Pathol. Oncol. Res. (2013) 19:229–235
DOI 10.1007/s12253-012-9573-1

RESEARCH

Selected Immuno-Histochemical Markers in Curettage Specimens and their Correlation with Final Pathologic Findings in Endometrial Cancer Patients

Basil R. Obeidat · Ismail I. Matalka · Alia A. Mohtaseb · Nabih S Al-Kaisi

Received: 17 January 2012 / Accepted: 18 September 2012 / Published online: 10 October 2012

Abstract To assess the immuno-histochemical expression of various markers in, endometrial biopsies of patients with endometrial cancer, and to correlate their expression with the final pathologic findings. Sixty-two patients with primary endometrial cancer who underwent surgical treatment were included in this study. Immuno-histochemical expression of estrogen receptor (ER), progesterone receptor (PR), p53, bcl-2, Her-2/neu and Ki-67 were assessed in curettage specimens, and review of the final pathology report from hysterectomy specimens was carried out. The expression of these markers in curettage was correlated with the final tumor characteristics obtained on hysterectomy specimens. Both ER and PR were significantly more expressed in endometrioid type (EC) than non-endometrioid type (NEC) (*P* value of 0.004 and 0.012). On the contrary, P53, Her-2 and Ki-67 showed higher positivity in NEC than EC (*P* value of 0.005, 0.025 and 0.002). Positive expression of ER and PR was significantly associated with low grade tumors and superficial myometrial invasion, whereas positive expression of Her-2 and Ki-67 was significantly associated with higher grade lesions, and deep myometrial invasion. Moreover, a statistically significant inverse relationship was observed between the positivity of P53, Her-2 and Ki-67 and the positivity of ER, PR. We found that determination of immuno-histochemical markers in curettage specimens might be helpful in predicting the final pathologic findings in patients with endometrial cancer. This might be helpful in planning the extensivity of the surgery.

Keywords Estrogen receptor · Progesterone receptor · p53 · Ki-67 · Her2/neu · Endometrial cancer

B. R. Obeidat (✉)
Department of Obstetrics and Gynecology,
Jordan University of Science and Technology,
P.O. Box 2954, Irbid 21110, Jordan
e-mail: b_obeidat@hotmail.com

I. I. Matalka · A. A. Mohtaseb
Department of Pathology and Laboratory Medicine,
Jordan University of Science and Technology,
Irbid, Jordan

N. S. Al-Kaisi
Department of Pathology, King Hussein Medical Centre,
Amman, Jordan

Introduction

Endometrial cancer is the most common gynecological cancer in the developed world [1]. Traditionally, endometrial cancer is divided into two groups: type 1(endometrioid) and type 2 (non endometrioid) according to their etiologic and pathologic features [2]. The majority of endometrial cancers is low grade, early stage and carries an excellent prognosis.

The initial diagnosis and subsequent management of endometrial cancer is usually based on the results of curettage specimens. The International Federation of Gynecology and Obstetrics implemented a surgical staging system for endometrial cancer [3]. The surgical procedure typically consists of hysterectomy, bilateral salpingo-oophorectomy, and retroperitoneal lymphadenectomy, including para-aortic nodes. However, lymphadenectomy, including para-aortic nodes in the surgical management of all Patients with endometrial cancer, remains controversial [4, 5].

The depth of myometrial invasion and tumor grade are the most important prognostic factors in endometrial carcinoma. They are usually used to determine the initial surgery and the need for lymphadenectomy. Different methods have been

used preoperatively and intraoperatively to assess the tumor grade and myometrial invasion. Computed tomography, ultrasonography, and magnetic resonance imaging (MRI) have all been used, with contrast enhanced MRI exhibiting the greatest efficacy [6]. Also, intraoperative gross examination of myometrial invasion [7], as well as frozen section to evaluate the tumor grade [8, 9] has been used, with controversial results [10].

Correlations between estrogen and progesterone receptors status in endometrial cancer and known prognostic parameters such as tumor grade, tumor stage and depth of myometrial invasion have been well documented [11–13]. The expression of these receptors is associated with well-differentiated tumors and correlates with disease stage and survival [14–17]. On the other hand, overexpression of P53, HER2/neu correlates with more aggressive tumors, advanced disease stage and decreased survival [18–23].

The Ki-67 nuclear antigen expression has been evaluated as a prognostic marker in endometrial cancer. Most endometrial carcinomas express low Ki-67 proliferation index and have a good prognosis. Correlation with tumor grade, disease stage and histologic types has been confirmed in several studies [24, 25].

The oncogene bcl-2 is another marker studied in endometrial cancer. Loss of expression of bcl-2 is associated with worse prognosis, deep myometrial invasion and more advanced disease stage [26, 27].

The expression of these markers has been commonly performed on hysterectomy specimens. Currently, there is an effort to use the expression of these biomarkers to identify high risk groups which would benefit from more radical treatment, and allowing more conservative therapy for patients with good prognosis, thus avoiding both overtreatment and under treatment. Therefore, the aim of this study was to evaluate the immunohistochemical expression of various markers (ER, PR, Her-2, P53, bcl-2 and Ki-67) in endometrial cancer and to correlate their expression in curettage specimens with final pathological findings at hysterectomy specimens. If we were able to preoperatively estimate the biologic behavior of endometrial cancer using the expression of these markers, this will help to decide on the extensivity of the surgical procedure required.

Materials and Methods

Between January 2003 and December 2010, all cases of primary endometrial cancers treated at King Abdullah University Hospital were identified. Cases were included in the study if both curettage and hysterectomy specimens were available for review. All patients diagnosed with endometrial cancer on curettage specimens underwent surgical staging including abdominal hysterectomy, bilateral salpingoopherectomy. Lymphadenectomy was performed selectively according to the surgeon's decision.

Both curettage and final surgical specimens were reviewed by two pathologists. According to the final hysterectomy specimens, tumors were classified into two histologic types: endometrioid carcinoma (EC) and non-endometrioid carcinoma (NEC). The clinical stage and tumor grade were assessed according to the International Federation of Gynecology and Obstetrics system of classification (FIGO 1988). Data collected included patient's age, depth of myometrial invasion, grade and stage of the tumor and the histologic type of the tumor.

Immunohistochemical staining was performed on curettage specimens according to standard protocol. Selected paraffin blocks were cut at 4 μm thickness and mounted on coated and charged slides (Super frost plus-Diapath). Sections were then dried in oven for one hour and then treated with two changes of xylene for dewaxing and finally hydrated through serial descending grades of ethyl alcohol. Antigen retrieval was performed using Nuve OT012 autoclave (Thermolabs industries). Slides were placed in citrate buffer and heated for 5 min at 121 C. After cooling slides were washed with PBS buffer and then covered by 3 % H2O2 for 10 min to block endogenous peroxidase activity. Slides are then washed again with PBS and covered by the selected primary antibodies for 45 min. The primary antibodies used in this study were: anti-ER mouse antihuman monoclonal antibodies 1D5, anti-PR mouse antihuman monoclonal antibodies clone pgR636, rabbit antihuman Ki-67 antigen monoclonal antibodies clone M1B-1, mouse antihuman p53 protein monoclonal antibodies DO-7, polyclonal rabbit anti-C-erbB-2 oncoprotein (Dako A0485) monoclonal mouse anti-bcl2 oncoprotein (clone 124), using original DAKO kit (DAKO Corporation, Denmark). After washing with PBS twice, the second layer (Dako dual link envision, Dako K4061) was placed for 30 min. Slides were then washed twice with PBS and signal detection was performed using liquid DAB substrate system. (Dako-K3468). Slides were finally counter stained with Mayers hematoxylin and mounted with DPX.

The analysis was performed with positive controls. Patients with normal or premalignant endometrium were excluded from the study.

Immunohistochemical analysis of tissue sections was done by one pathologist to reduce the amount of error in interpretation. The pathologist was blinded to the clinicopathologic features of the specimens. For estrogen receptors (ER) and progesterone receptors (PR), immunohistochemical results were evaluated for percentage of nuclear staining and intensity of staining. The results were recorded as 3+ for strong or weak nuclear staining in >50 % of cells, 2+ for strong or weak nuclear staining in >10 % to 50 % of cells, and 1+ for strong or weak nuclear staining in 10 % or less.

Cases with no evidence of nuclear staining were recorded as 0. HER2 expression was recorded as 3+ for complete membranous staining in greater than 10 % of tumor cells of strong intensity, 2+ for complete membranous staining in greater than 10 % of tumor cells with moderate intensity, 1+ for incomplete membranous staining or complete membranous staining in less than 10 % of tumor cells, and 0 for no staining or staining without membranous pattern. Expression for ER, PR and HER2 was designated as positive for 3+ and 2+, and negative for 1+ and 0.

Specimens were considered immunopositive for P53 and bcl-2 when 10 % or more of tumor cells showed clear evidence of nuclear or cytoplasmic staining respectively. For Ki-67, the actual percentage of cells stained was recorded. Specimens with nuclear staining of 50 % or higher of tumors cells were considered Ki-67 positive. Figure 1 shows examples of immuno-histochemical staining of curettage specimens.

Statistical analysis was performed using The Statistical Package for Social Sciences (SPSS) 17.0 for Microsoft Windows. Chi-square test and Fisher exact test were used

Table 1 Characteristics of endometrial cancer patients

Characteristic	No. (%)
All cases	62
Histologic type	
Endometrioid	53(85.5 %)
Non-endometrioid	9(14.5 %)
Stage	
Early	49(79 %)
Advanced	13(21 %)
Grade	
G1	29(46.8 %)
G2	21(33.9 %)
G3	12(19.4 %)
Myometrial invasion	
<50 %	40(64.5 %)
>50 %	22(35.5 %)

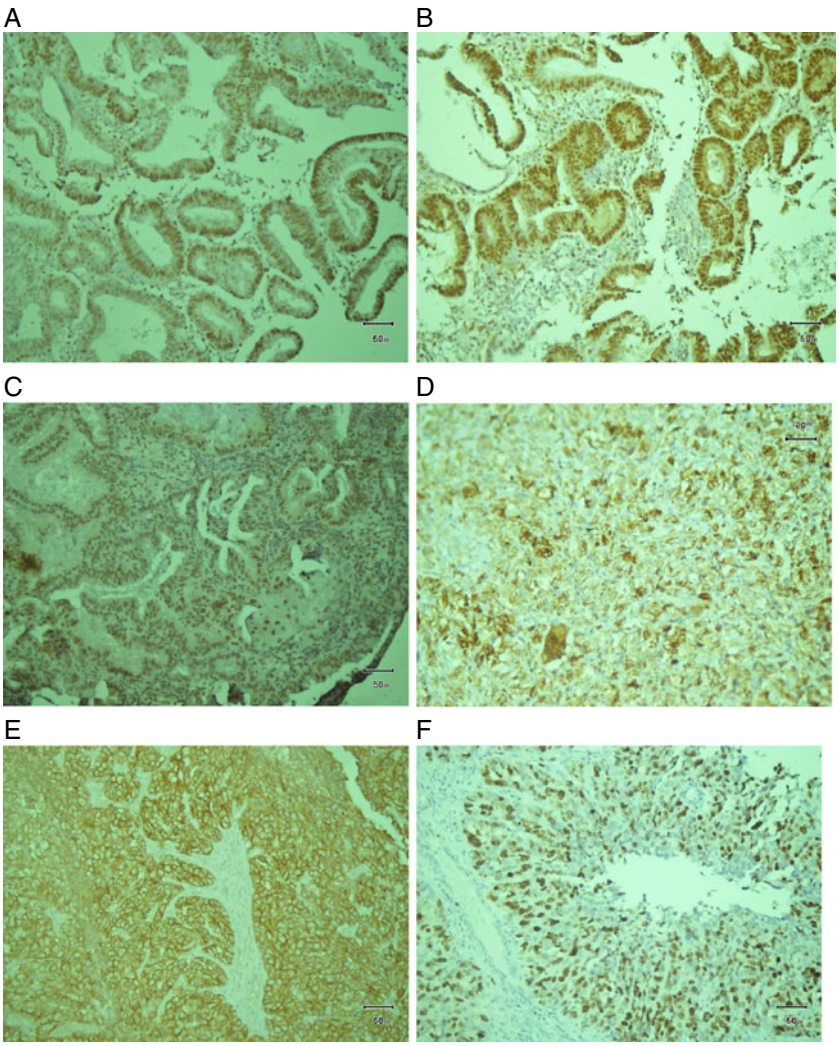

Fig. 1 Immunohistochemistry in curettage specimens of endometrial cancer, showing positive ER (**a**), positive PR (**b**), positive p53 (**c**), positive Bcl2 (**d**), positive HER/2 (**e**), positive ki67 (**f**)

Table 2 Markers positivity and histological types of endometrial cancer specimens

Marker	Endometrioid (n=53)	Non-Endometrioid (n=9)	Total (n=62)	P value
ER	48(90.6 %)	4(44.4 %)	52(83.9 %)	0.004
PR	49(92.5 %)	5(55.6 %)	54(87.1 %)	0.012
P53	14(26.4 %)	7(77.8 %)	21(33.9 %)	0.005
Bcl-2	27(50.9 %)	2(22.2 %)	29(46.8 %)	0.155
Her2/neu	14(26.4 %)	6(66.7 %)	20(32.3 %)	0.025
Ki-67	22(41.5 %)	9(100 %)	31(50 %)	0.002

to evaluate the relation between clinicopathological parameters and immunohistochemical markers, as well as the relation between immunohistochemical markers and steroid hormone receptor status. A P-value of <0.05 was considered significant. The study was approved by the local ethical committee.

Results

During the period of study, a total of sixty-two cases were identified. The curettage diagnoses were compared to those from the hysterectomy. Diagnostic agreement regarding histologic type and grade was observed in 92.5 % and 68 % respectively.

The mean age of the patients was 57.8 (range 30–80 years). The final hysterectomy specimens consisted of 53 cases (85.5 %) of endometrioid carcinoma (EC) and 9 cases (14.5 %) of non-endometrioid carcinoma (NEC). Clinico-pathological details of the patients are shown in Table 1. Non-endometrioid type of endometrial cancer was found to be significantly associated with more advanced stage, higher grade and deeper myometrial invasion compared with endometrioid type.

Immuno-Histochemical Analysis

Among all patients, positive expression of ER, PR, P53, bcl-2, Her2/neu and Ki-67 was observed in 52(83.9 %), 54 (87.1 %), 21(33.9 %), 29(46.8 %), 20(32.3 %) and 31 (50 %) of endometrial cancer specimens respectively. Both ER and PR were significantly more expressed in EC than NEC types (P value of 0.004 and 0.012). On the contrary, P53, Her-2/neu and Ki-67 showed higher positivity in NEC than EC (P value of 0.005, 0.025 and 0.002 respectively). There was no significant difference in the positivity of bcl2 between the two types, Table 2.

Positive expression of ER and PR was significantly associated with low grade tumors (P value of 0.000 and 0.000 respectively), whereas positive expression of P53, Her-2/neu and Ki-67 was significantly associated with higher grade lesions (P value of 0.015, 0.003 and 0.000 respectively). Bcl-2 was more expressed in low grade lesions, but the difference was not statistically significant, Table 3.

The expression of the markers in curettage specimens did not show any significant relation to the final disease stage as evaluated in the hysterectomy specimens. We observed a slight increase in Her-2 positivity in advanced stages (Table 4).

The relation between markers positivity and depth of myometrial invasion is shown in Table 5. Positive ER, and PR, bcl-2 expression was significantly associated with myometrial invasion of less than 50 %. However, high Ki-67 proliferative index was significantly associated with deep myometrial invasion. We also observed a significant increased Her-2 expression when myometrial invasion exceeded 50 %. Expression of P53 was not related to the depth of invasion.

Table 4 Markers positivity and endometrial stages

Marker	Early stage[a] (n=49)	Advanced stage[b] (n=13)	P value
ER	43(87.9%)	9(69.2 %)	0.196
PR	44(89.8 %)	10(76.9 %)	0.347
P53	16(32.7 %)	5(38.5 %)	0.748
Bcl-2	22(44.9 %)	7(53.8 %)	0.756
Her2/neu	13(26.5 %)	7(53.8 %)	0.094
Ki-67	22(44.9 %)	9(69.2 %)	0.211

[a] stage1-II

[b] stage III-IV

Table 3 Markers positivity and grades of endometrial cancer

Marker	Grade 1–2 (n=50)	Grade 3 (n=12)	P value
ER	47(94 %)	5(41.7 %)	0.000
PR	48(96 %)	6(50.0 %)	0.000
P53	13(26 %)	8(66.7 %)	0.015
BCL2	25(50 %)	4(33.3 %)	0.473
Her2	12(24 %)	8(66.7 %)	0.013
Ki 67	19(38 %)	12(100 %)	0.000

Table 5 Markers positivity and depth of myometrial invasion

Marker	<50 % thickness (n=40)	>50 % thickness (n=22)	P value
ER	38(95.0 %)	14(63.6 %)	0.003
PR	38(95.0 %)	16(72.7 %)	0.019
P53	13(32.5 %)	8(36.4 %)	0.785
BCL2	23(57.5 %)	6(27.3 %)	0.033
Her2	9(22.5 %)	11(50.0 %)	0.046
Ki 67	14(35.0 %)	17(77.3 %)	0.003

Table 6 Markers positivity in relation to ER,PR status

	Total no. (N=62)	P53 n (%)	Bcl-2 n (%)	Her-2/neu n (%)	Ki-67 n (%)
ER status					
Negative	10	7(70)	4(40)	7(70)	9(90)
Positive	52	14(26.9)	25(48.1)	13(25)	22(42.3)
P value		0.024	0.738	0.009	0.012
PR status					
Negative	8	7(87.5)	3(37.5)	6(75)	8(100)
Positive	54	14(25.9)	26(48.1)	14(25.9)	23(42.6)
P value		0.001	0.713	0.011	0.005

P53, bcl-2, Her-2 and Ki-67 in Association with Steroid Hormone Receptor Status

As shown in Table 6, a statistically significant inverse relationship was observed between the positivity of P53, Her-2 and Ki-67 and the positivity of ER, PR. The percentage of P53-positive group, Her-2 positive group and Ki-67 positive group were significantly higher in the ER-negative group than in the ER-positive group (P value of 0.024, 0.009 and 0.012 respectively). The same finding was observed in relation to the PR status.

On the contrary, no significant relation was observed between bcl-2 positivity and ER, PR positivity. However, a trend of higher bcl-2 positivity among ER, PR positive groups was observed.

Discussion and Conclusion

The expression of various immunohistochemical markers in different types of endometrial cancer has been addressed in several studies. Our study showed that both ER and PR were significantly more expressed in endometrioid (EC) than non-endometrioid types (NEC). On the contrary, P53, Her-2 and Ki-67 showed higher positivity in NEC than EC. Our results are in concordance with the work of Halperin et al. [15] who showed increased immunoreactivity for ER, PR and bcl-2 and low expression of Her-2/neu in grade 1 and 2 endometrioid carcinoma, whereas serous papillary endometrial cancers were characterized by immunonegativity for ER, PR and bcl-2 and high immunoreactivity for Her-2/neu. However, in his work, Halperin et al. demonstrated that the expression of Ki-67 did not differ significantly comparing the different types of endometrial cancer. Various authors confirmed a much higher P53 expression in serous and clear cell tumors than in endometrioid tumors [15, 28]. Our data showed similar higher P53 expression in non-endometrioid cancers compared to endometrioid type. Bcl-2 expression in our study was not statistically different between the two histologic types, which is in agreement with other reports [28].

Several studies have shown a correlation between estrogen and progesterone receptors status in endometrial cancer and known prognostic parameters such as tumor grade, tumor stage and depth of myometrial invasion [11–13]. The expression of these receptors is associated with well-differentiated tumors and correlates with disease stage and survival [14–17]. Our work showed that the expression of ER and PR was significantly associated with well- differentiated tumors and tumors with superficial myometrial invasion. This is in agreement with the above mentioned reports. Although, no significant relation to clinical stage was observed in our study, this can be explained by the predominance of low grade and early stage tumors in our study.

Overexpression of P53 in patients with endometrial cancer has been shown to be associated with unfavourable prognostic factors including poor differentiation, non endometrioid histologic type and advanced stage [15, 21, 25, 26, 28]. Moreover, P53 overexpression has been reported as the only marker able to predict distant metastases independent of other parameters [23]. Our results are in agreement with these reports. Moreover, positive P53 expression was found in our study to be inversely related to the ER, PR status of endometrial cancer.

Expression of the oncogene bcl-2 has been described to be increased in endometrial hyperplasia and decreased in endometrial cancer. Loss of expression of bcl-2 is associated with worse prognosis, deep myometrial invasion and more advanced disease stage [26, 27]. In our work, positive bcl-2 expression was significantly associated with myometrial invasion of less than 50 %. However, no significant relation was observed between bcl-2 expression and grade of tumor and stage of the disease. This is in agreement with the work done by other authors [29].

Increased expression of the oncogene Her-2/neu has been described to be associated with high grade, deep myometrial invasion and advanced disease stage [14]. Our results confirmed a significant relation between the expression of Her-2 and high grade lesions and deep myometrial invasion. We also observed a trend of increased expression with advanced stage disease. This is in agreement with the above report. Moreover, we confirmed a statistically significant inverse relationship between the positivity of Her-2 and the positivity of ER, PR. However, this is contrary to the results of other authors [14, 30] who confirmed no or positive relation between Her-2 expression and ER, PR positivity.

Ki-67 is a marker of cell proliferation. A number of studies have shown a correlation between elevated expressions of Ki-67 with grading, depth of myometrial invasion, disease stage and histologic type [20, 24, 30]. In our work,

we showed similar results. We confirmed that elevated expression of Ki-67 was significantly associated with higher grade lesion, deeper myometrial invasion and non-endometrioid histologic types. Moreover, an inverse relation between the expression of Ki-67 and ER, PR positivity was observed in our study.

Other potential markers in endometrial carcinoma have been described in recent reports. The tight junction (TJ) proteins claudin-3 and claudin-4 expression was found to be significantly higher in uterine serous papillary carcinoma (USPC) and clear cell endometrial cancer compared to endometrioid endometrial cancer. Moreover, expression of both tight junction proteins was also found to be significantly associated with poor clinical outcome [31]. The two types of endometrial adenocarcinomas were well distinguished by claudins 1 and 2 by immunohistochemistry [32]. Wilms tumour gene 1(WT1) over expression was found to be associated with advanced FIGO stage, myometrial invasion and high-grade histological differentiation [33, 34].

Despite The limitation of our study (small sample size and the relatively small proportion of high grade and advanced stage lesions), we have shown that, beside traditional pathologic features, immunohistochemical markers in curettage specimens might be beneficial in predicting final tumor characteristics. Therefore, endometrial cancers diagnosed on curettage specimens might be classified on molecular basis according to their immunohistochemical profile. Tumors positive for ER, PR and negative for P53, Her2/neu and Ki-67 could be regarded as low risk group which can be treated by less radical surgery. To achieve this purpose, large prospective comparative studies are needed.

Acknowledgments The authors would like to thank Dr Yousef Khader, Department Of public health, for his help in the analysis of the results.

Disclosure of Interests The authors declare that they have no conflicts of interest.

Funding The study was funded by the research Deanship at Jordan University of Science and Technology

References

1. Jemal A, Siegel R, Ward E, Hao Y, Xu J, Thun MJ (2009) Cancer statistics. CA Cancer J Clin 59(4):225–249
2. Bokhman JV (1983) Two pathogenetic types of endometrial carcinoma. Gynecol Oncol 15(1):10–17
3. Announcements (1989) FIGO stages-1988 revision. Gynecol Oncol 35:125–127
4. Yokoyama Y, Maruyama H, Sato S et al (1997) Indispensability of pelvic and paraaortic lymphadenectomy in endometrial cancers. Gynecol Oncol 64:411–417
5. Mariani A, Keeney GL, Aletti G et al (2004) Endometrial carcinoma: paraaortic dissemination. Gynecol Oncol 92:833–838
6. Kinkel K, Kaji Y, Yu KK et al (1999) Radiologic staging in patients with endometrial cancer: a meta-analysis. Radiology 212:711–718
7. Larson DM, Connor GP, Broste SK, Krawisz BR, Johnson KK (1996) Prognostic significance of gross myometrial invasion with endometrial cancer. Obstet Gynecol 88:396–398
8. Zorlu CG, Kuscu E, Ergun Y, Aydogdu T, Cobanoglu O, Erdas O (1993) Intraoperative evaluation of prognostic factors in stage I endometrial cancer by frozen section: how reliable? Acta Obstet Gynecol Scand 72:382–385
9. Sanjuán A, Cobo T, Pahisa J, Escaramís G, Ordi J, Ayuso JR, Garcia S, Hernández S, Torné A, Martínez Román S, Lejárcegui JA, Vanrell JA (2006) Preoperative and intraoperative assessment of myometrial invasion and histologic grade in endometrial cancer: role of magnetic resonance imaging and frozen section. Int J Gynecol Cancer 16(1):385–390
10. Vorgias G, Hintipas E, Katsoulis M, Kalinoglou N, Dertimas B, Akrivos T (2002) Intraoperative gross examination of myometrial invasion and cervical infiltration in patients with endometrial cancer: decision-making accuracy. Gynecol Oncol 85:483–486
11. Geisinger KR, Marschall RB, Kute TE, Homesley HD (1986) Correlation of female sex steroid hormone receptors with histologic and ultrastructural differentiation in adenocarcinoma of endometrium. Cancer 58:1506–1517
12. Kleine W, Maier T, Geyer H, Phleiderer A (1990) Estrogen and progesterone receptors in endometrial cancer and their prognostic relevance. Gynecol Oncol 38:59–65
13. Creasman WT, Soper JT, McCarty KS Jr, McCarty KS Sr, Hinshaw W, Clarke-Pearson DL (1985) Influence of cytoplasmic steroid receptor content on prognosis of early stage endometrial carcinoma. Am J Obstet Gynecol 151:922–932
14. Ferrandina G, Ranelletti FO, Gallotta V et al (2005) Expression of cyclooxygenase-2 (COX-2), receptors for estrogen (ER) and progesterone (PR), p53, Ki-67, and neu protein in endometrial cancer. Gynecol Oncol 98:383–389
15. Halperin R, Zehavi S, Habler L et al (2001) Comparative immunohistochemical study of endometrioid and serous papillary carcinoma of endometrium. Eur J Gynaecol Oncol 22:122–126
16. Kadar N, Malfetano JH, Homesley HD (1993) Steroid receptor concentrations in endometrial carcinoma: effect on survival in surgically staged patients. Gynecol Oncol 50:281–286
17. Morris PC, Anderson JR, Anderson B et al (1995) Steroid hormone receptor content and lymph node status in endometrial cancer. Gynecol Oncol 56:406–411
18. Geisler JP, Wiemann MC, Zhou Z et al (1996) p53 as a prognostic indicator in endometrial cancer. Gynecol Oncol 61:245–248
19. Cherchi PL, Marras V, Capobianco G et al (2001) Prognostic value of p53, c-erb-B2 and MIB-1 in endometrial carcinoma. Eur J Gynaecol Oncol 22:451–453
20. Lax SF, Pizer ES, Ronnett BM et al (1998) Clear cell carcinoma of the endometrium is characterized by adistinctive profile of p53, Ki-67, estrogen, and progesterone receptor expression. Hum Pathol 29:551–558
21. Ohkouchi T, Sakuragi N, Watari H et al (2002) Prognostic significance of Bcl-2, p53 overexpression, and lymph node metastasis in surgically staged endometrial carcinoma. Am J Obstet Gynecol 187:353–359
22. Lukes AS, Kohler MF, Pieper CF et al (1994) Multivariable analysis of DNA ploidy, p53, and HER-2/neu as prognostic factors in endometrial cancer. Cancer 73:2380–2385
23. Mariani A, Sebo TJ, Webb MJ et al (2003) Molecular and histopathologic predictors of distant failure in endometrial cancer. Cancer Detect Prev 27:434–441
24. Salvesen HB, Iversen OE, Akslen LA (1998) Identification of high-risk patients by assessment of nuclear Ki-67 expression in a

prospective study of endometrial carcinomas. Clin Cancer Res 4:2779–2785
25. Salvesen HB, Iversen OE, Akslen LA (1999) Prognostic significance of angiogenesis and Ki-67, p53, and p21 expression: a population-based endometrial carcinoma study. J Clin Oncol 17:1382–1390
26. Erdem O, Erdem M, Dursum A et al (2003) Angiogenesis, p53 and bcl-2 expression as prognostic indicators in endometrial cancer: comparison with traditional clinicopathological variables. Int J Gynecol Pathol 22:254–260
27. Sakuragi N, Ohkouchi T, Hareyama H et al (1998) Bcl-2 expression and prognosis of patients with endometrial carcinoma. Int J Cancer 79:153–158
28. Kounelis S, Kapranos N, Kouri E, Coppola D, Papadaki H, Jones MW (2000) Immunohistochemical profile of endometrial adenocarcinoma: a study of 61 cases and review of the literature. Mod Pathol 13(4):379–388
29. Appel MLM, Edelweis MI, Fleck J et al (2008) p53 and bcl-2 as prognostic markers in endometrial carcinoma. Pathol Oncol Res 14:23–30
30. Markova I, Duskova M, Lubusky M, Kudela M, Zapletalová J, Procházka M, Pilka R (2010) Selected immunohistochemical prognostic factors in endometrial cancer. Int J Gynecol Cancer 20 (4):576–582
31. Konecny GE, Agarwal R, Keeney GA, Winterhoff B, Jones MB, Mariani A, Riehle D, Neuper C, Dowdy SC, Wang HJ, Morin PJ, Podratz KC (2008) Claudin-3 and claudin-4 expression in serous papillary, clear-cell, and endometrioid endometrial cancer. Gynecol Oncol 109(2):263–269
32. Sobel G, Németh J, Kiss A, Lotz G, Szabó I, Udvarhelyi N, Schaff Z, Páska C (2006) Claudin 1 differentiates endometrioid and serous papillary endometrial adenocarcinoma. Gynecol Oncol 103(2):591–598
33. Dupont J, Wang X, Marshall DS, Leitao M, Hedvat CV, Hummer A et al (2004) Wilms Tumor Gene (WT1) and p53 expression in endometrial carcinomas: a study of 130 cases using a tissue microarray. Gynecol Oncol 94(2):449–455
34. Ohno S, Dohi S, Ohno Y, Kyo S, Sugiyama H, Suzuki N, Inoue M (2009) Immunohistochemical detection of WT1 protein in endometrial cancer. Anticancer Res 29(5):1691–1695