Prognostic biomarkers in endometrial adenocarcinoma

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Abstract
Endometrial carcinoma is the most common malignancy in the female genital system and has increased in incidence during the past years. Our study was retrospective and included 79 patients with diagnosed endometrial adenocarcinoma. The parameters investigated in the study included clinical status, menopause, history of estrogen intake, obesity, histological results, transvaginal ultrasonography. We evaluated the status of the common clinicopathological features and immunohistochemical biomarkers of endometrial carcinoma. The main type of carcinoma was endometrial endometrioid carcinoma (68 cases), followed by serous carcinoma (seven cases). Immunohistochemical study performed included the following antibodies: cytokeratin, vimentin, ER, PR, PTEN, p53, β-catenin, bcl-2, WT1 and Ki67. The immunohistochemical profile showed significant differences between the two subtypes. The majority of cases showed positivity for steroid hormones and the positivity correlated with the endometrioid subtype. We observed a correlation between p53 overexpression and specific histological alterations. A high percentage of Ki67 positivity tumors correlated with grade 3 tumors, as well as with a high percentage of p53 positivity. The study shows the importance of the use of biomarkers in the positive diagnosis and the guiding of therapeutic approach.

Keywords: endometrial adenocarcinoma, ER/PR, bcl-2, p53, Ki67, PTEN.

Introduction
Endometrial carcinoma is the most common malignancy in the female genital system [1] and it occupies the 7th place as cause of death by cancer in women in Western Europe (1–2% of all deaths from cancer) [2].

Endometrial cancer is symptomatic from early stages thus having a good prognosis in most patients, the overall five-year survival rate being relatively high [3]. Although it is not as studied or standardized as cervical preneoplasia, precursor lesions as complex hyperplasia without and with atypia, were identified. A study reported a cumulative risk of endometrial carcinoma during 20-year follow-up among women with endometrial hyperplasia of 28% in cases with atypia and only 5% in those without, with an average time to diagnosis of cancer of six years in all types of hyperplasia [4]. The diagnostic and treatment of premalignant lesions plays a very important role in prevention of the development of endometrial carcinoma, but the most common used classification [World Health Organization (WHO) system] is still considered as being poorly reproducible [5]. The WHO system classifies endometrial hyperplasia as simple, complex, typical and atypical based on the degree of architectural complexity and the cytological features [6]. An alternative classification system was proposed by an international group of gynecologic pathologists and defines two classes, endometrial hyperplasia and endometrial intraepithelial neoplasia, which do not correspond directly to the classes of the WHO system [7]. The counterpart of EIN is serous EIC (endometrial intraepithelial carcinoma), which is an early phase of serous adenocarcinomas of the endometrium.

The classification of endometrial carcinoma includes two classes, based on clinicopathological and molecular genetic features: type I, endometrioid being the most common and type II, non-endometrioid with a lower incidence but a poor prognosis. The etiology of this neoplasia differs between the endometrioid and non-endometrioid type. Endometrioid carcinoma is linked to increased sources of estrogen (endogenous and exogenous), while there is no connection between estrogen exposure and non-endometrioid carcinoma.

In this study, we evaluated the status of the common clinicopathological features and immunohistochemical biomarkers of endometrial carcinoma.

Materials and Methods
Our study was retrospective, based on the medical records of the patients and included 79 patients with diagnosed endometrial adenocarcinoma. The parameters investigated in the study included clinical status, menopause, history of estrogen intake, obesity, histological results, transvaginal ultrasonography.

The median time from the diagnostic biopsy to surgery was nine weeks (range, 8–39 weeks).

For all patients, we evaluated cervical and myometrial...
involvement and the presence of lymph node metastasis. The extent of the treatment relied on the estimated stage of the disease assessed on ultrasound. All patients underwent total hysterectomy with or without bilateral adnexectomy. Lymph node dissection was performed in 14 cases. Grading and staging were assessed according to the FIGO (International Federation of Gynecology and Obstetrics) criteria.

We performed ultrasound examination in all cases using a 2D ultrasound system with multifrequency transvaginal probes (3–9 MHz), both in B-mode and power Doppler (in order to assess vascularization). The cervical involvement and myometrial invasion were assessed subjectively by the observer.

After processing the specimen, sections were fixed in formalin and paraffin embedded according to standard procedures and then were stained with Hematoxylin and Eosin (HE). Slides were classified according to the WHO criteria.

Immunohistochemical study performed included the following antibodies: cytokeratin, vimentin, estrogen (ER) and progesterone (PR) receptors, PTEN (phosphatase and tensin homologue deleted on chromosome 10), p53, bcl-2, β-catenin, WT1 (Wilms tumor 1) and Ki67. The clones and dilutions used are listed in Table 1. Immunohistochemical results were either evaluated in a semi-quantitative manner and scored according to the percentages of positively staining cells or in a qualitative manner and appreciated as being positive or negative, paying attention to scoring only tumor cells stained in the appropriate nuclear/membrane position. The intensity of staining was not considered in the analyses.

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Clone</th>
<th>Dilution</th>
<th>Producer</th>
</tr>
</thead>
<tbody>
<tr>
<td>CK7</td>
<td>OV-TL 12-30</td>
<td>1:100</td>
<td>Dako</td>
</tr>
<tr>
<td>ER</td>
<td>1D5</td>
<td>1:1000</td>
<td>Novocastra</td>
</tr>
<tr>
<td>PR</td>
<td>1A6</td>
<td>1:25</td>
<td>Novocastra</td>
</tr>
<tr>
<td>PTEN</td>
<td>6H2</td>
<td>1:100</td>
<td>Dako</td>
</tr>
<tr>
<td>P53</td>
<td>DO7</td>
<td>1:50</td>
<td>Dako</td>
</tr>
<tr>
<td>β-catenin</td>
<td>17C2</td>
<td>1:100</td>
<td>Novocastra</td>
</tr>
<tr>
<td>WT1</td>
<td></td>
<td>1:200</td>
<td>Dako</td>
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<tr>
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<td>1:100</td>
<td>Dako</td>
</tr>
<tr>
<td>Bcl2</td>
<td>124</td>
<td>1:40</td>
<td>Dako</td>
</tr>
<tr>
<td>Ki67</td>
<td>MIB-1</td>
<td>1:50</td>
<td>Novocastra</td>
</tr>
</tbody>
</table>

Results

Most of the patients in the study group were post-menopausal (68 patients). The median age of the patients was 62-year-old (between 36 and 83-year-old). The majority of patients were over 40-year-old (68 cases) and 59 patients were already at menopause. Estrogen involvement was present in the form of intake either as supplements (five cases) or as treatment for a different malignancy – Tamoxifen for breast cancer in two cases. Also, more than 50% of the patients were obese (42 cases), with a BMI over 30 kg/m² (range, 17.2–42.3).

All patients presented with abnormal uterine bleeding for which they underwent transvaginal ultrasonography at which increased endometrial thickness was detected (Figure 1). The mean endometrial thickness was 17.5 mm. Cervical involvement was described in six cases. The estimation of the depth of myometrial invasion showed over 50% involvement in 24 cases and adnexal metastasis were suspected in two cases.

The types of surgical procedures performed depended on the initial diagnosis. In seven cases, in which an initial diagnosis of atypical endometrial hyperplasia was made and patients ranged from 34 to 39-year-old, a total hysterectomy with conservation of the ovaries was performed. In the rest of the cases, the surgical approach was total hysterectomy with bilateral adnexectomy in 58 cases and in 14 cases, this was accompanied by lymph node dissection.

The hysterectomy review showed endometrial endometrioid carcinoma in most of the cases (68 cases) and serous endometrial carcinoma in seven cases. All cases were graded and staged in accordance with the current recommendations of the International Federation of Gynecology and Obstetrics.

Biopsy results were available for all cases and we examined the concordance between them and the histological pattern in the hysterectomy specimen (Table 2). In 32 cases, the preoperative diagnosis was atypical endometrial hyperplasia.

<table>
<thead>
<tr>
<th>Biopsy</th>
<th>Resection specimen</th>
</tr>
</thead>
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<tr>
<td>Endometrioid hyperplasia</td>
<td>32</td>
</tr>
<tr>
<td>Mixed carcinoma</td>
<td>0</td>
</tr>
<tr>
<td>Serous carcinoma</td>
<td>4</td>
</tr>
<tr>
<td>Clear cell carcinoma</td>
<td>0</td>
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</tbody>
</table>

The main type of carcinoma that was diagnosed in hysterectomy specimens was endometrial endometrioid carcinoma (Figure 2), which showed a spectrum of histological features: different degrees of glandular formation with glandular overcrowding, papillary projections, intraglandular bridging and the presence of atypia and mitosis (68 cases), followed by serous carcinoma, which showed a predominantly papillary pattern with admixture of solid and glandular areas and cuboidal or hobnail-shaped tumoral cells with severe nuclear atypia (seven cases). In three cases, a mixed pattern was described but the major component was still the endometrioid carcinoma. One case showed the presence of cells with clear cytoplasm and marked nuclear atypia and was diagnosed as clear cell carcinoma.

After the histological grading of the cases, 24 cases were well differentiated with features similar to atypical complex hyperplasia (grade 1), with glandular and villoglandular pattern, 38 cases were moderately differentiated (grade 2) and in 17 cases the tumor consisted of solid nests and sheets of cells with no glandular formation (grade 3).

Postoperative staging showed that only 11 cases were confined to the endometrium, 68 cases were myoinvasive, and 26 cases involved the outer 50% of the myometrium. Out of the 14 cases, in which lymphadenectomy was performed, six cases had positive pelvic nodes.

The immunohistochemical findings revealed in 67
cases positive immunoreactivity confined to the nucleus for ER and PR (Figure 3). The degree of positivity varied between 30% and 90%. Most of the poorly differentiated cases of endometrioid carcinomas (11 cases) and the serous and clear cell carcinomas were negative. Cytokeratin 7 was positive in all cases and it also showed positivity for preneoplastic lesions (Figure 4) and normal glands.

PTEN showed diffuse positive staining in three cases, all non-endometrioid type. All endometrioid carcinomas showed absence of protein expression of PTEN. P53 expression was at a moderate to strong level in 24 cases and showed focal positivity in 13 cases. The normal adjacent areas of endometrium showed no reactivity for p53. In one case of serous carcinoma associated with EIC, p53 showed intense positivity in the area of serous EIC (Figure 5).

Beta-catenin was expressed in 21 cases of endometrioid adenocarcinomas, but also in the areas of normal endometrium, especially in glands in the proliferative phase. There was no positive reaction in the non-endometrioid cases.

WT1 was positive in only four cases, all of them being serous carcinomas. We did not identify any WT1 positive reaction in the endometrioid type tumors. Vimentin was positive in the tumoral cells in all cases of endometrioid carcinoma (Figure 6). Bcl-2 immunostaining was localized in the cytoplasm of tumoral cells in 24 cases (Figure 7) and also showed reaction in the areas of normal stroma and myometrium. The associated areas of endometrial hyperplasia were also positive.

Ki67, a marker of cell proliferation, was positive in all cases, but with various percentage of positivity between 15 and 80% (Figure 8). There was an increase in the number of positive cells in relation with the tumoral grade. All cases of non-endometrioid tumors showed a high value of the Ki67 index.

**Discussion**

Endometrial cancers, the most common cancer of the female reproductive tract, represents a health problem worldwide as it has increased in incidence 21% since 2008 and has doubled its death rate [8].

From a pathological point of view, endometrial carcinomas are classified by their histological profile, the most common subtype being endometrioid carcinoma. If we classify them by their clinical behavior, high-grade, clinically aggressive, endometrial carcinomas include FIGO grade 3 endometrioid carcinoma, serous carcinoma, clear cell carcinoma and undifferentiated carcinoma [9].
The data from the literature show a strong association between replacement estrogen therapy and the development of endometrial cancer in patients who use tamoxifen [10]. We had two cases of endometrial carcinomas in patients previously diagnosed with breast cancer and treated with Tamoxifen, both of them showing endometrioid histological features.

Obesity is a major risk factor for endometrial carcinoma, but the data from the literature are inconsistent in proving a relation between BMI and the histological subtype of endometrial carcinoma or between BMI and survival in patients with endometrial carcinoma [11]. Most of the cases of endometrial carcinoma in obese patients in our study were discovered in earlier stages, probably because of the more frequent medical check-ups due to associated comorbidities.

The normal endometrium is usually less than 1 mm in thickness in the postmenopausal period. The data from the literature describe a variation between 15 and 20 mm in endometrial carcinoma [12], data that are in concordance with the ones from our study. Although the measurement of endometrial thickness is an important tool in the evaluation of women with postmenopausal bleeding, it has low value as a screening test in asymptomatic postmenopausal women [13]. Transvaginal ultrasonography is an important noninvasive diagnostic method with increased sensitivity in the diagnosis of endometrial disease [14].

In the attempt to target distinct pathogenic pathways, multiple prognostic biomarkers have been reported for endometrial carcinoma because of the molecular and genetic events identified in the development of the disease [15].

ER and PR are normally present in the endometrium and their presence in endometrial tumors is associated with better prognosis and survival [16]. The majority of cases showed positivity for steroid hormones and the positivity correlated with the endometrioid subtype. Also, there was a correlation between the degree of positivity and the grade of the tumor.

PTEN, a tumor suppressor gene is the most frequently mutated gene in endometrial carcinoma. Loss of PTEN, early in carcinogenesis, activates several pathways related to proliferation and cell survival [17]. The use of immunohistochemistry to identify the cases with genetic PTEN loss was described in literature as a sensitive method [18]. In our study, we observed inactivation of PTEN in all cases of endometrioid endometrial carcinoma and most of the non-endometrioid type (95.5%).

Another tumor suppressor gene, p53, is involved in cell cycle arrest and the existence of cells bearing p53 mutation in endometrial carcinomas has already been extensively discussed in the literature. In our study, we
observed a correlation between p53 overexpression and specific histological alteration, characteristic of high-grade tumors like solid pattern of growth, cytological atypia, high nuclear grade. P53 as described in the literature as a biomarker for tumor aggressiveness and loss of differentiation of endometrial cancer [19].

Beta-catenin is a gene involved in both cell adhesion and invasion and plays important roles in cellular proliferation and differentiation [20]. We only identified positive reaction in 31% of the endometrioid endometrial carcinomas and in none of the type 2 cases. Nei et al. described nuclear positivity of β-catenin in 38% of endometrioid tumors, and thus suggesting that the Wnt-signaling pathway is mainly involved in the pathogenesis of endometrioid carcinomas [21].

WT1 is a tumor suppressor gene expressed in several normal adult cells, but also involved in various malignancies. [22]. We did not identify any immunostaining in the endometrioid subtype, which is consistent with the data from the literature [23].

Vimentin is an important biomarker in differentiation between cervical and endometrial adenocarcinoma, together with ER and CEA [24]. The distinction between cervical and endometrial adenocarcinomas is clinically important as different therapeutic approaches are used for these patients. All the cases in our study showed positivity for vimentin.

Bcl-2 is an oncogene involved in apoptosis and its loss of expression was related to more aggressive disease (higher grade, advanced stage and lymph node invasion) [25]. We did not find in our study a correlation between expression of bcl-2 and grade or stage of the endometrial cancer, data previously described in the literature by Markova et al. [15].

A high percentage of Ki67 positivity tumors correlated with grade 3 tumors, as well as with a high percentage of p53 positivity. Ki67 is a well-known marker of proliferation and several studies have described highly significant correlation between the percentage of the proliferating tumor cells and the survival [26]. Grade 3 endometrioid tumors showed similar clinical and immunohistochemical features with type 2, non-endometrioid carcinomas. Some studies in the literature have shown similar prognoses in the two entities and recommended similar therapeutic approach [27].

Conclusions

Many benign, but also preneoplastic and malignant lesions, are encountered during routine examination of the endometrium and may give rise to diagnostic difficulties. In this study, we observed changes in the morphological and biological spectrum of different entities, sometimes difficult to diagnose with certainty. The correct classification of the diseases is a prognostic factor, having a major impact on quality of life. Early detection is crucial for the development of endometrial malignancies and thus a thorough investigation of endometrial tumorigenesis is a critical step in the development of strategies for screening and early diagnosis.

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References


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