Contribution of Immunohistochemical Profile in Assessing Histological Grade of Endometrial Cancer

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Abstract. *Background: The aim of this study was to correlate immunostaining expression profiles with histological grade using a predictive model. Patients and Methods: Samples were collected from 69 women with endometrial cancer. Immunostaining for expression of estrogen receptor (ER), progesteron receptor (PR), Ki67 and p53 in grade 1 or 2 and grade 3 tumors were compared. After determining optimal immunostaining cut-offs, we built a model to predict the final histological grade. Results: Higher immunostaining of ER and PR was found in grade 1 or 2 (p=0.01) compared with grade 3 tumors. Higher immunostaining for Ki67 (p<0.0001) and p53 (p<0.001) was found in grade 3 than in grade 1 or 2 tumors. The recursive partitioning model predicted a grade 1 or 2 tumor in 98% of cases when Ki67 and p53 were underexpressed. The misclassification rate was 13%. Conclusion: Our results show that integrating immunohistochemical profiles in a simple predictive model could help predict the final histological grade of endometrial tumors, especially for grade 1 or 2.*

Endometrial cancer is the most common pelvic gynecological cancer in France, with an estimated 6,560 new cases in 2010, and causing approximately 1900 deaths per year (1). Almost 80% of endometrial cancer cases are diagnosed at an early stage (tumor restricted to the *corpus uteri*) with an overall survival of 95% (1). Patients are classified as being at low-, intermediate- or high-risk of recurrence according to histological type, grade and depth of myometrial invasion (2).

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Diagnosis of histological type and grade of endometrial cancer is based on preoperative endometrial biopsy, determining the surgical management (*i.e.* pelvic with/without para-aortic lymphadenectomy) in association with imaging techniques. However, previous studies have underlined discrepancies between preoperative histological grade assessed by biopsy and final histology (3-5). In a retrospective study including 153 patients, Frumovitz *et al.* showed that 56% of preoperative grade 2 and 23% of preoperative grade 1 endometrial cancer cases were discrepant with final histology (4). Similarly, a retrospective study by Ballester *et al.* including 89 patients with presumed low- or intermediate-risk endometrial cancer, found that 21.4% of the patients at presumed low-risk and 21.2% of those at presumed intermediate-risk were up-staged by final histology (3). These important discrepancies are a major source of inaccurate initial surgical staging leading to unnecessary lymphadenectomy in the case of an overestimated grade or imposing further surgery or the use of adjuvant therapies in the case of an underestimated grade.

Routine histology, thus, needs to be supported by additional tools to improve diagnosis of endometrial cancer. Previous studies have shown that immunohistochemical expression of estrogen receptor (ER) and progesteron receptor (PR) (6-14), Ki67 (6, 7, 15, 16) and p53 (17-28) is associated with clinicopathological characteristics of endometrial cancer (7, 29-34). Moreover, Obeidat *et al.* (35) recently found that the immunohistochemical expression of ER, PR, p53, B-cell lymphoma-2 (BCL-2), Human Epithelial Growth Factor-2 (HER2/neu) and Ki67 in curettage specimens might be helpful in predicting the final pathological findings in patients with endometrial cancer. However, there are currently no validated detection thresholds for such markers and no reliable expression profiles associated with the final histological grade.

Hence, the aims of this prospective study were to assess the immunohistochemical expression of ER, PR, Ki67 and p53 in endometrial cancer and to correlate expression profiles with final histological grade using a predictive model.

Patients and Methods

Patients. This cohort study included 69 patients with primary endometrial cancer who underwent surgical treatment with at least total hysterectomy with bilateral salpingo-oophorectomy from June 2006 to December 2012 at the Department of Gynecology of Tenon Hospital, Paris, France. All women had undergone a preoperative endometrial biopsy and preoperative magnetic resonance imaging (MRI) to assess the disease stage. Patients were staged on the basis of final pathological findings according to the 2009 International Federation of Gynecology and Obstetrics (FIGO) classification (36).

Medical records were reviewed to determine age, surgical procedure, histological type and tumor grade, lymphovascular space invasion (LVSI) and myometrial invasion on final histology. All the tissue samples were obtained with full and informed patient consent. The research protocol was approved by the Consultative Committee for Protection of Persons in Biomedical Research of Paris 6 (France).

Histological type and grade. As previously reported, histological type I corresponds to endometrioid tumor, whatever the histological grade; histological type 2 includes clear cell carcinomas, papillary/serous carcinomas and carcinosarcomas (CSC) (37).

Grade 1 was defined by 5% or less non-squamous, non-morular growth pattern; grade 2 by 6% to 50% non-squamous, non-morular growth pattern; and grade 3 by more than 50% non-squamous, non-morular growth pattern (1).

Immunohistochemistry. Tissues were immediately fixed in formalin (10%) and then processed as paraffin blocks. Three micrometer-thick sections of formalin-fixed tissues were de-paraffinated in a xylene substitute (EZ Prepw 1× ref 950102; Ventana Medical Systems, Tucson, Arizona, USA) and rehydrated through a graded series of ethanol solutions. Immunohistochemical staining was performed on paraffin sections with rabbit monoclonal antibodies directed against ER (prediluted, SP1; Ventana Medical Systems, Tucson, Arizona, USA) and PR (prediluted, 1E2; Ventana Medical Systems, Tucson, Arizona, USA), mouse monoclonal antibody against Ki67 (1/150, MIB1; DAKO, Les Ulis, France) and mouse monoclonal antibody against p53 (1/75, DO7; DAKO, Les Ulis, France). The sections were immunostained using a Ventana Benchmark XTw automated immunohistochemistry system (Ultra-ViewTM, Universal DAB-Ventana; Ventana Medical Systems, Tucson, Arizona, USA). An antigen retrieval procedure was run (Dako Target Retrieval Solution; 98˚C, 20 min; DAKO, Les Ulis, France) prior to ER, PR, Ki67 and p53 staining. This automated procedure is based on an indirect biotin-avidin system. A universal biotinylated immunoglobulin was used as the secondary antibody, diaminobenzidine as the substrate and hematoxylin as the counterstain. Positive controls were sections of human breast tissue for ER and PR (2), colonic adenocarcinoma for Ki67 (according to the manufacturer's instructions), and serous ovarian carcinoma for p53 (according to the manufacturer's instructions). The primary antibody was replaced by an irrelevant antibody of the same IgG subtype for negative controls. The negative control was run without either the primary antibody or the secondary antibody. These procedures resulted in negative staining.

Statistical analysis. For purposes of comparison, we separated the histological grades into two groups, grade 1 or 2 *vs*. grade 3 endometrial cancer.

For semi quantitative analysis, the percentage of stained epithelial cells was calculated by two independent observers. Each observer examined the slides at least twice. Differences in opinion between the observers were resolved by discussion.

For qualitative analysis, we calculated optimal cut-offs for each marker to correlate semi-quantitative expression and final histological grade. The optimal cut-off for immunostaining level was determined by a minimal *p*-value approach. This involved dichotomizing the immunostaining level into dummy variables with a cut-off every five units of its range of values. Chi-square tests comparing the rate of grade 1 or 2 and grade 3 endometrial cancer for every dummy variable were then calculated. The cut-off with the minimal *p*-value was chosen as the optimal cut-off for this variable. Only samples with semi-quantitative expression (percentage of stained epithelial cells) equivalent to or greater than optimal cut-off were considered positive.

We developed a recursive partitioning (RP) model to determine optimal ER, PR, Ki67 and p53 thresholds in predicting final histological grade. RP is a technique that can be applied to mine large datasets in order to uncover hidden patterns within the data and to reveal statistically significant sub-groupings. In our case, at each step, the RP program optimally separated women into homogeneous groups corresponding, in this case, to biological marker profiles. We internally validated our RP model using a bootstrap method which is based on a re-sampling obtained by drawing randomly with replacement from the original dataset (1,000 re-samplings were performed). To build the RP model, we analyzed grade 1 or 2 *vs*. grade 3 endometrial carcinomas.

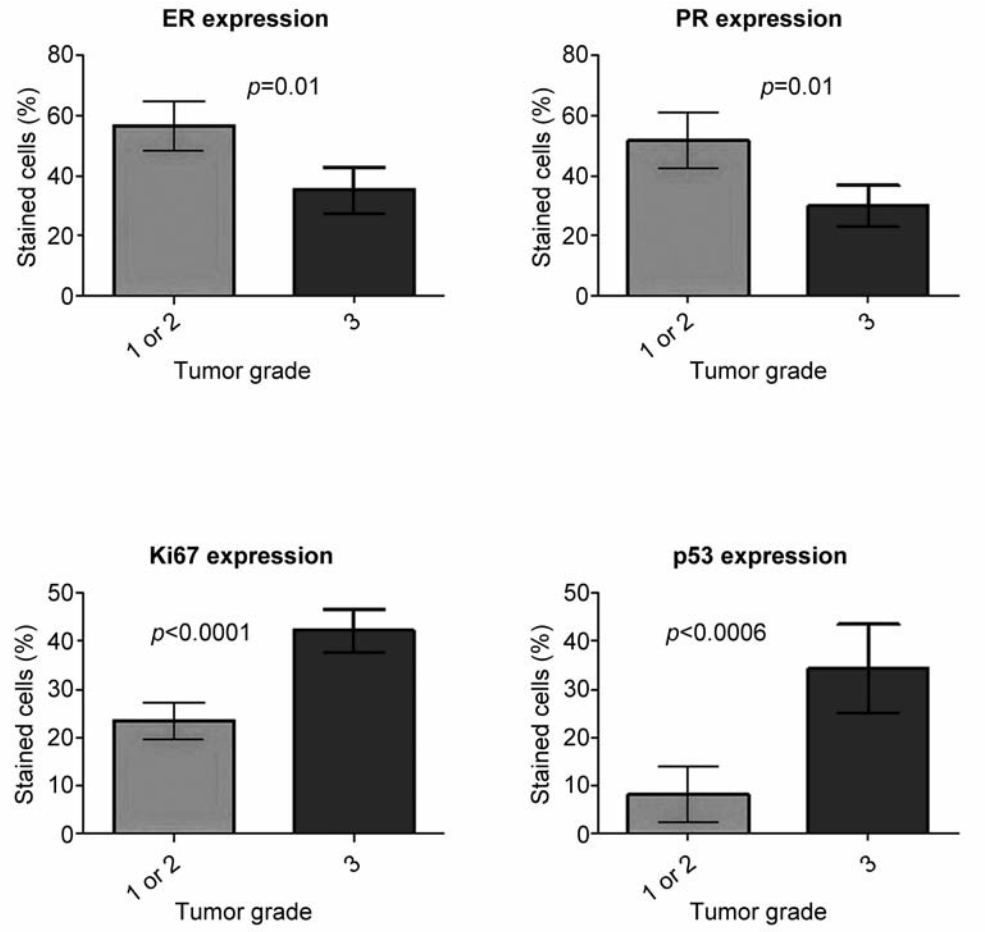
Statistical analysis was based on Student's *t*-test and the Mann–Whitney test for parametric and non-parametric continuous variables, respectively, and the Chi-square test or Fisher's exact test, as appropriate, for categorical variables. Values of $p<0.05$ were considered to denote significant differences.

Data were managed with an Excel database (Microsoft, Redmond, WA, USA) and analyzed using R 2.15 software, available online at http://cran.r-project.org/.

Results

Epidemiological characteristics of the population. A total of 69 patients were included in the study. Their median age was 68 years (range: 58 to 77). Final histology found type 1 endometrial cancer in 56 patients (81 %) and type 2 in 13 (19 %). Final histological grade was 1 or 2 in 52 cases (75%) and 3 in 17 cases (25%). Final International Federation of Gynecology and Obstetrics (FIGO) stage was IA in 29 cases (42%), IB in 14 cases (29%), II in six cases (9%), III in 17 cases (25%) and IV in three cases (4%). Depth of myometrial invasion was <50% in 41 cases (59%) and ≥ 50% in 28 (41%). Lymphovascular space involvement (LVSI) was present in 21 cases (30%), absent from 38 cases (55%) and not available in 10 cases. A pelvic lymphadenectomy was performed in 47 patients. Among them, 15 (32%) had disease-positive lymph nodes.

Semi quantitative immunostaining of ER, PR, Ki67 and p53. The results for semi-quantitative immunostaining of ER, PR, Ki67 and p53 are summarized in Figure 1. The semi-quantitative expression of each marker was significantly



Figue 1. *Comparison of semi quantitative estrogen receptor (ER), progesterone receptor (PR), Ki67 and p53 immunostaining between grade 1 or 2 and grade 3 endometrial cancer.*

correlated with the final histological grade. Higher immunostaining of ER and PR was found in grade 1 or 2 (p=0.01) compared with grade 3 endometrial cancer. Higher immunostaining of Ki67 (p<0.0001) and p53 (p<0.001) was found in grade 3, compared with grade 1 or 2 endometrial cancer. Figure 2 illustrates the immunostaining expression profiles of grade 1 or 2 and grade 3 endometrial cancer.

Optimal cut-offs denoting the strongest correlation between semi quantitative expression of each marker and final histological grade are summarized in Figure 3. The positivity cut-offs defined were 20%, 50%, 40% and 50% for ER, PR, Ki67 and p53, respectively.

Using the determined cut-offs, we compared qualitative ER, PR, Ki67 and p53 expression between grade 1 or 2 and grade 3 tumors: ER≥20% immunostaining was more common in grade 1 or 2 (87%, p=0.03) compared with grade 3 endometrial cancer (53%); PR≥50% immunostaining was more common in grade 1 or 2 (61%, p=0.01) compared with grade 3 (24%); Ki67≥40% immunostaining was more common in grade 3 (75%, p<0.0001) compared with grade 1 or 2 (14%); and p53≥50% immunostaining was more common in grade 3 (44%, p=0.004) compared with grade 1 or 2 (7%).

Recursive partitioning model. The RP model based on the previously-determined cut-offs is reported in Figure 4. We found that grade 1 or 2 and grade 3 tumors had different ER, PR, Ki67 and p53 immunohistochemical expression profiles. Indeed, the RP model predicted a grade 1 or 2 tumor in 98% of cases when Ki67 and p53 were underexpressed (*i.e.* below 40% and 50%, respectively). In contrast, the RP model found that the probability of grade 3 tumor was 80% when ER was underexpressed (*i.e.* below 20%).

We found a mis-classification rate of 13% in the training cohort; among the 45 grade 1 or 2 tumors that were predicted by the RP model, one (2.2%) case in fact had a grade 3 tumor. Among the 24 grade 3 tumors predicted by the RP

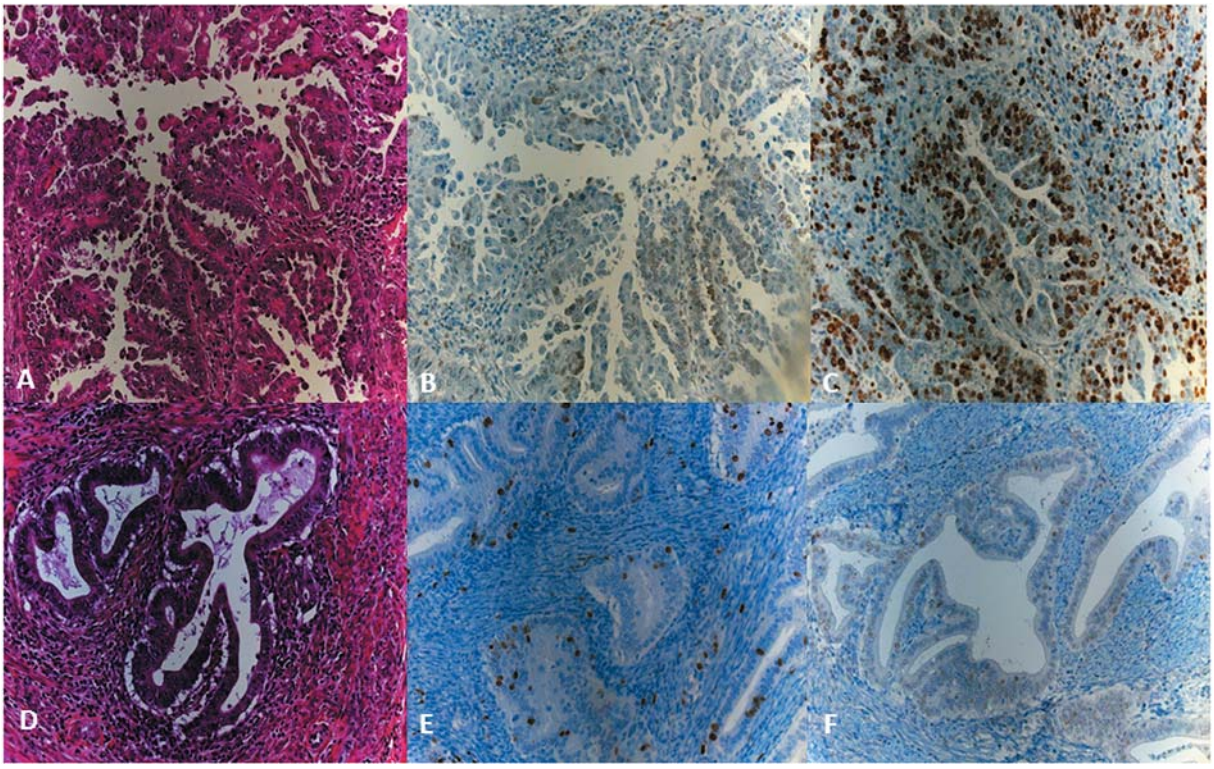


Figure 2. *Representative examples of immunostaining profiles of grade 1-3 endometrial cancer. Hematoxylin eosin staining in type 2 endometrial cancer (A). Estrogen receptor (ER) underexpression (B) and Ki67 overexpression (C) in type 2 endometrial cancer. Hematoxylin eosin staining in type 1 grade 1 or 2 endometrial cancer (D). Ki67 (E) and p53 (F) underexpression in type 1 grade 1 or 2 endometrial cancer.*

model, eight (33%) had a grade 1 or 2 tumor corresponding to type 1 endometrial cancer in all cases.

To internally validate the RP model, we used the bootstrap method with 1,000 re-samplings. The mis-classification rate was 19.2% (95% confidence interval=18.8 to 20.3%).

Discussion

Our results show that grade 1 or 2 endometrial carcinomas have different ER, PR, Ki67 and p53 immunostaining profiles compared with grade 3. Moreover, we found that final histological grade 1 or 2 can be accurately predicted using a combination of these routine immunohistochemical markers.

We chose to analyze histological grade, as it currently represents one of the major limitations to preoperative tumor staging along with depth of myometrial invasion. Histological grade, coupled with depth of myometrial invasion and histological subtype, is an independent prognostic factor for disease recurrence (3) and determines the surgical strategy (1). According to the current international guidelines, a patient with preoperative FIGO stage IB endometrioid grade 2 tumor should undergo total hysterectomy with bilateral salpingo-oophorectomy, while in the case of a grade 3 tumor, concomitant pelvic and para-aortic lymphadenectomy is recommended (1). However, many studies have demonstrated that considerable discrepancies exist between the preoperative and final histological grade (3, 4) which can lead to over- or undertreatment (37). Moreover, Werner *et al*. recently found that patients with discordant preoperative biopsy with final histology were at higher risk for disease spread and poorer prognosis (40).

We showed that grade 1 or 2 tumors can be predicted in almost 98% of cases by an RP model. This is a crucial point as the impact on surgical management is strongly relevant. Patients with predicted grade 1 or 2 tumors and presumed early-stage endometrial cancer at preoperative MRI might undergo total hysterectomy and bilateral salpingo-oophorectomy without lymphadenectomy, with a low risk of surgical downstaging (*i.e*. 2%). In contrast, we found that among the 24 grade 3 tumors predicted by the RP model, 33% had a grade 1 or 2 tumor. However, this result has to be interpreted with caution as we did not distinguish type 2 from type 1 grade 3 endometrial carcinomas. Indeed, Alkushi *et al*. previously demonstrated that type 1 grade 3 tumors have a different immunohistochemical profile from type 2 (30).

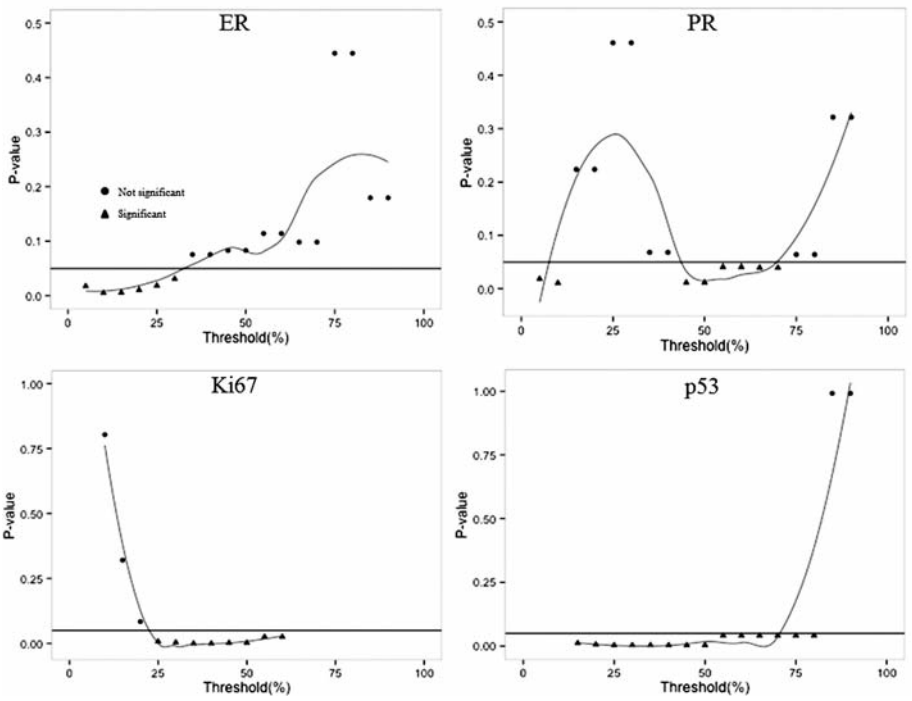


Figure 3. *Optimal cut-offs denoting a correlation between semi-quantitative immunostaining for estrogen receptor (ER), progesterone receptor (PR), Ki67 and p53 and histological grade.*

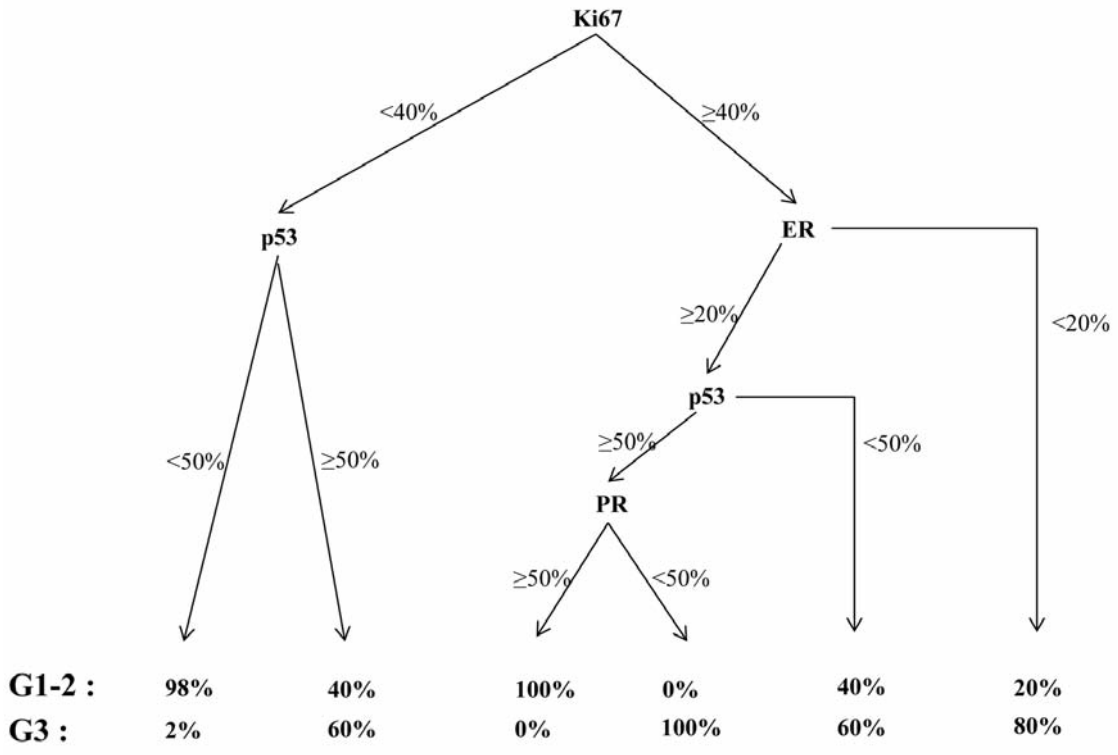


Figure 4. *Recursive partitioning model illustrating immunostaining profiles to predict final histological grade.*

However, all the patients with type 2 endometrial cancer, requiring systematic pelvic and paraaortic lymphadenectomy, were correctly classified by the RP model.

Our study demonstrates a significant correlation between ER, PR, Ki67 and p53 immunostaining and histological grade in endometrial cancer. We chose to assess these four markers as they are frequently used in routine practice to evaluate the aggressiveness of cancer. Our results are in agreement with those reported in the literature. Several studies have previously focused on the interest of additional ER, PR, Ki67 and p53 immunostaining to better-characterize histological endometrial cancer phenotypes. Indeed, a correlation has been found between ER and PR immunostaining and good differentiation with better prognosis (7, 8, 13, 14, 29, 32). In the same way, some authors have suggested that PR immunostaining is an independent prognostic factor for survival (11, 41). Moreover, positive p53 or Ki67 immunostaining is associated with more malignant phenotypes (29). Stefansson *et al*. reported that tumor cell proliferation measured by Ki67 was an independent prognostic factor, and that it could be of value in identifying tumors of the endometrioid subtype with poor prognosis (42). Finally, previous studies have shown that p53 is overexpressed in 70-90% of uterine serous carcinomas and in 10-35% of endometrioid carcinomas (43). Among patients with the endometrioid subtype, p53 overexpression was associated with histological grade 3 (8, 18, 33, 34). Many other markers correlating with histological type and grade of endometrial cancer have been reported. Cherchi *et al*. found that the overexpression of Human epithelial growth factor-2 (HER2/Neu) is correlated with a more malignant phenotype (18). More recently, Alkushi *et al*. reported that Phosphatase and tensin homolog (PTEN) and p16, which can help to distinguish between grade 3 endometrioid and serous carcinoma, are correlated with poorer disease-specific survival (30).

Two important points need to be underlined concerning the correlation between immunostaining profiles and histological grade. Firstly, in previous studies on ER and PR expression, cut-offs determining positive immunostaining were chosen arbitrarily or according to previously published data and varied from 1% to 50% according to the series (8, 29, 30, 34). In contrast, in our study, optimal cut-offs were calculated for each biological marker based on the strongest correlation between semi quantitative expression and final histological grade. This is of major importance as it allows for greater strength and better reproducibility. In addition, although we used a statistical approach to establish the optimal cut-offs, it appears that these cut-offs (especially for Ki67 and p53) are consistent with those reported in previous series (7, 30). Secondly, in most of the studies, immunohistochemical markers were reported as being independent factors. However, as it has been previously suggested, a panel of markers is required as no single marker is completely sensitive or specific for a given cell type (43). To our knowledge, only Alkushi *et al*. used two-marker combinations (p16 and PTEN) to distinguish grade 3 endometrioid carcinomas and serous adenocarcinomas and assessed the performance of the test by the area under the receiving operating characteristic (ROC) curve (30). This, therefore, justifies the use of a predictive model to establish immunostaining profiles with greater sensitivity and specificity.

Several limits of the current study should be underlined. Firstly, we cannot exclude any bias linked to the sample size and the high incidence of type-2 endometrial cancer. Secondly, immunostaining profiles and histological grade were assessed on final histology. To our knowledge, to date only Obeidat *et al*. have assessed immunohistochemical markers on biopsy specimens with encouraging results (35). However, we believe our study to be the first to predict histological grade by determining immunostaining profiles. Hence, we believe that this is a crucial step that cannot be omitted before applying the predictive model to biopsy specimens in order to assess its accuracy and reproducibility. Finally, although tumor cell type and histological grade can be diagnosed based on routine histology in most cases, we show that additional immunohistochemical markers can help improve the diagnosis of histological grade, especially for type 1 grade-1 or 2 endometrial cancer, and thus can contribute to the selection of the most appropriate surgical strategy.

Conclusion

Because of discrepancies between preoperative and final histology and the impact on the management of endometrial cancer, our results demonstrate that the additional immunohistochemical profile of ER, PR, p53 and Ki67 integrated into a simple predictive model, could help predict the final histological grade. Further studies are needed to validate these data on biopsy specimens used in routine practice.

References

1 Querleu D, Planchamp F, Narducci F, Morice P, Joly F, Genestie C, Haie-Meder C, Thomas L, Quénel-Tueux N, Daraï E, Dorangeon PH, Marret H, Taïeb S, Mazeau-Woynar V; Institut National duCancer; Societe Francaise d'Oncologie Gynecologique: Clinical practice guidelines for the management of patients with endometrial cancer in France: recommendations of the Institut National du Cancer and the Societe Francaise d'Oncologie Gynecologique. Int J Gynecol Cancer *21(5)*: 945-950, 2011.

2 Creasman WT, Morrow CP, Bundy BN, Homesley HD, Graham JE and Heller PB: Surgical pathologic spread patterns of endometrial cancer. A Gynecologic Oncology Group Study. Cancer *15;60(8 Suppl)*: 2035-2041, 1987.

3 Ballester M, Naoura I, Chereau E, Seror J, Bats AS, Bricou A and Daraï E: Sentinel node biopsy upstages patients with presumed low- and intermediate-risk endometrial cancer: results of a multicenter study. Ann Surg Oncol *20(2)*: 407-412, 2012.

4 Frumovitz M, Singh DK, Meyer L, Smith DH, Wertheim I, Resnik E and Bodurka DC: Predictors of final histology in patients with endometrial cancer. Gynecol Oncol *95(3)*: 463-468, 2004.

5 Eltabbakh GH, Shamonki J and Mount SL: Surgical stage, final grade, and survival of women with endometrial carcinoma whose preoperative endometrial biopsy shows well-differentiated tumors. Gynecol Oncol *99(2)*: 309-312, 2005.

6 Chakravarty D, Gupta N, Goda JS, Srinivasan R, Patel FD and Dhaliwal L: Steroid receptors, HER2/neu and Ki-67, in endometrioid type of endometrial carcinoma: Correlation with conventional histomorphological features of prognosis. Acta Histochem *112(4)*: 355-363, 2010.

7 Ferrandina G, Ranelletti FO, Gallotta V, Martinelli E, Zannoni GF, Gessi M and Scambia G: Expression of cyclooxygenase-2 (COX-2), receptors for estrogen (ER), and progesterone (PR), p53, ki67, and neu protein in endometrial cancer. Gynecol Oncol *98(3)*: 383-389, 2005.

8 Halperin R, Zehavi S, Habler L, Hadas E, Bukovsky I and Schneider D: Comparative immunohistochemical study of endometrioid and serous papillary carcinoma of endometrium. Eur J Gynaecol Oncol *22(2)*: 122-126, 2001.

9 Morris PC, Anderson JR, Anderson B and Buller RE: Steroid hormone receptor content and lymph node status in endometrial cancer. Gynecol Oncol *56(3)*: 406-411, 1995.

10 Creasman WT, Soper JT, McCarty KS Jr., McCarty KS, Sr., Hinshaw W and Clarke-Pearson DL: Influence of cytoplasmic steroid receptor content on prognosis of early stage endometrial carcinoma. Am J Obstet Gynecol *151(7)*: 922-932, 1985.

11 Fukuda K, Mori M, Uchiyama M, Iwai K, Iwasaka T and Sugimori H: Prognostic significance of progesterone receptor immunohistochemistry in endometrial carcinoma. Gynecol Oncol *69(3)*: 220-225, 1998.

12 Jazaeri AA, Nunes KJ, Dalton MS, Xu M, Shupnik MA and Rice LW: Well-differentiated endometrial adenocarcinomas and poorly differentiated mixed mullerian tumors have altered ER and PR isoform expression. Oncogene *20(47)*: 6965-6969, 2001

13 Jongen V, Briet J, de Jong R, ten Hoor K, Boezen M, van der Zee A, Nijman H and Hollema H: Expression of estrogen receptor-alpha and -beta and progesterone receptor-A and -B in a large cohort of patients with endometrioid endometrial cancer. Gynecol Oncol *112(3)*: 537-542, 2009.

14 Zannoni GF, Monterossi G, De Stefano I, Gargini A, Salerno MG, Farulla I, Travaglia D, Vellone VG, Scambia G and Gallo D: The expression ratios of estrogen receptor alpha (ERalpha) to estrogen receptor beta1 (ERbeta1) and ERalpha to ERbeta2 identify poor clinical outcome in endometrioid endometrial cancer. Hum Pathol (in press), 2012.

15 Salvesen HB, Iversen OE and Akslen LA: Identification of high-risk patients by assessment of nuclear Ki-67 expression in a prospective study of endometrial carcinomas. Clin Cancer Res *4(11)*: 2779-2785, 1998.

16 Salvesen HB, Iversen OE and Akslen LA: Prognostic significance of angiogenesis and Ki-67, p53, and p21 expression: a population-based endometrial carcinoma study. J Clin Oncol *17(5)*: 1382-1390, 1999.

17 Geisler JP, Wiemann MC, Zhou Z, Miller GA and Geisler HE: p53 as a prognostic indicator in endometrial cancer. Gynecol Oncol *61(2)*: 245-248, 1996.

18 Cherchi PL, Marras V, Capobianco G, Ambrosini G, Piga MD, Fadda GM, Rosas N and Dessole S: Prognostic value of p53, c-erb-B2 and MIB-1 in endometrial carcinoma. Eur J Gynaecol Oncol22(6): 451-453, 2001.

19 Lax SF, Pizer ES, Ronnett BM and Kurman RJ: Clear cell carcinoma of the endometrium is characterized by a distinctive profile of p53, Ki-67, estrogen, and progesterone receptor expression. Hum Pathol *29(6)*: 551-558, 1998.

20 Ohkouchi T, Sakuragi N, Watari H, Nomura E, Todo Y, Yamada H and Fujimoto S: Prognostic significance of Bcl-2, p53 overexpression, and lymph node metastasis in surgically staged endometrial carcinoma. Am J Obstet Gynecol *187(2)*: 353-359, 2002.

21 Appel ML, Edelweiss MI, Fleck J, Rivero LF, Rivoire WA, Monego HI and Dos Reis R: P53 and BCL-2 as prognostic markers in endometrial carcinoma. Pathol Oncol Res *14(1)*: 23-30, 2008.

22 Coronado PJ, Vidart JA, Lopez-asenjo JA, Fasero M, Furio-bacete V, Magrina J and Escudero M: P53 overexpression predicts endometrial carcinoma recurrence better than HER-2/neu overexpression. Eur J Obstet Gynecol Reprod Biol *98(1)*: 103-108, 2001.

23 Dupont J, Wang X, Marshall DS, Leitao M, Hedvat CV, Hummer A, Thaler H, O'Reilly RJ and Soslow RA: 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, 2004.

24 Erdem O, Erdem M, Dursun A, Akyol G and Erdem A: Angiogenesis, p53, and bcl-2 expression as prognostic indicators in endometrial cancer: comparison with traditional clinico-pathologic variables. Int J Gynecol Pathol *22(3)*: 254-260, 2003.

25 Koivisto-Korander R, Butzow R, Koivisto AM and Leminen A: Immunohistochemical studies on uterine carcinosarcoma, leiomyosarcoma, and endometrial stromal sarcoma: expression and prognostic importance of ten different markers. Tumour Biol *32(3)*: 451-459, 2011.

26 Nout RA, Bosse T, Creutzberg CL, Jurgenliemk-Schulz IM, Jobsen JJ, Lutgens LC, va der Steen-Banasik EM, van Eijk R, Ter Haar NT and Smit VT: Improved risk assessment of endometrial cancer by combined analysis of MSI, PI3K-AKT, Wnt/beta-catenin and P53 pathway activation. Gynecol Oncol *126(3)*: 466-473, 2012.

27 Soong R, Knowles S, Williams KE, Hammond IG, Wysocki SJ and Iacopetta BJ: Overexpression of p53 protein is an independent prognostic indicator in human endometrial carcinoma. Br J Cancer *74(4)*: 562-567, 1996.

28 Zheng W, Cao P, Zheng M, Kramer EE and Godwin TA. p53 overexpression and bcl-2 persistence in endometrial carcinoma: comparison of papillary serous and endometrioid subtypes. Gynecol Oncol *61(2)*: 167-174, 1996.

29 Markova I, Duskova M, Lubusky M, Kudela M, Zapletalova J, Prochazka M and Pilka R: Selected immunohistochemical prognostic factors in endometrial cancer. Int J Gynecol Cancer *20(4)*: 576-582, 2010.

30 Alkushi A, Kobel M, Kalloger SE and Gilks CB: High-grade endometrial carcinoma: serous and grade 3 endometrioid carcinomas have different immunophenotypes and outcomes. Int J Gynecol Pathol 29(4): 343-350, 2010.

31 Darvishian F, Hummer AJ, Thaler HT, Bhargava R, Linkov I, Asher M and Soslow RA: Serous endometrial cancers that mimic endometrioid adenocarcinomas: a clinicopathologic and immunohistochemical study of a group of problematic cases. Am J Surg Pathol *28(12)*: 1568-1578, 2004.
32 Kounelis S, Kapranos N, Kouri E, Coppola D, Papadaki H and Jones MW: Immunohistochemical profile of endometrial adenocarcinoma: a study of 61 cases and review of the literature. Mod Pathol *13(4)*: 379-388, 2000.
33 Yamauchi N, Sakamoto A, Uozaki H, Iihara K and Machinami R. Immunohistochemical analysis of endometrial adenocarcinoma for bcl-2 and p53 in relation to expression of sex steroid receptor and proliferative activity. Int J Gynecol Pathol *15(3)*: 202-208, 1996.
34 Zhu C, Luo J, Shi H, Xie X and Ding Z: Expression of tubulin, p53, ki67, receptors for estrogen, and progesterone in endometrial cancer. Eur J Gynaecol Oncol *30(5)*: 514-517, 2009.
35 Obeidat BR, Matalka, II, Mohtaseb AA and Al-Kaisi NS: Selected Immuno-Histochemical Markers in Curettage Specimens and their Correlation with Final Pathologic Findings in Endometrial Cancer Patients. Pathol Oncol Res (in press), 2012.
36 Petru E, Luck HJ, Stuart G, Gaffney D, Millan D and Vergote I: Gynecologic Cancer Intergroup (GCIG) proposals for changes of the current FIGO staging system. Eur J Obstet Gynecol Reprod Biol *143(2)*: 69-74, 2009.
37 Colombo N, Preti E, Landoni F, Carinelli S, Colombo A, Marini C and Sessa C: Endometrial cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol *22(Suppl 6)*: vi35-39, 2011.
38 Bevitt DJ, Milton ID, Piggot N, Henry L, Carter MJ, Toms GL, Lennard TW, Westley B, Angus B and Horne CH: New monoclonal antibodies to oestrogen and progesterone receptors effective for paraffin section immunohistochemistry. J Pathol *183(2)*: 228-232, 1997.
39 Zaki MA, Robbins JR, Fatteh S, Mahan MG, Hanna RK and Elshaikh MA: Histological grade predicts for recurrence in patients with uterine endometrioid carcinoma without myometrial involvement. Anticancer Res *32(9)*: 4061-4065, 2012.
40 Werner HM, Trovik J, Marcickiewicz J, Tingulstad S, Staff AC, Engh ME, Oddenes K, Rokne JA, Tjugum J, Lode MS, Amant F and Salvesen HB: A discordant histological risk classification in preoperative and operative biopsy in endometrial cancer is reflected in metastatic risk and prognosis. Eur J Cancer *49(3)*: 625-632, 2012.
41 Nyholm HC, Christensen IJ and Nielsen AL: Progesterone receptor levels independently predict survival in endometrial adenocarcinoma. Gynecol Oncol *59(3)*: 347-351, 1995.
42 Stefansson IM, Salvesen HB, Immervoll H and Akslen LA: Prognostic impact of histological grade and vascular invasion compared with tumour cell proliferation in endometrial carcinoma of endometrioid type. Histopathology *44(5)*: 472-479, 2004.
43 Clarke BA and Gilks CB: Endometrial carcinoma: controversies in histopathological assessment of grade and tumour cell type. J Clin Pathol *63(5)*: 410-415, 2010.

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