≥ 2.0), it was 27.3%, and the odds ratio for recurrence was significantly higher in patients with an NLR ≥ 2 (103). Furthermore, our findings are supported by the observation made by Zhai et al. that lower NLR levels were associated with a protective effect, while higher levels were associated with an increased risk. In contrast, the PLR and SII showed only a slight correlation (104).

Overall, it can be said that as the severity of CIN progresses, NLR values show a gradual increase. The importance of the NLR as a prognostic marker in cases of transcriptionally active, high-risk HPV infections has been unanimously emphasized. The ROC analysis of our study suggests that the NLR is more effective than the PLR and the LMR at distinguishing between p16-positive and p16-negative cases. This means that, even with its moderate diagnostic performance, the NLR has proven to be a promising supplementary diagnostic tool.

Nevertheless, it should be noted that both the first and second SCOPE studies highlight the difficulty of establishing universal cut-off values for the NLR. In the first study, all patients with an NLR value above 1.31 were HPV-positive; it was not possible to determine a clear cutoff value for a normal NLR. This also reflects the discrepancies among the values reported in the literature. Building on previous findings, the second SCOPE study evaluates the neutrophil-to-lymphocyte ratio (NLR) as a potential predictive biomarker for cervical cancer among patients undergoing cytology and LEEP conization. Similarly, NLR values were significantly higher in patients with a malignant histological diagnosis ($p = 0.001$), and an NLR cutoff value of 1.865 proved to be significant ($p = 0.045$) in predicting malignancy.

These results are comparable to the meta-analysis by Zhuang et al., which demonstrated a significant association between elevated NLR levels and poorer survival outcomes in patients with cervical cancer and identified an NLR cutoff of 3 as a critical prognostic threshold (105). Furthermore, highlighting its significance, the logistic regression analysis showed that each unit increase in the NLR increases the probability of a tumor by 37.2% ($p=0.008$). This is supported by logistic regression analyses conducted in a large cohort of men who underwent a prostate biopsy with PSA levels between 4 and 10

ng/ml, which showed that each unit increase in the NLR was associated with a 37% higher likelihood of detecting cancer (adjusted OR = 1.372, $p = 0.038$) (106).

Although the NLR showed some prognostic significance, its association with cytological abnormalities remained weak in our study and was not statistically significant. The Kruskal–Wallis test ($\chi^2 = 5.785$; $p = 0.123$) revealed no significant differences among the cytological categories. Nevertheless, the Mann–Whitney U test indicated a marginally significant difference between stages II and IV ($p = 0.046$), suggesting that higher NLR values may be associated with more advanced lesions. Even though no concrete limit could be established, a gradual increase in the NLR was observed in parallel with more severe cytological findings. These results may also be explained by the fact that cytology provides less definitive results than conization, and there may be discrepancies between cytology results and the definitive histological findings from conization (107, 108).

When presenting the results, it is important to note that, with regard to high-risk HPV status, the majority of patients in the second study were HPV-positive (61%), which limited variability and may explain why HR-HPV did not remain a significant, independent predictor in the models. However, due to its clinical relevance, we continued to include it in the adjusted analyses so as not to compromise the overall evaluation. A cohort study by Tian and colleagues, corroborating our findings, reported that high-risk HPV infection, positive surgical margins, and elevated PLR and NLR values all increased the likelihood of recurrence or the development of residual CIN lesions following LEEP treatment (109).

The area under the curve (AUC = 0.734) indicated moderate discriminatory power, which further reinforces the importance of the NLR in risk assessment. At the optimal NLR cutoff of ≥ 1.865 , determined using the Youden index, the model's sensitivity was 87.0% and its specificity was 53.8%. In addition, the optimal cutoff value of ≥ 2.041 , determined using the Closest Top Left method, resulted in a sensitivity of 78.3% and a specificity of 61.0%, suggesting that the NLR may be useful as an adjunctive parameter in the diagnostic evaluation of cervical cancer. To examine the predictive value of the NLR, we used a logistic regression model, which showed that the NLR is a significant predictor of a malignant conization outcome ($\chi^2(1) = 8.012$, $p = 0.005$).

These results provide further evidence that the NLR is worth further investigation as a potential predictive biomarker for cervical cancer. However, the explanatory power of our model—as indicated by the Cox–Snell R^2 (0.021) and Nagelkerke R^2 (0.057) coefficients—was relatively low, indicating that, although the NLR has some predictive value, other factors would need to be incorporated in order to improve predictive accuracy. The NLR alone explains only a portion of the risk variance; multivariate models perform better when the NLR is combined with clinical and molecular markers. In a study by Oh and colleagues, the inclusion of the NLR in a prostate cancer risk model resulted in a moderate but statistically significant improvement in the model’s discriminatory power, suggesting that the NLR provides additional value when used in combination with other predictors (106). In the case of cervical CIN, the combined use of NLR and surgical margin status significantly improved the accuracy of recurrence prediction: the AUC value of the logistic model reached ~0.87, and it demonstrated higher sensitivity and specificity compared to the use of NLR alone (109).

Taken all together, our results support the notion that systemic inflammation is closely associated with active HPV infection: higher NLR values indicate an inflammatory environment that promotes viral persistence and progression to CIN and cervical cancer. Furthermore, the clear association between NLR and p16 positivity further supports the hypothesis that systemic inflammation plays a key role in the persistence of high-risk, transcriptionally active HPV infections and in promoting malignant transformation. The results of the conization highlight the potential clinical utility of NLR as part of an integrated screening approach, as a component of a broader strategy aimed at identifying high-risk patients. It should be noted that, based on our NLR results, it is advisable to evaluate this marker in combination with other biomarkers (e.g., p16 and Ki-67, high-risk HPV genotyping) and clinical parameters.

5.1. Strengths and Limitations

The strengths of our study include the large sample size (395 and 374 participants), which allows for statistically reliable analyses. Both the Kruskal-Wallis test and the Mann-Whitney U test help in accurately assessing the diagnostic utility of the NLR. In addition, the use of ROC curve analysis quantifies the NLR's sensitivity and specificity in predicting p16 positivity and cervical cancer. However, one limitation of the study is that the explanatory power of the logistic regression model in the second analysis is relatively low. This suggests that, although the neutrophil-to-lymphocyte ratio is a significant predictor of the outcome of cancer conization, it has limited predictive value on its own.

Another power of the results is that the combined evaluation of hematological and immunohistochemical markers provides valuable insights into the prognostic potential of the NLR. The use of real-world clinical data reinforces the practical applicability of the NLR in everyday clinical practice, as it can be obtained from routine tests alone. Moreover, they present the NLR in a comprehensive manner, taking potential confounding factors into account. We examined the NLR in relation to smoking, alcohol consumption, diabetes, and immunological disorders, and its significance remained substantial in all of these cases. This suggests that the observed associations are not product of independent inflammatory conditions, but rather reflect a biological link between systemic inflammation and HPV-related cervical pathology.

However, it should be mentioned that while NLR values tended to increase in parallel with disease severity, but the 95% confidence intervals for the median NLR values showed significant overlap across several categories, particularly for the intermediate grades. This indicates that, although we found statistically significant differences in certain comparisons with invasive carcinoma, the distinction between the groups is not clear-cut.

Besides, it should be noted that, since this is a retrospective study, the analysis has inherent limitations. This means that, due to the nature of the study design, the possibility of selection and information bias cannot be ruled out with complete certainty; indeed, the study does not involve randomization or prospective data collection. Ultimately, these factors may affect the generalizability of the results. Although the study identified several

significant associations, cause-and-effect relationships cannot yet be determined with complete certainty due to the study design.

5.2. Practical Implications

Based on our findings, NLR may serve as a promising supplementary diagnostic marker for the early detection of high-grade lesions, particularly in settings where modern cervical screening programs are not available and reliance remains firmly on the sensitivity of the low-sensitivity Pap test (110-112). A major advantage of the NLR is that it is cost-effective, as it does not entail any additional costs. These blood tests are already part of standard preoperative, routine laboratory work. Considering this, it is important to emphasize that this is a simple, reproducible, minimally invasive, and easily accessible screening method. When integrated into cervical cancer screening protocols, the NLR appears to be a promising tool for risk assessment, early detection of cervical cancer, and treatment follow-up.

5.3. Future Prospects

Our findings highlight the need for future prospective, multimodal, multicenter studies to examine different patient populations. Additionally, based on our results, it would be also important to conduct further studies incorporating additional factors or biomarkers in order to refine the performance of the integrated model.

Future research should include longitudinal studies so that we can more clearly see the accuracy of the NLR's predictive value as it changes over time.

To support therapeutic decisions, it is important to understand additional associations between causes and effects—for example, how interventions targeting systemic inflammation can provide valuable information on how to influence the course of HPV-related cervical pathology.

6. CONCLUSIONS

In tandem with exploring the interactions between systemic inflammation, human papillomavirus (HPV) infection, and the progression of cervical lesions, this research provides important insights into the pathophysiology of HPV-related cervical diseases and their potential prognostic biomarkers.

Our study showed that the neutrophil-to-lymphocyte ratio (NLR) has meaningful diagnostic and predictive value in cervical cancer. Based on the results of the two retrospective patient cohorts, the NLR showed a consistently increasing trend in cases of cytology and a significant association with HPV-related molecular markers as well as with histopathological outcomes following conization. It is worth noting that our study was the first to define an NLR cutoff value that reliably predicted a malignant conization result, providing a very high negative predictive value and considerable sensitivity. Taken together, these findings confirmed the diagnostic and predictive value of the NLR in the context of cervical cancer for the first time.

Based on our study, we concluded that the integration of the NLR can improve personalized cervical cancer management. Overall, our results suggest that the NLR can be used as an easily accessible and cost-effective adjunct in risk stratification and early detection, particularly in settings where advanced diagnostic methods are not available. Additional advantages include its reproducibility, ease of use, and minimally invasive nature, which pave the way for innovative approaches to the prevention and treatment of cervical cancer. The novelty and strength of our findings underscore the need for further multicenter, prospective studies to validate standardized cutoff values and refine the integration of the NLR into multimodal diagnostic frameworks.

7. SUMMARY

The aim of this doctoral thesis was to identify a new screening method for cervical cancer, which causes a significant global health problem. Experience shows that this method could supplement the currently available, often limited-access options in low-income countries, where the disease—which is otherwise easily treatable—often becomes fatal due to late-stage diagnosis. Thus, we sought to find a method that is reliable, easily accessible, simple to perform, minimally invasive and cost-effective.

To address this aim, in the first part, using data from 395 patients who underwent LEEP conization, we conducted a retrospective analysis of the relationship between inflammatory indices derived from routine peripheral blood samples—the neutrophil-to-lymphocyte ratio (NLR), the platelet-to-lymphocyte ratio (PLR), and the lymphocyte-to-monocyte ratio (LMR) with the presence of cervical disease-causing HPV. Among these, NLR values showed a significant association with the p16 marker, which indicates HPV-related cervical disease. These findings highlighted the potential significance of the NLR in the detection of cervical cancer. Thus, in a subsequent study, we sought to elucidate the previously unclear diagnostic and predictive role of the NLR in patients who underwent conization for cervical cancer. The second retrospective SCOPE study compared preoperative NLR values with cytological and histopathological results from conization in 374 patients. Our findings showed that NLR values were significantly higher in cases with malignant conization results. Crucially, the analysis revealed high negative predictive value, robust sensitivity, significant specificity with a definable cutoff value.

Overall, these associations suggest that systemic inflammation contributes to HPV persistence and cervical carcinogenesis, and that NLR reflects underlying biological processes that promote neoplastic progression.

In summarizing, our study is the first to demonstrate that the NLR has shown promising practical value as a supplementary diagnostic method for risk stratification and early detection of cervical cancer. Our findings suggest that NLR may influence the reconsideration of cervical cancer screening protocols, particularly in settings where advanced diagnostic tools are not available.

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9. BIBLIOGRAPHY OF PUBLICATIONS

9.1. Publications related to the thesis

Balázs Vida, **Emese Keszthelyi**, Zsófia Tóth, Lotti Lőczi, Barbara Sebők, Petra Merkely, Balázs Lintner, Ferenc Bánhid, Attila Keszthelyi, Szabolcs Várbíró, Richárd Tóth, Márton Keszthelyi. The Neutrophil-to-Lymphocyte Ratio (NLR) as A Potential Predictor in Conization Outcomes for Cervical Cancer.

CANCERS 17 : 11 Paper: 1856 , 16 p. (2025)

Zsófia Tóth, Lotti Lőczi, Barbara Sebők, Petra Merkely, **Emese Keszthelyi**, Balázs Lintner, Nándor Ács, Attila Keszthelyi, Szabolcs Várbíró, Richárd Tóth, Márton Keszthelyi. Neutrophil/Lymphocyte Ratio (NLR) as a Predictive Marker for p16 Positivity and Cervical Cancer Progression: Insights from the SCOPE Study

CANCERS 17 : 6 Paper: 921 , 13 p. (2025)

9.2. Publications not related to the thesis

Balázs Lintner, Zsófia Havrán, Gabriella Vajda, Lotti Lőczi, Marianna Török, Petra Merkely, Ferenc Bánhid, **Emese Keszthelyi**, Richárd Tóth, Márton Keszthelyi. Robot-Assisted Hysterectomy Provides Higher Sentinel Node Detection and Lower Conversion Rates Compared to Laparoscopy in Endometrial CancerLIFE-BASEL 16 : 2 Paper: 244 , 12 p. (2026)

Emese Ágnes Keszthelyi, Judit Forrai, Márton Keszthelyi

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