PhD THESIS
ABSTRACT

THE ROLE OF IMMUNOHISTOCHEMICAL EXAMINATION IN EARLY AND DIFFERENTIAL DIAGNOSIS OF CERVICAL CANCER

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*Keywords: cervical adenocarcinoma, invalidity, histopathological variants, immunohistochemical markers.*
INTRODUCTION

Cervical carcinoma is the second most common cancer in women worldwide and it remains a leading cause of cancer-related death for women in developing countries.

The diagnosis of cervical adenocarcinoma is clinically very important because of its poorer prognosis and lower sensitivity to radiotherapy and chemotherapy in comparison with squamous cell carcinoma [1, 2].

The purpose of this thesis is to supplement existing data in the specialized literature on clinicomorphological aspects of cervical cancer and to highlight immunohistochemical examination implications in early and differential diagnosis of cervical adenocarcinomas, in order to apply corresponding therapeutic management.

In this respect, we performed a histopathological and immunohistochemical study on 16 cases of endocervical adenocarcinoma selected from the casuistry of the MorphoPathology Laboratory from the Slatina Clinical Hospital within 2005-2010.

We have also highlighted the adverse impact of cervical cancer at socioeconomic level, particularly on disability in the county of Olt.

STATE OF KNOWLEDGE

CHAPTER I
HISTOLOGY AND UTERUS HISTOPHYSIOLOGY

The uterus develops along with the other female internal genital organs (fallopian tubes and vagina) of Müller's channels (paramesonephrotic channels).

The Müller channels have three portions: one portion of skull, dilated (ostium), an intermediate portion which opens through several orifices in the cloacal cavity and caudal portion, in which the two Müller channels approach but remain separated by the septum that will reabsorb in the 3rd month of intrauterine life, when it will form the uterovaginal canal, through the union of the two channels.

Thus, the skull portion and from the area from the beginning of the intermediate portion will form the fallopian tube, the intermediate bottom portion, the body and the cervix uteri, and lower portion of the uterovaginal canal will form the vagina.

Anatomically, the uterus has three parts: the upper section and the most dilated is the body of the uterus, the isthmus, a narrow middle zone and the uterine cervix a lower portion of cylindrical shape. The cervix is divided by insertion of the vagina in two par areas: supra-vaginal and sub-vaginal (vaginal) that protrudes into the vagina.

At the level of these segments, there are three cavities: the cavity of the body of the uterus, the isthmus canal and the cervical canal or uterine cervix canal [3].

Uterine wall has three tunics: tunica mucosa (endometrium), tunica musularis (myometrium), tunica serosa (perimeter).

The cervix is the lower portion of the uterus and it has cylindrical shape. The wall has three tunics: mucosa, muscular-elastic (smooth muscle fibers and connective tissue rich in elastic fibers) and adventives.

The cervix has two areas: exocervix and endocervix. The exocervix is the portion visible in the vaginal cavity, being covered by a nonkeratinized stratified squamous epithelium and an aglandular chorion and the endocervix or endocervical canal, which
continues the uterine isthmus, consisting of a simple cylindrical epithelium with numerous mucous cells and glandular chorion.

CHAPTER II

EPIDEMIOLOGY AND PATHOGENESIS OF CERVICAL CANCER

Cervical carcinoma is the second most common cancer among women worldwide. Global burden is enormous, with over 500,000 new cases of cervical cancer diagnosed each year and 280,000 registered deaths [4].

While in developed countries screening programs have significantly reduced the incidence of the disease, about 80% of cervical cancers still occur in developing countries.

Epidemiological studies conducted in the last 30 years have shown that the risk of cervical cancer is strongly influenced by the following factors: sexual activity, smoking, number of births, diet, infection with HPV, the use of birth control pills for a long time.

HPV strains present most frequently in precancerous lesions of the cervix and cervical cancer are types 16 and 18.

CHAPTER III

HALLMARKS OF CANCER

Vastul catalog de genotipuri de celule canceroase este determinat de şase modificări esenţiale în fiziologia celulei, care determină creşterea malignă The broad catalog of cancer cell genotypes is determined by six essential alterations in cell physiology, determining the malignant growth [5]:

- self-sufficiency in growth signals;
- insensitivity to anti-growth signals;
- evading apoptosis;
- limitless replicative potential;
- sustained angiogenesis;
- tissue invasion and metastasis.

CHAPTER IV

DISABILITY CAUSED BY CERVICAL NEOPLASM IN OLT COUNTY

Cervical cancer is the main cause of death among women aged between 15 and 44 years from Romania, when they are professionally active, this having a negative socioeconomic impact.

I have conducted a clinicoepidemiological study and about the disability in Olt County between 2005-2010.

The following materials were used: population cancer registries, dispensary records of the patients with cervical cancer and statistics from the Department of Medical Expertise and Work Capacity Recovery Olt, between 2005-2010.

The results obtained reveal that the majority of female patients are uninsured, come from rural areas, have advanced disease stages and working capacity lost.
CHAPTER V
HISTOPATHOLOGICAL STUDY OF ENDOCERVICAL ADENOCARCINOMA

In the histopathological study we investigated the main clinicomorphological features of endocervical adenocarcinoma that might be involved in the prognosis of these patients.

Histopathological material came from the casuistry of the MorphoPathology Laboratory of the Emergency Hospital from Slatina County and was represented by 16 archived paraffin blocks.

In the morphological study we used the classic histological techniques including paraffin. As staining methods we used:

- Hematoxylin eosin (HE) for diagnostic evaluation according to the criteria for the classification of cervical tumors set by WHO (2003) [6];
- Masson trichrome of aniline blue to assess the degree of fibrosis tumor;
- Alcian Blue Periodic Acid Schiff (PAS) to assess the profile of mucins (neutral versus acidic) secreted by tumor cells.

In the table below are presented the main clinicopathological features of investigated casuistry (Table V.1).
Tabelul V.1 The major clinicopathological features of the investigated cervical adenocarcinoma

<table>
<thead>
<tr>
<th>Cervical adenocarcinoma type</th>
<th>Age (years)</th>
<th>Tumor size (cm)</th>
<th>Differentiation</th>
<th>Depth of stromal invasion</th>
<th>Depth of muscular invasion</th>
<th>Parametrial involvement</th>
<th>Lymph node metastasis</th>
<th>Vaginal margins</th>
<th>TNM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mucinous endocervical</td>
<td>45</td>
<td>≤4</td>
<td>W-M</td>
<td>≤1/2L</td>
<td>&lt;1/2L</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>IB1</td>
</tr>
<tr>
<td>Mucinous endocervical</td>
<td>56</td>
<td>&gt;4</td>
<td>P</td>
<td>&gt;1/2L</td>
<td>&gt;1/2L</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>IB2</td>
</tr>
<tr>
<td>Mucinous endocervical</td>
<td>69</td>
<td>≤4</td>
<td>W-M</td>
<td>≤1/2L</td>
<td>≤1/2L</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>IB2</td>
</tr>
<tr>
<td>Mucinous endocervical</td>
<td>56</td>
<td>&gt;4</td>
<td>P</td>
<td>&gt;1/2L</td>
<td>&gt;1/2L</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>IB2</td>
</tr>
<tr>
<td>Mucinous endocervical</td>
<td>64</td>
<td>≤4</td>
<td>W-M</td>
<td>≤1/2L</td>
<td>≤1/2L</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>IB2</td>
</tr>
<tr>
<td>Mucinous endocervical</td>
<td>55</td>
<td>&gt;4</td>
<td>P</td>
<td>&gt;1/2L</td>
<td>&gt;1/2L</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>IB2</td>
</tr>
<tr>
<td>Mucinous endocervical</td>
<td>61</td>
<td>≤4</td>
<td>W-M</td>
<td>≤1/2L</td>
<td>≤1/2L</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>IB2</td>
</tr>
<tr>
<td>Mucinous endocervical</td>
<td>57</td>
<td>&gt;4</td>
<td>P</td>
<td>&gt;1/2L</td>
<td>&gt;1/2L</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>IB2</td>
</tr>
<tr>
<td>Mucinous endocervical</td>
<td>37</td>
<td>≤4</td>
<td>W-M</td>
<td>≤1/2L</td>
<td>≤1/2L</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>IB1</td>
</tr>
<tr>
<td>Mucinous endocervical</td>
<td>40</td>
<td>&gt;4</td>
<td>P</td>
<td>&gt;1/2L</td>
<td>&gt;1/2L</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>IB2</td>
</tr>
<tr>
<td>Endometroid</td>
<td>59</td>
<td>≤4</td>
<td>W-M</td>
<td>≤1/2L</td>
<td>≤1/2L</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>IIB</td>
</tr>
<tr>
<td>Endometroid</td>
<td>62</td>
<td>&gt;4</td>
<td>P</td>
<td>&gt;1/2L</td>
<td>&gt;1/2L</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>IIB</td>
</tr>
<tr>
<td>Endometroid</td>
<td>58</td>
<td>≤4</td>
<td>W-M</td>
<td>≤1/2L</td>
<td>≤1/2L</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>IIB</td>
</tr>
<tr>
<td>Endometroid</td>
<td>63</td>
<td>&gt;4</td>
<td>P</td>
<td>&gt;1/2L</td>
<td>&gt;1/2L</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>IIB</td>
</tr>
<tr>
<td>Serous</td>
<td>47</td>
<td>≤4</td>
<td>W-M</td>
<td>≤1/2L</td>
<td>≤1/2L</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>IB2</td>
</tr>
<tr>
<td>Serous</td>
<td>67</td>
<td>&gt;4</td>
<td>P</td>
<td>&gt;1/2L</td>
<td>&gt;1/2L</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>IIB</td>
</tr>
</tbody>
</table>

- tumor degree of differentiation: W-M= well- moderate; P= poor; L= Layer

Fig. V.1 Cervical adenocarcinoma - mucinous endocervical type. HE stain, X100

Fig. V.2 Cervical adenocarcinoma - mucinous intestinal type. Malignant cells with goblet cell morphology that apically present a pool of acidic mucins. AB – PAS stain, X200.
In the investigated casuistry prevailed mucinous carcinoma cases (62.5%), with the endocervical subtype as most common (43.75% from all cases investigated and 70% from the all mucinous endocervical tumors). The second most common histopathological type of endocervical carcinoma was the endometroid type which accounted for 25% of all investigated cases.

Other variants diagnosed include: serous adenocarcinoma (12.5%), mucinous villoglandular type (12.5%) and intestinal mucinous adenocarcinoma (6.25%).

The most aggressive form of endocervical adenocarcinoma investigated proved to be the endometroid, the vast majority (75%) being diagnosed in advanced clinical stages IIIA and IIIB.

A better prognosis had the serous, respectively the villoglandular varieties, which were diagnosed in less advanced clinical stages (IB and IIB).

CHAPTER VI
IMMUNOHISTOCHEMICAL STUDY OF ENDOCERVICAL CARCINOMAS

The aim of immunohistochemical study was to assess the expression of immunohistochemical markers: CD105, VEGF, EGFR and c-erbB2 and their prognosis in different types of endocervical adenocarcinoma.

In many situations, especially when a tumor involves both the lower uterine segment and upper endocervix, the distinction between a primary endometrial and a primary endocervical adenocarcinoma may be difficult. This differential diagnosis is
imperative because the treatment plans and adjuvant therapies are totally different for the two forms of adenocarcinoma [7, 8].

This problem is partially solved by using appropriate panels of antibodies. Several studies have reported that typical immunoprofile of the primitive endocervical adenocarcinoma seems to be ER-/PR-/Vim-/CEA+, whereas for primitive endometrial carcinoma the characteristic profile is ER+/PR+/Vim+/CEA-[9,10,11,12].

Our investigations proved that this panel specific to the endocervical adenocarcinoma was recorded in 62.5% of our cases [13].

In all investigated cases, we observed that the angiogenesis process identified by the determination of MVD CD105 was more intensive in peritumoral area, regardless of clinicomorphological features of theses cases (the degree of differentiation, depth of muscular invasion or TNM stage).

For the first time, we revealed that the angiogenesis process seems to be dependent on histopathological subtype, with the highest values of MVD CD105+ recorded in the mucinous variant of endocervical adenocarcinoma and the lower values in serous type.

Investigation of VEGF expression showed that regardless of histopathological variant, the highest values of VEGF reactivity corresponded to higher MVD CD105 values.

Reactivity for markers CD105, VEGF, EGFR-1 and C-erbB2 allow the stratification of the patients with endocervical adenocarcinoma in order to determine the most effective treatment options that will target these molecular markers.
CONCLUSIONS

Cervical cancer remains a serious public health problem worldwide with high mortality in developing countries, especially in Romania.

In our study, we highlighted that the clinical stage is the most important prognostic factor and that to same extent, same histopathological features of these tumors can condition their biological behavior.

We also noticed that in endocervical adenocarcinomas there is an intense process of angiogenesis, which is controlled by the interrelation between VEGF, EGFR and c-erbB2.

Further studies are needed to elucidate whether specific angiogenic molecular profiles exist in different histopathological subtype of uterine adenocarcinomas and which is their impact on prognosis and therapeutic outcomes for these patients.

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