Prognostic Significance of E-Cadherin Protein Expression in Pathological Stage I-III Endometrial Cancer

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ABSTRACT

Purpose: **Decreased expression of E-cadherin in endometrial cancer cells is associated with adverse prognostic features. This study aimed to evaluate the prognostic significance of decreased E-cadherin expression in patients with endometrial cancer.**

Experimental Design: **Between 1992 and 1999, 102 endometrial cancer patients with stage I-III disease underwent primary surgery at the University of Chicago. Representative tissue specimens were immunostained with a monoclonal antibody to E-cadherin. A semiquantitative evaluation scale was developed based on the percentage of endometrial cancer cells with membranous E-cadherin staining. Tissue sections were scored as "3" if >75%, "2" if 25–75%, "1" if 5–25%, and "0" if <5% of cells stained. E-Cadherin staining was correlated with overall survival (OS), cause-specific survival (CSS), progression-free survival (PFS), and extrapelvic progression. Multivariate Cox proportional hazards modeling was used to estimate hazard ratios, controlling for clinicopathological characteristics and adjuvant treatment. Median follow-up for the study group was 58.5 months.**

Results: **E-Cadherin staining was scored as 0, 1, 2, and 3 in 29.4%, 18.6%, 26.5%, 25.5% of cases, respectively. E-Cadherin expression was positively correlated with myometrial invasion (Kendall τ: 0.30, $P < 0.01$), and negatively correlated with grade (Kendall τ: -0.13, $P = 0.15$) and papillary serous or clear cell histology (Kendall τ: -0.14, $P = 0.12$). Five-year actuarial OS, CSS, PFS, and extrapelvic recurrence rates for negative (score = 0), heterogeneous (score = 1–2), and positive (score = 3) staining were as follows: OS, 69.2 *versus* 75.7 *versus* 81.0% ($P = 0.64$); CSS, 78.8 *versus* 91.2 *versus* 95.5% ($P = 0.19$); PFS, 69.1 *versus* 88.6 *versus* 92.2% ($P = 0.079$), and extrapelvic progression, 20.8 *versus* 7.3 *versus* 4.0% ($P = 0.17$). On multivariate Cox regression, a higher E-cadherin expression score was associated with decreased overall mortality [hazard ratio (HR), 0.59; 95% confidence interval (CI), 0.34–1.03; $P = 0.066$), and statistically significant decreases in endometrial cancer mortality (HR, 0.23; 95% CI, 0.055–0.94; $P = 0.040$), disease progression (HR, 0.28; 95% CI, 0.10–0.77; $P = 0.014$), and extrapelvic recurrence (HR, 0.24; 95% CI, 0.062–0.97; $P = 0.045$).**

Conclusions: **Decreased E-cadherin expression is an independent prognostic factor for disease progression and mortality in pathological stage I-III endometrial cancer. Evaluation of E-cadherin expression may aid in the selection of patients for more aggressive adjuvant therapy.**

INTRODUCTION

Endometrial cancer is the most common malignancy of the female genital tract, with an annual incidence of ~24.6 cases per 100,000 women (1). In the majority of patients, the prognosis for long-term survival after primary therapy, typically total abdominal hysterectomy and bilateral salpingo-oophorectomy, is excellent (2). However, patients with tumor invasion deep into the myometrium, poor tumor differentiation, papillary serous or clear cell histology, or extension of disease to other organs or lymph nodes within the pelvis, are at higher risk for disease recurrence (2–4).

Adjuvant radiation therapy to the pelvis has been used to treat residual disease and reduce the risk of pelvic recurrence in such patients (5–9). Unfortunately, many women with high-risk pathological features ultimately develop extrapelvic metastases, despite receiving adjuvant radiation therapy (10–15). In such cases, extrapelvic recurrence may occur either because unrecognized distant metastatic cells were present at the time local therapy was administered, or because local therapy was inadequate to eliminate clinically relevant tumor cells from the pelvis.

The addition of adjuvant chemotherapy has, therefore, been considered as a way to reduce the risk of extrapelvic recurrence in high-risk patients (16–18). However, because the majority patients in this high-risk group will not develop distant metastases, and some patients may fail despite receiving chemotherapy, a strategy of treating all high-risk patients with chemotherapy would expose many women to unnecessary toxicity. Better prognostic indicators are, therefore, needed to identify which patients are most likely to develop extrapelvic metastases and, thus, potentially benefit from chemotherapy.

Proteins involved in oncogenesis and metastasis are natural candidate molecular markers the expression of which can be analyzed in archived surgical samples and correlated with tumor

Received 6/17/03; revised 4/15/04; accepted 4/26/04.

Grant support: This research was supported in part by a generous grant from the Wendy Will Memorial Cancer Fund.

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recurrence and mortality in retrospective studies. Molecular markers have been studied intensely in endometrial cancer in attempt to predict metastatic potential or clinical outcome (19–27). Several studies have demonstrated a potential role for the transmembrane protein E-cadherin, a regulator of cell–cell adhesion, in predicting risk for metastasis in breast cancer (28–32). Recent immunohistochemical analyses have indicated that decreased expression of E-cadherin on the tumor cellular membrane is correlated with known adverse prognostic features and lower overall survival (OS) in patients with endometrial carcinoma (33–37). The present study used immunohistochemistry to examine the relationship between E-cadherin protein expression and clinical outcome in patients with pathological stage I-III endometrial carcinoma.

MATERIALS AND METHODS

Case Selection. This study was approved by the Institutional Review Board at the University of Chicago. We analyzed 112 women diagnosed with pathological stage I-III endometrial cancer at the University of Chicago between 1992 and 1999. Initial diagnoses were made by pathological review of endometrial biopsy or curettage specimens. As primary therapy, 107 women underwent total abdominal hysterectomy and bilateral salpingo-oophorectomy (BSO), 2 women underwent radical hysterectomy, and 1 had a total vaginal hysterectomy with BSO. Two women who received preoperative radiation therapy were excluded from the analysis.

Pathological stage, grade, and histological subtype (endometrioid, papillary serous, and so forth) were determined for each surgical specimen according to 1988 International Federation of Gynecology and Obstetrics (FIGO) criteria. Slides from each case were re-reviewed by a gynecological pathologist (A. G. M.) to verify the diagnosis, stage, grade, and histological type. Four women with sarcoma or spindle cell carcinoma of the uterus, 3 women with synchronous ovarian cancer, and 1 woman for whom follow-up could not be obtained were also excluded, leaving 102 women available for analysis.

Fifty-two of 102 women were surgically staged and had no pathological evidence of disease outside the pelvis. Forty-nine of the 52 women who underwent lymph node sampling or dissection were found to have no nodal disease. Three women with stage IIIA disease had tumor extending to the serosa or adnexa but had negative peritoneal washings. Three women with stage IIIC disease had tumor involving the pelvic but not para-aortic lymph nodes. The remaining 50 women did not undergo surgical staging; of these, 24 had grade 1, 16 had grade 2, and 10 had grade 3 disease. Lymph node sampling or dissection was generally performed in patients having tumors with deep myometrial invasion and/or high-grade or aggressive histological features. Obesity, advanced age, and excessive comorbidity were mitigating factors against full surgical staging.

Immunohistochemistry. Surgical specimens were fixed in 10% buffered formalin and were embedded in paraffin. H&E-stained slides for each case were reviewed to identify sections likely to contain both tumor and normal adjacent endometrium. Paraffin specimens were cut into 4-μm sections and were mounted on positively charged slides. The slides were deparaffinized and rehydrated in xylene followed by graded alcohols, then washed in Tris-buffered saline. Antigen unmasking was performed in a microwave oven in 0.01 M sodium citrate buffer (pH 6.0). Slides were then incubated in 0.03% hydrogen peroxide for 5 min to block endogenous peroxidase activity, followed by incubation for 20 min in a protein-blocking solution (Protein Block Serum-free solution, DAKO Corp. cat X090989, Carpinteria, CA) to reduce nonspecific background. A primary antihuman E-cadherin mouse monoclonal antibody (clone 36B5, 1:25 dilution; Vector Laboratories, Burlingame, CA) was applied for 1 h at room temperature. Slides were then incubated for 30 min at room temperature with goat antimouse IgG conjugated to a horseradish peroxidase-labeled polymer (DAKO Envision+ System; DAKO Corp., Carpinteria, CA), treated for 5 min with 3–3′-diaminobenzidine (DAB) chromogen, counterstained with hematoxylin, and coverslipped. Hysterectomy specimens for 10 women with benign uterine disease were selected to ascertain E-cadherin protein expression in benign endometrial tissue. Both benign endometrium and tonsillar squamous epithelium exhibited strong staining and were used as positive controls. Appropriate negative controls for the immunostaining were prepared by omitting the primary antibody step.

Evaluation of Immunostaining. Slides were examined jointly by two scorers (A. G. M., J. J. M.), who were blinded to patient outcome. For each slide, a minimum of 10 fields with well-preserved tumor was examined at ×100 magnification. An average was taken over these fields to identify the percentage of tumor cells demonstrating membranous E-cadherin expression. Intensity of staining and extent of cytoplasmic staining were not considered in this scoring system. Slides were assigned a score of 3, 2, 1, or 0, according to a predefined scoring system, depending on whether membranous E-cadherin expression was found in >75%, 25–75%, 5–25%, or <5% of cells, respectively (Fig. 1). E-Cadherin expression was considered "negative" if a score of 0, "heterogeneous" if a score of 1 or 2, and "positive" if a score of 3 was assigned. Expression was considered "low" if a score of 0–2 was assigned. A subset of slides (97 of 102) was reviewed independently by a third scorer (M. T.), also blinded to clinical outcome, to test interobserver agreement of the scoring system.

Clinical Data. Patient data were obtained from three sources: hospital tumor registry, automated database, and chart review. The tumor registry systematically collects baseline data, including demographic, diagnosis, and treatment information on all cancer patients who are diagnosed or treated at the University of Chicago. Patients are contacted annually by registry personnel to ascertain status of disease, survival, and cause of death. Automated records and, when available, charts (63 of 102) for each patient were reviewed to verify diagnosis and presence or absence of radiographic or pathological evidence of disease recurrence. All cases of recurrence had radiographic evidence of disease or biopsy-proven progression of disease.

Statistical Analysis. Frequencies were compared with the Pearson χ^2 test. The outcomes analyzed were OS, cause-specific survival (CSS), progression-free survival (PFS), and extrapelvic progression (EPP). Univariate analysis was performed using the method of Kaplan-Meier, applying the log-rank test to test the equality of survival functions. Prognostic models used multivariate Cox proportional hazards, adjusting for age, race, stage, myometrial invasion, grade, lymphovascular

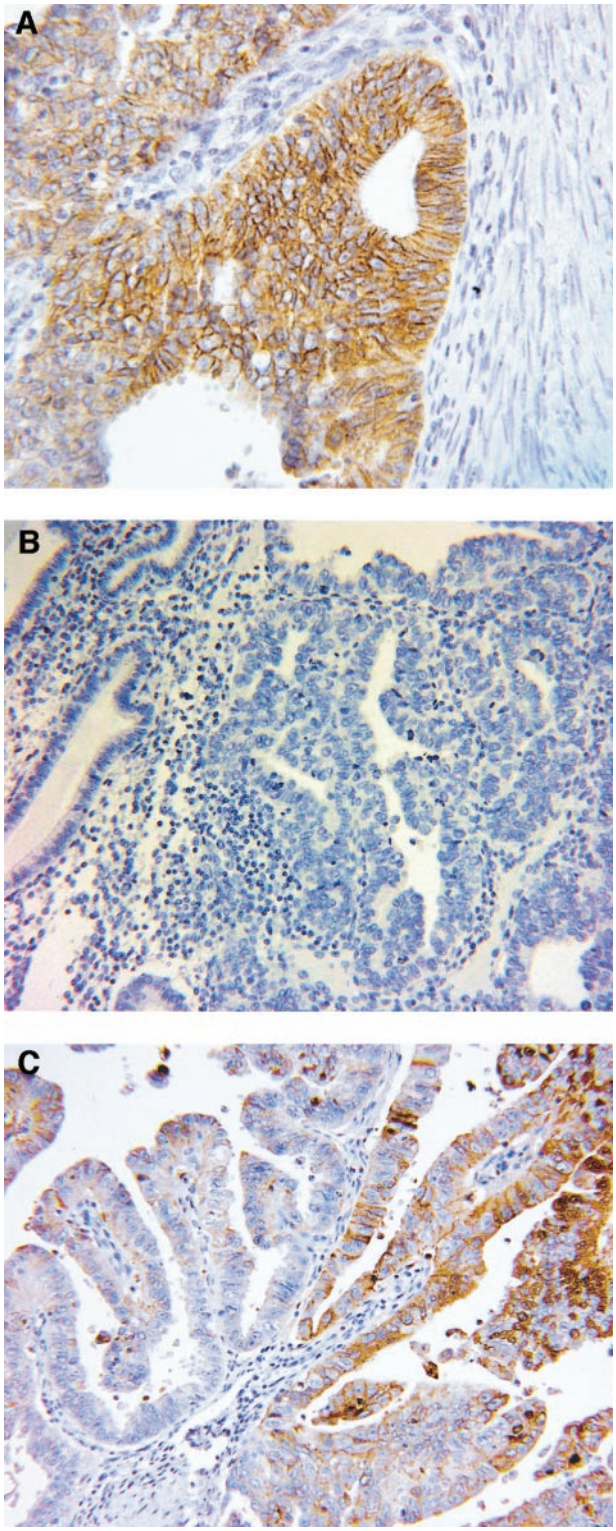

Fig. 1 Classification of E-cadherin staining. *A*, >75% of tumor cells stained with monoclonal antibody to E-cadherin (positive); *B*, <5% of tumor cells stained (negative); *C*, heterogeneous staining.

space invasion, unfavorable histology (papillary serous or clear cell), chemotherapy, and radiation therapy. The proportional hazards assumption was tested with the Schoenfeld residual method. The Kendall τ-b correlation coefficient was used to evaluate the association between E-cadherin expression and grade, unfavorable histology, and myometrial invasion. Agreement between observers' scores was tested with the weighted kappa statistic. All of the statistical analyses were conducted with SAS (SAS Corp., Cary, NC) and STATA (STATA Corp., College Station, TX) software.

RESULTS

The clinical characteristics of our sample are summarized in Table 1. The majority of patients were white (63.7%) and had pathological stage I disease (77.4%). Eight women had involvement of extrauterine sites limited to the pelvis. Grade 3 histology, deep (outer two-thirds) myometrial invasion, and unfavorable histology were seen in 16.7%, 17.6%, and 9.8% of patients, respectively. Adjuvant chemotherapy and radiation therapy were administered in 9.8% and 19.6% of patients, respectively.

E-Cadherin staining was scored as 0, 1, 2, and 3 in 29.4%, 18.6%, 26.5%, and 25.5% of cases, respectively. Inter-observer agreement was 82.8% [weighted kappa = 0.57; 95% confidence interval (CI) 0.46–0.67]. Strong membranous E-cadherin expression was observed in all 10 specimens of benign endometrial tissue. Overall, low E-cadherin expression was seen in 74.5% of endometrial cancer patients. Associations between low expression and clinicopathological features are summarized in Table 2. A more detailed analysis of the association between E-cadherin expression and grade, histology, and myometrial

Table 1 Demographics and clinical characteristics (N = 102)

Median age at diagnosis (range)	62.5 (17–85)
Ethnicity	
White	65 (63.7%)
Black	33 (32.4%)
Other	4 (3.9%)
Stage	
IA	10 (9.8%)
IB	60 (58.8%)
IC	9 (8.8%)
IIA	7 (6.9%)
IIB	8 (7.8%)
IIIA	3 (2.9%)
IIIB	2 (2.0%)
IIIC	3 (2.9%)
Grade	
1	45 (44.1%)
2	40 (39.2%)
3	17 (16.7%)
Deep myometrial invasion (≥1/2)	18 (17.6%)
Lymphovascular space invasion	10 (9.8%)
Histology	
Endometrioid	69 (67.6%)
Adenosquamous	11 (10.8%)
Mucinous	3 (2.9%)
Papillary serous	11 (10.8%)
Clear cell	7 (6.9%)
Mixed papillary serous/clear cell	1 (1.0%)
Chemotherapy	10 (9.8%)
Radiation therapy	20 (19.6%)

Table 2 E-Cadherin staining by clinical characteristics

Characteristic	Low[a] E-cadherin staining (%)	P^b
Age (yrs)		
<65	28/39 (71.8%)	
≥65	48/63 (76.2%)	0.62
Ethnicity		
White	50/65 (76.9%)	
Other	26/37 (70.3%)	0.46
Stage		
IA–IB	55/70 (78.6%)	
IC–IIB	16/24 (66.7%)	
III	5/8 (62.5%)	0.37
Grade		
1	34/45 (75.6%)	
2	27/40 (67.5%)	
3	15/17 (88.2%)	0.25
Myometrial invasion		
<1/2	65/84 (77.4%)	
≥1/2	11/18 (61.1%)	0.15
Lymphovascular space invasion		
No	70/92 (76.1%)	
Yes	6/10 (60.0%)	0.27
Histology		
Favorable	59/83 (71.1%)	
Unfavorable	17/19 (89.5%)	0.10

[a] Staining score of 0–2.
[b] Pearson χ^2 test.

invasion is exhibited in Table 3. E-Cadherin expression was negatively correlated with grade (Kendall τ, -0.13; $P = 0.15$) and papillary serous or clear cell histology (Kendall τ, -0.14; $P = 0.12$), but these associations were not statistically significant. E-Cadherin expression was found to be positively correlated with myometrial invasion in this sample (Kendall τ, 0.30; $P < 0.01$).

The median follow-up for all patients was 58.5 months (range, 4–152 months). Seventeen patients (16.7%) relapsed (6 pelvic, 10 extrapelvic, 1 both). Nine relapses occurred in stage I patients, 6 occurred in stage II patients, and 2 occurred in stage III patients. The median time to disease recurrence was 10 months (range, 3–79 months). Overall, 11 patients (10.8%) died of endometrial cancer, and 21 patients (20.6%) died of other causes. The 5-year actuarial OS, CSS, PFS, and EPP rates for all patients were 75.2, 88.7, 83.5, and 10.7%, respectively. Fig. 2 illustrates univariate outcomes based on E-cadherin staining. Five-year OS for negative, heterogeneous, and positive E-cadherin staining was 69.2, 75.7, and 81.0%, respectively ($P = 0.64$). Five-year CSS for negative, heterogeneous, and positive E-cadherin staining was 78.8, 91.2, and 95.5%, respectively ($P = 0.19$). Five-year PFS for negative, heterogeneous, and positive E-cadherin staining was 69.1, 88.6, and 92.2%, respectively ($P = 0.079$). The 5-year EPP rates for negative, heterogeneous, and positive E-cadherin staining were 20.8, 7.3, and 4.0%, respectively ($P = 0.17$).

On multivariate Cox regression adjusting for stage, grade, deep myometrial invasion, lymphovascular space invasion, unfavorable histology, adjuvant therapy, age, and race, increased E-cadherin expression was associated with statistically significant decreases in endometrial cancer mortality, disease progression, and EPP, with a trend toward decreased overall mortality (Table 4). In each model the proportional hazards assumption was satisfied (global $P > 0.05$), except the model for OS. The hazard ratios for the continuous variable reflecting E-cadherin staining were as follows: overall mortality: HR, 0.59; 95% CI, 0.34–1.03; $P = 0.066$); endometrial cancer death: HR, 0.23; 95% CI, 0.055–0.94; $P = 0.040$; disease progression: HR, 0.28; 95% CI. 0.10–0.77; $P = 0.014$); and extrapelvic recurrence: HR. 0.24; 95% CI, 0.062–0.97; $P = 0.045$. Deep myometrial invasion and unfavorable histology were associated with increased overall mortality, endometrial cancer death, disease progression, and extrapelvic recurrence. Black race was also associated with poor outcomes, and a statistically significant increase in disease progression (HR, 3.98; 95% CI, 1.31–12.1; $P = 0.016$).

DISCUSSION

This study adds to the increasing body of evidence implicating E-cadherin in the pathogenesis of invasive and metastatic endometrial cancer. E-Cadherin is a transmembrane protein the extracellular domain of which regulates cell–cell adhesion via a Ca^{2+}-dependent mechanism. Its role in inhibiting invasion and metastasis is related to its function in prohibiting the first step in the metastatic cascade, namely local invasion (38–39). Decreased expression of E-cadherin in tumor cells has been shown to correlate with increased invasiveness *in vitro*, increased metastasis in murine models, and adverse clinicopathological characteristics in numerous human cancers (40–53).

In the 102 patients studied here, decreased E-cadherin expression was found to be associated more commonly with higher grade and with papillary serous and clear cell histology, albeit these relationships were not statistically significant. Interestingly, decreased E-cadherin expression was found to be correlated with decreased myometrial invasion, in contrast to two preceding studies that have analyzed this relationship (33, 37). This may be related to decreased E-cadherin expression in the initial invasive stage, and subsequent "re-expression" at metastatic sites, as has been found in prostate cancer (51–52) and perhaps breast cancer (53). Endometrial carcinoma cells that have invaded deeply into the myometrium may behave much like prostate cancer cells at sites distant from their origin and may require normal expression of cell adhesion molecules to survive in their new environment. With the first step in the metastatic cascade surpassed, survival of metastatic or deeply invasive cells may then depend on re-establishing normal cell adhesiveness.

This study provides evidence that decreased membranous E-cadherin expression is predictive for endometrial cancer mortality, disease progression, and extrapelvic recurrence, independent of known prognostic factors such as stage, grade, and histological subtype. Moreover, an inverse relationship between E-cadherin expression and adverse outcomes was observed in

Table 3 Association between E-cadherin staining and grade, histology, and myometrial invasion

Characteristic	Kendall τ	P
Grade	−0.13	0.15
Papillary serous/clear cell histology	−0.14	0.12
Myometrial invasion	0.30	<0.01

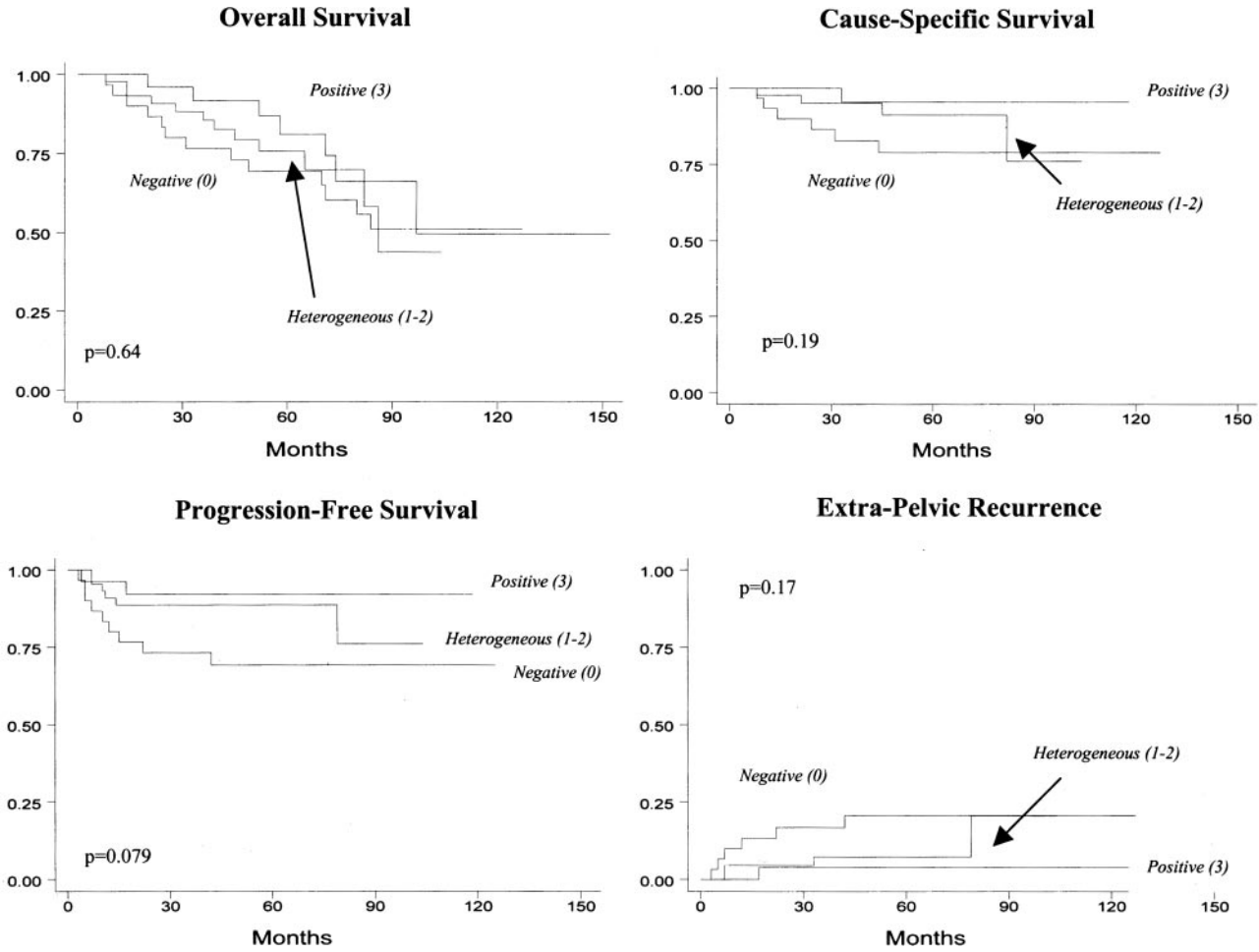

Fig. 2 Kaplan-Meier curves of clinical outcomes by E-cadherin score

this study, with successively lower levels of expression corresponding to higher risk of adverse outcomes. One previous study of 33 patients with stage I-IV endometrial carcinoma examined the prognostic significance of E-cadherin expression on clinical outcomes (34). The authors in that study reported a statistically significant decrease in OS in patients having tumors with decreased E-cadherin expression. The present study, however, is the first to demonstrate increased endometrial cancer

Table 4 Clinical outcomes[a]

	Hazard ratio (95% confidence interval)			
	Overall mortality	Endometrial cancer mortality	Disease progression	Extrapelvic failure
E-Cadherin expression score	0.59 (0.34–1.03)	**0.23 (0.055–0.94)**	**0.28 (0.10–0.77)**	**0.24 (0.06–0.97)**
Stage	1.09 (0.83–1.41)	1.14 (0.71–1.85)	1.26 (0.84–1.90)	1.18 (0.72–1.92)
Grade	1.20 (0.65–2.20)	1.48 (0.42–5.20)	1.42 (0.60–3.41)	1.46 (0.43–4.96)
MMI[b]	**3.34 (1.10–10.2)**	**12.6 (1.61–98.5)**	2.78 (0.56–13.7)	**9.92 (1.25–78.4)**
Papillary serous/clear cell	1.95 (0.70–5.47)	**7.26 (1.17–45.1)**	**3.76 (1.01–14.0)**	**8.74 (1.55–51.4)**
LVSI	0.61 (0.18–2.07)	0.99 (0.19–5.17)	1.84 (0.44–7.76)	1.08 (0.20–5.77)
Adjuvant radiotherapy	0.73 (0.27–1.97)	0.62 (0.14–2.71)	0.54 (0.15–1.97)	0.57 (0.12–2.65)
Adjuvant chemotherapy	1.14 (0.32–4.18)	0.31 (0.03–3.16)	0.52 (0.08–3.19)	0.30 (0.03–3.03)
Age	**1.04 (1.00–1.08)**	0.97 (0.91–1.03)	0.99 (0.95–1.03)	0.97 (0.92–1.03)
Black population	1.18 (0.54–2.56)	3.94 (0.83–18.7)	**3.98 (1.31–12.1)**	3.42 (0.73–16.0)

[a] Multivariate Cox regression hazard ratio estimates. Bold-face type indicates statistically significant ($P < 0.05$) estimate. E-Cadherin score, stage, grade, MMI, and age modeled as continuous variables. Histologic subtype, LVSI, and adjuvant therapy modeled as dichotomous variables.
[b] MMI, mymetrial invasion; LVSI, lymphovascular space invasion.

mortality and distant recurrence in patients with decreased E-cadherin expression. An advantage of our study was the ability to examine a large cohort of patients with local disease, with risk adjustment to isolate the predictive effect of E-cadherin expression independent of other prognostic factors. We were also able to obtain long-term follow-up to ensure the accuracy of our recurrence data.

Several limitations of this study should be considered. First, despite the selection of patients with local disease, the range of stages and pathological risk factors made this a heterogeneous group. Whether E-cadherin staining has a prognostic role in specific subgroups is a matter for further study. Second, some patients with high-grade disease did not undergo full surgical staging, leaving open the possibility that extrapelvic disease was present in these patients at the time of diagnosis. However, because the use of systemic therapy in such patients is not universally recommended, insofar as E-cadherin staining would be used to guide the clinical management of such patients, their inclusion in this study was rational. Third, the low number of events made precise estimation of the prognostic value of E-cadherin staining impossible, and studies with greater power would be beneficial to more precisely define the clinical predictive value of this molecular marker. In particular, there was a moderate degree of association between E-cadherin staining and both higher grade and unfavorable histology. Although the confounding effects of these factors were taken into account in our models, it would be worthwhile to analyze the effects of decreased E-cadherin protein expression in separate histological subgroups. Lastly, the semiquantitative immunohistochemical scoring system used in this study should be externally validated. Moderate interobserver agreement was observed here, and, if scoring from institution to institution were highly variable, the predictive value of this prognostic test would decrease.

Finally, the retrospective nature of this study makes it primarily hypothesis-generating. Numerous molecular markers have been shown to correlate with clinicopathological outcomes, yet few translate into widespread clinical use. Single proteins studied via immunohistochemistry may not have the predictive capability of the molecular profiling made possible by technologies such as cDNA microarray analysis and serial analysis of gene expression (SAGE) profiles, which can evaluate the expression of thousands of genes; these studies, however, require very large sample sizes (54–60). Such studies can be refined to look for combination "strong feature" gene sets of two to three genes that can both better classify tumors into diagnostic categories and also, potentially, yield useful information concerning the aggressive potential of a tumor (61). Such sets can then be feasibly analyzed by an immunohistochemical panel on a given patient's surgical specimen. Other groups have recently used tissue microarray analysis with a related "combination biomarker" approach applied to prostate carcinoma (62).

Whether the predictive power of E-cadherin is significant enough to be helpful in guiding the clinical management of patients with endometrial cancer remains to be seen. Our results suggest a potential clinical role for E-cadherin as a molecular marker of endometrial cancer severity. Given the results seen here, its potential use would likely be to risk-stratify women already considered at high risk for disease recurrence. Extrapelvic recurrence was extremely rare in patients with early-stage and low-grade disease, whereas low E-cadherin expression was common. However, among women with high-risk clinicopathological features, decreased E-cadherin expression was associated with an elevated rate of extrapelvic recurrence. This may be relevant for selecting patients at very high risk of extrapelvic recurrence, for whom a strategy of treating with adjuvant chemotherapy would be most beneficial. Conversely, women in a "high-risk" category according to traditional prognostic methods may really have a low probability of extrapelvic recurrence, and may be unlikely to benefit from adjuvant chemotherapy. Future studies to evaluate the role of E-cadherin and other molecular markers in predicting for extrapelvic recurrence to guide administration of adjuvant chemotherapy are, therefore, warranted.

ACKNOWLEDGMENTS

We would like to thank Terry Kelley and Jennifer Lewis for their assistance with this research.

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