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Clinical, Morphological and Biomolecular Entities of Cervical Carcinoma

DOCTORAL THESIS ABSTRACT

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LIST OF PUBLISHED ARTICLES

1. Lucian Şerbănescu; Sebastian Mirea; Paris Ionescu; **Laura-Andra Petrica**; Ionut Ciprian Iorga; Monica Surdu; Traian Virgiliu Surdu; Vadym Rotar. Involuntary Urine Loss In Menopause – A Narrative Review. J. Clin. Med. 2025, 14(21), 7664. ISSN: 2077-0383. ISI (Factor de Impact 2,9). https://pubmed.ncbi.nlm.nih.gov/41227060/. doi: 10.3390/jcm14217664
2. **Laura-Andra Petrica**; Mariana Deacu; Georgeta Camelia Cozaru; Anca Florentina Mitroi; Gabriela Izabela Bălţătescu; Manuela Enciu; Oana Cojocaru; Anca-Antonela Nicolau; Andrei Radu Baz; Lucian Şerbănescu; Mariana Aşchie. Interplay Between Immune Microenvironment $CD8^+$ Tumor-Infiltrating Lymphocytes And PDL-1 Expression as Prognostic Markers in Invasive Cervical Squamous Cell Carcinoma. Medicina. 2025, 61(11), 2007. ISSN: 1648-9144. ISI (Factor de Impact 2,4). https://pubmed.ncbi.nlm.nih.gov/41303843/. doi: 10.3390/reports8040243
3. **Laura-Andra Petrica**; Mariana Deacu; Georgeta Camelia Cozaru; Gabriela Izabela Bălţătescu; Mariana Aşchie. Clinically Advanced Warty Invasive Squamous Cell Carcinoma of the Cervix with p16 Overexpression—Case Study and Literature Review. *Reports*. 2025; 8(4):243. **ISSN: 2571-841X (Online). ISI (Factor de Impact 0,6).** https://pubmed.ncbi.nlm.nih.gov/41283611/**. doi:** 10.3390/reports8040243

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CURRENT STATE OF KNOWLEDGE

1. STRUCTURE AND DEVELOPMENT OF THE CERVIX

The uterus is a hollow organ with a thick and contractile wall, whose role is to host the fertilized ovum during its developmental stages and to expel the fetus once it has completed its development. The uterus is a median, unpaired organ, composed of the uterine body and the uterine cervix, also called the cervix uteri [1,2].

The cervix (uterine cervix) has the shape of a cylindrical barrel and presents two orifices/ostia: internal and external. The external orifice is punctiform in nulliparous women and appears as a transverse slit in multiparous women. Between the two orifices lies the cervical canal (clinically called the endocervix). The vagina inserts onto the cervix along an oblique line, oriented supero-inferiorly and postero-anteriorly; thus, the vaginal insertion divides the cervix into two portions: supravaginal and intravaginal. The external cervical orifice is bordered by two lips: anterior and posterior, united by two lateral commissures. The average cervical dimensions are about 3 cm in length and 2 cm in width, and its consistency is firm. The axis of the cervix is oblique, running from anterosuperior to posteroinferior [1,2].

The internal configuration of the cervix consists of a muscular layer that is much less developed compared to that of the uterine body and a mucosal layer made up of two types of epithelial tissue: single-layered columnar epithelium and non-keratinized stratified squamous epithelium. The transition between these two epithelial types is called the transformation zone or the squamocolumnar junction [1].

These parameters vary depending on constitutional factors, parity, age, physiological state, or associated pathology.

As mentioned above, the cervix has two portions: the exocervix (also called the ectocervix by some authors) and the endocervix (also called the endocol by some authors).

The *exocervix* is normally covered by non-keratinized stratified squamous epithelium. The thickness of the epithelium is influenced by the amount of available hormones (from endogenous production or exogenous intake) and varies depending on age and hormonal stimulation.

During reproductive life, the epithelium is thick and well-differentiated. It consists of:

• Basal layer — cells with large nuclei containing dense chromatin and scant cytoplasm; these cells are usually mitotically inactive and do not express proliferation markers such as Ki-67 and PCNA on immunohistochemistry [3-6].

• Parabasal layer — formed of cells slightly larger compared to those in the basal layer due to a greater amount of cytoplasm and with nuclei containing less dense chromatin; at this level, mitoses are usually present but are neither abnormal nor numerous in normal epithelium. The cells in this layer express proliferation markers [3–6].

• Intermediate layer — composed of cells with smaller nuclei and more abundant cytoplasm, which contains abundant glycogen [3–6].

• Superficial layer — contains mature, non-keratinized cells, although keratohyalin granules may be present. The nuclei of these cells are small and pyknotic, and the cytoplasm is abundant; cells in this layer are considered mature and are those that exfoliate [3–6].

During the maturation process, cells change their shape (becoming progressively flatter), the nucleus becomes smaller, and the cytoplasm more abundant.

Using immunohistochemical techniques, endocrine cells have been identified in the squamous epithelium of the exocervix. Their function is unknown, but they are believed to give rise to rare cervical carcinoid tumors [6–13].

Langerhans cells are also present in the exocervical epithelium and in the transformation zone. They are involved in presenting antigens to T lymphocytes [14–16]. Melanin-containing cells have been identified in the cervical epithelium, providing a plausible explanation for the origin of cervical melanomas and blue nevi (both rare entities at this site) [17].

The exocervical epithelium rests on a basal membrane that separates it from the underlying stroma.

The *endocervix* – it anatomically extends from the external cervical os to the internal os, but the endocervical glandular epithelium is not strictly confined to the anatomical endocervical region described above. The endocervical epithelium occupies significant areas of the anatomical exocervix during childhood and after menarche.

The endocervix consists of a single layer of tall columnar epithelial cells with small, basal nuclei and supranuclear cytoplasm containing mucin. The nuclei are small, elongated, and have dense chromatin. When the endocervical epithelium is injured and regenerates, the nuclei may become larger and rounder, but mitoses are rarely observed in

non-neoplastic endocervical cells. Nucleoli are usually not prominent in resting endocervical cells but may become so during regeneration, pregnancy, and neoplastic transformation. Reserve cells located beneath have the potential to differentiate into ciliated or mucus-secreting cells, although differentiated mucus cells are capable of division without the participation of reserve cells. Endocrine cells are also present within the endocervical epithelium [3–6]. Their function remains unclear, but they are believed to give rise to endocrine neoplasms, such as carcinoid tumors or neuroendocrine carcinomas, which are occasionally found in the cervix [7,11].

The *squamocolumnar junction (SCJ)* of the cervix is defined as the boundary between the non-keratinized stratified squamous epithelium and the mucus-secreting columnar epithelium of the endocervix. Two types of squamocolumnar junction are described:

- Original squamocolumnar junction — the site where the native squamous epithelium and the mucus-secreting columnar epithelium meet; it is present from birth. Its exact location varies individually and depending on developmental stages in life.
- New squamocolumnar junction — the site where the metaplastic zone meets the mucus-secreting columnar epithelium. This is an important landmark and is relevant for the proper evaluation of the transformation zone.

2. GENERAL CONSIDERATIONS REGARDING CERVICAL CARCINOMA

Cervical cancer (CC) ranks fourth worldwide in terms of morbidity and mortality among women, according to data from the International Agency for Research on Cancer (IARC – Global Cancer Observatory) [18]. In 2020, more than 600,000 new cases were recorded globally (~6.5% of all cancers) and 342,000 deaths (7.5%) [18,19]. Cervical cancer is the most frequently diagnosed malignant pathology in 23 countries and the leading cause of cancer-related mortality in 36 countries [20,21].

Mortality caused by cervical cancer is unevenly distributed, ranging from 5.2 deaths per 100,000 people in highly developed countries to 12.4 deaths per 100,000 in less developed countries [22]. Statistical data clearly demonstrate that both incidence and mortality from cervical cancer vary according to income level. Thus, in low- and middle-

income countries, its incidence is almost twice as high, and mortality rates are three times higher than in high-income countries.

According to Globocan, in 2020, in Romania, cervical cancer ranked third among newly diagnosed cancers in the female population (after breast and colorectal cancer) [23]. An interesting comparison can be made between Romania and the United Kingdom, which has a functional screening program: the incidence of cervical cancer in the general population in Romania is 3.4% with a mortality of 3.3%, while in the United Kingdom it is 0.83% with a mortality of 0.62% [23,24].

In 2022, in Romania, cervical cancer held the third position among cancers diagnosed in women (after breast and colorectal cancer) and the fourth position in terms of mortality (after breast, colorectal, and lung cancer) [25].

According to a notice issued by the National Institute of Public Health (INSP) evaluating overall mortality in the population in 2023, cervical cancer represents the third cause of death among women, after respiratory and breast cancer. The same bulletin notes a decrease in the number of deaths caused by cervical cancer in 2023 compared to 2022. In 2022, cervical cancer caused 1,290 deaths, corresponding to 5.9 deaths per 100,000 inhabitants, while in 2023 it caused 1,177 deaths, corresponding to 5.4 deaths per 100,000 inhabitants [25].

Cervical cancer is a disease that can affect women of any age, being most frequently diagnosed in women aged 35 to 44 years [68]. Despite efforts and progress in both the detection of cervical cancer and in identifying and applying optimal therapies to improve disease-free survival and overall survival, there are still many cases where these measures are not effective. It is estimated that, in the absence of adequate measures, between 2018 and 2030, the annual number of new cases of cervical cancer will increase from 570,000 to 700,000, and the annual number of deaths will rise from 311,000 to 400,000 [69]. Therefore, continued research is needed to identify new therapeutic targets that can lead to better quality of life and longer survival.

Ideally, the diagnosis of cervical carcinoma should be easily achieved through routine screening examinations. The current screening method is described in the latest SOGR guideline [26].

Regarding gynecological examination, in the case of invasive cervical carcinoma, the following findings can be observed:

- Speculum examination reveals abnormal appearance of the cervix, which is transformed by the tumor and may present as exophytic, polypoid, ulcerated, or endophytic. Depending on the stage, the tumor may extend to the vaginal walls.
- Vaginal palpation (bimanual examination) evaluates the size of the cervical tumor, the extension of the tumor to the vaginal walls, as well as the presence of any associated pathologies (such as adnexal or uterine masses).
- Rectal examination assesses the extension of cervical carcinoma to the parametrium and rectovaginal space. In the past, the staging of cervical carcinoma was exclusively clinical.

Pretreatment evaluation of cervical carcinoma includes the assessment of hematological, hepatic, and renal function, transvaginal ultrasound, chest X-ray or thoracic CT scan. Starting from stage IB, radiological imaging by CT or MRI of the chest, abdomen, and pelvis is mandatory [27,29]. Bladder or rectal invasion requires biopsy confirmation [27,28].

From 2019 until present time, the Romanian Society for Obsterics and Gynecology (SOGR) has issued three clinical guidelines (2019, 2022, and 2024) establishing appropriate management for cervical carcinoma. Therapeutic management is decided after the case is discussed within a multidisciplinary committee and considers several aspects, such as tumor stage, the patient's desire to preserve fertility, inability or refusal to undergo surgery, pregnancy status, and lymphovascular space invasion.

The differences between the approaches outlined in these guidelines are minor, except for the increased emphasis on the sentinel lymph node technique in the 2022 and 2024 guidelines and the therapeutical change for stage IA2 cervical carcinoma. In the 2019 guideline, the standard indication for this stage was modified radical hysterectomy with pelvic lymphadenectomy; this recommendation was not maintained in the later guidelines [26].

3. MORPHOPATHOLOGICAL ASPECTS OF CERVICAL CARCINOMA

The World Health Organization (WHO) provides a standardized classification system for cervical cancer, which assists in the diagnosis, staging, and treatment of this disease [30].

According to this classification, cervical tumors are divided into:

- squamous epithelial tumors
- glandular tumors
- mixed and mesenchymal tumors
- germ cell tumors

The grading of squamous cell cervical carcinomas is performed according to two systems:

- the Broders system, which uses four grades (well, moderately, poorly differentiated, and undifferentiated)
- the FIGO system, which uses three grades (well, moderately, and poorly differentiated)

The grading of cervical adenocarcinomas has been a subject of long-standing debate, and to date there is no validated system that can be universally applied. This may also be due to the lack of consensus regarding the prognostic value of grading.

The macroscopic appearance of cervical carcinoma has a very wide spectrum. It also includes early forms that are not accompanied by macroscopic changes; any suspicious lesions are often detected following a Babeș–Papanicolaou cytobacteriological examination and subsequent colposcopy.

Microscopic aspects are highly variable and depend on the tumor type.

For squamous cervical carcinoma, several histological growth patterns have been described: non-keratinizing, keratinizing, basaloid, condylomatous (verrucous), papillary, and lymphoepithelioma-like.

For HPV-dependent cervical adenocarcinoma, two major histological types have been described: the usual (classic) type and the mucinous type.

4. TUMOR MICROENVIRONMENT

The understanding of malignant disease development has evolved considerably over the last decade. It is now recognized that malignant disease is not strictly a genetic disorder but a complex ecosystem involving numerous non-cancerous cells and their interactions with malignant cells.

It is clear that genetic alteration is necessary but not sufficient to initiate and sustain the malignant process and its progression. The complexity of malignant disease becomes

apparent through microscopic examination, which shows that the tumor microenvironment is a highly specialized system containing malignant cells surrounded by various non-cancerous cells embedded in a vascularized and modified extracellular matrix.

The *tumor microenvironment* contains a remarkable diversity of immune cells, endothelial cells, pericytes, fibroblasts, and other cell types that vary depending on the tissue, such as neurons or adipocytes. Until relatively recently, these cells were perceived as mere bystanders in tumorigenesis; however, several studies have demonstrated that the tumor microenvironment and the molecules secreted within it are critical to malignant pathogenesis and are therefore considered attractive therapeutic targets.

Depending on the organ of tumor origin, the intrinsic characteristics of malignant cells, the tumor stage, and the individual patient's particularities, the cellular composition and functional status of the tumor microenvironment will vary, and some cells within it may either suppress or support tumor development.

The presence of tumor-infiltrating lymphocytes (their type and quantity) within the tumor reflects the interaction between the host's immune cells and the tumor antigens as well as the tumor microenvironment. Analyzing the type of tumor-infiltrating lymphocytes — their number and ratio — can provide valuable information about tumor progression and therapeutic prognosis.

Usually, the tumor-infiltrating lymphocytic infiltrate in cervical cancer consists of several immune cell subtypes such as CD4+ and CD8+ T cells, natural killer (NK) cells, and regulatory T cells; in some reports, macrophages are also described. These immune cells are unable to eliminate the tumor, and the reasons for this are complex. Two main reasons should be mentioned: either these immune cells are insufficient in number, aged, or there are immunosuppressive cells within the tumor-infiltrating lymphocyte population [31], or the tumor microenvironment itself inhibits their activity [32].

The evaluation of tumor-infiltrating lymphocytes is becoming increasingly important in the search for a biomarker that could help select patients with the highest probability of benefiting from immunotherapy.

Neoangiogenesis represents the process through which new blood vessels are formed, developing from pre-existing vessels. This phenomenon occurs both under physiological conditions, such as wound healing or embryonic development, and under pathological conditions, such as tumor growth, chronic inflammation, or ischemic diseases.

Currently, protocols measuring microvascular density (MVD) are considered the gold standard for assessing neovascularization in malignant tumors. The method uses specific markers for vascular endothelium — most commonly CD31 and CD34 — and immunohistochemical techniques to highlight microvessels [33]. The microvascular density of the primary tumor correlates significantly with the development of metastases and prognosis in some tumors and is considered a valuable predictive factor in tumors that induce considerable angiogenesis, especially breast carcinomas, prostate carcinomas, and hematologic malignancies [34].

5. CERVICAL CARCINOMA IN THE AGE OF ARTIFICIAL INTELLIGENCE AND DIGITAL PATHOLOGY

Artificial intelligence represents a revolution in many fields, and digital pathology is among them. In short, artificial intelligence has increased the analytical validity of traditional cytopathological techniques, improved diagnostic accuracy and sensitivity, and resulted in greater clinical efficiency. Methods that use artificial intelligence are divided into two main categories: those using machine learning protocols and those using deep learning protocols [35].

The inclusion of artificial intelligence systems in the management of cervical carcinoma offers enhanced possibilities for diagnostic accuracy, standardization of screening mechanisms, and personalized case management.

Regarding cervical cancer screening, the use of artificial intelligence has the potential to improve the detection of precancerous lesions and malignantly transformed cells. Studies have shown that systems using artificial intelligence can reach an accuracy of 70–100% in identifying malignant cervical lesions [36]. Another multicenter study demonstrated that artificial intelligence using deep learning protocols achieved a sensitivity of 94.6% and a specificity of 89% in the interpretation of digital cytology slides [37]. The same study showed that a cytologist assisted by artificial intelligence had a 13.3% increase in diagnostic sensitivity compared to a cytologist without artificial intelligence assistance [37].

Histopathology is another essential component of pathological diagnosis and involves the evaluation of tissue samples from various organs to detect and identify abnormalities or existing pathology. Similar to cytopathological evaluations, histopathology has advanced significantly through digital methods. In a histopathological

analysis, bioinformatics was used to examine unknown histopathological samples, identifying the original source of malignancy [38]. A systematic review of the specialized literature on artificial intelligence and histopathological samples from ovarian cancer found several models that could be implemented in real-world practice [39].

Digital pathology represents the entirety of actions related to the acquisition, management, sharing, and interpretation of pathological information, including both slides and data, in a digital environment. Digital slides are created by capturing glass microscopic slides with a scanning device that produces a high-resolution image, which can then be viewed on a computer or mobile device. This type of examination offers several benefits: improved analysis of the examined slide, reduced error rates, enhanced collaboration between physicians, opportunities for remote work, increased opportunities for medical education and training, and facilitation of research and scientific development [40,41].

Digital pathology has advanced significantly, especially through the visualization and exploration of the entire histological slide image (WSI – whole slide imaging). Practice guidelines have been reported describing the applications of whole slide imaging evaluation, and studies have been conducted to validate the interpretation of breast and gynecological pathology, showing improved intra-observer variability [42].

Immunohistochemical testing involves the evaluation of collected specimens using immunohistochemical markers. These markers are antibodies that detect specific antigens in the examined tissue. This method allows the pathologist to visualize and evaluate certain cells or proteins and contributes to the diagnosis and classification of diseases, particularly malignant pathologies. The utility of immunohistochemistry has since expanded and currently includes the evaluation of predictive and prognostic markers in many malignant conditions [43,44]. Although there are guidelines for standardized analytical validation of immunohistochemical tests, the number of available markers continues to increase, and for some, reporting and interpretation may vary [44–46].

The integration of artificial intelligence methods with immunohistochemical testing improves the performance of the pathologist [47–52].

QuPath is an open-source software program designed for digital pathology and the analysis of biological images, characterized by an easy-to-use interface and extensibility. Although, to date, few studies on cervical cancer have used QuPath, related evaluations support the method — a study on inter-platform reproducibility found “excellent reproducibility between three different digital image analysis platforms in quantifying the

Ki-67 score" [53–56]. The application of QuPath for detecting immune cell markers [54] and digital image analysis in various tumor types improves reproducibility and decreases inter-observer variability [57,58].

PERSONAL CONTRIBUTION

6. MOTIVATION, PURPOSE AND OBJECTIVES OF THE STUDY

Cervical carcinomas remain a major cause of morbidity and mortality worldwide, with a more pronounced impact in countries without functional screening programs. Although these tumors generally do not present particular diagnostic challenges, except for early adenocarcinomas developing high in the endocervical canal, their diagnosis is often established at advanced stages of the disease, when therapeutic options can sometimes be limited.

In Romania, there are few studies on cervical carcinoma, and the existing ones mainly refer to screening — participation of the general population or certain ethnic groups in screening programs, challenges in implementing screening, the evolution of the incidence of various gynecological malignancies, high-risk HPV testing in the population, and the level of public knowledge about HPV.

The tumor microenvironment (TME) has become an active area of research in recent years due to its major influence on tumor progression and metastasis. The TME is largely composed of immune cells, fibroblasts, endothelial cells, stromal cells, and extracellular matrix (ECM). Interactions between tumors and immune cells are more complex and dynamic than previously thought. Many subtypes of immune cells infiltrating the TME possess strong tumor-promoting abilities. However, the impact and interplay of these physiological processes with the development, invasion, and metastatic potential of malignant cells remain not fully elucidated.

The immune mechanisms involved in cervical cancer can be evaluated by analyzing the tumor-infiltrating lymphocyte (TIL) population and by assessing the immunohistochemical expression of the CD8 biomarker. These findings form the basis for establishing further correlations with the expression of PD-L1 (Programmed Death-Ligand 1), an essential biomarker in cancer immunotherapy, playing a significant role in

the personalized treatment of various cancers. The immunohistochemical expression of PD-L1, both in malignant neoplastic cells and in immune cells within the tumor microenvironment, can influence the response to immune checkpoint inhibitors (ICI), such as PD-1 and PD-L1 inhibitors, which are used to stimulate the body's immune system to attack cancer cells.

Another important topic is the study of factors that influence angiogenesis, the process of forming new blood vessels, and the metastasis of malignant cells. Vascular endothelial growth factors (VEGF) are often overexpressed in tumors and are associated with an unfavorable prognosis. In addition, hypoxia, or lack of oxygen, is a common feature of the tumor microenvironment and is associated with aggressive tumor behavior. Hypoxia induces the transcription factor HIF-1α, which regulates genes involved in angiogenesis, cellular metabolism, and cell survival. Hypoxic tumors are often more resistant to conventional therapies such as radiotherapy and/or chemotherapy.

A deep understanding of the complex interactions within the tumor microenvironment and of the factors contributing to the development of lymphatic metastases is essential for the creation of new therapeutic strategies for cervical cancer. Anti-angiogenic therapy aims to inhibit the formation of new blood vessels in tumors, and Bevacizumab, a VEGF inhibitor, has demonstrated efficacy in treating advanced cervical cancer as well as other malignant diseases.

The process of neoangiogenesis is initially analyzed by immunohistochemical evaluation of the biomarker CD34 and quantification of microvascular density (MVD), establishing correlations with key prognostic factors. The next step involves analyzing the expression of the endoglin biomarker (CD105) in cervical cancer — a current marker and a therapeutic target. CD105 is a molecule with broad potential in tumor exploration and therapy due to its role in the TGF-β signaling system and its increased expression in proliferating endothelial cells.

Thus, considering the high incidence of cervical cancer in the absence of effective screening programs and the therapeutic limitations in advanced stages of the disease, a deeper understanding of the biological mechanisms involved in tumor progression is necessary. The study of the tumor microenvironment — with a focus on the interactions between immune cells, angiogenesis factors, and immune evasion — provides valuable insights for identifying prognostic biomarkers. In the context of modern therapies, such as immunotherapy and anti-angiogenic treatments, the characterization of these mechanisms

becomes essential for developing personalized and effective therapeutic strategies for cervical cancer.

The *main objective* of the doctoral study is to characterize the tumor microenvironment in cervical cancer by analyzing the relationships between clinical-biological, histopathological, immunological, and neoangiogenic parameters and by identifying factors with prognostic potential for stratifying patients with cervical cancer.

The *secondary objectives* of the doctoral study are:

I. Integrative evaluation of the clinical-biological parameters and the histopathological and proteomic characteristics of the cervical carcinoma cases included in the present study.

II. Characterization of the distribution of tumor-infiltrating lymphocytes (TILs) in conjunction with the analysis of the distribution and expression of CD8+ TILs in the tumor microenvironment, in order to assess their immunological and prognostic role.

III. Investigation of the density and distribution of tumor microvascularization in cervical cancer by analyzing the immunohistochemical expression of the endothelial markers CD34 and endoglin (CD105) and their correlation with clinico-pathological parameters.

IV. Evaluation of the immunohistochemical expression of the PD-L1 biomarker in cervical cancer and analysis of its correlations with immunological and angiogenic parameters in the tumor microenvironment, in order to better understand the mechanisms of immune evasion and its potential therapeutical prognostic value.

Purpose of the doctoral study: by simultaneously investigating tumor-infiltrating lymphocytes, neoangiogenesis processes, and immune evasion mechanisms, this doctoral study contributes to a deeper understanding of the interactions between invasive malignant neoplastic cells and their microenvironment. This knowledge can support the development of modern therapeutic strategies, such as immunotherapy and anti-angiogenic treatments. Moreover, correlating these data with the clinical and histopathological profiles of the patients provides a valuable framework for future research and for patient stratification aimed at implementing personalized treatment in medical practice.

7. MATERIAL AND METHOD

The doctoral study was conducted within the Clinical Service of Pathological Anatomy of the County Emergency Clinical Hospital "Saint Andrew the Apostle" Constanța in collaboration with the Research and Development Center for Morphological and Genetic Studies in Malignant Pathology (CEDMOG) of Ovidius University Constaţa.

This retrospective study includes 50 female patients and is based on information obtained both from the electronic database of the County Emergency Clinical Hospital "Saint Andrew the Apostle" Constanța and from patient medical records. The study cohort comprised 50 cases morphopathologically diagnosed with cervical carcinoma within the Clinical Service of Pathological Anatomy of the County Emergency Clinical Hospital "Saint Andrew the Apostle" Constanța between January 2019 and December 2021.

Inclusion criteria:

1. Patients admitted and investigated in the obstetrics and gynecology clinical departments of the County Emergency Clinical Hospital "Saint Andrew the Apostle" Constanța;
2. Newly diagnosed patients with cervical cancer, without neoadjuvant therapy (chemotherapy or radiotherapy prior to diagnosis);
3. Patients with a morphopathological diagnosis of primary cervical carcinoma, established through paraffin-embedded examination of surgical specimens (cervical biopsy or total/radical hysterectomy) within the Clinical Service of Pathological Anatomy of the County Emergency Clinical Hospital "Saint Andrew the Apostle" Constanța;
4. Availability of sufficient biological material to perform all subsequent immunohistochemical investigations;
5. Patients without immunosuppressive or biological therapy at the time of diagnosis or within the previous 12 months.

Exclusion criteria:

1. Cases with anatomo-pathological diagnosis performed outside the County Emergency Clinical Hospital "Saint Andrew the Apostle" Constanța;
2. Cases with histopathological diagnosis of benign cervical lesions;
3. Cases with morphopathological diagnosis of malignant cervical tumors of non-epithelial origin;

4. Cases representing secondary cervical tumors or metastases;
5. Cases with insufficient biological material or with extensive tumor necrosis.

This study was conducted after obtaining approval from the Ethics Committee of the County Emergency Clinical Hospital "Saint Andrew the Apostle" Constanța (42006/02.09.2020), in accordance with the Declaration of Helsinki and internationally recognized guidelines [59]. For each patient, the signed "Informed Consent Form" at the time of hospital admission was identified.

The biological samples of these cases, represented by the paraffin blocks of the patients archived in the histopathology archive of the County Emergency Clinical Hospital "Saint Andrew the Apostle" Constanța, were retrieved and examined for quality in order to perform immunohistochemical tests.

Each case was analyzed, both in terms of morphopathological features and the results of immunohistochemical examinations, by two pathologists from the Clinical Service of Pathological Anatomy of the County Emergency Clinical Hospital "Saint Andrew the Apostle" Constanța.

Immunohistochemical evaluation was performed for the biomarkers Ki67, p53, CD8, CD34, CD105 (endoglin), and PD-L1. Stromal TILs (tumor-infiltrating lymphocytes) were also assessed.

8. INTEGRATIVE EVALUATION OF CLINICO-BIOLOGICAL PARAMETRS, HISTOPATHOLOGICAL AND PROTEOMIC CHARACTERISTICS OF THE CERVICAL CARCINOMA CASES INCLUDED IN THE STUDY

This initial study aims to provide a comprehensive characterization of the cohort of patients diagnosed with cervical carcinoma in terms of age, place of residence, clinical, paraclinical, morphological, and immunohistochemical parameters, and to identify elements related to aggressive tumor behavior.

The secondary objectives of this study are:

- to identify biological markers with a potential prognostic role in recognizing cases with severe disease progression;
- to identify statistically significant correlations among different clinical, paraclinical, morphological, and immunohistochemical parameters (Ki67 and p53) that may have prognostic value in detecting cases with poor prognosis.

The study includes a total of 50 cases diagnosed between January 2019 and December 2021, most of them from urban areas (52%), with ages ranging from 32 to 83 years at the time of admission and cervical cancer diagnosis, with a mean age of 55.54 years. The majority of patients belonged to the 45–69 years age group, with a frequency of 66% (33/50), followed by patients aged 44 years or younger with a frequency of 22% (11/50). These data are consistent with the recent statistical data provided by the National Institute of Public Health [60]. Regarding the place of residence, most patients came from urban areas (52%), and the majority of cases were within the 50–69 years age group.

From the perspective of clinical staging, statistical analysis revealed a higher frequency of cases in stage III (36%), followed by stage II (34%), with no significant differences observed between urban and rural origin. Furthermore, in the study cohort, there was a slightly higher frequency of advanced-stage disease — 52% of patients were diagnosed at stage III–IV compared to 48% of patients diagnosed at stage I–II.

No statistically significant associations were observed between age, place of residence, disease stage, or histological grade.

Regarding morphopathological aspects, most of the cases included in the present study were diagnosed with squamous cell carcinoma — 96% (48/50), and two cases were diagnosed with cervical adenocarcinoma — 4% (2/50). Among the squamous cell carcinoma cases, the majority were represented by the keratinizing squamous cell carcinoma subtype — 52% (26/48), followed by non-keratinizing squamous cell carcinoma — 32% (16/48).

Among the cases included in the present study, the diagnosis of endocervical adenocarcinoma was identified in two patients, both being moderately differentiated (G2), of the usual non-mucinous type with a destructive invasion pattern — pattern C — according to the Silva classification system. The Silva system, which emphasizes the growth and invasion pattern of malignant neoplastic cells, is associated with the risk of lymph node metastases, tumor recurrence, and disease-free survival [61,62].

Following the analysis of tumor grade, a high proportion of cervical cancer cases with histological grade G3 (poorly differentiated) was observed — 70%, most of them identified in patients aged between 45 and 69 years. No associations were found between disease stage and histological grade.

Statistically significant results were observed regarding disease stage and blood leukocyte levels, as well as serum creatinine levels.

In the studied cohort, lymphovascular invasion was present in 19 cases (38%) and absent in 31 cases (62%). Statistical analysis showed a significant association between clinical stage and lymphovascular space invasion, indicating that the values of one variable clearly influence the values of the other.

In the studied cohort, the Ki67 proliferation index was classified into three categories: <30% (low proliferation), 30–50% (moderate proliferation), and >50% (high proliferation), with relatively balanced distribution rates (18%, 40%, 42%).

Statistical analysis demonstrated a significant association between the Ki67 proliferation index and age, clinical stage, histological grade, and lymphovascular invasion (LVI), showing a slight increasing trend of nuclear proliferation rates with advancing patient age.

In the current study, a high frequency (64%) of cases showed immunohistochemical expression of the p53 antibody with a mutational pattern, while the remaining cases displayed a non-mutational “wild-type” pattern. Among the cases with a mutational pattern, the most frequent were the “null-type” with a rate of 42% (21/50), followed by 22% (11/50) showing a mutational pattern with p53 protein overexpression. The p53 expression pattern was significantly associated with disease stage, histological grade, lymphovascular invasion, and Ki67 proliferation index.

The conducted study provides a characterization that integrates clinico-biological, histopathological, and immunohistochemical parameters of cervical carcinoma, with emphasis on the expression of the markers Ki67 and p53. The analysis of the 50 cases included in the study demonstrated a series of clinically and prognostically significant correlations:

(1) Lymphovascular invasion and high Ki67 proliferative index values are associated with advanced FIGO stages and poorly differentiated histological grade (G3), thus outlining a tumor phenotype with increased aggressiveness.

(2) The mutational pattern of p53 expression was more frequently observed in poorly differentiated tumors and advanced FIGO stages and showed correlation with a

high proliferative index, suggesting a link between genetic instability and the aggressive biological behavior of the evaluated tumors.

Clinically, these findings confirm the value of Ki67 and p53 as prognostic markers for the evolution of cervical tumors and provide a basis for their inclusion in risk stratification algorithms. Additionally, the association between lymphovascular invasion and advanced FIGO stages highlights the importance of their systematic assessment in routine practice.

9. CHARACTERIZATION OF THE DISTRIBUTION OF TUMOR-INFILTRATING LYMPHOCYTES (TILs) IN CONJUNCTION WITH THE ANALYSIS OF THE DISTRIBUTION AND EXPRESSION OF $CD8^+$TILs IN THE TUMOR MICROENVIRONMENT TO EVALUATE THEIR IMMUNOLOGICAL AND PROGNOSTIC ROLE

This study has as its main objective the evaluation of the immune component within the tumor microenvironment by analyzing the distribution levels of TILs and cytotoxic T lymphocytes $CD8^+$ ($CD8^+$ TILs) in cervical cancer, correlating their density with clinical, paraclinical, morphological, and immunohistochemical parameters in order to identify elements related to aggressive tumor behavior.

The secondary objectives of this study are:

1. Analysis of the density of TILs and $CD8^+$ TILs according to demographic parameters (age and place of residence), clinical parameters (FIGO stage), and morphopathological parameters (tumor grade, lymphovascular invasion, Ki67 and p53 expression), in order to identify associations with possible prognostic implications.
2. Evaluation of the prognostic value of stromal tumor-infiltrating lymphocytes (TILs) and intratumoral $CD8^+$ T lymphocytes in cervical cancer.

For a detailed immunological characterization of the tumor microenvironment, morphological and immunohistochemical analysis of tumor-infiltrating lymphocytes (TILs) and cytotoxic T lymphocytes $CD8^+$ ($CD8^+$ TILs) was performed for all 50 cases included in the study, based on the previously described criteria.

In the studied cohort, 25 out of 50 patients (50%) showed high TILs, 15 out of 50 patients (30%) showed moderate TILs, and only a minority — 10 out of 50 patients

(20%) — showed low TILs. Statistical analysis demonstrated that TILs were associated with FIGO stage.

The study demonstrated that increased TIL density, particularly $CD8^+$ TILs, correlates with early FIGO stages, absence of lymphovascular invasion, low tumor proliferation rate assessed by the Ki67 marker, and the non-mutational expression pattern of p53, reinforcing their favorable prognostic value. Regarding survival, we found that patients with a high immune infiltrate had a significantly longer overall survival and a reduced risk of death. Thus, $CD8^+$ TILs prove to be a biological marker with prognostic clinical value.

10. EVALUATION OF NEOANGIOGENESIS IN THE CERVICAL CANCER MICROENVIRONMENT THROUGH ANALYSIS OF VASCULAR DENSITY USING IMMUNOHISTOCHEMICAL EXPRESSION OF THE BIOMARKERS CD34 AND CD105 (ENDOGIN) AND THEIR CORRELATION WITH CLINICO-PATHOLOGICAL PARAMETERS

This study aims to evaluate tumor microvascular density by analyzing the immunohistochemical expression of the endothelial markers CD34 and CD105 (endoglin) and correlating these with clinical, paraclinical, morphological, and immunohistochemical parameters in order to identify prognostic elements related to aggressive tumor behavior.

The secondary objectives of this study are:

1. Investigation of the correlations between microvascular density evaluated by the markers CD34 and CD105 and clinico-morphological and immunohistochemical parameters with prognostic relevance.
2. The role of the biomarkers CD34 and CD105 in risk stratification of patients with cervical cancer.

Table 1. Classification of the values for the vascular markes used

Parameter	**CD34**		**CD105**	
Values obtained on the average of 5 HPF	Mean: 19,304 ± 8,35		Mean: 12,702 ± 4,87	
	Median: 18 (minimum 5 – maximum 34)		Median: 13 (minimum 3 – maximum 24,2)	
Microvascular density (MVD)	Low (<18)	High (≥18)	Low (<13)	High (≥13)
Cases (N)	23	27	24	26
Cases (%)	46%	54%	48%	52%

Tumors with high CD105-MVD more frequently show lymphovascular invasion, a high proliferation index, p53 expression with a mutational pattern, and low-density $CD8^+$ TIL distribution — all elements that characterize tumor aggressiveness and predict poor prognosis. Additionally, increased microvascular density was associated with a higher risk of death for both markers, confirming their unfavorable prognostic value. In the present study, both CD34 and CD105 (endoglin) highlighted that increased microvascular density correlates with reduced overall survival. CD105 proved to be more sensitive in its correlation with FIGO stage and other parameters such as lymphovascular invasion, proliferation index, p53 pattern, and CD8 distribution.

It is particularly important that angiogenic analysis has as its ultimate goal clinical implementation to stratify patients who may benefit from anti-angiogenic therapy, alone or combined with immunotherapy, thus improving prognosis and quality of life.

Future research directions may focus on: prospective studies evaluating the correlation of microvascular density with the response to anti-angiogenic therapies, with or without immunotherapy; exploring serum markers (circulating endoglin or plasma VEGF) as a non-invasive alternative or surrogate test; and investigating differences between histological subtypes (squamous cervical carcinoma versus adenocarcinoma).

11. EVALUATION OF THE IMMUNOHISTOCHEMICAL EXPRESSION OF THE BIOMARKER PD-L1 IN CERVICAL CANCER AND ANALYSIS OF ITS CORRELATIONS WITH IMMUNOLOGICAL AND NEOANGIOGENIC PARAMETERS IN THE TUMOR MICROENVIRONMENT, TO UNDERSTAND THE IMMUNE-EVASIVE ROLE AND ITS POTENTIAL THERAPEUTICAL PROGNOSTIC VALUE

The main objective of this study is to evaluate the immunohistochemical expression of PD-L1 in cervical cancer and to identify correlations with immunological markers ($CD8^+$ TILs) and angiogenic markers (MVD-CD34/CD105).

The secondary objectives are:

1. Exploration of the functional relationship between neoangiogenesis (CD105/CD34) and PD-L1 expression in the context of tumor immune evasion. This objective investigates the hypothesis that a hypoxic, highly vascularized tumor microenvironment can stimulate PD-L1 expression, limiting lymphocytic infiltration and facilitating tumor progression.
2. Determining the clinico-prognostic significance of the combined expression of PD-L1 with angiogenic markers (CD34 and CD105) and immunological markers (TILs and $CD8^+$ TILs) in cervical cancer, with the goal of identifying the most effective method for stratifying patients by risk and therapeutic response.

Among the 50 patients diagnosed with cervical cancer included in the analysis, 34 (68%) showed positive PD-L1 expression with a CPS score >1, while 16 (32%) were classified as PD-L1 negative with a CPS score <1. The mean age of PD-L1 positive patients was 55.56 ± 14.26 years, and for PD-L1 negative patients it was 55.0 ± 11.96 years.

In the present study, positive PD-L1 expression was identified in a high percentage of patients with cervical cancer, most frequently in the 45–69 age group, and it may have prognostic and therapeutic relevance (anti-PD-1/PD-L1 immunotherapy). A positive PD-L1 status correlated with advanced FIGO stage (III–IV), lymphovascular invasion, and a high Ki67 index, all reflecting aggressive tumor biology.

The mutational p53 pattern was significantly more frequent in PD-L1 positive cases, suggesting a genomically unstable tumor phenotype associated with unfavorable prognosis.

Furthermore, positive PD-L1 expression was associated with shorter overall survival, thus being considered a negative prognostic factor, while high-density $CD8^+$ TILs were associated with favorable prognosis, once again confirming the important role of the antitumor immune response.

Following statistical analysis, a statistically significant association was identified between PD-L1 expression and clinico-morphological and immunohistochemical parameters with a negative impact on prognosis (Table 2).

Tabel 2. Distribution of cases according to PD-L1 expression and FIGO stage, histological grade, lymphovascular invasion, Ki-67 index, and p53 expression pattern

Parameter	**PD-L1 negative n (%)**	**PD-L1 pozitive n (%)**	**Test χ^2/Fisher; p**	**ρ Spearman p**
Stage FIGO (I–II / III–IV)	I–II: 14 (87,5%); III–IV: 2 (12,5%)	I–II: 10 (29,4%); III–IV: 24 (70,6%)	$\chi^2(1)=14,708$; p<0,001	ρ=0,542; p<0,001
Histological grade (G1–G2 / G3)	G1–G2: 7 (43,8%); G3: 9 (56,3%)	G1–G2: 8 (23,5%); G3: 26 (76,5%)	Fisher p=0,191	ρ=0,206; p=0,152
LVSI (absent / present)	Absent: 12 (75,0%); Prezent: 4 (25,0%)	Absent: 7 (20,6%); Prezent: 27 (79,4%)	$\chi^2(1)=13,672$; p<0,001	ρ=0,523; p<0,001
Ki67 score (<42% / ≥42%)	<42%: 11 (68,8%); ≥42%: 5 (31,3%)	<42%: 9 (26,5%); ≥42%: 25 (73,5%)	$\chi^2(1)=8,104$; p=0,004	ρ=0,403; p=0,004
p53 (non-mutational / mutational)	Non-mut.: 12 (75,0%); Mut.: 4 (25,0%)	Non-mut.: 6 (17,6%) Mut.: 28 (82,4%)	$\chi^2(1)=15,533$ p<0,001	ρ=0,557; p<0,001

Evaluation of the prognostic role of the PD-L1 biomarker

The prognostic role of the PD-L1 biomarker was assessed through overall survival (OS) analysis. Univariate Cox regression analysis confirmed that negative PD-L1 status is associated with a significantly reduced risk of death, suggesting a protective role of negative PD-L1 status for patients with cervical cancer. In the multivariate Cox regression model, variables that had shown statistical significance in the univariate analysis, as well as clinically relevant variables, were included in order to evaluate the independent effect of PD-L1 expression on overall survival (Table 6).

It was found that the parameters influencing OS are age, FIGO clinical stage, Ki67 nuclear proliferation rate, $CD8^+$ TILs distribution, microvascular density quantified by CD34 and CD105, and the mutational or non-mutational pattern of the p53 protein.

Overall survival analysis showed that positive PD-L1 status and a high level of tumor inflammatory $CD8^+$ cells (CD8-DC) were associated with favorable prognosis.

In contrast, older age, advanced FIGO stage (III–IV), increased microvascular density (CD34, CD105), high Ki67 expression, and the mutational status of p53 were correlated with significantly reduced survival.

Multivariate analysis: PD-L1 and TILs/$CD8^+$ TILs distribution levels

The multivariate Cox regression analysis evaluating the impact of PD-L1 status (positive or negative) and the level of tumor-infiltrating lymphocytes (TILs — low, moderate, high) on overall survival showed no significant association between PD-L1 status and overall survival within this model.

The multivariate Cox regression model that included PD-L1 expression status and the distribution level of $CD8^+$ TILs, to investigate the independent impact of PD-L1 protein expression and $CD8^+$ lymphocytic infiltration on patient overall survival, demonstrated that positive PD-L1 expression was associated with a significantly increased risk of events. This association indicates that PD-L1 expression acts as a negative prognostic factor. In parallel, $CD8^+$-DC lymphocytic infiltration was associated with a significant protective effect on survival.

These data highlight the potential independent and complementary role of PD-L1 expression and $CD8^+$ infiltration distribution as prognostic markers in the analyzed pathology. Their combined use may contribute to better risk stratification and to selecting patients eligible for specific immunomodulatory therapies.

Multivariate analysis: PD-L1 and CD34/CD105

The multivariate Cox regression model, which evaluated PD-L1 expression in combination with microvascular distribution marked by CD34 (MVD CD34), showed a significant effect of PD-L1 on survival, while the effect of MVD CD34 did not reach the threshold of statistical significance. This indicates a significant association between positive PD-L1 expression and increased risk of death. These data suggest that PD-L1 remains a negative prognostic marker, independent of tumor vascularization status as assessed by CD34.

In a similar model, the impact of PD-L1 expression in association with microvascular distribution marked by CD105 (MVD CD105) was analyzed, but the results indicated no significant effect for either PD-L1 or CD105.

Based on the results obtained from the multivariate analysis, where it was observed that both the presence of PD-L1 expression and the level of tumor-infiltrating $CD8^+$ lymphocytes significantly influence prognosis: positive PD-L1 status was associated with an increased risk of death, while the presence of a high $CD8^+$ TIL infiltrate ($CD8^+$-DC) was correlated with a lower risk. These results suggest that each marker plays an important role in disease evolution. To better understand the interaction between these factors, patients were grouped as follows: PD-L1 negative / $CD8^+$-DS; PD-L1 negative / $CD8^+$-DC; PD-L1 positive / $CD8^+$-DS; and PD-L1 positive / $CD8^+$-DC.

This stratification allowed the evaluation of survival differences between specific combinations of these two biomarkers. Kaplan-Meier results showed statistically significant differences between groups (Log-Rank, $p < 0.001$), confirming the usefulness of this approach. The PD-L1 positive / $CD8^+$-DS group had the highest number of events with a survival rate of only 25%. At the opposite end, the PD-L1 negative / $CD8^+$-DC group recorded no events, with all patients alive at the end of the follow-up period (100% survival). Thus, we can conclude that positive PD-L1 expression is a major risk factor for reduced overall survival, while an abundant $CD8^+$ TIL infiltrate (DC) has a protective effect, improving prognosis even in the presence of positive PD-L1 status.

12. GENERAL CONCLUSIONS

The present study demonstrates the important role that immunological and angiogenic markers play in cervical cancer of epithelial origin.

In addition, we have shown that among the evaluated clinico-biological parameters, lymphovascular invasion and high values of the Ki67 proliferative index are associated with advanced FIGO stages and poorly differentiated histological grade (G3), thus outlining a tumor phenotype with increased aggressiveness. Furthermore, the mutational pattern of p53 expression was more frequently observed in poorly differentiated tumors and advanced FIGO stages and correlated with a high proliferative index, suggesting a link between genetic instability and the aggressive biological behavior of the evaluated tumors.

Practically, the nuclear proliferation index Ki67 and the p53 pattern remain important reference points for tumor characterization, but their correlation with TILs and neoangiogenesis markers (CD34, CD105) adds additional value regarding prognosis and prediction.

A high density of tumor-infiltrating lymphocytes (TILs), especially the $CD8^+$ subset, which correlates with early FIGO stages, absence of lymphovascular invasion, low Ki67 proliferation index, and non-mutational p53 pattern, represents elements with favorable prognostic value.

Patients with intense immune infiltrate have better overall survival and a reduced risk of death, which supports the importance of the antitumor immune response in controlling tumor progression.

PD-L1 expression was associated with tumor aggressiveness factors such as advanced FIGO stage, lymphovascular invasion, high Ki67 proliferation index, and mutational p53 pattern. However, PD-L1 interpretation is much more relevant when evaluated in relation to the presence of $CD8^+$ infiltration. The association of PD-L1 positivity and high-density $CD8^+$ defines a subgroup of patients with an immunological profile that has direct implications for response to anti–PD-1/PD-L1 immunotherapies.

Assessment of microvascular density using CD34 and CD105 (endoglin) demonstrated correlations with clinical and histological aggressiveness parameters, supporting the prognostic role of neoangiogenesis and the potential use of these markers as therapeutic targets through anti-angiogenic therapy.

These results, derived from an analysis that integrated multiple factors, encourage the incorporation of immunological and vascular parameters into risk stratification algorithms and the development of personalized therapeutic strategies to complement current traditional approaches (surgical therapy, neoadjuvant/adjuvant chemotherapy and radiotherapy).

In conclusion, we propose a risk stratification protocol based on the cohort of patients included in the study, which aims to integrate clinical-morphological parameters with assessed biomarkers to delineate risk categories and biological subgroups useful in the discussion in the multidisciplinary committee.

The proposed stratification is carried out in two steps:

- the first step establishes the baseline clinical risk using FIGO clinical stage, histological grade, presence of lymphovascular invasion and lymph node status (when available), with the classification of patients into 3 clinical risk groups (Rc): low (Rc-0) = early FIGO stage (I or II) + ILV absent + G1/G2 ± LN0; intermediate (Rc-1) = early FIGO stage (I or II) + ILV present ± G3 ± LN0; high (Rc-2) = advanced FIGO stage (III or IV) + ILV present ± G3 ± LN1;

- the second step analyzes the tumor biological profile and consists of the evaluation of 3 components:1) proliferation rate/genomic profile = “high” Ki67 index and/or aberrant p53 (absent – 0 points, present – 1 point); 2) immunological profile = low TILs and/or CD8 (absent – 0 points, present – 1 point); neoangiogenesis = increased DMV, especially CD105 or CD34 (absent – 0 points, present – 1 point). The sum of the points (0–3) represents the biological risk score (Rb).

The final risk (Rf) is made up of the clinical risk combined with the biological risk: low risk (Rc-0 and Rb 0-1); intermediate risk when there are moderate discordances between clinical and biological (Rc -0 but Rb 2–3 or Rc-1 with Rb 0–1); high risk (Rc-2 or Rc-1 is associated with Rb 2–3).

For the protocol to be reproducible, the report must specify the type of sample (biopsy/surgical specimen), the areas analyzed and the methods and cut-off values for biomarker evaluation and, when applicable, intratumoral heterogeneity (focal or diffuse, predominantly peripheral or intratumoral distribution of the immunolabel).

The findings confirm that malignant cervical pathology should not be viewed solely through the lens of traditional morphology but requires an extended characterization — molecular and immunological — to provide patients with treatments adapted to the

biological profile of the tumor. Such therapeutic interventions may significantly influence survival time and, equally important, the quality of life of patients.

The strengths of this study include its complex design, integrating multiple clinical, morphological, and immunohistochemical data, and the analysis of several relevant markers such as Ki67, p53, CD8, CD34, CD105, and PD-L1, providing a comprehensive view of the tumor microenvironment and prognostic mechanisms.

The limitations of the study are related to the small number of cases (a cohort of 50 patients — 48 cases of squamous carcinoma and two cases of adenocarcinoma); its retrospective design; the absence of evaluation of therapeutic response to immunotherapy; and the lack of molecular data (e.g., HPV infection status, genetic analyses).

We consider that future research directions should focus on multicenter and prospective studies with the possible integration of HPV infection status and dynamic evaluation before and after therapy, in order to more accurately identify patients who could benefit from immunotherapy with PD-1/PD-L1 inhibitors. Equally important is the investigation of the interaction between neoangiogenesis — which can be assessed using multiple markers — and immune evasion (PD-L1/$CD8^+$) for the development and application of combined therapies that could positively influence prognosis and quality of life in patients with cervical cancer.

13. ORIGINALITY OF THE THESIS

The present doctoral thesis represents an original contribution both in the field of pathological anatomy and in the field of gynecological oncology, through the approach of immunological and angiogenic parameters in epithelial cervical cancer.

The elements of novelty and originality consist of the following:

1. Integrative analysis of the immunohistochemical markers Ki67, p53, PD-L1, tumor-infiltrating lymphocytes (TILs and $CD8^+$), and vascular markers (CD34 and CD105), correlated with clinico-pathological data, based on modern computer-assisted image analysis techniques. This study is unique both due to the complexity of the simultaneously analyzed parameters and the use of modern digital pathology methods.

2. Reporting the prognostic value of the $CD8^+$ TIL subset, which has an impact on survival time and risk of death.

3. Establishing the interactions between PD-L1 expression and $CD8^+$ TILs, an aspect that allows more precise prognostic stratification and shows potential applicability in selecting patients eligible for immunotherapies.

4. Highlighting the role of neoangiogenesis in the process of tumor progression and the importance of the endothelial markers CD34 and CD105 in risk assessment. These are analyzed together for the first time in cervical cancer.

5. Inclusion of immunological and vascular parameters within a unified framework, which may have practical value for personalizing therapeutic management and developing risk stratification algorithms.

I consider that through its chosen topic, applied methodology, and interpretation of results, this thesis brings an original and significant contribution to a deeper understanding of the pathogenic mechanisms of cervical cancer and encourages the perspective of applying biomarkers in current clinical practice to improve patient outcomes.

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