Immunohistochemical evaluation and lymph node metastasis in surgically staged endometrial carcinoma

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Summary

Objective: To assess the expression of immunohistochemical markers in surgically staged endometrial cancer patients. *Methods:* We studied 107 cases of primary untreated endometrial carcinoma in which the p53, bcl-2, her-2/neu, Ki-67, estrogen receptor (ER) and progesterone receptor (PR) antigens were investigated by an immunohistochemical method. In the last 50 consecutive patients immunoreactivity for MMP-7 and MMP-26 was assessed as well. We evaluated the correlations among the immunohistochemical staining assessed by histoscore, and the age, grading, depth of invasion, stage of the neoplasia and extrauterine disease. *Results:* Mean age was 65 years (range 34-88). All patients were submitted to total abdominal or modified radical vaginal hysterectomy plus bilateral salpingo-oophorectomy and systematic pelvic lymphadenectomy; p53, bcl-2, her-2/neu, Ki-67, MMP-7, MMP-26, estrogen and progesterone receptors were positive in 36 (43%), 71 (86%), 13 (16%), 80 (96%), 65 (78%), 80 (96%), 61 (73%) and 71 (86%) patients, respectively. p53 overexpression was found to be related to poor grade of differentiation and deep myometrial invasion. Immunostaining for ER was inversely related to the histopathological differentiation of the tumors. Decreased expression of PR was related to advanced stage, poor histopathologic differentiation and extrauterine spread of disease. *Conclusion:* The overexpression of p53 seems to indicate more malignant phenotype, while PR expression correlates with parameters of better clinical outcome.

Key words: Endometrial cancer; Immunohistochemistry; Prognostic factor.

Introduction

Adenocarcinoma of the endometrium is the most common malignancy of the female genital tract in developed countries and represents the seventh leading cause of death for cancer in women [1]. The prognostic impact of traditional clinicopathological variables in endometrial cancer patients, such as International Federation of Gynecology and Obstetrics (FIGO) stage, histologic type, depth of invasion, histologic grade and lymph node metastasis, is well established [2-5]. Correlations between steroid hormone receptor status (estrogen receptor – ER, progesterone receptor – PR) of endometrial carcinoma and known prognostic parameters such as tumor stage, tumor grade, and depth of myometrial infiltration have been documented [6-9]. Endometrial cancers expressing ER and PR seem to characterize clinically less aggressive tumors with a better chance of response to endocrine treatment [10, 11]. Recently, some other factors have been proven to offer additional data about the biologic behavior of the endometrial carcinoma. The mutation of the p53 tumor suppressor gene has been documented in endometrial cancer, although the prognostic significance of its overexpression is still conflicting [12-14]. Likewise, the oncogene her-2/neu, the apoptosis indicator bcl-2, and the cell proliferation indicator Ki-67 have also been shown to be prognostic factors of endometrial cancer [15-20]. However, the majority of patients in these studies did not undergo surgical staging with lymphadenectomy. Increased expression of certain matrix metalloproteinases (MMPs) in advanced tumors and the ability of these enzymes to degrade extracellular matrix barriers suggest a role for them not only in the initial steps of tumor development, but also in tumor metastasis [21]. MMP-7 and MMP-26 are two members of the matrilysin subfamily, containing the minimal domain organization. and belonging to the few MMPs with epithelial expression. Both heve been reported to be expressed in endometrial tumors [22-24]. However, the prognostic value of MMP-7 and MMP-26 expression in endometrial carcinoma is controversial [25, 26].

The aim of this study was to evaluate the prognostic significance of p53, bcl-2, her-2/neu, Ki-67, MMP-7, MMP-26, ER and PR antigens in patients with endometrial carcinoma who underwent surgical staging including lymphadenectomy.

Materials and Methods

Tissue samples were collected from women ranging in age from 34-88 years undergoing surgery for endometrial cancer between September 2001 and March 2009 at the Department of Gynecology and Obstetrics, Olomouc University Hospital, Czech Republic. None of the patients received chemotherapy or radiotherapy before surgery, which consisted of total hysterectomy, bilateral salpingo-oophorectomy, peritoneal cytology, and pelvic and paraaortic sampling as necessary. Two pathologists reviewed all the tumors to confirm the diagnosis and to determine histologic characteristics.

The 107 formalin-fixed, paraffin-embedded tumor samples were from patients with endometrioid carcinoma. The histologic grade, the depth of myometrial invasion, and the clinical stage were classified as recommended by the FIGO [27]. Epidemiologic and histologic features of the 107 patients are shown in Table 1.

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Table 1. — *Clinicopathological characteristics*.

Characteristics	No. of patient (%)
All cases	107
Age (years)	
≤ 65	63 (58.9)
> 65	44 (41.1)
FIGO stage	
I	72 (67.3)
II	20 (18.7)
III-IV	15 (14.0)
Grade	
G1-2	81 (75.7)
G3	26 (24.3)
Myometrial invasion	
Absent < 50%	63 (58.9)
> 50%	44 (41.1)
Lymph node status	
Negative	93 (86.9)
Positive	14 (13.1)

Immunohistochemistry

The processed material included endometrial cancer samples obtained from the abdominal hysterectomy after previous diagnosis confirmation from diagnostic curretage. The samples were standardly processed 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 tumor structures were then selected for subsequent immunohistological examination and processed into tissue slices 5-8 μm thick on Vectabond (Vector) coated slides. For 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). The activity of peroxidase was visualized by diaminobenzidine. Positive control, a sample of endometrial tissue with known immunoreactivity, was included in each set samples analyzed, as well as negative control, i.e. slides incubated with Tris buffer instead of the appropriate antibodies. The analysis of all tissue sections was done by two pathologists without any prior knowledge of the clinical and biological parameters. ER and PR staining was scored in a minimum of 300 histologically identified neoplastic cells showing nuclear reaction. According to Sivridis *et al*. tumors with positive ER or PR nuclear staining in > 5% of tumor cells were defined as ER or PR positive, respectively [11].

Scoring of Ki-67, p53 and bcl-2 was expressed as the percentage of cells with positive nuclear staining. Tumors with positive Ki-67 staining in > 40% (median value) tumor cells or positive p53, and bcl-2 staining in > 20% (median value) tumor cells were considered Ki-67, p53, and bcl-2 positive, respectively.

Scoring of her-2/neu protein was expressed as membrane positivity. Samples with positive her-2/neu staining in > 10% tumor cells were considered as positive.

Scoring of MMP-7 and MMP-26 was expressed as the percentage of immunopositive tumor cells. Tumors with positive MMP-7 staining in > 65% (median value) tumor cells or positive MMP-26 in > 40% (median value) tumor cells were considered MMP-7, and MMP-26 positive, respectively.

Statistical analysis

Fischer's exact test of chi-square test was used to analyze the distribution of cases considered positive for the biological parameters examined according to several clinicopatological features; p values below 0.05 were considered significant. Statistical analysis was carried out using SPSS version 15 statistical software (SPSS Inc., Chicago, USA).

Results

Correlation with clinicopathological parameters

In Table 2 we present the distribution of positive immunohistochemical staining in relation to individual clinicopathological parameters in 107 cases of endometrial cancer. MMP-7 and MMP-26 were evaluated in only 50 patients with endometrial cancer. A statistically significant dependence between p53, grading, and myometrial invasion was observed. In the G3 group, there was significantly greater p53 positivity compared to groups G1-G2 (42.3% vs 22.2%, $p = 0.045$). Similarly, p53 expression was higher when myoinvasion exceeded 50% compared to myoinvasion less than 50% of myometrial thickness (38.6% vs 19.0%), p = 0.025. In the group of ER and PR positive tumors, correlation with grading was noted; in group G3 a significantly lower ER and PR positivity was seen compared with groups G1-G2 (53.8% vs 80.2% and 69.2% vs 88.9%, respectively, $p = 0.008$ and 0.028, respectively). The only marker correlating with FIGO stage was PR, where in the group of patients FIGO Stage I-II a significantly higher positivity of this marker was observed in comparison to patients FIGO Stage III-IV (87.9% vs 62.5%, $p = 0.02$). Furthermore, PR positivity correlated inversely with lymph node status: PR positivity was higher in patients with negative lymph nodes (87.2% vs 61.5%, $p = 0.032$).

Although no statistically significant dependence between bcl-2, c-erbB-2, Ki-67, MMP-7, MMP-26 and any clinicopathological parameter was observed, we noted a trend of increased c-erbB-2 and Ki-67 expression when myoinvasion exceeded 50% of myometrial thickness, as well as a trend of increased MMP-7 expression in the group of patients aged less than 65 years. Contrarily, with bcl-2 a trend of decreased expression was observed in clinically advanced tumors (FIGO III-IV).

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, c-erb-2, Ki-67, MMP-7 and MMP-26 dependent on ER or PR positivity was observed. Contrarily, the percentage of bcl-2 positive endometrial tumors was significantly higher in the ER positive group than in the ER negative group (77.2% vs 46.4%, $p = 0.002$). Also, significantly greater bcl-2 positivity was seen in the PR positive group than the PR negative group (75.6% vs 35.3%, $p = 0.001$).

Table 2. — *Distribution of tumor positivity for several biological markers according to clinickopathological characteristics in endometrial cancer.*

Parameter	No. of cases	p53 positive no. (%)	bcl-2 positive no. (%)	neu positive no. (%)	Ki-67 positive no. (%)	ER positive no. (%)	PR positive no. (%)	No. of cases	MMP-7 positive no. (%)	MMP-26 positive no. (%)
All cases	107	29 (27.1)	74 (69.2)	32 (29.9)	50 (46.7)	79 (73.8)	90 (84.1)	50	25 (50.0)	29 (58.0)
Age (years)										
≤ 65	58	16 (27.6)	39 (67.2)	21 (36.2)	26 (44.8)	43 (74.1)	48 (82.8)	32	19 (59.4)	16 (50.0)
> 65	49	13 (26.5)	35 (71.5)	11 (22.4)	24 (49.0)	36 (73.5)	42 (85.7)	18	6 (33.3)	13 (72.2)
Significance		$p = 0.903$	$p = 0.640$	$p = 0.121$	$p = 0.668$	$p = 0.938$	$p = 0.677$		$p = 0.077$	$p = 0.126$
FIGO stage										
I-II	91	25 (27.4)	66 (72.5)	27 (29.7)	40 (44.0)	69 (75.8)	80 (87.9)	42	21 (50.0)	26 (61.9)
III-IV	16	4 (25.0)	8 (50.0)	5 (31.3)	10 (62.5)	10 (62.5)	10 (62.5)	8	4 (50.0)	3 (37.5)
Significance		$p = 1.000$	$p = 0.084$	$p = 1.000$	$p = 0.170$	$p = 0.354$	$p = 0.020$		$p = 1.000$	$p = 0.255$
Grade										
G1-G2	81	18 (22.2)	56 (69.1)	24 (29.6)	35 (43.2)	65 (80.2)	72 (88.9)	37	19 (51.3)	23 (62.2)
G3	26	11 (42.3)	18 (69.2)	8 (30.8)	15 (57.7)	14 (53.8)	18 (69.2)	13	6 (46.2)	6 (46.2)
Significance		$p = 0.045$	$p = 0.993$	$p = 0.912$	$p = 0.198$	$p = 0.008$	$p = 0.028$		$p = 0.747$	$p = 0.314$
Myometrial invasion										
Absent < 50%	63	12 (19.0)	42 (66.7)	15 (23.8)	25 (39.7)	47 (74.6)	54 (85.7)	29	13 (44.8)	16 (55.2)
> 50%	44	17 (38.6)	32 (72.7)	17 (38.6)	25 (56.8)	32 (72.7)	36 (81.8)	21	12 (57.1)	13 (61.9)
Significance		$p = 0.025$	$p = 0.504$	$p = 0.099$	$p = 0.080$	$p = 0.828$	$p = 0.587$		$p = 0.390$	$p = 0.634$
Lymph node status										
Negative	94	26 (27.7)	66 (70.2)	29 (30.9)	42 (44.7)	71 (75.5)	82 (87.2)	43	22 (51.2)	26 (60.5)
Positive	13	3 (23.1)	8 (61.5)	3 (23.1)	8 (61.5)	8 (61.5)	8 (61.5)	7	3 (42.9)	3 (42.9)
Significance		$p = 1.000$	$p = 0.534$	$p = 0.751$	$p = 0.254$	$p = 0.318$	$p = 0.032$		$p = 1.000$	$p = 0.434$

Statistically significant.

Table 3. — *Distribution of tumor positivity for several biological parameters according to ER and PR status*.

	No. of cases	p53 positive no. (%)	bcl-2 positive no. (%)	c-erb/neu positive no. (%)	Ki-67 positive no. (%)	No. of cases	MMP-7 pos no. (%)	MMP-26 positive no. (%)
All cases	107	29 (27.1)	74 (69.2)	32 (29.9)	50 (46.7)	50	25 (50.0)	29 (58.0)
ER status								
Negative	28	10 (35.7)	13 (46.4)	6 (21.4)	13 (46.4)	7	5 (71.4)	5 (71.4)
Positive	79	19 (24.1)	61 (77.2)	26 (32.9)	37 (46.8)	43	20 (46.5)	24 (55.8)
Significance		$p = 0.233$	$p = 0.002$	$p = 0.254$	$p = 0.970$		$p = 0.417$	$p = 0.684$
PR status								
Negative	17	7 (41.2)	6 (35.3)	3 (17.6)	8 (47.1)	6	3 (50.0)	3 (50.0)
Positive	90	22 (24.4)	8 (75.6)	29 (32.2)	42 (46.7)	44	22 (50.0)	26 (59.1)
Significance		$p = 0.232$	$p = 0.001$	$p = 0.229$	$p = 0.976$		$p = 1.000$	$p = 0.686$

Statistically significant.

Discussion

In endometrial carcinoma, conventional clinicopathological factors such as FIGO grade and stage, histologic type, lymph-vascular invasion, or depth of myometrial invasion are well-known predictors of prognosis. However, identification of molecular genetic alterations that could add relevant prognostic information to the traditional factors has become one of the goals of recent research. Our results show a correlation between certain biological markers and clinicopathological prognostic factors in surgically staged primary endometrial cancer.

Elevated p53 expression significantly correlated with poor differentiation and deep myometrial invasion in endometrial tumors. A number of works confirm association between elevated p53 expression and unfavorable prognostic factors in women with primary endometrial cancer [28-31]. This is in accordance with our finding of p53 overexpression in endometrial tumors with deep endometrial invasion. Contrary to the present series where p53 positivity was similar in lymph node positive and lymph node negative tumors, Mariani *et al*. describe p53 as the only molecular marker able to predict distant metastases independent of other histopathological, molecular and cytokinetic parameters [18]. Furthermore, various authors confirm a much higher p53 expression in serous tumors and clear cell tumors than in endometrial cancer, which supports the hypothesis of mutation of gene p53 as a late event in endometrial cancer and contrarily an early event during the development of rare endometrial tumors [32-34]. The results of Halperin *et al*. regarding p53 immunoreactivity in G3 tumors and papillary serous tumors demonstrate the uniqueness of G3 tumors, which are histopathologically more similar to papillary serous tumors than to endometrial cancer. G3 tumors probably follow a different path of carcinogenesis than G1 and G2 tumors [32, 35]. Our results assume the same conclusion.

The antiapoptotic gene bcl-2 regulates programmed cell death and thus lengthens cell survival which aids in

the spreading 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 [16, 28, 29, 36, 37]. 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*., we did not observe a correlation between bcl-2 expression and degree of tumor differentiation, depth of myometrial invasion and lymph node involvement [38]. In accordance with works by other authors, we demonstrated a significant positive correlation between bcl-2 expression and positivity of hormonal representation of ER and PR [16, 32, 36], which could be an important prognostic factor for a negative prognosis.

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 [30, 39-41]. Contrarily, Pansare *et al*. did not show any correlation between Ki-67, histological type, grade or clinical stage of the disease [42]. We observed a trend of elevated Ki-67 expression in tumors with deep invasion. Our results are partly in accordance with works by Laxe *et al*. and Salvesen *et al*., who demonstrated correlations between elevated expression of Ki-67 with grading, depth of myometrial invasion and risk of recurrence [13, 43].

The fact that increased expression of 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 [44-47]. Morrison *et al*. recently in their extensive study (483 cases) demonstrated that increased expression of c-erbB-2 as an independent prognostic factor correlated with worse survival [48]. Contrarily, Coronado *et al*. and Czerwenka *et al*. did not confirm a significant dependence with traditional prognostic factors of endometrial cancer [49, 50]. A trend of increased expression in patients with deep invasion was observed in our series, which correlates with the above-mentioned study results. Due to often opposing study results, the utilization of this factor remains ambiguous.

ER and PR are present in both normal endometrial tissue and endometrial cancer. Based on the results of various authors, the presence and amount of steroid receptors correlate with the clinical stage of the disease, histological grade and survival. The absence of steroid receptors is considered a negative prognostic factor of aggressive growth and poor prognosis [51-54]. Expression of ER in our work reached statistical significance in dependence on histopahological grading. PR expression correlated inversely with clinical Stage III-IV, poor tumor differentiation and extrauterine spread of tumor. This result is in accordance with the above-mentioned works; in addition, it seems that PR may be a stronger prognostic factor than ER, as supported by other authors [54-56].

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 is a limited number of published studies involving expression of MMP-7 in endometrial cancer. Ueno *et al*. demonstrated that increased MMP-7 expression correlated with a worse clinical disease stage and with presence of lymphatic metastases [24]. A similar trend was described by Graesslin *et al*. and Wang *et al*. [24, 57]. Contrary to these results, our work showed a trend of higher MMP-7 expression in patients under age 65.

Another member of the subfamily of matrilysin enzymes was described as MMP-26 (matrilysin-2). MMP-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 mid-cycle levels and in hyperplastic endometrium and contrarily low levels in the late phase of the cycle and in endometrial cancer point to an association with estrogen receptors. Isaka *et al*. and Pilka *et al*. demonstrated a significantly decreased MMP-26 expression in endometrial cancer, which is in discordance with the results of Tunuguntla *et al*., who describe increased immunohistochemical expression of MMP-26 in poorly differentiated endometrial cancer [22, 23, 26]. Our study did not show dependence on classical prognostic factors of endometrial cancer.

Conclusion

In conclusion, our findings suggest that there is no simple relationship between the determination of various immunohistochemical parameters and the biological aggressiveness of endometrial cancer. 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. For the eventual practical diagnostically therapeutic use of molecular biological factors, additional studies are needed.

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