Morphological, imaging and surgical aspects in endometrial endometrioid adenocarcinoma

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Abstract
Endometrioid endometrial adenocarcinomas (EECs) are frequent genital tumors for which the clinical, imaging and histopathological integrated analysis is the basis of differential diagnosis and therapeutic attitude. This research represents a tertiary multicenter study including 58 cases examined histopathologically and immunohistochemically, surgically treated, on a five years period. The main characteristics of the patients in the study group are represented by the average age of 66 years, associated with obesity, hypertension, diabetes, history of infertility, early menopause, nulliparity or long-time oral contraception. The most important clinical sign was the menopausal or postmenopausal vaginal bleeding. The golden standard in the diagnosis of endometrial carcinoma is the dilation and curettage of the uterine cavity, followed by morphological, imaging and surgical aspects of endometrial endometrioid carcinoma, surgery, immunophenotype, invasive malignancy.

Keywords: endometrial endometrioid carcinoma, surgery, immunophenotype, invasive malignancy.

Introduction
It is widely acknowledged today that epidemiological, clinical and molecular studies converge to the idea of endometrial adenocarcinomas classification in two basic subclasses: type I, in which tumors are endometrioid adenocarcinomas in most cases, whereas type II includes non-endometrioid forms as serous, clear-cell or squamous carcinomas [1].

The malignant pathology of the endometrium is the most common invasive malignancy of the female genital tract. Most of these cases are represented by the endometrioid adenocarcinoma (type I), which is the most common histological type [2]. The histopathological grade and stage are the most important parameters for prognosis and, in many cases, for the therapeutic management of endometrial adenocarcinomas [3].

Most patients seek the advice of a health professional when vaginal hemorrhage, the most important symptom of this pathology, occurs and the tumor is usually confined to the uterus at the time of diagnosis [4–7].

Risk factors for endometrial cancer include obesity and old age [8]. Other affecting risk factors are: polycystic ovary syndrome, estrogen therapy especially at menopause, early menarche, early menopause, a history of infertility, nulliparity, irregular menarche, white race, tamoxifen, diabetes, hypertension, gallbladder disease, long-term combined oral contraception and smoking [8–11]. There have been numerous studies in the last two decades, on endometrial carcinoma, but equally true is the fact that there have been significant changes in the patterns of distribution of pathological characteristics within these entities with rather broad variability [2, 8]. The last histopathological classifications of endometrial adenocarcinomas, use criteria related to tumor cellularity and architecture, with a role in the differential diagnosis of various subtypes as well with tumors in other locations [8, 12–14]. Nevertheless, the used criteria are occasionally insufficient, being necessary to analyze the tumor immunophenotype [12].

The aim of this study was to evaluate the integrated morphological, imaging and surgical aspects of endometrial endometrioid adenocarcinomas, in relation with the tumors immunophenotype, which can contribute to improve the patients’ diagnosis and therapy.
Materials and Methods

The study includes 58 cases examined histopathologically and immunohistochemically, operated on between January 2011–January 2016, diagnosed with endometrioid endometrial adenocarcinoma (EEC). The study was conducted in five tertiary university centers: Emergency University Hospital of Craiova, University of Medicine and Pharmacy of Craiova, “St. Pantelimon” Emergency Clinical Hospital (Bucharest), “Nicolae Malaxa” Clinical Hospital (Bucharest), “Carol Davila” University of Medicine and Pharmacy, Bucharest and “Dominic Stanca” Clinic of Obstetrics and Gynecology, “Iuliu Hatieganu” University of Medicine and Pharmacy, Cluj-Napoca, Romania. All the patients in the study group were Caucasians.

The diagnosis of endometrial carcinoma was established based on clinical, imaging and histopathological criteria. Paraclinically, Pap test (PAP) in liquid medium was a sign of diagnostic suspicion by identifying the atypical endometrial or glandular cells on the result of this test. In selected cases with atypical glandular abnormalities, colposcopy was used. The endometrial biopsy, either by hysteroscopy or by the classical multistage instrumental check-up of the uterine cavity was done in all cases. Laboratory tests included routine biological screening and determining the CA125 value. Transvaginal ultrasonography (TVUS) has been used as a routine imaging scan in all patients. Computed tomography (CT) and magnetic resonance imaging (MRI) were used in selected cases.

The surgical specimens resulting from total hysterectomy with bilateral salpingo-oophorectomy, and lymphadenectomy performed for curative purposes were fixed in 10% buffered neutral formalin, processed by paraffin embedding and Hematoxylin–Eosin (HE) staining. The immunohistochemical study included the criteria established by World Health Organization (WHO) in 2014 [12]. After establishing the diagnosis of EEC, the specimens were immunohistochemically processed in order to identify the tumors immunophenotype (Table 1).

<table>
<thead>
<tr>
<th>Differentiation degree</th>
<th>No. of cases</th>
</tr>
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<tbody>
<tr>
<td>Well differentiated</td>
<td>31</td>
</tr>
<tr>
<td>Moderate differentiated</td>
<td>18</td>
</tr>
<tr>
<td>Poorly differentiated</td>
<td>9</td>
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<thead>
<tr>
<th>Lymph node metastasis</th>
<th>No. of cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>N0</td>
<td>55</td>
</tr>
<tr>
<td>N1</td>
<td>3</td>
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<thead>
<tr>
<th>Depth of invasion/Stage</th>
<th>No. of cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>pT1/Stage I</td>
<td>46</td>
</tr>
<tr>
<td>pT2/Stage II</td>
<td>8</td>
</tr>
<tr>
<td>pT3/Stage III</td>
<td>4</td>
</tr>
</tbody>
</table>

The immunohistochemical study included the following panel of antibodies: cytokeratin 7 (CK7, clone OV-TL 12/30, 1:75, Dako); estrogen receptor (ER, clone 1D5, 1:1000, Novocastra), progesterone receptor (PR, clone 1A6, 1:25, Novocastra), vimentin (Vim, clone V9, 1:100, Dako), Ki67 (clone MIB-1, 1:50, Novocastra), p16 (clone E6H4, 1:800, MTM Laboratories), p53 (clone DO7, 1:50, Dako), carcinoembryonic antigen staining (CEA, clone II-7, 1:50, Dako). The antigenic retrieval and working systems were used according to manufacturers, for visualization being used 3,3'-diaminobenzidine tetrahydrochloride (DAB, Dako, code 3467). Also, in order to validate the immunoreactions, internal and external negative controls were used for each marker.

The immunohistochemical reactions were analyzed in terms of the signals intensity (low, high) and the percentage of labeled cells expressed as the index of positivity (IP) obtained by dividing the number of positive cells per 1000 cells/case counted on 20× microscopic objective. The threshold positivity value of reactions was 5% labeled tumor cells.

Statistical analysis used average values, standard deviations and comparison tests (ANOVA, Pearson’s) within SPSS 10 software.

The local ethical committee approved the study and from all the patients was obtained a written informed consent.

Results

The main characteristics of the patients in the study group are represented by the average age of 66 years (53–79 years), associated with obesity, hypertension, diabetes, history of infertility, early menopause, nulliparity or long-time oral contraception. Treatment with tamoxifen for previous breast cancers, hormone replacement therapy at menopause and smoking are risk factors identified in the study patients.

The clinical signs were indisputably dominated by menopausal or postmenopausal vaginal bleeding. Abnormal leucorrhea also represented a clinically important criterion, along with pelvic pressure and pain.

The paraclinical imaging diagnosis of uterine formations by TVUS revealed less characteristic aspects as a screening method. The diagnostic pathway in our study was represented by TVUS followed by the classical endometrial biopsy or hysteroscopy. EEC occurred as a localized endometrial lesion, irregular, polypoid or polyp-like appearance, or as a diffuse thickening of the endometrium. The tumor was usually hyperecogenous, fairly homogeneous, surrounded by a hypoecogenic strip through the peritumoral myometrium. By the presence of the hematometra, appearance becomes non-homogeneous, with anechogenic areas or hypoecogenic areas through tumor necrosis. Spectral, color, power or power high-definition (HD) flow Doppler examination provides additional information (Figure 1, A and B).

The identified gross aspects were various, the tumors frequently being unique (93.1%), ranging in size between 2–30 cm located in the uterine fundus (60.3%). More frequently, the tumors had an exophytic, polypoid growth pattern (68.9%), sometimes in combination with ulcerated areas, which on the surface section indicated varying degrees of wall invasion. Necrosis, hemorrhage, heterogeneous appearance and friability were common aspects of EEC (Figure 2, A and B).
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Figure 1 – Endometrioid carcinoma: (A) Sagittal sonogram demonstrating severely enlarged uterine cavity (red arrows), heterogeneous echogenic mass (green arrow) on the rear wall of the uterine cavity, irregular outline, clear edges. Note hypoechogenic posterior band and the underlying formation (blue arrow) representing the peritumoral myometrium; (B) Sagittal sonogram demonstrating hematometra, with inhomogeneous, heterogeneous appearance, with anechogenic or hypoechogenic areas by tumor necrosis (yellow arrows). Note the intramural leiomyoma on the anterior uterine wall (green asterisk).

Figure 2 – Endometrioid carcinoma: (A) Hysterectomy specimen without section. Uterus appears enlarged, with irregular surface, leiomyomatous, with three subserosal leiomyomas on the anterior surface. Uterine cervix appears elongated and hypertrophic. Both ovaries with climacteric atrophy; (B) Hysterectomy specimen after midsagittal section. Note the three subserosal and intramural leiomyomas with the typical white, whorled tendency to pop up from the surrounding myometrium when cut. The intraluminal softer structure in the center is an endometrial endometroid carcinoma, with exophytic polyp-like appearance.

The histopathological analysis indicated the predominance of well and moderately EEC, respectively in 53.4% and 31% of cases, without regional lymph node metastases (94.8%) and limited invasion to myometrum (79.3%) (Table 1).

Invasion of the myometrium internal half (pT1a) was observed in 73.9% of pT1 cases, for 26.1% of cases the invasion being present in the external half of the myometrium. In 13.8% of EEC cases, the cervical stroma was invaded (pT2) and for 6.9% of cases, the tumors lesions invaded the uterine serosa, annexes or regional lymph nodes.

The analyzed EEC indicated a histopathological typical pattern. We observed different degrees of glandular structures, partly confluent, taped by epitheliums with a variable degree of atypia, papillary projections, intraglandular bridging and the presence mitotic activity. In poorly differentiated EEC, by the absence of glandular structures, polymorphic neoplastic cells were arranged in compact islands or beaches with less obvious tumor stroma. The cytoplasm of neoplastic cells has been reduced, basophilic, with unique nuclei, hyperchromatic, round or ovoid, enlarged in volume, often prominent nucleoli and high mitotic activity.

In 25.8% of EEC cases were identified particular aspects, with the presence of squamous metaplasia areas and mucin-rich areas (Figure 3, A and B).

The immunohistochemical analysis indicated particular and classical EEC aspects (Table 2).

Table 2 – Immunohistochemical EEC evaluation

<table>
<thead>
<tr>
<th>Antibodies panel/Immunoreaction</th>
<th>Positive cases [%]</th>
<th>Intensity</th>
<th>IP%</th>
</tr>
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<tbody>
<tr>
<td>CK7</td>
<td>100</td>
<td>high</td>
<td>75–100</td>
</tr>
<tr>
<td>ER</td>
<td>82.7</td>
<td>high</td>
<td>35–90</td>
</tr>
<tr>
<td>PR</td>
<td>81</td>
<td>high</td>
<td>45–75</td>
</tr>
<tr>
<td>Vimentin</td>
<td>100</td>
<td>high</td>
<td>35–90</td>
</tr>
<tr>
<td>Ki67</td>
<td>100</td>
<td>variable</td>
<td>10–45</td>
</tr>
<tr>
<td>p16</td>
<td>15.5</td>
<td>low</td>
<td>10–30</td>
</tr>
<tr>
<td>p53</td>
<td>25.8</td>
<td>variable</td>
<td>10–45</td>
</tr>
<tr>
<td>CEA</td>
<td>6.9</td>
<td>low</td>
<td>5–15</td>
</tr>
</tbody>
</table>

CK: Cytokeratin; ER: Estrogen receptor; PR: Progesterone receptor; CEA: Carcinoembryonic antigen; IP: Index of positivity.
CK7 immunostaining was observed in all cases in the tumor cells cytoplasm, the reactions being diffuse, with high intensity and a CK7 IP mean value of 86.2±12.9 (Figure 4A). ER and PR immunostaining was found in the nucleus of tumor cells in 82.7% and respectively 81% of analyzed EEC, the negative cases belonging to some moderate poorly differentiated tumors invading beyond the internal myometrium or cervical stroma. The hormone receptors reactions were diffuse, with high intensity, and the medium IP values of 60.2±32.1 in case of ER and 60.2±32.1 in case of PR (Figure 4, B and C). The high values of ER IP and PR IP were associated with well-differentiated adenocarcinomas with incipient invasion in the myometrium (p<0.05, ANOVA test).

Vimentin immunostaining was identified in the cytoplasmic of tumor cells in all analyzed cases, with high intensity and a mean IP value of 60.3±14.6 (Figure 4D).

Ki67 immunostaining was identified in all investigated cases at nuclear level, variable intensity and Ki67 IP medium value of 17.5±7.7, with significantly higher differences in poorly differentiated and advanced stage adenocarcinoma (p<0.05, ANOVA test) (Figure 4E).

P16 and p53 immunoreactions were observed in 15.5% and 25.8% of analyzed EEC, the positive being represented by moderately/poorly differentiated EEC, with deep invasion into the myometrium. In the case of p16, the reactions were nuclear and cytoplasmic, focally, with low intensity and an IP p16 medium value of 18.7±7 (Figure 4F). For p53, the reactions were observed in nuclei, variable intensity and a IP p53 medium value of 26.2±11.9 (Figure 4G).

In four (6.9%) analyzed EECs, we found focally apical cytoplasmic positivity with low levels for CEA immunoreactions. The cases belonged to EEC with varying degrees of differentiation and mucus-rich areas, and IP CEA medium value was 10.5±4.2 (Figure 4H).

In this study, we found no statistical relationship of p53, p16 and CEA with clinicopathological parameters. In the case of ER, PR, and vimentin we found a positive linear correlations of IP values (p<0.05, Pearson’s test).

**Discussion**

It has been shown in numerous studies that tumors of higher grade are associated with a deeper myometrial invasion and therefore with increased rates of metastasis in pelvic and para-aortic lymph nodes and therefore with a more reserved prognosis and reduced survival [15, 16].

In many cases, the presence and degree of myometrial invasion cannot be assessed preoperatively, but it is encouraging that an MRI scan can sometimes provide very accurate information about tumor extension and therefore about the surgical technique to be used [17–19].

Another important observation on the prognosis is linked to the presence of estrogen and progesterone receptors (ER, PR) in the tumor tissue. They are often located in low-grade carcinomas, and seem to indicate a high probability of positive response to subsequent hormonal therapy [20, 21]. In this study, the high immunostaining values of ER and PR were associated with well/moderately differentiated carcinomas, with incipient invasion in the myometrium.

Ren et al. showed that inhibition of PR methylation results in restoration of PR expression and induction of apoptosis in endometrial cancer cells, suggesting a role for inhibition of methylation for therapy in endometrial cancers [22, 23].

Estrogens have been shown to stimulate the proliferative capacity of endometrial cancer cells through activation of the alpha isof orm of the ER. Nuclear co-activators and co-repressors control the nuclear functions of ER. Several co-activators have been shown to be overexpressed in high grade endometrial cancers compared to low grade ones [23–25].

Uterine bleeding is the main sign of endometrial carcinoma in over 90% of cases. Therefore, it is widely agreed that any vaginal bleeding in postmenopausal women should be suspicious for endometrial carcinoma [26].

TVUS and endometrium measurement in patients with abnormal bleeding is a feasible method to estimate endometrial hypertrophy and its possible correlations [27–29].

The golden standard in the diagnosis of endometrial carcinoma is the dilation and curettage of the uterine cavity, followed by histopathological assessment. The association between TVUS and endometrial biopsy increases to 100% the diagnostic sensitivity [30].
Figure 4 – Endometrioid endometrial adenocarcinoma (EEC): (A) Poorly differentiated EEC, CK7 immunostaining, ×100; (B) EEC with mucin-reach areas, ER immunostaining, ×40; (C) EEC with mucin-reach areas, PR immunostaining, ×100; (D) Poorly differentiated EEC, vimentin immunostaining, ×100; (E) Moderate differentiated EEC, Ki67 immunostaining, ×100; (F) Moderate differentiated EEC, p16 immunostaining, ×100; (G) Moderate differentiated EEC, p53 immunostaining, ×100; (H) Moderate differentiated EEC, CEA immunostaining, ×100.
Bansal et al. and Hecht & Mutter considers that endometrial cancer is, from the biological and histological viewpoints, a group of diverse cancers through a dualistic model of pathogenesis, EEC type I are low-grade, estrogen dependent, and they are derived from atypical endometrial hyperplasia. Type II EEC are serous or clear cell histologically, they have no precursor lesions and they are more aggressive. These clinical and morphological differences are in parallel with the genetic differences [31, 32].

The surgical treatment in endometrial cancer should be established using the revised FIGO system [8, 12, 33]. The surgical treatment of endometrial cancer diagnosed in stage I through extrafascial total hysterectomy is generally sufficient, but in advanced endometrial cancer, radical hysterectomy is indicated [8, 34–36].

Mariani et al. and Mayo Clinic protocol considers that the surgical treatment of endometrial cancer should be hysterectomy, bilateral salpingo-oophorectomy, peritoneal cytology, bilateral pelvic and para-aortic lymphadenectomy up to renal vessels. Lymphadenectomy can be omitted if there is no myometrial invasion, endometrioid histology and no evidence of tumor outside corpus. Lymphadenectomy can also be omitted if there is an endometrioid carcinoma grade 1 or 2, myometrial invasion <50%, tumor diameter less than 2 cm, and no evidence of tumor outside corpus. If non-endometroid, complete omentectomy and peritoneal biopsies should be added [26, 37].

The histopathological analysis of EEC by determining the degree and tumor extension, stay based on the final diagnosis and subsequent therapeutic protocols, with major impact on prognosis. However, sometimes immunohistochemical reactions are required for differential diagnosis with other carcinomas with endometrial (ovary, endocol) or non-endometrioid (serous adenocarcinoma) pattern.

EEC are positive for ER, PR, and vimentin in most cases and specialized studies indicate the immunostaining decreasing in tumors with histopathological parameters of aggression [38, 39], aspect which was emphasized in this study. Also, in this study, high Ki67, p53 and p16 immunoreactions were associated with high grade, invasive EEC.

P53 and p16 are diffuse immunoreactions are usually associated serous non-endometrioid type adenocarcinomas, but some studies indicate the presence of focal reactions in some EEC, which can pose problems for the differential diagnosis [38, 40, 41]. Moreover, p16 immunoexpression is usual diffuse in endocervical adenocarcinoma and vimentin sometimes is focally expressed in ovarian or endocervical primitive adenocarcinomas [42]. In these situations, it may be necessary to include cytokeratins 7 and 20 in immunohistochemical analysis, most of EEC having a CK7+/CK20- immunophenotype. Although expression of CEA is not specific for EEC, in our study we found focally reaction in some cases, which required differential diagnosis with endocervical adenocarcinoma, which is frequently positive for this marker and in addition can be positive and ER [42].

The use of an expanded panel of antibodies is sometimes necessary to differentiate EEC from other endometrioid or non-endometrioid adenocarcinomas. This panel should include Ki67, p53 and p16, which appear to be associated with high-grade and invasive lesions and give information about the EEC aggressiveness.

Prognosis and risk of recurrence in EEC are influenced by multiple factors including the stage of the disease, old age, histology, high tumor grade, myometrial invasion, invasion in the lymphovascular space, positive peritoneal cytology for cancer cells, the increased size of the tumor, elevated levels of tumor expression for ER and PR [8].

Obesity is widely recognized as a risk factor for EEC. As this factor becomes more prevalent, the incidence of EEC is expected to increase proportionately [8].

This is also an argument to the fact that every post-menopausal woman with vaginal bleeding should be considered to have endometrial cancer until proven otherwise [26].

There is currently no role of routine screening for endometrial cancer in medium or high-risk women [43].

Burke et al. and Smith et al. believe that annual screening through endometrial biopsy should begin at age 35 for women at high risk of endometrial cancer due to Lynch syndrome or hereditary non-polyposis colorectal cancers (HNPCC) [43, 44].

The risk of endometrial cancer in women with hereditary nonpolyposis colorectal cancer (HNPPC) lifetime is estimated at 40–60%, and starting from this consideration, prophylactic hysterectomy may be an option [45].

The recommendation for genetic counseling may additionally clarify what the risk of developing EEC is for these women [46, 47].

Conclusions

The integrated analysis of clinical, imaging and histopathological EEC data is usually required. The clinical diagnosis is based on the menopausal or postmenopausal woman’s vaginal bleeding and the association TVUS and endometrial biopsy increases diagnostic sensitivity very close to maximum. The surgical treatment consists of total hysterectomy and bilateral salpingo-oophorectomy with rigorous surgical staging and lymphadenectomy imposed by very well standardized criteria and widely recognized. The use of an antibodies panel that includes CK, ER, PR, vimentin can guide the diagnosis to EEC and the analysis of Ki67, p16, p53 and CEA immunoreactions can be useful both for EEC differential diagnosis and to assess the tumor aggressiveness. The interrelation between the accurate histopathological diagnosis and the adequate surgical staging is the cornerstone of curative therapy in the EEC. General health, endogenous and exogenous hormonal status of the patient, old age and, increasingly importantly, body mass index are factors that contribute to the therapeutic management adapted to each patient according to the individual tumor.

Conflict of interests

The authors declare that there is no conflict of interests regarding the publication of this paper. All authors read and approved the final manuscript.

References


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