Selected Immunohistochemical Prognostic Factors in Endometrial Cancer

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Objective: The objectives of this study were to assess the immunohistochemical expression of *p53*, *bcl-2*, *c-erbB-2*, Ki-67, estrogen (ER) and progesterone (PR) receptors, matrix metalloproteinase-7 and -26 (MMP-7 and MMP-26) in endometrial cancer patients and to assess the relation between steroid receptor positivity and other markers.

Design: Experimental prospective study.

Setting: Department of Obstetrics and Gynecology, Department of Genetics, Department of Pathology, Palacký University Medical School and University Hospital Olomouc.

Methods: We studied 144 cases of primary untreated endometrial carcinoma in which the *p53*, *bcl-2*, *c-erbB-2*, Ki-67, ER, PR, MMP-7, and MMP-26 antigens were investigated with the use of immunohistochemical methods. We evaluated the correlations among immunohistochemical staining and the age, International Federation of Gynecology and Obstetrics stage, grading, depth of invasion, and metastatic spread to lymph nodes.

Results: Mean age was 65.7 years (range, 34–90 years). *p53*, *bcl-2*, *c-erbB-2*, Ki-67, ER, and PR were positive in 35 (24.3%), 100 (69.4%), 41 (28.4%), 65 (45.1%), 115 (79.8%), and 127 (88.1%) cases, respectively. Matrix metalloproteinases were evaluated in a group of 70 patients, wherein MMP-7 was positive in 33 patients (47.1%) and MMP-26 was positive in 40 patients (57.1%). The expression of MMP-7 decreased with higher patient age. *p53* and Ki-67 overexpression was found to be related to poor differentiation. Immunostaining for *bcl-2* correlated with the positivity of steroid receptors status, whereas immunostaining for *c-erbB-2* correlated inversely with ER-positive group of cases.

Conclusions: The overexpression of *p53* and Ki-67 seems to indicate a more malignant phenotype, whereas *bcl-2* expression in dependence of steroid receptor positivity could contribute to the identification of high-risk tumors.

Key Words: Endometrial cancer, Immunohistochemistry, Prognostic factors

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Gynecologic malignant tumors represent approximately 15% of all female tumors in the Czech Republic. Its incidence is rising worldwide. In 2005, the incidence in the Czech Republic reached 33.2 per 100,000 women, which, in absolute numbers, represents 1739 women.[1]

The reasons for the increasing incidence are multifactorial. At diagnosis, the course of the disease is dependent on age, histological type, status of hormonal receptors, clinical stage, and other factors (dietary and hormonal influences and increasing life expectancy in the female population).[2] Among the most important risk factors are prolonged unopposed endometrial stimulation, obesity, age, nulliparity, diabetes, hypertension, tamoxifen therapy, and, in many cases, their combination.[2–6]

To improve treatment and dispensary of patients with endometrial cancer, a number of prognostic factors were studied. Well-known and routinely used prognostic factors include

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age, disease stage, histological type, histological grade, depth of myometrial invasion, and lymph node involvement.[2,3,7,8] Currently, great efforts are being made to identify high-risk groups, which could enable conservative therapy in patients with good prognosis as well as reserve effective and more radical treatment for patients with more aggressive forms of the disease.

An abnormally increased expression of the *p53* tumor suppressor gene in endometrial cancer correlates with aggressive histological types, advanced disease stage, and decreased survival.[9–12]

Increased expression of oncogene *bcl-2* is described in endometrial hyperplasia; on the contrary, loss of expression is associated with a worse prognosis, greater depth of myometrial invasion, a more advanced disease stage, and a greater probability of lymph node metastases.[12–15]

Amplification and increased expression of oncogene *c-erbB-2* (HER-2/*neu*) is present in 10% to 40% of endometrial carcinomas and correlates with a worse prognosis and more aggressive tumor behavior.[16,17]

One of the most common markers of cell proliferation is Ki-67. Most endometrial carcinomas express a low Ki-67 proliferation index and have a good prognosis, although most serous and clear cell tumors have a high proliferation index. Correlation with grading, disease stage, and histopathological tumor type has been confirmed in numerous studies.[18–20]

It seems that estrogen (ER) and progesterone (PR) expression plays a significant role in endometrial carcinogenesis. Based on the results of numerous studies, the expression of these receptors is associated with well-differentiated tumors and correlates with tumor stage and survival.[18,21–23]

Matrix metalloproteinase 7 (MMP-7) was immunolocalized in tumor cells in 73% of endometrial cancer cases, and higher levels were associated with a more advanced disease stage and the presence of lymph node metastases.[24]

High levels of MMP-26 were described in hyperplastic endometrium, whereas its expression in endometrial cancer decreases with histological dedifferentiation. Similarly to MMP-7, this enzyme is also limited to only epithelial and tumor cells of the endometrium.[25] The significance of MMP-7 and MMP-26 for the prognosis of women with endometrial cancer has not been studied to date.

There is an effort to use the information obtained from changes in the expression of tumor biomarkers to decrease the radicality of surgical and radiation therapy. The aim of this work was to assess the immunohistochemical expression of molecular biological markers, the relation between steroid receptor positivity and other markers, and the correlation of all biomarkers with clinicopathological characteristics of endometrial cancer.

PATIENTS AND METHODS

Characteristics of the Patient Set

Between September 2001 and March 2009, 154 cases of primarily untreated endometrial cancer were studied at the Department of Obstetrics and Gynecology at the University Hospital Olomouc. Postoperative staging was performed using the International Federation of Gynecology and Obstetrics (FIGO) classification (1988). Detailed characteristics of the patient set are shown in Table 1. The mean age of the patients was 65.7 years (range, 34–90 years). The group of 10 rare samples (6.5%) consisted of 6 cases of papillary serous adenocarcinoma, 2 cases of clear cell carcinoma, 1 carcinosarcoma, and 1 stromal sarcoma, which were all excluded from the study.

Abdominal hysterectomy with bilateral adnexectomy was performed on all patients. Systematic pelvic lymphadenectomy was performed in 107 high-risk patients (69.4%).

Postoperatively, high-risk patients underwent actinotherapy and brachytherapy based on individual choice at the Oncology Department at the University Hospital Olomouc.

Our study was confirmed by the ethical committee, and all patients obtained and signed the informed consent.

Immunohistochemistry

The processed material included endometrial cancer samples obtained from the abdominal hysterectomy after previous diagnosis confirmation from diagnostic curettage. The immunohistochemical analysis of all tissue sections was done by one pathologist (M.D.) to reduce the amount of error in interpretation. The samples were processed based on standard after a 24-hour fixation in 10% formaldehyde and embedded into paraffin blocks followed by routine staining with hematoxylin-eosin to establish histopathological diagnosis. Samples with sufficient amounts of well-preserved

TABLE 1. Clinicopathological characteristics of endometrial cancer patient set

Characteristic	No. Patients (%)
All cases	154
Age, yr	
<65	79 (51.2)
>65	75 (48.7)
FIGO stage	
I	105 (68.1)
II	28 (18.1)
III–IV	21 (13.6)
Histological type	
Endometrial carcinoma	144 (93.5)
Rare	10 (6.5)
Grade	
1–2	120 (77.9)
3	34 (22.0)
Myometrial invasion	
Not present/<50%	91 (59.0)
>50%	63 (40.9)
Lymph node involvement	
Negative	130 (84.4)
Positive	24 (15.5)

tumor structures were then selected for subsequent immunohistological examination and processed into tissue slices 5 to 8 μm thick on Vectabond (Vector)-coated slides. For the detection of individual markers, a standard indirect immunohistochemical technique was used with a set of rabbit or mouse antibodies (for *p53*, *c-erbB-2*, Ki-67, ER, and PR, DAKO; for *bcl-2*, BIOGENEX; for MMP-7 and MMP-26, Abcam).[24–28,30] The analysis was performed with positive controls.

Quantification of positivity was expressed in percentages. The median value was the established positivity limit for *p53*, *bcl-2*, *c-erbB-2*, Ki-67, MMP-7, and MMP-26. Samples with nuclear staining of at least 20% of tumor cells were considered *p53*- and *bcl-2*–positive, and samples with 40% or higher were considered Ki-67–positive. Samples with c-erbB-2 membrane positivity were considered positive in 10% and higher, and the positivity limit for MMP-7 was set at 65% and higher, whereas that for MMP-26 was set at 40% and higher. The positivity limit for ER and PR was set to 5% based on numerous previous studies.[29–31]

Statistical Evaluation

The χ^2 test and Fisher exact test were used to evaluate the relation between clinicopathological parameters and immunohistochemical markers as well as the relation between immunohistochemical markers and status of hormonal receptors. The level of statistical significance was established at $P < 0.05$. The statistical program SPSS version 15 (SPSS, Inc, Chicago, IL) was used to process the results.

RESULTS

Correlation With Clinicopathological Parameters

In Table 2, we present the distribution of positive immunohistochemical staining in relation to individual clinicopathological parameters in 144 cases of endometrial cancer. Matrix metalloproteinase-7 and -26 were evaluated in only 70 patients with endometrial cancer.

A statistically significant dependence between grading and *p53* was observed. In the group with grade 3 tumors, there was a significantly greater *p53* positivity compared with the groups with grades 1 and 2 (42.3% vs 20.3%, $P = 0.018$). Similarly, in the group of Ki-67–positive tumors, correlation with grading was noted; in the group with grade 3 tumors, a significantly higher Ki-67 positivity was seen compared with the groups with grade 1 and 2 tumors (65.3% vs 40.6%, $P = 0.022$). The only marker correlating with the patient's age was MMP-7; in the group of patients younger than 65 years, a significantly higher positivity of this marker was observed compared with the patients older than 65 years (52.2% vs 38.4%, $P = 0.027$).

Although no statistically significant dependence among *bcl-2*, *c-erbB-2*, ER, PR, MMP-26, and any clinicopathological parameter was observed, we observed a trend of increased *c-erbB-2* and Ki-67 expression when myoinvasion exceeded 50% of myometrial thickness. On the contrary, with PR, a trend of decreased expression was observed in clinically

TABLE 2. Correlation of individual immunohistochemical markers with clinicopathological parameters

	Total No.	*p53*, n (%)	*bcl-2*, n (%)	*c-erbB-2*, n (%)	Ki-67, n (%)	ER, n (%)	PR, n (%)	Total No.	MMP-7, n (%)	MMP-26, n (%)
All endometrial carcinoma	144	35 (24.3)	100 (69.4)	41 (28.4)	65 (45.1)	115 (79.8)	127 (88.1)	70	33 (47.1)	40 (57.1)
Age <65 yr	76	16 (21.0)	53 (69.7)	25 (32.8)	34 (44.7)	60 (78.9)	67 (88.1)	44	23 (52.2)	22 (50.0)
Age >65 yr	68	19 (27.9)	47 (69.1)	16 (23.5)	31 (45.5)	55 (80.8)	60 (88.2)	26	10 (38.4)	18 (69.2)
P		0.336	0.936	0.214	0.918	0.773	0.989		0.027	0.740
FIGO stage I–II	128	31 (24.2)	91 (71)	38 (29.6)	57 (44.5)	104 (81.2)	115 (89.8)	63	29 (46.0)	37 (58.7)
FIGO stage III–IV	16	4 (25)	9 (56.2)	3 (18.7)	8 (50)	11 (68.7)	12 (75)	7	4 (57.1)	3 (42.8)
P		0.754	0.255	0.558	0.679	0.317	0.099		0.762	0.557
Grade 1–2	118	24 (20.3)	82 (69.4)	34 (28.8)	48 (40.6)	97 (82.2)	107 (90.6)	57	27 (47.3)	33 (57.8)
Grade 3	26	11 (42.3)	18 (69.2)	7 (26.9)	17 (65.3)	18 (69.2)	20 (76.9)	13	6 (46.1)	7 (53.8)
P		0.018	0.979	0.847	0.022	0.135	0.085		0.937	0.790
Myometrial invasion <50%	87	17 (19.5)	59 (67.8)	20 (22.9)	34 (39)	71 (81.6)	77 (88.5)	45	18 (40.0)	25 (55.5)
Myometrial invasion >50%	57	18 (31.5)	41 (71.9)	21 (36.8)	31 (54.3)	44 (77.1)	50 (87.7)	25	15 (60.0)	15 (60.0)
P		0.100	0.600	0.072	0.071	0.518	0.886		0.108	0.719

TABLE 3. Distribution of immunopositivity of *p53*, *bcl-2*, *c-erbB-2*, Ki-67, and MMP in association with hormonal receptor status

	Total No. (N = 144)	*p53*, n (%)	*bcl-2*, n (%)	*c-erbB-2*, n (%)	Ki-67, n (%)	Total No. (N = 70)	MMP-7, n (%)	MMP-26, n (%)
ER status								
Negative	29	5 (17.2)	9 (31.0)	3 (10.3)	9 (31.0)	8	6 (75.0)	6 (75.0)
Positive	115	26 (22.6)	88 (76.5)	35 (30.4)	54 (46.9)	62	27 (43.5)	34 (64.8)
P		0.530	<0.0001	0.028	0.122		0.136	0.452
PR status								
Negative	17	7 (41.1)	4 (23.5)	2 (11.7)	7 (41.1)	5	3 (60.0)	2 (40.0)
Positive	127	28 (22.0)	96 (75.5)	39 (30.7)	58 (45.6)	65	30 (46.1)	38 (58.4)
P		0.128	<0.0001	0.152	0.727		0.661	0.645

P value < 0.05.

advanced tumors (FIGO stage III-IV) and in poorly differentiated tumors (grade 3).

p53, *bcl-2*, *c-erbB-2*, Ki-67, and MMP in Association With Hormonal Receptor Status

As seen in Table 3, no significant difference in staining positivity of *p53*, Ki-67, MMP-7, and MMP-26 dependent on ER or PR positivity was observed. On the contrary, the percentage of *bcl-2*–positive endometrial tumors was significantly higher in the ER-positive group than in the ER-negative group (76.5% vs 31.0%, $P < 0.0001$). Also, a significantly greater *bcl-2* positivity was seen in the PR-positive group than the PR-negative group (75.5% vs 23.5%, $P < 0.0001$). Surprisingly, a statistically significant dependence between the ER group and *c-erbB-2* was discovered, where the ER-positive group had a significantly higher positivity of this marker compared with the ER-negative group (30.4% vs 10.3%, $P = 0.028$).

DISCUSSION

Our results show correlation between certain biological markers and clinicopathological prognostic factors in primary endometrial cancer.

Elevated *p53* expression significantly correlated only with poor differentiation of endometrial tumors. A number of works confirm an association between elevated *p53* expression and unfavorable prognostic factors in women with primary endometrial cancer.[12,13,20,32] Mariani et al[17] described *p53* as the only molecular marker able to predict distant metastases independent of other histopathological, molecular, and cytokinetic parameters. Furthermore, various authors confirm a much higher *p53* expression in serous tumors and clear cell tumors than in endometrial cancer, which support the hypothesis on the mutation of gene *p53* as a late event in endometrial cancer and, on the contrary, an early event during the development of rare endometrial tumors.[21,33,34] The results of Halperin et al[21] regarding *p53* immunoreactivity in grade 3 tumors and papillary serous tumors demonstrate the uniqueness of grade 3 tumors, which are histopathologically more similar to papillary serous tumors than to endometrial cancer. Grade 3 tumors probably follow a different path of carcinogenesis than grades 1 and 2 tumors. Our results are in accordance with the data of Halperin et al and Inoue,[35] although we have not included papillary serous tumors in our study.

The antiapoptotic gene *bcl-2* regulates programmed cell death and thus lengthens cell survival that aids in the spread of the tumor process. A number of studies have confirmed that *bcl-2* expression increases in endometrial hyperplasia and is decreased in endometrial cancer. This loss of expression correlates with worse prognosis, worse clinical stage, depth of myometrial invasion, and lymph node involvement.[12–15,36] The relation between loss of *bcl-2* expression and biological aggressiveness of endometrial cancer seems paradox; the mechanism is not yet fully understood. Similarly to Appel et al,[37] we did not observe a correlation between *bcl-2* expression and degree of tumor differentiation, depth of myometrial invasion, and lymph node involvement. In accordance with works by other authors, we demonstrated a significantly positive correlation between *bcl-2* expression and positivity of hormonal representation of ER and PR,[14,21,36] which could be an important prognostic factor for a negative prognosis.

An elevated expression of Ki-67 indicates increased cellular mitotic activity and proliferation. A number of studies have shown that Ki-67 is an independent prognostic indicator of survival.[9,20,38,39] On the contrary, Pansare et al[40] did not show any correlation among Ki-67, histological type, grade, and clinical stage of the disease. An elevated Ki-67 expression in our work significantly correlated with poor differentiation, and we also observed a trend of elevated expression in tumors with deep invasion. Our results are partly in accordance with works by Lax et al[11] and Salvesen et al,[19] who demonstrated a correlation among elevated expression of Ki-67 with grading, depth of myometrial invasion, and risk of recurrence.

The fact that increased expression of the oncogene *c-erbB-2* correlates with worse prognosis has already been confirmed in various malignant tumors. According to certain authors, increased expression correlates with grading, depth of myometrial invasion, and advanced disease stage.[41–44] Recently in their extensive study (483 cases), Morrison et al[45] demonstrated that the increased expression of *c-erbB-2* as an independent prognostic factor correlated with worse survival. Our work did not confirm a significant dependence with traditional prognostic factors of endometrial cancer, similarly to Coronado et al[46] or Czerwenka et al.[47] Nonetheless, a trend of increased expression with deep invasion was observed, which correlates with the abovementioned study results. On the contrary, in terms of *c-erbB-2* expression independent of hormonal receptor status, a significantly higher expression in the ER-positive group was observed. Owing to the often-opposing study results, the use of this factor remains ambiguous.

Estrogen and progesterone receptors are present in both normal endometrial tissue and endometrial cancer. Based on the results of various authors, the presence and the amount of steroid receptors correlate with the clinical stage of the disease, the histological grade, and survival. The absence of steroid receptors is considered a negative prognostic factor for aggressive growth and poor prognosis.[18,22,48,49] Expression of ER and PR in our work did not reach statistical significance independent of clinical stage, grading, myometrial invasion or metastatic spreading; however, a trend of inverse correlation between PR and clinical stage III to IV and poor tumor differentiation is apparent. This result is in accordance with the abovementioned works; in addition, it seems that PR may be a stronger prognostic factor than ER, as supported by other authors.[23,49,50]

An important member of the family of metalloproteinases with epithelial expression is MMP-7 (matrilysin-1), whose expression was captured in both normal and malignant epithelial cells. There are a limited number of published studies involving the expression of MMP-7 in endometrial cancer. Ueno et al[24] demonstrated that increased MMP-7 expression correlated with a worse clinical disease stage and with the presence of lymphatic metastases. A similar trend was described by Graesslin et al[51] and Wang et al.[52] Our work showed a statistically significant dependence only between age and MMP-7, that is, in patients older than 65 years, the expression of MMP-7 was significantly lower.

Another member of the subfamily of matrilysin enzymes was described as MMP-26 (matrilysin-2). Matrix metalloproteinase-26 is also expressed in various tissues, including endometrial cancer. It has been established that MMP-26 expression specifically fluctuates during the menstrual cycle. Findings of elevated levels midcycle and in hyperplastic endometrium and, on the contrary, low levels in the late phase of the cycle and in endometrial cancer point to an association with estrogen receptors. Isaka et al[53] and Pilka et al[25] demonstrated a significantly decreased MMP-26 expression in endometrial cancer, which is in discordance with the results of Tunuguntla et al,[54] who described an increased immunohistochemical expression of MMP-26 in poorly differentiated endometrial cancer. Our study did not show dependence on classic prognostic factors of endometrial cancer.

CONCLUSIONS

The significance of various immunohistochemical parameters for the prognosis of patients with endometrial cancer has not yet been fully established. The results of our work show that besides clinicopathological factors, molecular biological prognostic factors may contribute to better tumor characterization and thus more precisely determine its clinical behavior. A goal for the future should be further classification of endometrial cancer subtypes based on their genetic alterations, especially those with prognostic significance. It is probable that future histological classifications will rely more on the molecular basis. For the eventual practical diagnostically therapeutic use of molecular biological factors, additional studies are needed.

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