Comparative immunohistochemical study of endometrioid and serous papillary carcinoma of endometrium

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Summary

Objective: The aim of this study was to determine whether immunohistochemical analysis of molecular parameters can provide an alternative method for classification of endometrial cancer cases according to their aggressiveness.

Methods: Sixty-four cases of endometrial carcinoma were assigned to three groups: group I - 28 cases of endometrioid well and moderately differentiated (G_1-G_2) carcinoma; group II - 14 cases of endometrioid poorly differentiated (G_3) carcinoma; group III - 22 cases of serous papillary endometrial cancer. Immunohistochemistry was used to determine the existence of estrogen receptors (ER), progesterone receptors (PR), and the expression of bcl-2, p53, HER-2/neu and Ki-67.

Results: There was a significant difference in the immunohistochemical profile of the studied molecular parameters comparing the three study groups. The endometrioid G_1-G_2 cases (group I) were characterized by increased immunoreactivity for ER and PR (85.7% and 78.6%, respectively), increased immunoreactivity for bcl-2 (42.8%) and low expression of p53 (14.3%) and HER-2/neu (14.3%). In contrast to group I cases, the serous papillary endometrial cancer cases (group III) were characterized by immunonegativity for ER, PR and bcl-2 and high immunoreactivity for p53 (81.8%) and HER-2/neu (45.4%). The endometrioid G_3 cases (group II) demonstrated an intermediate immunoprofile, characterized by immunonegativity for ER, PR and HER-2/neu, low immunoreactivity for bcl-2 (7.1%) and high expression of p53 (57.1%). The expression of Ki-67 did not differ significantly comparing the different cases of endometrial cancer.

Conclusion: This study provides evidence that the immunohistochemical analysis of endometrial carcinoma differentiates between different grades and histological types, thus being useful in the distinction of high risk cases.

Key words: Endometrioid carcinoma of endometrium; Serous papillary carcinoma of endometrium; Grade; Immunohistochemistry; Molecular markers.

Introduction

In 1983, Bokhman [1] proposed dividing endometrial carcinoma into two broad categories that reflected two different pathogenetic pathways. One pathway was associated with low-grade tumors and was related to estrogenic stimulation (type I), and the other pathway was associated with high-grade tumors and unrelated to estrogenic stimulation (type II). According to Sherman [2], endometrioid carcinoma, known as type I, develops from atypical hyperplasia as a result of unopposed estrogenic stimulation, while serous carcinoma, known as type II, develops in atrophic endometrium. According to previous publications, there has been great variability in immunohistochemical expression of molecular parameters, such as estrogen and progesterone receptors (ER, PR) [3-5], bcl-2 [6, 7], p53 [5, 6, 8, 9], HER-2/neu [10-12] and Ki-67 [5, 13], comparing different histological types of endometrial carcinoma. Moreover, immunohistochemical studies were performed to examine the prognostic significance of steroid receptors [14, 15], oncoproteins p53 and HER-2/neu [16-19], and cell proliferation marker Ki-67 [13, 20, 21] in identifying high risk endometrial carcinoma cases. Yet, no extensive study examining the immunohistochemical status of endometrioid carcinoma in relation to it's grade has been reported. The current study was undertaken to evaluate the immunohistochemical expression of ER and PR, bcl-2, p53, HER-2/neu and Ki-67 in endometrioid G_1-G_2 carcinoma compared with endometrioid G_3 and serous papillary endometrial carcinoma. The wide range of molecular markers examined in this study may provide an immunohistochemistry based method for classification of endometrial carcinomas according to their aggressiveness, as expressed by immunohistochemical analysis.

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Materials and Methods

Selection of Cases

All cases of endometrial cancer, diagnosed between January 1995 and January 2000 were reviewed by a single pathologist (S.Z.). The histologic subtype of the tumor specimens was assessed according to the guideliness of the 1994 WHO Classification of Tumors of the Female Genital Tract [22], and the nuclear grade was classified according to Kurman *et al.* [23]. Sixty-four cases were assigned to three groups: group I - 28 cases of endometrioid well and moderately differentiated (G_1 and G_2) carcinoma; group II - 14 cases of endometrioid poorly differentiated (G_3) carcinoma; group III - 22 cases of serous papillary endometrial cancer.

All cases were staged using the criteria of the International Federation of Gynecology Oncology (FIGO) [24]. Twenty-eight cases in study group I included 26 in stage I and 2 cases in stage II. Fourteen cases in group II consisted of two cases in stage I, seven in stage II and five cases in stage III. Twenty-two cases in group III consisted of one case in stage I, two in stage II, 18 in stage III and one case in stage IV.

Immunohistochemical analysis

Two paraffin-embedded blocks from each case were selected for staining. Three micron tissue sections, placed on positive ion-charged slides, were stained. The slides were then deparaffinized, treated with 3% hydrogen peroxide for 20 min to block endogenous peroxidases, and then washed in distilled water. A microwave antigen retrieval procedure using citrate buffer, pH=6, was performed on all slides, besides those used for immunostaining with anti HER-2/neu. All slides, excluding those used for immunostaining with anti bcl-2, were incubated with primary antibody in Ventana autoimmunostainer Es (Ventana Medical Systems, S.A., Strasbourg, Cedex, France). For ER, the monoclonal mouse antibody-clone 6F11, prediluted from Zymed (Zymed Laboratories, Inc. San Francisco, Ca) was used. The mouse monoclonal antibody - clone PR-2C5, prediluted (Zymed) was used for PR immunostaining. For bcl-2 staining, the monoclonal mouse antibody-clone bcl-2-100 (Zymed) (dilution1:50) was added using the LSAB-2 (Dako, Glostrup, Denmark). For demonstration of Ki-67 and p53, the slides were incubated with monoclonal mouse antibodies: anti Ki-67-clone 7B11 (Zymed) (dilution 1:40) and anti p53-clone D07 (Dako A/S, Glostrup, Denmark) (dilution 1:100), respectively. For HER-2/neu immunostaining, the slides were incubated with mouse monoclonal anti HER-2/neu antibody-clone TAB 250 (Zymed) (dilution 1:100) and with Protease I in Ventana autoimmunostainer ES for 8 min. Slides were then developed with diaminobenzidine chromogen, lightly counterstained with Mayer's hematoxylin, and mounted.

A hematoxylin and eosin stained section was examined for each block, and a negative control slide, using nonspecific mouse IgG substituted for the primary antibody, was performed on all blocks. Background staining was negligible. The immunostained slides were compared with positive controls.

Immunostaining score

The immunostaining score was based on the percentage of stained cells out of 500 cells counted (0 = <10%, 1=10-25%, 2=26-50% and 3 = ≥50%), intensity (1=weak, 2=moderate, 3=strong) and heterogeneity (1=marked, 2=intermediate, 3=mild). Heterogeneity was defined as non-uniform or sporadic immunostaining patterns in tumor sections. The final score was calculated by adding the three parameters, as described by Zheng *et al.* [6]. The staining was defined positive when the final score was ≥7. Ki-67 index was expressed as percentage of positively stained cells per total 500 cells counted.

Statistical analysis

The percentage of immunoreactive cases regarding each molecular parameter was statistically compared between the study groups using the chi-square test and the two-tailed student's test; $p<0.05$ was considered statistically significant.

Results

The three study groups differed regarding patient age (group I - mean 58.7; group II - mean 67; group III - mean 69.3).

Immunohistochemical analysis of endometrial carcinoma cases according to the study groups is presented in Table 1. Nuclear specific staining with monoclonal antibodies, recognizing ER and PR (Figures 1C, D), was measured in 85.7% and 78.6% of cases in group I, respectively, versus none of the staining in groups II and III (p=0.00001). There was also a significant difference in the cytoplasmic expression of bcl-2 (Figure 1E) comparing group I (42.8%) versus group II (7.1%) (p=0.018) and group III (0%) (p=0.0004). Nuclear specific staining for p53 (Figure 1F) demonstrated an inverted ratio, being significantly more predominant in group III (81.8%) and group II (57.1%) than in group I (14.3%) (p=0.0001 and p=0.004, respectively) (Table 1). Membrane specific staining with monoclonal antibody directed toward the HER-2/neu gene product (Figure 1G) was demonstrated in 14.3% of cases in group I, in none of the cases in group II (0%) and in 45.4% of cases in group III (p=0.13 and p=0.014, respectively). Ki-67 proliferation index, expressed as percent of stained nuclei (Figure 1H), was not significantly different comparing the cases in group I (32%), group II (50%) and group III (52%).

Table 1. — *Comparison of immunohistochemical analysis of endometrial carcinoma cases according to the study groups.*

Molecular Parameter	Group I Endometrioid G_1-G_2 (n=28)	Group II Endometrioid G_3 (n=14)	Group III Serous Papillary (n=22)	Significance		
				P[a]	P[b]	P[c]
ER	24 (85.7%)	0 (0%)	0 (0%)	0.00001	0.00001	NS[d]
PR	22 (78.6%)	0 (0%)	0 (0%)	0.00001	0.00001	NS
bcl-2	12 (42.8%)	1 (7.1%)	0 (0%)	0.018	0.0004	NS
p53	4 (14.3%)	8 (57.1%)	18 (81.8%)	0.004	0.0001	NS
HER-2/neu	4 (14.3%)	0 (0%)	10 (45.4%)	NS	0.014	0.003
Ki-67	32%	50%	52%	NS	NS	NS

[a]P value between groups I and II; [b]P value between groups I and III; [c]P value between groups II and III; [d]NS = not significant.

Discussion

The aim of the current study was to perform immunohistochemical analysis and to define the aggressiveness of endometrial cancer according to different molecular parameters. We examined the immunoreactivity for ER and PR, bcl-2, p53, HER-2/neu and Ki-67 and 64 cases of endometrial carcinoma, divided into three groups according to the grade of differentiation and histological subtype. Previous studies have suggested that the status of ER and PR could be a significant prognostic parameter in endometrial carcinoma [14, 15, 25, 26]. Our study demonstrated an increased immunostaining for ER and PR in cases of group I, while there was no immunoreactivity for hormone receptors in cases assigned to study groups II and III. Thus, the cases of endometrioid G_3 carcinoma could be ascribed to the pathogenetic pathway associated with tumors unrelated to estrogenic stimulation. Another theoretical possibility could be that further development into poorly differentiated neoplastic tissue might cause the disappearance of steroid hormone receptors. Nevertheless, this theory is yet to be proved.

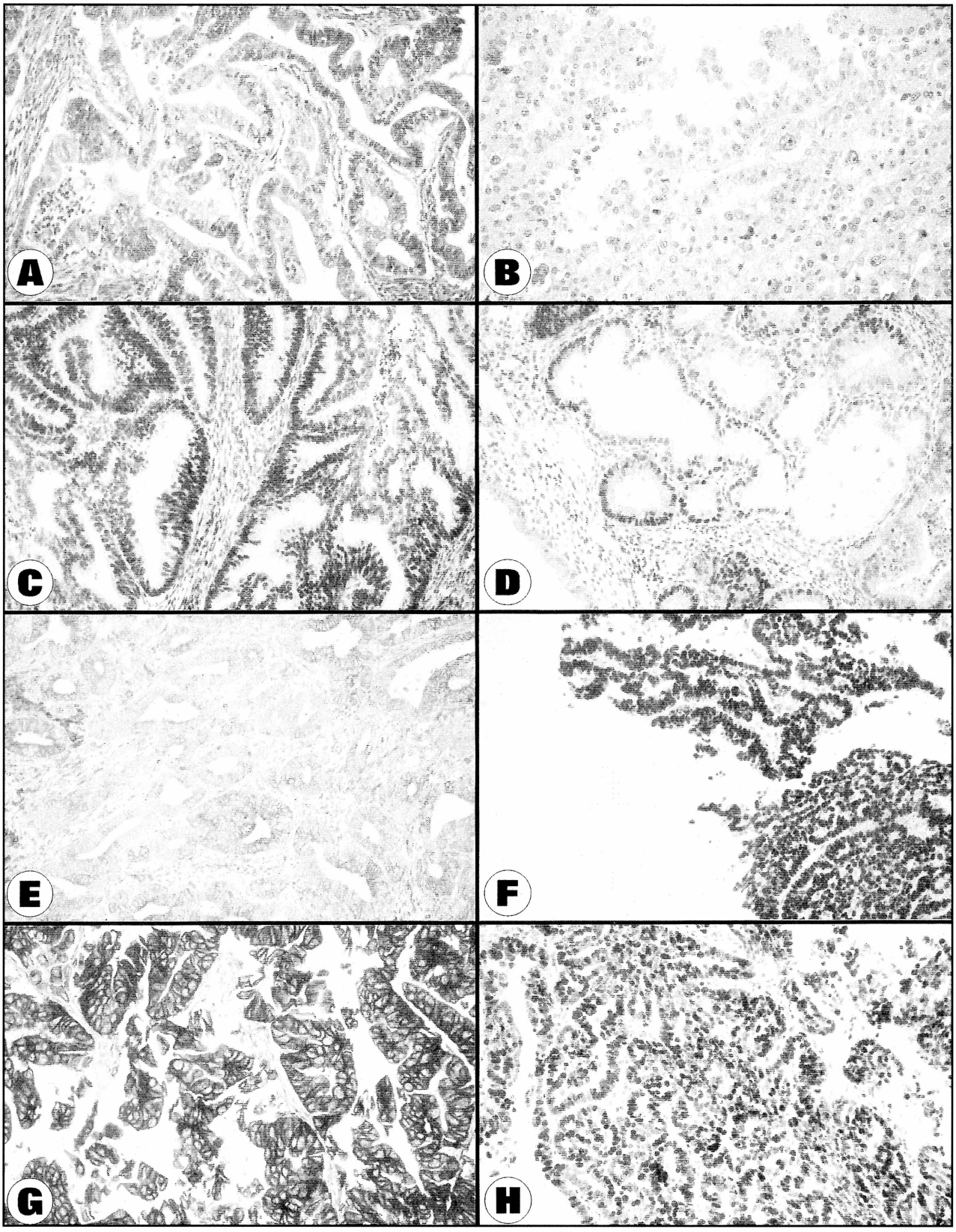

Figure 1. — Immunohistochemical staining for different molecular parameters in endometrioid and serous papillary endometrial carcinomas (EC). Hematoxylin and eosin stain in A) endometrioid and B) serous papillary EC. Nuclear specific staining for C) estrogen receptor (ER) and D) progesterone receptor (PR) in endometrioid EC. E) Cytoplasmic bcl-2 immunoreactivity in endometrioid EC. F) Nuclear specific staining for p53 in serous papillary EC. G) Membrane specific staining for HER-2/neu in serous papillary EC. H) Nuclear specific staining for Ki-67 in serous papillary EC. (Original magnifications x 200).

The role of bcl-2 protein in endometrial cancer has not been well studied. In a study by Zheng *et al.* [6], bcl-2 expression progressively diminished from proliferative endometrium to hyperplastic endometrium and to endometrial cancer. Henderson *et al.* [27] reported a down regulation of bcl-2 in cases of endometrial cancer, and observed no association of bcl-2 with grade, stage, ER or PR status. According to our study, bcl-2 expression correlated with histological subtype and grade (Table 1), demonstrating immunoreactivity in 42.8% of endometrioid G_1-G_2 cases, in 7.1% of endometrioid G_3 and in none of the serous papillary cases ($p=0.018$ and $p=0.0004$, respectively). Mitursky *et al.* [28] also demonstrated bcl-2 immunopositivity in women with early clinical disease. Histological grade, surgical stage and PR accumulation were strongly related with bcl-2 expression in endometrial neoplasia, as reported by Taskin *et al.* [29]. According to Saegusa *et al.* [30], bcl-2 was positively correlated with PR status in tubular but not in solid components of endometrial carcinoma, and no association with ER was reported. Our results strengthen the assumption that immunoreactivity for bcl-2 is related with hormonal receptor status as well as with the grade of differentiation of endometrioid endometrial tumors.

Overexpression of the tumor suppressor gene p53 in endometrial carcinoma has previously been reported [5-9, 31]. The immunohistochemical evidence of nuclear accumulation of mutant p53 protein has been in the range of 10% to 86% according to the literature [8, 9, 32]. We demonstrated a significant correlation between p53 overexpression and the histological grade and subtype of endometrial carcinoma. It has previously been demonstrated that the expression of p53 protein is an important event in the development of serous tumors [9], while p53 mutations are not usually found in endometrioid carcinomas [8, 33], associated with hyperestrogenism and endometrial hyperplasia. Still, according to our data, there was a significant increase in the frequency of p53 overexpression in endometrioid carcinomas G_3 compared with endometrioid carcinomas G_1-G_2 ($p=0.004$). The group of serous papillary carcinomas presented 81.8% positive immunostaining for p53, thus emphasizing the difference in tumor aggressiveness between the endometrioid and the serous histological type.

Data, concerning the relationship between bcl-2 and p53 expression in endometrial carcinomas, is contradictory and not fully understood. Saegusa *et al.* [34] demonstrated an inverse correlation between bcl-2 immunostaining and p53 accumulation in specimens of the neoplastic endometrium. Also Taskin *et al.* [29] reported an inverse relationship between bcl-2 and p53, but only in G_1 and not in G_2 or G_3. No relationship between bcl-2 expression and p53 accumulation in endometrial neoplasia has been demonstrated in other studies [6, 28, 35]. Our results demonstrated an inverse correlation between bcl-2 immunostaining and p53 accumulation in studied specimens of endometrioid (all grades) and serous papillary endometrial carcinoma (Table 1).

Positive immunostaining for HER-2/neu was observed mainly in the group of serous papillary carcinomas, being probably the best differentiating marker between the endometrioid (including G_3) and serous papillary endometrial cancers. The HER-2/neu proto-oncogene, encoding a transmembrane tyrosine kinase growth factor receptor protein [36], has been associated with increased recurrency and aggressive behavior in breast and ovarian tumors [37, 38]. As in previous publications [11, 12], our results demonstrated no correlation between HER-2/neu expression and tumor grade, yet there was a significant correlation with histological subtype ($p=0.003$) (Table 1). This data strengthens the association of positive HER-2/neu immunostaining with tumor aggressiveness and with decreased survival [39].

The nuclear Ki-67 antigen is present in all stages of the cell cycle except G_0 [40], and the development of antibodies against Ki-67 has enabled the detection of proliferating cells in microwave-processed sections obtained from formalin-fixed and paraffin-embedded specimens [41]. Our results, regarding the expression of Ki-67, demonstrated no correlation with histological type or grade. This data is in contradiction with previously published reports [42], presenting a significant association between the expression of Ki-67 and the histological type and grade of endometrial carcinoma.

Our study demonstrates the uniqueness of endometrioid G_3 endometrial carcinoma. The identification of high risk endometrial cancer cases can be based on an array of molecular markers in addition to the histological distinction. Our study presents the aggressiveness of endometrioid carcinoma G_3 endometrial cancer, being related much more closely to serous papillary carcinoma than to endometrioid carcinoma G_1-G_2. Only the expression of HER-2/neu differs between endometrioid G_3 endometrial cancer and the serous papillary type, placing the endometrioid carcinoma G_3 in an intermediate place between endometrioid G_1-G_2 and serous carcinoma. The importance of this point may be in the consideration of adjuvant therapy. The similarity of endometrioid G_3 carcinoma to the serous papillary type may raise the question of adjuvant chemotherapy. However, this issue is yet to be studied.

The absence of ER and PR expression and the infrequent association with endometrial hyperplasia suggest that endometrioid carcinoma G_3 is hormone independent. Moreover, the distinctive immunoprofile of endometrioid G_3 suggests that the underlying molecular genetic events, responsible for its development, may differ from those of endometrioid carcinoma G_1-G_2 on one hand, and those of serous carcinoma on the other hand. Thus, there might be another pathway in endometrial carcinogenesis, leading to the development of poorly differentiated endometrioid carcinoma.

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