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Clinical and morphological correlations in early diagnosis of endometrial cancer

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Abstract

The prevalence of endometrial cancer among asymptomatic women is rather reduced also due to the absence of a cost-efficient test, as there are no ideal screening examinations for endometrial cancer. Several methods were proposed in medical practice to discover endometrial neoplasia at an early stage, among which: endometrial biopsy, endovaginal ultrasound, targeted biopsy hysteroscopy. This study was made on a group of 38 patients monitored for metrorrhagia in the interval between July 1, 2014–July 1, 2016. All patients were monitored clinically and by ultrasound, endometrium samples were taken by biopsied uterine curettage, and a histopathological examination was performed, completed by immunomarking, whenever necessary. The clinical and paraclinical methods allowed for the creation of a protocol by which patients were monitored. This protocol allowed for the modulation and effectiveness of the treatment, establishing the best therapeutic conduct and the remote supervision.

Keywords: endometrial adenocarcinoma, screening, early diagnosis, hormonal receptors.

Introduction

The endometrium is a variety of epithelial and mesenchymal tissue, with great proliferative and differentiation potential, strong hormone-dependent, subject to cyclic alterations due to steroid hormones in the reproductive period and later under hormonal stimulus, throughout the woman's lifetime. The endometrium shows a very varied pathology, but the most serious is endometrial cancer.

The endometrial cancer occupies the third place among neoplasias encountered in women, after breast cancer and after colorectal cancer. In Eastern Europe, the incidence of endometrial cancer is of 14.6 cases in 100 000 women, and it increases by age [1]. After some studies, it appears that approximately 320 000 new cases and approximately 76 000 patients are diagnosed each year [2, 3].

The improvement of diagnosis methods and the definition of the notion of screening have determined the increase of the number of cases detected in incipient stages [4–6].

The causes of occurrence of endometrial cancer are not fully known, but most risk factors are associated with alterations of the hormonal pattern throughout the woman's life. Estrogens are the main factors proven to have an effect on the proliferation of endometrial cells. Estrogens make bonds in their own receptors at endometrial level and generate cellular proliferation, while progesterone has the opposite effect. Due to the high presence of these types of receptors at malignant tissue level, they are considered to be important therapeutic targets and markers to determine therapeutic variance.

The human epidermal growth factor receptor 2 (HER2) is a member of the family of tyrosine-kinase growth factors that regulate the processes involved in the proliferation and survival of tumor cells. The HER2 path that interacts with the estrogen receptor path is one of the most important physiopathological aspects that are involved, and in the development of endocrine resistance in breast cancer.

The abnormal uterine bleeding is the most frequent clinical symptom in case of endometrial neoplasia. When it occurs in the pre- or peri-menopause period, it is difficult to interpret, as it may be assigned to the deficit of luteal phase [7].

To avoid the delay in setting the diagnosis of endometrial cancer, it is imperative to undergo a fractioned biopsied uterine curettage in all the cases of meno-metrorrhagia occurring in the perimenopause period in women showing high risk, in all the cases of metrorrhagia in menopause and, depending on the clinical examination and on the transvaginal ultrasound (US), in any patient with abnormal uterine bleeding [8]. Clinical data must be completed with those provided by transvaginal US. This may provide information concerning the stage of the intrauterine disease and the probable risk of extra-

uterine dissemination in case of neoplasia. As it is considered to be a method of screening, transvaginal US measures the thickness of the endometrium and may appreciate with accuracy the myometrial invasion [9].

Aim

The aim of this study was to investigate, from the echographic, histopathological (HP) and immunohisto-chemical (IHC) perspective, 38 woman patients diagnosed with metrorrhagia, aged between 35–70 years. We targeted by this study the correlations between the classification criteria based on echography and the HP exam and IHC examinations, allowing an improved prediction of prognostic as well as the substantiation of a standard protocol of diagnostic and supervision of the selected pathology. The evolution of cases was followed under standard hormonal treatment.

⊟ Patients, Materials and Methods

The study group included 38 patients in the interval between July 1, 2014–July 1, 2016, with symptomatology suggesting endometrial cancer, where the US aspects of endometrial thickness were monitored (transvaginal US), as well as the pathological examination of the product obtained by uterine curettage, hysteroscopy or surgical piece. The patients were hospitalized, investigated and treated according to the protocol, at the Department of Obstetrics–Gynecology, Ilfov Emergency County Hospital, Bucharest, Romania. In all the cases, an IHC examination was performed, for estrogen receptor (ER), progesterone receptor (PR), and proliferating cell nuclear antigen (PCNA). For all the antibodies that were used, the immunohistochemistry reactions were analyzed in the glandular epithelium and in the stromal cells.

For ER, PR and PCNA, the positivity index (PI) was calculated, represented by the percentage of positive cells out of the total of counted cells. The cells that had nuclei of doubtless coloration from brown to black were considered positive.

The patients were revaluated every six months.

The study included patients that came to the doctor due to metrorrhagia, aged between 35–70, that showed willingness to undergo the method proposed for monitoring.

Out of the 38 studied cases, 29 have benefited from hormonal treatment (20–40 mg Progesterone/day, 10 days/ month for group A and 40–100 mg Progesterone/day, 15 days/month for group B), three from hormonal and surgical treatment, and six from surgical treatment.

⊟ Results

From the distribution per age groups of the patients taken into account in the study, a maximum incidence was noticed at the age group between 41–50 years old (29 patients, representing approximately 76%).

Out of the 38 samples of endometrium obtained by uterine curettage or by hysteroscopy, the HP examination outlined:
▪ simple hyperplasia without abnormalities – 15 (39.47%) cases;
▪ simple hyperplasia with abnormalities – two (5.26%) cases;
▪ complex hyperplasia without abnormalities – 14 (36.84%) cases;
▪ complex hyperplasia with abnormalities – four (10.52%) cases;
▪ endometrial carcinoma – three (7.89%) cases.

The calculation of PI for ER was higher in the endometrium in the proliferative phase than in the secretory phase.

The highest values of PI for ER were found in the complex hyperplasia without abnormalities (72%), followed by the complex hyperplasia with abnormalities (57%) and simple hyperplasia without abnormalities (50%). In the endometrial carcinoma, PI for ER was of 28%. It is noticed that the complex hyperplasia without abnormalities has the highest level of ER, and the endometrial carcinoma, the smallest.

The analysis of the IHC expression of PR consisted in similar results as those obtained for ER: complex hyperplasia without abnormalities 76% and endometrial carcinoma 24%.

The monoclonal anti-PCNA antibody determines an immunomarking with heterogenic intensity, probably reflecting the phases of the cellular cycle. In order to appreciate the cellular proliferation degree, the PCNA index was calculated, and it was found that the high number of positive cells is correlated with a high histological degree and with an unfavorable prognosis: endometrial carcinoma 36%, complex hyperplasia with abnormalities 34%, complex hyperplasia without abnor-malities 17%, simple hyperplasia with abnormalities 11% and simple hyperplasia without abnormalities 13%.

Out of the 38 studied cases, three groups were differentiated, according to the response to treatment.

Of the 38 cases studied, at the first examination, three groups were differentiated according to the US and HP evaluation. All 38 patients included in the study were assessed every six months. Also, the therapeutic protocol was applied according to the assessment (Table 1).

After six months, the patients were reevaluated. Reevaluation after six months allowed the adjustment of treatment (Table 2):
▪ 14 patients (from group A) were no longer provided hormonal treatment, remaining under clinical observation;
▪ six patients (from group A) were provided treatment with progestative 20–40 mg/day, 15 days/month;
▪ 11 patients (from group B) were provided treatment with progestative 40–100 mg/day, 15 days/month (refusing surgical treatment);
▪ one patient required surgical treatment.

Reevaluation after one year showed the following: After reevaluation, the treatment of the supervised patient supervised was the following (Table 3):
▪ 20 patients (from group A) were no longer provided hormonal treatment, remaining under clinical observation;
▪ nine patients (from group B) were provided treatment with progestative 10 mg/day, 10 days /month, remaining under observation – on next evaluation, the clinical, echographic and HP parameters allowed the stop of hormonal treatment, the patients remaining under clinical observation;
▪ two patients underwent hysterectomy.

Table 1 – *Initial distribution of our patients*

Patients	Group A – 20 cases (20–40 mg Progesterone/day)	Group B – 12 cases (40–100 mg Progesterone/day)	Group C – 6 cases (hysterectomy)
Clinical symptoms	Abundant metrorrhagia	Abundant metrorrhagia	Abundant metrorrhagia
Ultrasound	Endometrium 12–14 mm RI 0.5	Endometrium ≥14 mm RI ≤0.45	Endometrium ≥16 mm RI 0.4
HP exam	Simple and/or complex hyperplasia without abnormalities	Simple and complex hyperplasia with and without abnormalities	Simple and/or complex hyperplasia with abnormalities; endometrial carcinoma
IHC exam	ER high PI PR high PI PCNA low PI	ER high PI PR high PI PCNA high PI	ER low PI PR low PI PCNA high PI

HP: Histopathological; IHC: Immunohistochemical; RI: Resistive index; PI: Positivity index; ER: Estrogen receptor; PR: Progesterone receptor; PCNA: Proliferating cell nuclear antigen.

Table 2 – *Distribution of our patients at six months*

Patients	Group A – 20 cases	Group B – 12 cases	Group C – 6 cases (hysterectomy)
Clinical symptoms	Absent or moderate metrorrhagia	Moderate and abundant metrorrhagia	–
Ultrasound	Endometrium 8–10 mm RI 0.5	Endometrium ≥14 mm RI ≤0.45	–
HP exam	Normal endometrium; simple and/or complex hyperplasia without abnormalities	Simple and/or complex hyperplasia with and/or without abnormalities	–
IHC exam	ER high PI PR high PI PCNA low PI	ER high PI PR high PI PCNA low PI	–

HP: Histopathological; IHC: Immunohistochemical; RI: Resistive index; PI: Positivity index; ER: Estrogen receptor; PR: Progesterone receptor; PCNA: Proliferating cell nuclear antigen.

Table 3 – *Patient distribution one year after the start of treatment*

Patients	Group A – 20 cases	Group B – 11 cases	
		9 cases	*2 cases*
Clinical symptoms	Absent metrorrhagia	Absent or moderate metrorrhagia	Moderate metrorrhagia
Ultrasound	Endometrium 8–9 mm RI 0.5	Endometrium ≥10 mm RI 0.5	Endometrium ≥14 mm RI ≤0.45
HP exam	Normal endometrium	Simple and/or complex hyperplasia without abnormalities	Complex hyperplasia with abnormalities
IHC exam	ER high PI PR high PI PCNA low PI	ER high PI PR high PI PCNA low PI	ER high PI PR high PI PCNA high PI

HP: Histopathological; IHC: Immunohistochemical; RI: Resistive index; PI: Positivity index; ER: Estrogen receptor; PR: Progesterone receptor; PCNA: Proliferating cell nuclear antigen.

⊟ Discussions

As regards the US, regardless of the HP shape, the hyperplasic endometrium appears thickened, hyperechogenic, sometimes with dilatation of endometrial glands that may be suggested by the US aspect [10–12].

Limits beyond normal are represented by an endometrial thickness of 10–12 mm in a genitally active woman in the proliferative phase, and of 15–16 mm in the secretory phase, and less than 5 mm in a woman at menopause [6].

As regards the pre-neoplasic lesions, the thickness of the endometrium may exceed 16 mm in peri-menopause, and 8 mm in menopause [10]. The non-uniform, heterogeneous aspect is a characteristic of malignant lesions.

Because in endometrial hyperplasia (EH), angiogenetic alterations occur, the Doppler US may bring additional data of diagnosis. During the examination of the blood flow, by calculating the resistive index (RI), its average value is identified as 0.5, significantly higher than in relation to the endometrial carcinoma, when the average value of RI is of 0.4 [13].

The two-dimensional (2D) transvaginal US examination and the Doppler represent an initial stage of diagnosis, which allows for the avoidance of invasive examinations, sometimes useless.

The US aspect of the endometrium is a good indicator of EH in women in peri- and post-menopause, allowing for their monitoring especially in case of co-morbidities, such as: diabetes, arterial hypertension, obesity, etc.

The examination by computed tomography (CT) or by nuclear magnetic resonance imaging (MRI) of the pelvis and of the genital apparatus may be necessary to complete the data, providing information on the volume of the ganglions invaded by neoplasm, allowing for the detection of intraperitoneal and remote metastases [14–18].

The endometrial biopsy has great diagnosis sensitivity, representing the election method for the diagnosis of endometrial cancer. It may be performed by several methods [9]: suction, fractioned biopsy curettage, hysteroscopy, hysterectomy–biopsy.

Hysteroscopy is the method that allows for the viewing of the uterine cavity by means of hysteroscope. Its basic principle consists in the artificial distension of the uterine cavity, followed by the global visualization of the uterine cavity and of the cervical duct. The method allows to highlight the lesion, to perform guided biopsy, to evaluate the area extension and to limit the tumor in relation to the uterine isthmus and cervix [7, 19]. The major advantage of the method is that it allows for the early identification of the endometrial adenocarcinoma (ADK), specifying the location of the lesion and highlighting a possible

extension towards the cervix. The main disadvantage of the method is the risk of dissemination of the malignant cells in the peritoneal cavity due to the extension fluid.

The biopsy sample must provide information both on the functional layer, and on the basal layer of the endometrium. As examination methods of those samples, we specify: extemporaneous HP examination, classical examination of sections under paraffin, examination by electron microscopy.

The IHC examination of the endometrium is a method by which the specific antigens are localized in tissues or cells based on the antigen–antibody reaction. This method allows the pathologist to confirm a diagnosis assumed by morphology, and it considerably reduces the series of differential diagnoses. A battery of antibodies will be always used to confirm a diagnosis [20].

The expression of ER and PR is a modern aspect and it is currently considered indispensable for the appraisal of the prognosis of malignancy and for the determination of the therapeutic conduct. Their presence is associated with a better clinical prognosis, even if the association with the existence of metastases or the risk of recurrence has not been studied yet. The high levels of ER and PR are associated with a high survival rate, and the high levels of HER2 indicate an unfavorable prognosis.

In our study, the significant decrease of the ER and PR expression at endometrial level may be suspicious for an evolution towards malignancy. The higher the level of ER or PR, the more efficient may be the treatment with progestative drugs. Also, the high PCNA index is correlated with a high histological degree and with an unfavorable prognosis.

From the IHC point of view, the expression of PCNA was studied at endometrial level during a menstrual cycle and in women in post-menopause. During a menstrual cycle, in the basal layer of the endometrium, a PNCA high PI is noticed in the epithelial cells in the menstrual phase. PI reaches its maximum value in the proliferative phase and it decreases in the secretory phase. No alteration of stromal cells is observed in the basal layer. In the functional layer of the endometrium, the PIs of epithelial cells reach the peak in the late proliferative phase, they decrease in the secretory phase and they remain unchanged afterwards. At post-menopause, PI must be reduced, its higher values being associated to endometrial neoplasia.

The cervicovaginal cytology has reduced sensitivity in the diagnosis of endometrial cancer.

This is why methods of endocavitary cytological sampling were created, by grating or suction, with a much higher sensitivity of diagnosis. The smears of atypical glandular cells with undetermined significance (AGCUS) may suggest endometrial pathology. Most of the times, a positive Babeş–Papanicolau cytology, suggesting an endometrial ADK, is associated with a high risk of extra-uterine dissemination of the disease [21, 22].

⊟ Conclusions

The endometrium may be the location of various pathological processes. The endometrial ADK is a pathology that occurs frequently at perimenopause, and it is less aggressive than breast or cervical cancer, so an early diagnosis and a correct treatment will ensure a good prognosis.

The HP examination is essential in identifying hyperplasia coexisting with ADK or hyperplasia with abnormalities progressing towards carcinoma.

The intra-vaginal US and Doppler US allow a good monitoring of the endometrium.

Immunohistochemistry explains the preservation of the response to hormonal therapy and allows its modulation, trying to differentiate between benign and malignant, achieving prognosis and rendering the hormonal therapy more efficient.

By corroborating HP, IHC, hysteroscopy and imagery data, we established a therapeutic algorithm, which allowed the monitoring of hormonal therapy in patients included in the working batch and of treatment efficiency.

Based on doctor–patient cooperation, one of the types of therapy is established, after explaining all the therapeutic possibilities to the patient, with their advantages and disadvantages.

Conflict of interests

The authors declare that they have no conflict of interests.

Author contribution

Adrian Neacşu and Mădălina Lucia Marcu contributed equally to this work.

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