ORIGINAL PAPER

RJME
Romanian Journal of
Morphology & Embryology
http://www.rjme.ro/

Endometrial carcinomas: correlation between ER, PR, Ki67 status and histopathological prognostic parameters

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Abstract

We present a retrospective histopathological study that included a total of 22 endometrial carcinomas, from patients that were operated in the Obstetrics and Gynecology Clinics of the Emergency County Hospital of Craiova. This current study investigates these cases in order to assess the prognostic value of the correlations between the expression of estrogen and progesterone receptors, Ki67 expression and the histological stage, tumor stage, the degree of myometrial and vascular invasion. The study showed that estrogen and progesterone positive receptors correlate significantly with early stage and well differentiated tumors. The invasion of more than half the thickness of the myometrium and the vascular invasion were associated with decreased expression of the analyzed receptors and an increased proliferation index.

Keywords: endometrial carcinomas, ER, PR, Ki67.

Introduction

The endometrial carcinoma is the most common malignant tumor of the female genital tract, with an increasing incidence in socio-economic developed countries [1, 2]. The investigation of the immune markers that are involved in the endometrial carcinogenesis and establishing possible correlations between the studied parameters, may influence the early detection and treatment of these important lesions [3, 4].

The endometrial carcinoma is formed and develops in close relation to the plasma and tissue levels of sex steroidal hormones and their receptors. Also, the connection with the atypical endometrial hyperplasia is recognized, associated with a prolonged estrogen stimulation, of endogenous or exogenous origin, not being counterweight by progesterone [4, 5].

Historically, estrogen has been seen as a direct promoter of endometrial carcinogenesis *via* the stimulation of rapid proliferation of epithelial cells, which is confirmed by the results of several studies [5, 6].

The estrogen, on the one hand, can bind to nuclear estrogen receptors (ER), thus initiating the gene expression, and on the other hand, the estrogen can increase the mutational rate by stimulating cell proliferation [7, 8]. Also, the expression of the progesterone receptors (PR) completes the picture for the hormonal levels in endometrial carcinoma, representing independent prognostic factors in several studies, alongside with the Ki67 proliferation index [9, 10].

Out of the histological parameters with prognostic potential, the tumor stage, the tumor grade and the extent of myometrial and vascular invasion proved most useful in this regard [11, 12].

The purpose of this study was to analyze the immunoexpression of ER, PR and Ki67 in endometrial carcinomas, according to the histopathological parameters with prognostic value.

Materials and Methods

We performed a retrospective study, in which we used post-hysterectomy specimens from 22 patients operated in the Obstetrics and Gynecology Clinics of the Emergency County Hospital of Craiova, that were histopathologically diagnosed as endometrial carcinomas.

The specimens were processed for histopathological and immunohistochemical examination. The histological preparation was performed by the classical method for inclusion in paraffin, followed by Hematoxylin–Eosin staining. The histopathological analysis was used in histological staging, and also for the assessment of myometrial and vascular invasion, the staging process following the FIGO system. The degree of the myometrial invasion was expressed as a percentage of the thickness of the myometrium.

The immunohistochemical analysis was performed on serial sections, using an immunoenzymatic soluble

complex method. The staining system was the LSAB+ System-HRP (code K0690, Dako). The antibodies that were used for the specific antigen retrieval are shown in Table 1.

Table 1 – *Antibodies used, clone, dilution and antigen unmasking*

Antibody	Clone	Dilution	Antigen retrieval
ER	1D5	1:50	
PR	pgr636	1:50	EDTA, pH 9
Ki67	MIB1	1: 100	

The immunostaining of the estrogen receptor (ER) and progesterone (PR) was determined by calculating the positivity index (PI) which represented the percentage of positive cells per 1000 cells counted on 40× power field. The cases in which the nuclear marker was present in at least 5% of cells were considered positive [4].

In the current study, only the epithelial nuclear receptor expression was assessed, and not of the stromal receptors. Ki67 immunoreactivity was quantified with the proliferation index, the percentage reporting the number of marked cells by the total number of cells counted in 40× power field [13]. The threshold for significant expression of Ki67 proliferation index was 15% of the normal glandular endometrium, which is in a proliferative phase [14]. DAB (3,3'-diaminobenzidine tetrahydrochloride) was used to visualize the reaction, followed by counterstaining with Hematoxylin.

Also, a negative external control was used by omitting the primary antibody and positive external control, represented by sections of breast carcinoma. Image acquisition was performed with Nikon Eclipse E600 with Lucia 5 software. Statistical analysis used the results of chi-square test, Student *t*-test and the Pearson index.

⮐ Results

The histopathological analysis showed the presence in the selected batch of 11 cases in which well differentiated endometrial carcinoma was observed (G1), seven cases moderately differentiated (G2) and four cases of poorly differentiated endometrial carcinoma (G3) (Figure 1).

The myometrial invasion was limited to the internal half of the thickness of the myometrium in eight cases; in three cases, the invasion was present in half of its external layer, reaching the serous and the cervix in five and six cases, respectively. Thus, we found 11 cases in stage I, six cases in stage II, and five cases in stage III of endometrial carcinoma.

Vascular space invasion was present in four cases, associated with moderately and poorly differentiated tumors.

The analysis of the hormone receptors indicated the presence of ER in 54.5% of cases investigated and of the PR in 68.1% of them. Thus, the immunoexpression ER was present in 19 cases (86.3%) and absent in three cases (13.4%), while the immunoexpression PR was present in 18 cases (81.1%) and absent in four cases (18.9%) (Table 2).

Table 2 – *Positivity index (PI) and the number of positive cases for ER and PR, according to the tumor grade, the myometrial invasion and the tumor stage*

Positivity index	IR-ER [%]/number of positive cases	IR-PR [%]/number of positive cases	IP-Ki67 [%]
Tumor grade			
G1	39/11	49/11	16
G2	22/6	28/5	24
G3	9/2	12/2	39
Myometrial invasion			
Internal half	41/8	51/8	15
External half	33/3	43/3	18
Tumor stage			
I	39/11	49/11	16
II	18/6	22/6	26
III	5/2	5/1	34

The negative cases for both receptors corresponded to moderate endometrial carcinoma (one case) and poorly differentiated endometrial carcinoma (two cases).

ER and PR immunoreactivity was identified at the nuclear level on the positive external control sections and on those analyzed. The average values of the positivity index according to IP-ER/PR in the analyzed histopathological parameters are shown in Table 2.

Well-differentiated tumors had a higher number of receptors for estrogen and progesterone, which was not the case in poorly differentiated tumors. Thus, G1 IP-ER was on average 39% in endometrial carcinomas, in G2 forms of IP-ER endometrial carcinoma it was 22%, while in G3 endometrial carcinomas this index was only 9% (Figure 2).

PR-IP analysis showed in the G1 endometrial carcinomas an IP-PR average value of 49%, in the forms of IP-PR G2 endometrial carcinomas it was 28%, while the index in G3 endometrial carcinoma was only of 12% (Figure 3).

The statistical analysis showed a significant correlation between IP-ER/PR and tumor grade, especially for PR ($p < 0.05$ and $p < 0.005$).

The index of positivity for estrogen and progesterone was determined considering the depth of myometrial invasion. The endometrial carcinomas that reached the internal half of the myometrium had higher IP-ER/PR values compared with those that have reached the external half (41/51% *vs.* 33/43%).

Regarding the immune expression of ER and PR, according to the tumor stage, a decrease in value was noted in the positivity index for the tumors in stage III (5%) compared to stage II (IP-ER/PR: 18/22%), and first stage tumor (IP-ER/PR: 39/49%), respectively. The statistical analysis showed significant correlations of IP-ER/PR with the tumor stage, especially in the case of PR ($p < 0.05$ and $p < 0.005$).

The immune expression of Ki67 was identified at the nuclear level in the positive external control sections and in all the examined cases, the proliferation index values ranging between 11% to 42%. The average values of Ki67 proliferation index based on the histopathological parameters analyzed are shown in Table 2.

We found that Ki67 proliferation index had on

average the highest values in poorly differentiated carcinomas (39%) with the invasion of the external half of the myometrium (18%), and those in stage III tumor (34%). Statistical analysis showed a significant correlation with the degree of differentiation marker ($p<0.005$) and stage of lesion ($p<0.005$) (Figure 4).

For the tumors that have invaded the vascular space, the IP-ER/PR values were negative or reduced. The ER and PR expression was present in all cases that showed no vascular invasion. Also, IP-Ki67 was higher in the tumors that had vascular invasion (35%) compared with those in which this issue was not present (20%). Chi-square test showed a statistically significant correlation of the vascular invasion with IP-PR ($p<0.005$), IP-Ki67 ($p<0.005$) and insignificant with IP-ER ($p>0.05$).
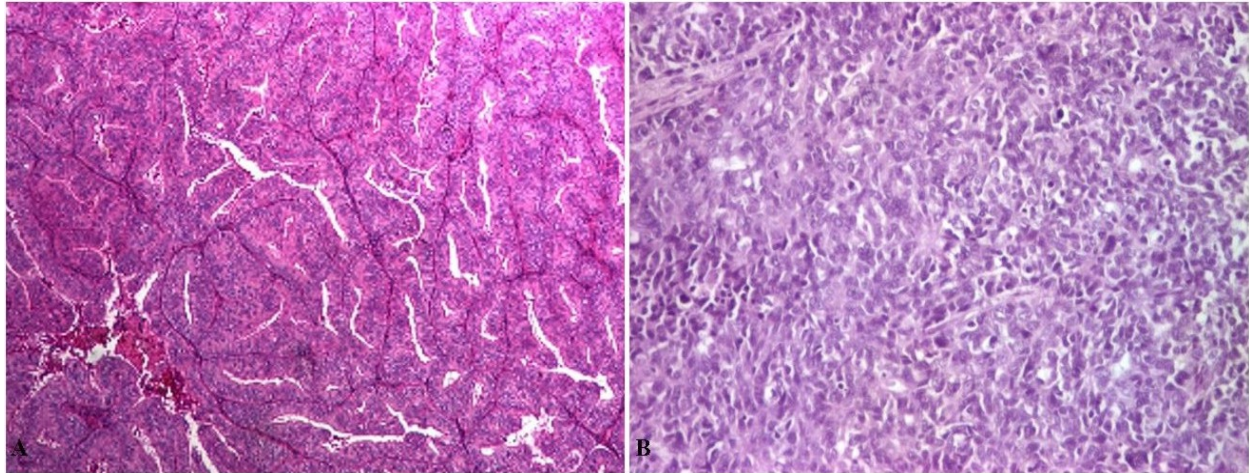

Figure 1 – *(A) Well-differentiated endometrial adenocarcinoma, HE stain, ×100. (B) Poorly differentiated endometrial adenocarcinoma, HE stain, ×100.*
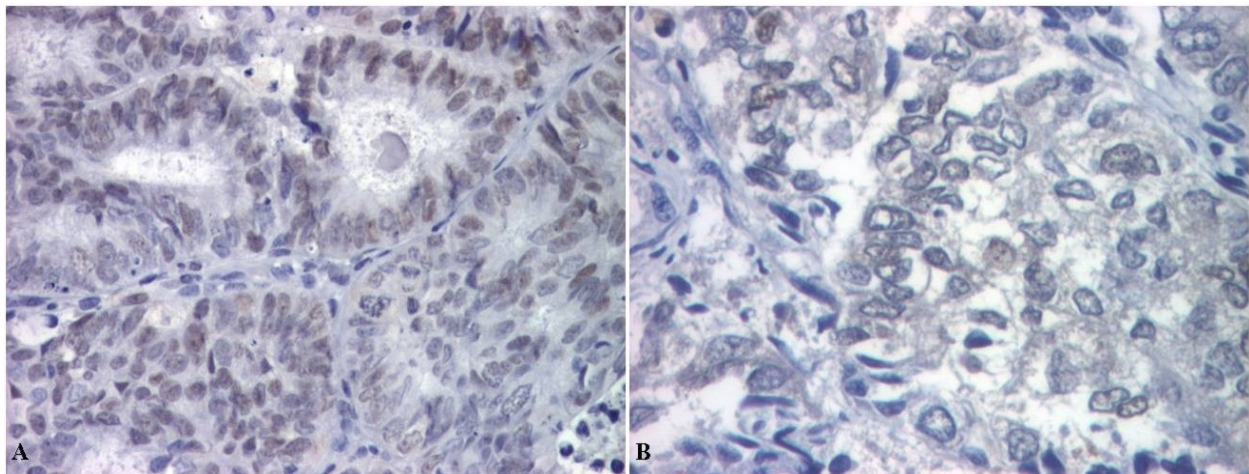

Figure 2 – *Immune marking of ER, ×200. (A) Well-differentiated endometrial adenocarcinoma, IP-ER: 45%. (B) Poorly differentiated endometrial adenocarcinoma, IP-ER: 12%.*
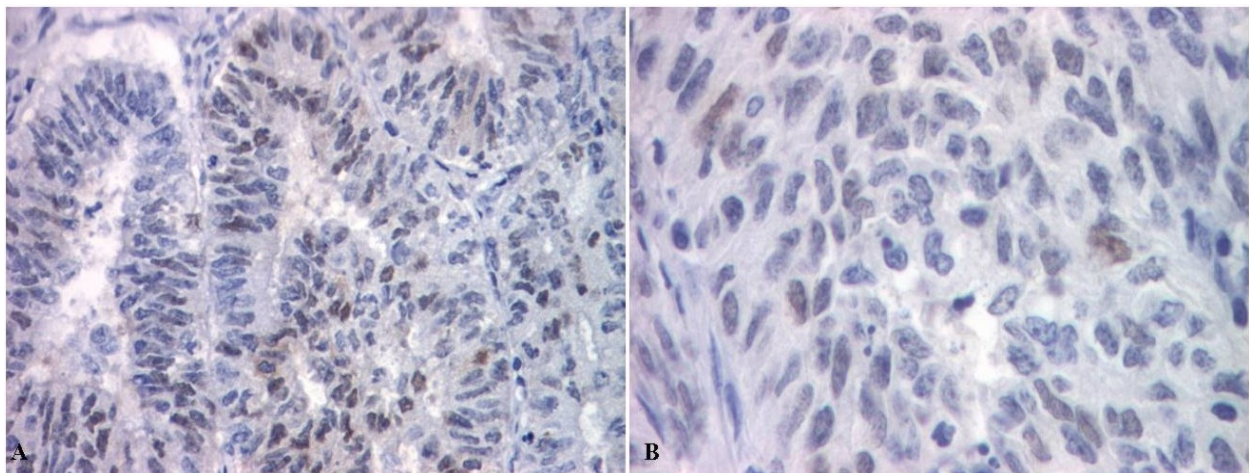

Figure 3 – *Immune marking of PR, ×100. (A) Well-differentiated endometrial adenocarcinoma, IP-PR: 54%. (B) Poorly differentiated endometrial adenocarcinoma, IP-PR: 17%.*
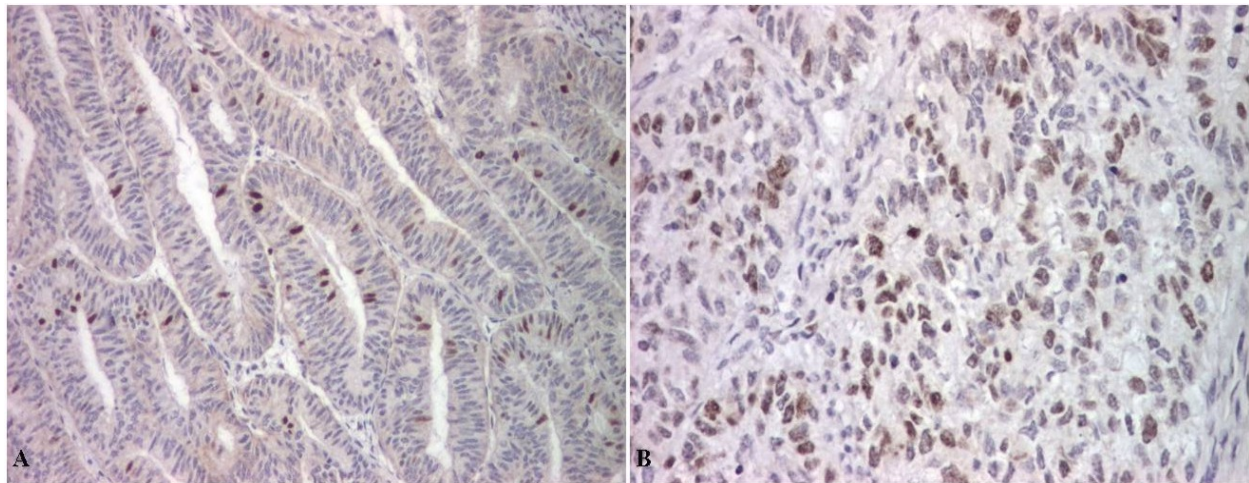
Figure 4 – *Immune marking of Ki67, ×100. (A) Well-differentiated endometrial adenocarcinoma, IP-Ki67: 10%. (B) Poorly differentiated endometrial adenocarcinoma, IP-Ki67: 52%.*

In our study, we found a positive linear variation for ER and PR positivity index (Pearson index 0.95), and a negative linear variation with a Ki67 proliferation index (Pearson index -0.90, -0.88 respectively) (Figure 5).
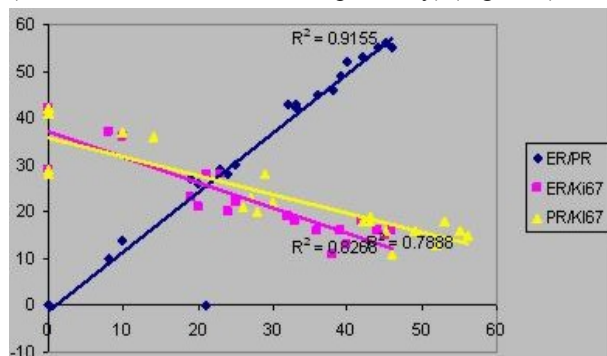

Figure 5 – *Linear variation of IP-ER/PR and IP-Ki67.*

Student *t*-test also showed a significant correlation between ER and PR expression ($p < 0.005$), no correlation being present between ER and Ki67 ($p > 0.05$) or PR ($p > 0.05$).

⊟ Discussion

The endometrial carcinoma is one of the malignant tumor lesions, for which there are histopathological parameters that have definite prognostic value. Thus, numerous studies in the literature consider the stage and histopathologic tumor grade as being the most relevant features for subsequent therapeutic management [11, 12]. Other studies have assigned the depth of the myometrial invasion and the vascular invasion as being prognostic parameters in terms of survival and recurrence rate of the endometrial carcinomas [10, 15]. In our study, well-differentiated carcinomas corresponded to lesions in stage I, compared with the moderately or poorly differentiated ones, in advanced stages. Also, vascular invasion and of the external half of the myometrium, were identified in advanced tumor stages.

The hormone receptor state, alongside with the histopathological features in the tumor tissue are important prognostic factors. They are ligand-dependent transcription factors and belong to a superfamily of steroid nuclear receptors [16]. Data from literature indicates the positivity for ER and PR in 35–90% of the endometrial carcinomas [13, 16–18].

In our study, ER and PR were positive in 86.3% and 81.1% of the analyzed cases. The positivity index values for estrogen and progesterone decreased with the degree of differentiation, with the myometrial invasion and the stage of the tumor, with significant statistical correlation in this respect ($p < 0.05$). Chi-square test value was higher in statistical correlation in the case of the progesterone receptors, being also the only hormone immune marker whose value was correlated with the vascular invasion.

Many studies have found that the hormone dependence, and thus the response to the hormonal therapy or the chemotherapy for the endometrial carcinoma decreases in aggressive tumors, the survival rate improves at every stage in the case of the patients with receptor-positive tumors compared with tumors that are receptor-negative [4, 7, 13, 17].

Similar studies found the correlation of the hormone receptors content (estrogen and progesterone) with several histopathological features, and especially the tumor differentiation. The well-differentiated tumors are more frequently positive for the estrogen and the progesterone receptors than the poorly differentiated lesions [19]. The same results were obtained by other authors, which by making a comprehensive statistical analysis showed that the ER expression is decreasing in the highly differentiated carcinomas with a statistically significant difference to those with low differentiation [20–22].

Other studies have researched the existence of a demonstrable correlation between the estrogen and the progesterone receptor status and the staging of the endometrial cancer. The authors reached the following conclusions: for the stage I endometrial cancers, 65% of cases are ER/PR positive; for the stage II endometrial cancers, 50% of cases are ER/PR positive; for the stage III endometrial cancer 17% of cases are ER/PR positive and stage IV endometrial cancers are ER/PR negative [23]. Likewise, Ferrandina G *et al.* (2005) [24] found that ER and PR positive tumors had a statistically

significant association with the clinicopathological parameters, which correlates with a more favorable prognosis. In addition, the hormone receptor status appears to correlate with the tumor response to the progesterone therapy. This finding may be of particular clinical importance, since almost half of poorly differentiated endometrial carcinomas contain estrogen/ progesterone receptors and progesterone therapy could be beneficial in these cases [25].

Other studies dispute the importance of the ER and PR immune expression, which failed to show direct correlations with the tumor grade or the stage of the differentiation, but only with the myometrial and the vascular invasion [4, 26, 27]. Some authors also suggest a better prognosis impact separately for the ER and PR expression, comparing these with tumor hormonal status viewed as a whole, while others think that only some isoforms ER and PR correlate significantly with the histopathologic parameters that have a prognostic value [16, 28]. Moreover, other studies question the full function of the hormone receptors identified by immunohistochemistry methods, but these studies recognize the prognostic value of the tumor hormonal status [18].

The study showed a significant correlation between the immune expression of the estrogen and the progesterone receptors, the result being similar to the one of other studies [4].

Ki67 antibodies recognize a nuclear antigen of the cell proliferation l, which has been shown in many studies to reflect the tumors aggressiveness.

In our study, Ki67 proliferation index was significantly correlated with the tumor grade, the myometrial invasion and so with the stage and the vascular invasion. Salvesen HB *et al.* (1999) [29] found that survival for endometrial carcinoma is significantly related to Ki67 expression, low survival rates were present in the category of the tumors with the highest Ki67 values (>35%). Gassel AM *et al.* (1998) [30], investigating 224 cases, found a highly significant correlation between the percentage of the proliferating tumor cells and the survival, independent of other associated factors such as tumor grade and stage.

The finding corresponds with other studies that have found significant correlation of the antigens expression associated to the proliferation (PCNA and MIB-1) with the survival [31, 32].

⊡ Conclusions

Our study certifies the prognostic importance, the therapeutic aspect of investigating the hormonal status and the proliferation in the endometrial carcinomas, considering the direct correlation between the level of the hormone receptors and the Ki67 index in relation to the grade, the myometrial and the vascular invasion and the tumor stage. The value of the statistical significance was higher for the progesterone *vs.* the estrogen receptors. We found a significant positive and important linear variation between the hormone receptors, and a negative and insignificant correlation between them and the Ki67 proliferation index.

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