Published in final edited form as:

Hum Pathol. 2008 June ; 39(6): 866–874. doi:10.1016/j.humpath.2007.09.023.

Biopsy Histomorphometry Predicts Uterine Myoinvasion By Endometrial Carcinoma: A Gynecologic Oncology Group (GOG) Study

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Abstract

Purpose—A barrier to nonsurgical management of premalignant endometrial disease is the need to perform hysterectomy to exclude concurrent myoinvasive endometrioid adenocarcinoma. Occult adenocarcinoma rates for premalignant disease diagnosed by biopsy or curettage are approximately 40%. We applied the histomorphometric 4-Class rule ("4C," which measures epithelial abundance, thickness, and nuclear variation) to diagnostic biopsies to predict myoinvasive cancer outcomes at hysterectomy.

Experimental Design—Women with endometrial biopsies or curettages having a community diagnosis of atypical endometrial hyperplasia were enrolled in a clinical trial in which subsequent hysterectomy was scored for endometrial adenocarcinoma, and 4C rule ability to predict cancer outcomes was measured. Qualifying biopsies were stratified into high and low risk histomorphometric subgroups.

Results—Two-hundred thirty-three women had biopsies suited to morphometry and scorable hysterectomy outcomes, of which 46% contained adenocarcinoma. Assignment to a high-risk category by the 4-Class Rule was highly sensitive in predicting any (71%) or deeply (92%) myoinvasive adenocarcinoma at hysterectomy, and assignment to a low-risk group had a high negative predictive value for absence of any (90%) or deeply (99%) myoinvasive disease. Volume percentage epithelium was associated with myoinvasive cancer outcomes above a threshold of 50% ($p < 0.001$), and a measure of nuclear pleomorphism was significantly increased ($p = 0.004$) in deeply myoinvasive cancers.

Conclusions—Formal histomorphometry of endometrial biopsies using the 4-Class rule has been validated as a means to identify a subset of women with premalignant disease who are unlikely to have concurrent myoinvasive adenocarcinoma, and who may qualify for alternative nonsurgical therapies.

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Keywords

endometrial carcinoma; Endometrial Intraepithelial Neoplasia; Histomorphometry

INTRODUCTION

Pathologists have used a variety of terms and diagnostic criteria to refer to premalignant endometrial lesions, including atypical endometrial hyperplasia, which confers an approximately 14-fold increased risk of carcinoma[1]. Outcome prediction improves when using modified diagnostic criteria for premalignant endometrial lesions, as Endometrial Intraepithelial Neoplasia (EIN), which incorporates discrete lesion architectural (gland area exceeds that of stroma) and size (>1mm dimension) criteria in addition to use of an internal relative rather than absolute standard for identification of significant cytologic change[2,3]. Women with a new diagnosis of EIN have a long term (beyond one year) risk for endometrial cancer 45 times that of women without EIN.[4] The diagnostic boundary between benign and premalignant disease is thus defined by emergence of EIN, an event which requires therapeutic intervention.

Therapeutic ablation of precancerous lesions is an effective strategy for prevention of the most common form of endometrial carcinoma, endometrioid endometrial adenocarcinoma.[5] The therapeutic window for cancer prevention by ablation of EIN is as long as the length of time required to transit from a premalignant to malignant phase of disease. Average observed progression time in individual monitored patients without concurrent cancer at time of initial presentation is estimated as four years.[4,6] Ruling out a concurrent cancer, however, is problematic. Thirty-nine percent of women with a new diagnosis of EIN and 43% with a diagnosis of atypical endometrial hyperplasia, who undergo clinical surveillance by repeat biopsy and/or hysterectomy will be diagnosed with adenocarcinoma within one year.[4,7] These are unlikely to represent short term progression events, but rather pathologist interpretive errors or cancers missed during sampling. Tissue sampling devices, which access the endometrium via the uterine lumen cannot obtain access to blind luminal pockets, and have a tendency to under represent tissues deep to the surface lining. Myoinvasive cancers are easily missed if the bulk of tumor is below the endometrial-myometrial interface. Furthermore, tumor lineage mapping studies have shown that myoinvasion is accompanied in about one third of cases by selection of aggressive disease subclones that are poorly represented on the surface. [8] In current practice, only hysterectomy provides a definitive pathology specimen for exclusion of myoinvasive adenocarcinoma.

A particular concern is the possibility that co-existing carcinoma missed at the time of initial pre-cancer diagnosis would also be under treated by uterus-sparing hormonal therapies. Although current practice favors hysterectomy for premalignant endometrial lesions in women past childbearing age, there is an active interest in developing uterus-sparing treatments for younger women and those who present unfavorable surgical risks. Progestin-based hormonal therapies offer the best alternative to surgery, but the optimal regimens, expected clinical response, and attendant risks remain to be defined. Progestin delivery by hormone-impregnated intrauterine devices is an example of an intervention that locally delivers supraphysiologic hormonal doses capable of effectively treating even early stage endometrial carcinomas.[9] Exclusion of myoinvasive disease is a desirable qualifying criterion for nonsurgical therapy.

Histomorphometric analysis of endometrial biopsy specimens has been successful in stratification of those clinical outcome groups which correspond to particular management options. The 4-class rule is a morphometric classification system performed on routine hematoxylin and eosin (H&E) slides designed to distinguish between premalignant, and

myoinvasive malignant endometrial disease.[10–13] It was originally developed by morphometric analysis of luminal surface endometrial morphology in women with and without myoinvasive endometrial carcinoma, and identification of those discrete independent variables associated with myoinvasion. The 4-Class rule component variables include measurements of nuclear variation, epithelial thickness, gland branching, and epithelial abundance. An appropriate application of this algorithm would be to identify women unlikely to have myoinvasive disease that may safely be managed by nonsurgical modalities.

We applied the quantitative histomorphometric 4-Class rule to a series of enrollment endometrial biopsies (or curettages) diagnosed in the community as atypical endometrial hyperplasia, and correlated membership in risk groups to actual hysterectomy cancer outcomes. All enrollment biopsies were also diagnosed subjectively using the EIN schema to determine a concordance between subjective and histomorphometric group assignments. All patients were part of Gynecologic Oncology Group (GOG) trial #167A, in which hysterectomy was performed within three months of initial biopsy. Hysterectomy outcomes were obtained by centralized review in all patients, and predictive value of diagnostic classification of antecedent biopsies calculated.

MATERIALS AND METHODS

Case selection

All patients were accrued between November 1998 and February, 2003 by the multi-center cooperative GOG under trial GOG167A, as administered by the Cancer Prevention Committee of the GOG. This trial was designed to determine concurrent endometrial cancer rates in women with premalignant endometrial disease. In brief, participating institutions consented and entered women with atypical endometrial hyperplasia diagnosed by dilation and curettage, Novak curettage, Vabra aspirate, or Pipelle endometrial biopsy. Hysterectomy was performed within three months and examined pathologically. Pathologists at each source institution selected representative routinely stained (H&E) tissue sections from the initial biopsy and hysterectomy specimens, and sent these for central processing as part of this study.

From a total of 305 eligible women entered in the study, H&E stained sections of endometrial biopsies from 299 women with a community diagnosis of atypical endometrial hyperplasia were available for histomorphometry. These were reviewed by a single gynecologic pathologist (GLM) and cases excluded which either demonstrated known confounders for histomorphometric analysis (endometrial polyp or secretory change, 18 cases), or lacked sufficient intact tissue fragments to evaluate lesion architecture (excessive fragmentation artifact in 30 cases and inadequate or very scanty sampling of endometrial tissues in seven cases). After these exclusions, all patients had provided 244 cases suited to morphometry.

Pathologist Diagnosis of Enrollment Biopsies

The results of centralized diagnostic biopsy review of these study cases using World Health Organization (WHO) endometrial hyperplasia criteria have been published elsewhere, as has the cancer predictive value of a subjective diagnosis of atypical endometrial hyperplasia[14, 7] Subjective pathologist review as benign, EIN (premalignant), or carcinoma, was performed by a gynecologic pathologist (GLM) using published EIN criteria.[3,6] The reviewing pathologist was blinded to all prior pathology reviews, hysterectomy outcomes, and selection of areas for histomorphometry.

Histomorphometry and diagnostic class assignment of Enrollment Biopsies

Enrollment biopsy H&E stained sections were reviewed by a gynecologic pathologist (GLM) to identify a discrete region representative of the lesion, and this area marked by an ink

perimeter on the coverslip. Measurement of four quantitative variables needed for lesion classification according to the morphometric 4-Class rule was performed by histomorphometry of the previously demarcated region using the QProdit 6.1 system (Leica, Cambridge, UK) as previously described.[12] For each lesion the 4-Class score was calculated, incorporating the variables volume percentage epithelium (VPE), standard deviation diameter nucleus (SDDN, μm), outer surface density (OUTSD, mm^2/mm^3), and thickness epithelium (THICKEPI, μm) into a probability of assignment to each of four possible classes: low grade endometrial hyperplasia (LGH), high grade endometrial hyperplasia (HGH), Low grade endometrial adenocarcinoma (LGA), high grade endometrial adenocarcinoma, (HGA). In brief, architectural features (VPE, OUTSD, and THICKEPI) are measured on a computer screen overlay of histologic images (40 $\times$ objective magnification, field diameter 450 μm) with a 20 point graticule (Weibel grid with 2-point length 67.52 μm). Tissue underlying each point is scored visually from the monitor as stroma, epithelium, or gland lumen and results from a total of 300–400 points are tallied and the VPE calculated as the number of epithelial points divided by the total points counted. Intersections of gland outer surfaces with calibrated horizontal lines of the Weibel grid were tallied and the outer surface density of glands calculated by the program according to the formula $\text{OUTSD} = (4000 \times \text{number of outer surface intersections}) / (2 \text{ point length} \times \text{total number of points}) \text{ mm}^2/\text{mm}^3$. Epithelial thickness is calculated according to the formula $(\text{Diameter glands} - \text{Diameter lumina} / 2) = (40 * (\text{Volume percentage glands} / \text{OUTSD}) - 40 * (\text{Volume percentage lumen} / \text{Inner Surface Density} / 2)) / 2$. Nuclear morphometry is performed on at least 150 randomly selected nuclei and the shortest nuclear axis calculated by the program from nuclear perimeter outlines. Nuclear measurement was tracked for each area in a running mean graph, and terminated when the coefficient of variation went below 5%.

Classification probabilities of each histologic sample to the four discrete histologic classes was achieved by the 4-Class rule using the above variables applied to a linear discriminant analysis algorithm.[12,13] The variables in the 4-class classification program (VPE, SDDN, OUTSD, and THICKEPI) were selected by a multivariate discriminant analysis program as the smallest combined set of features able to discriminate between non-myoinvasive and invasive endometrial disease. Features were then combined in a linear discriminant function (implemented as a module of the QProdit system), and the resultant algorithm validated on several different independent patient groups.[10,13]

The QProdit 4-class rule algorithm was applied to the study materials to assign a probability value between 0 and 1 for each of the four possible classes, always summing to a value of 1 (100%) for all four classes combined. Each tissue was then assigned to a single class, identified as that class with the greatest probability assignment. A collapsed version of the 4-class rule with only two assignment options of hyperplasia vs. cancer was developed by summing the hyperplasias (=low risk group, LGH+HGH) and cancer (high risk group, LGA+HGA) probability assignments.

Morphometry reproducibility was evaluated by repeat evaluation of 50 cases.

Hysterectomy Outcomes

All patients underwent hysterectomy within three months of the initial biopsy, and representative hysterectomy tissue sections submitted by the source institution underwent consensus group review by a panel of four gynecologic pathologists (George Mutter, Cornelia Trimble, Steven Silverberg, Richard Zaino) blinded to the enrollment biopsy interpretation. At review, hysterectomies were scored as positive or negative for adenocarcinoma, any myoinvasive adenocarcinoma, and deep myoinvasive (exceeding half of myometrial thickness) adenocarcinoma.

Statistics

Sensitivity, specificity, Positive and Negative predictive values were computed, along with their corresponding 95% exact confidence intervals for the combined 4-Class Rule for the endpoints of any cancer, invasive cancer, and deeply invasive cancer. The 4-Class Rule assignment versus the outcome variables were plotted graphically in Systat v.11 (Systat Software Inc, Richmond, CA).

RESULTS

Correlations of enrollment biopsy subjective with objective morphometric diagnoses (Table 1)

Of the 244 cases classified as atypical endometrial hyperplasia by community pathologists, 36% (87/244) were judged to be carcinoma upon subjective central review using the EIN schema. Comparison of enrollment biopsy morphometric diagnostic class assignments with subjective pathologist diagnoses provides some indication of how morphometric carcinomas are likely to be diagnosed by a central reviewing pathologist. The majority of these subjectively diagnosed cancers were classified as carcinoma by the 4-class histomorphometric rule (86%, 75/87). Histomorphometric carcinomas, however, included many cases subjectively judged to be premalignant (EIN). Almost half (45%, 62/137) of 4-Class morphometric carcinomas were judged subjectively to be something less than carcinoma.

Hysterectomy Outcomes

Of the 299 patients with available endometrial biopsies, hysterectomy outcomes were not scored in three cases, and seven more cases were excluded by protocol violation (evidence of progestin therapy), leaving 289 cases for outcome review. Forty-three percent (124/289) of all accepted hysterectomies contained adenocarcinoma on review. Myoinvasion status was not recorded in five cases that had adenocarcinoma, leaving 199 cases with myoinvasion data complete regarding depth of involvement. Myoinvasive adenocarcinoma was seen in 33% (39/119) of these hysterectomies with adenocarcinoma.

Of the 244 patients with histomorphometry-adequate enrollment biopsies (this excludes 55 patients with morphometry suitability exclusions, as specified above in Methods Section), two hysterectomy outcomes were excluded due to failure to record a diagnosis at review, five due to presence of superimposed progestin therapy effect, and four because of failure to record myoinvasion status. Complete histomorphometry and hysterectomy outcome data was thus available for 233 patients.

Morphometry Reproducibility

Morphometry reproducibility was assessed by repeat analysis of 50 samples (15 random, and 35 repeats pre-selected by assignment probabilities near the hyperplasia-carcinoma threshold, or cases with poorly preserved histology) of the 244 enrollment biopsies suited to morphometry. These reproducibility estimates are therefore weighted to problematic examples. Measured variables of VPE, SDDN, OSD, and EPITHIC had Pearson correlation coefficients of 0.94, 0.77, 0.88, and 0.83 respectively. Pearson correlation coefficient of 4-Class rule probability of assignment into the combined carcinoma groups was 0.81. Eighty-four percent (42/50) of cases had probability assignments from the replicate determination which did not change across the hyperplasia (LGH or HGH) to carcinoma (LGA or HGA) boundary.

Biopsy histomorphometric prediction of hysterectomy outcomes (Table 2)

Histomorphometric assignment of an enrollment biopsy to a carcinoma class was associated with hysterectomy outcomes of carcinoma, myoinvasive carcinoma, and deep myoinvasive

carcinoma (Figure 1). A morphometric biopsy diagnosis of adenocarcinoma (4C diagnosis of LGA or HGA) predicted cancer in the hysterectomy with sensitivity of 69% (95% CI, 59.2–77.3%). Specificity of hysterectomy cancer prediction was 55% (95% CI, 45.6–63.5%). Positive predictive value of a biopsy cancer diagnosis in predicting carcinoma at hysterectomy is 56% (95% CI, 47.5–65.0%). The negative predictive value of a non-cancer biopsy diagnosis in predicting a benign (no carcinoma) hysterectomy is 67% (95% CI, 57.4–76.2%).

A biopsy diagnosis of adenocarcinoma (4C diagnosis of LGA or HGA) predicted myoinvasive cancer (any depth) in the hysterectomy with sensitivity of 71% (52.5, 84.9%) and specificity of 46% (95% CI, 39.2–53.4%). The positive predictive value of a biopsy cancer diagnosis in predicting myoinvasive carcinoma at hysterectomy is 18% (95% CI, 12.1–26.0%). The respective negative predictive value of a non-cancer biopsy diagnosis in predicting absence of myoinvasive carcinoma at hysterectomy is 90% (95% CI, 82.7–95.2%).

A biopsy diagnosis of adenocarcinoma (4C diagnosis of LGA or HGA) predicted deep myoinvasive cancer (>50% of myometrial thickness) in the hysterectomy with sensitivity of 92% (95% CI, 61.5–99.8%) and specificity of 46% (95% CI, 39.0–52.5%). Positive predictive value of a biopsy cancer diagnosis in predicting deep myoinvasive carcinoma at hysterectomy is 8% (95% CI, 4.3–14.5%), and respective negative predictive value of a non-cancer biopsy diagnosis in predicting absence of deep myoinvasive carcinoma at hysterectomy is 99% (95% CI, 94.7–100%).

Component morphometric variable correlation with hysterectomy outcomes

Four variables were measured to calculate 4-Class group assignments. Their level of significance in predicting relevant hysterectomy outcomes varies as shown in Table 3 and Figure 2. Presence of carcinoma in the hysterectomy is associated with an increase in VPE, OSD, and EPITHC in the biopsy. VPE and EPITHC remain significant associations with myoinvasive carcinoma. Predictive variables switch at the threshold of deep myoinvasion, where only the cytologic variable of SDDN achieves individual significance.

DISCUSSION

When applied to a diagnostic biopsy, the 4-class rule was specifically designed to distinguish between those lesions likely to invade (the high risk carcinoma categories) compared to those unlikely to be accompanied by invasive disease (the low risk hyperplasia categories). In our series, we confirmed that histomorphometric stratification of "atypical endometrial hyperplasia" using the 4-class rule is capable of identifying a subset at high risk for deep myoinvasion at subsequent hysterectomy with 92% (11/12) sensitivity and 46% (101/221) specificity. In contrast, subjective central review re-diagnosis of biopsies as adenocarcinoma (Table I) was much less sensitive (57%, 8/14) in prediction of deeply myoinvasive cancers at hysterectomy. 4-Class assignment into a low risk category has a very high negative predictive value for myoinvasive carcinoma, with 90% (92/102) being free of any myoinvasive cancer and 99% (101/102) free of deep myoinvasive cancer at hysterectomy. Because only 18% (24/131, positive predictive value) of women with high risk lesions at biopsy are confirmed at hysterectomy to have myoinvasive malignant disease, further diagnostic evaluation is required to rule this possibility in or out in an individual patient.

4-Class histomorphometric groups do not rigidly correspond to individual subjective diagnostic categories (Table 1). The majority (87%, 82/94, overall) of 4C high grade hyperplasias (HGH) were subjectively interpreted as EIN, being composed of cytologically altered tightly packed individual glands (Figure 3, Panels C, D). The 4C low grade carcinoma class (LGA), however, was more heterogeneous, being split evenly into subjective EIN and adenocarcinoma diagnostic groups (Figure 3, Panels E–H, respectively). Subjective EIN

reclassification by the 4C rule as adenocarcinoma occurs when glands are sufficiently tightly packed such that the volume percentage epithelium exceeds 50% (Figure 3, panel E), but otherwise lack the solid, cribriform, or mazelike architecture required for a subjective diagnosis of carcinoma. Other 4C carcinomas demonstrate overt malignant architectural features such as mazelike interconnected glands or cribriform architecture (Figure 3, Panels F,G,H). Another barrier to tighter morphometric-subjective concordance is poor diagnostic reproducibility of the subjective hyperplasia evaluations. All of the enrollment biopsies were originally diagnosed as atypical endometrial hyperplasia by community pathologists, but upon central review by a panel of GOG expert pathologists, only 38% of community diagnoses of atypical hyperplasia were confirmed, and 29% upgraded to well differentiated adenocarcinoma with interobserver kappa scores of 0.40 overall.[14] This is representative of a more general experience.[15,16] In contrast, a hospital-based study has reported that subjective EIN diagnostic reproducibility is better, with 75% of pathologists agreeing on the initial diagnosis, and interobserver kappas of 0.54–0.62.[6] Morphometry yields even more consistent results, with 84% of 4-Class assignments in this study replicated exactly upon repeat evaluation.

Different histomorphometric variables are individually associated with any cancer, compared to deeply myoinvasive, cancer outcomes. When the surface area of neoplastic epithelium in a histologic section exceeds that of the combined gland lumen and stromal area (Volume percent epithelium, VPE>50%) the likelihood of finding any type of carcinoma (with or without myoinvasion) in the hysterectomy increases (Figure 2, Panel A, dashed line). In contrast, aggressive, deeply myoinvasive adenocarcinomas are associated with nuclear pleomorphism as measured by an increase in the standard deviation of the nuclear diameter (SDDN, Figure 2, Panel B). This is objective confirmation of a previous report that subjectively scored high nuclear grade independently predicts disease progression and survival in patients with endometrial adenocarcinoma.[17]

Histomorphometry is informative only if two conditions are met: 1) standards of technical preparation are adequate, and 2) confounding benign conditions are excluded by a trained pathologist. Poor tissue preparation, including extreme fragmentation or very poor staining compromises both subjective and histomorphometric diagnosis. Pathologists, however, are able to individually assess relative importance of individual cell (cytology) compared to larger scale (architecture) features for each case and may be willing to render an opinion even in suboptimal specimens. An example is carcinoma diagnosed from characteristic cytologic findings despite fragmentation. In contrast, a fixed morphometric algorithm that requires measurement of large scale architectural features is not capable of rendering a result with incomplete data. Another limitation of the 4C rule is that certain conditions readily diagnosed by an experienced pathologist, such as normal secretory endometrium, endometrial polyps, and squamous change consistently are over interpreted as high risk. In those cases a subjective, rather than histomorphometric, diagnosis is of greatest clinical utility. For these reasons, in our study only 5% of specimens were inadequate for pathologist diagnosis, whereas 18% were inadequate for morphometry.

Our reproducibility estimates are based upon repeat analysis of a pre-selected area, and omits any variation that might be contributed by the process of subjectively identifying a representative region for morphometry. In fact, selection of an area for analysis occurred in two stages. First, materials were generated in a large number of hospitals within a cooperative research group, and different local pathologists chose representative slides for centralized collection as part of this clinical trial. It is very possible that in some instances relevant lesional tissue was not included in that subset of slides sent to the GOG from the source institution. Secondly, in the centralized laboratory all available slides were reviewed by a gynecologic pathologist who chose a single representative tissue fragment for morphometry. It is likely that two pathologists when faced with the same slides might have chosen different tissue fragments

for morphometry, and this could have a negative impact on overall reproducibility. Although these variables were not all subject to complete control, they were equally present between compared groups.

Those morphometric features of a biopsy specimen which predict myoinvasive carcinoma do not follow a bimodal distribution corresponding to presence or absence of myoinvasive disease, but rather behave as a continuous and gradual spectrum. This raises the possibility that mutually exclusive labels of pre-cancer compared to cancer, although clinically desirable, may not conform to similarly sharply defined, discrete biologic entities. In contrast, the benign-premalignant distinction is a clean threshold event as defined in the endometrium by genetic, histomorphometric, and clinical outcome data and implemented clinically as the EIN schema. [18,2,3].

While hysterectomy remains the primary therapy for patients with premalignant disease (atypical endometrial hyperplasias or EIN) diagnosed by endometrial sampling, delays in reproductive planning, and aging of the population are increasingly responsible for a larger fraction of women who wish to retain fertility or otherwise are poor surgical candidates. This study has validated performance of the 4-Class histomorphometric algorithm as useful for identification of a subset of patients with premalignant endometrial disease who are unlikely to have concurrent or occult myoinvasive carcinoma (strong negative predictive value). Although our results do stratify patients with premalignant endometrial disease into differing risk groups for myoinvasive disease, the predictive value is imperfect. Even amongst patients in the low risk group, approximately 10% have some form of myoinvasive disease, and 1% deeply myoinvasive disease. Our data provides a basis for improved risk estimation, allowing it to be incorporated into informed discussions between individual patients and their physicians considering nonsurgical therapy.

There are several possible scenarios to extend our observations to a broader clinical environment. The analysis as described takes approximately 30 minutes technician time at a histomorphometry workstation and 10 minutes pathologist time per case. At present, a need for specialized equipment and minimum case volume to maintain staff expertise is most suited to a centralized reference laboratory engaged in histomorphometry for a variety of diagnostic applications. Alternatives for the future include the development of automated image analysis software and its application in a digital pathology diagnostic environment where "virtual slides" are routine. Not all histomorphometric algorithms, however, need to be implemented as formal morphometry routines. Individual variables associated with poor outcome might be subjectively recognized by a trained pathologist, including presence of extreme nuclear pleomorphism and fractional abundance of neoplastic epithelium (exceeding that of combined stroma and lumen). These evaluations may be of assistance in counseling patients of the risks of occult carcinoma, a key consideration in balancing the benefits of surgical compared to nonsurgical therapy.

ACKNOWLEDGEMENTS

This study was supported by National Cancer Institute grants to the Gynecologic Oncology Group Administrative Office (CA 27469), the Gynecologic Oncology Group Statistical and Data Center (CA 37517). The following Gynecologic Oncology Group member institutions participated in this study: Duke University Medical Center, Walter Reed Army Medical Center, University of Mississippi Medical Center, University of Pennsylvania Cancer Center, Milton S. Hershey Medical Center, University of North Carolina School of Medicine, University of Iowa Hospitals and Clinics, University of Texas Southwestern Medical Center at Dallas, Wake Forest University School of Medicine, University of California Medical Center at Irvine, Tufts-New England Medical Center, University of Kentucky, The Cleveland Clinic Foundation, State University of New York at Stony Brook, Southwestern Oncology Group, Washington University School of Medicine, Memorial Sloan-Kettering Cancer Center, Columbus Cancer Council, University of Massachusetts Medical School, Fox Chase Cancer Center, Women's Cancer Center, University of Oklahoma, University of Virginia Health Sciences Center, University of Chicago, Tacoma General Hospital, Gynecologic Oncology Network, Ellis Fischel Cancer Center, Fletcher Allen Health Care, and Ozark Regional CCOP.

The authors wish to thank those GOG pathologists who contributed their expertise during central review of the hysterectomy specimens (Cornelia Trimble of Johns Hopkins Hospital, Steven Silverberg of University of Maryland Hospital, and Richard Zaino of Hershey Medical Center), and Jan Barnes of the GOG statistical office for her constant support in triaging materials used in this study. Research technicians within Dr. Mutter's laboratory who participated in image analysis of the histologic sections include Maryann Ogden and Cecil Rise.

This work was supported by the following grants: NIH-NCI # RO1-CA92301 to GLM, and NIH-NCI #U10CA101165 to the Gynecologic Oncology Group.

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Figure 1. Biopsy Morphometry Predicts Hysterectomy Cancer Outcome

The distribution of enrollment biopsy cancer likelihood (Y axis, Cancer likelihood, percent) is shown for each hysterectomy cancer outcome group (X axis: no cancer, non (myo)invasive cancer, superficial (inner half) and deep (outer half) myoinvasive cancers). Scattergram shows, by outcome group, distribution of cancer likelihood's calculated for each enrollment biopsy using the 4-class rule (likelihood of membership in LGA or HGA groups). Cancer likelihood on biopsy increases with all cancer outcomes, most strongly with deep myoinvasive cancers.

Figure 2. Epithelial Density and Nuclear Pleomorphism In a Biopsy Correlate with Hysterectomy Cancer Outcomes

Morphometric variables of Volume Percent Epithelium (VPE, left) and standard deviation of nuclear axis (SDDN, Panel A) as measured in enrollment biopsies are plotted for each hysterectomy outcome group (x axis). As the area of neoplastic epithelium exceeds combined luminal and stromal area (VPE>50%, dashed line, left panel) the likelihood of finding carcinoma in the hysterectomy increases. Acquisition of nuclear pleomorphism (high values of SDDN, Panel B) is most pronounced in deeply myoinvasive carcinoma outcomes.

Figure 3. Examples of 4C Rule hyperplasias and adenocarcinoma

4C Rule morphometric rule reclassification of eight community "atypical endometrial hyperplasias" as low grade hyperplasia (LGH, Panels A–B), high grade hyperplasia (HGH, Panels C–D), low grade adenocarcinoma (LGA, Panels E–F), or high grade adenocarcinoma (HGA, Panels G–H). In general, higher proportion of epithelium and increased nuclear pleomorphism elevate the 4C rule assignment to worse categories. The low grade adenocarcinoma category contains examples judged subjectively to be either EIN (Panel E, individual glands) or well differentiated adenocarcinoma (Panel F, mazelike glands). High grade adenocarcinomas have very little remaining stroma, and pleomorphic tumor nuclei (Panels G–H). Patients with samples shown in Panels E, F, and G were found to have deeply myoinvasive disease at hysterectomy.

Table 1
Biopsy concordance of Subjective EIN-schema review diagnosis (columns) with 4-Class Rule Morphometric (Rows) Classification
Carcinoma as diagnosed subjectively in the EIN schema (“cancer” column) do not necessarily correspond to carcinomas diagnosed using the histomorphometric 4-Class (LGA/HGA) rule.

Abbreviation	4-Class Group	Subjective Diagnosis			Total
		benign	EIN	cancer	
LGH	Hyperplasia, Low	10	3	0	13
HGH	Hyperplasia, High	0	82	12	94
LGA	Carcinoma, Low	0	59	59	118
HGA	Carcinoma, High	0	3	16	19
Total		10	147	87	244

Hysterectomy Outcomes of All 299 Biopsies

Hysterectomy outcomes (columns) are shown by enrollment biopsy diagnosis (rows). Enrollment biopsies were classified using subjective EIN, and histomorphometric 4-class rules. A collapsed 4-class rule categorization in which subtypes (LGH and HGH) of hyperplasia and (LGA and HGA) carcinoma are combined is also shown.

Table 2

Biopsy Diagnosis		Totals, n	Hysterectomy				Hysterectomy			
Schema	Dx Class		Study Panel	Diagnosis	Cancer	Myoinvasion	not reported	inner	outer	
Subjective EIN	Inadequate	15	0	8	7	2	1	2	2	
	Benign	36	1	31	4	3	0	1	1	0
	EIN	153	4	92	56	37	1	14	4	
	Cancer	95	2	34	57	38	3	8	8	
4-Class Rule	Inadequate	55	1	37	15	9	1	3	2	
	LGH	13	0	12	1	0	0	1	0	
	HGH	94	1	58	33	22	2	8	1	
	LGA	118	1	50	64	45	1	9	9	
Collapsed 4-Class Rule	HGA	19	0	8	11	4	1	4	2	
	Inadequate	55	1	37	15	9	1	3	2	
	low risk (hyperplasia, LGH or HGH)	107	1	70	34	22	2	9	1	
	high risk (cancer, LGA or HGA)	137	1	58	75	49	2	13	11	
TOTAL	ALL	299	3	165	124	80	5	25	14	

Morphometric component variables predict cancer outcomes
The relative cancer outcome predictive value of component variables VPE, SDDN, OSD, and EPITHIC is greatest for the architectural parameter Volume Percent Epithelium (VPE). The cytologic variable SDDN, a measure of nuclear pleomorphism, assumes significance in prediction of deep myoinvasive outcomes. Probabilities reflect 2-group t-tests. Tabular values are shown as Mean (SD).

				Biopsy Variable Measured			
				VPE(%)	SDDN	OSD	EPITHIC
			n	volume percentage epithelium, %	Standard deviation diameter of nucleus, μm	Outer surface density, mm^2/mm^3	Epithelial thickness, μm
Any Cancer	Present		109	55(11.1)	1.2(0.20)	21.2(5.88)	51.1(22.19)
	Absent		128	45(12.3)	1.2(0.17)	18.8(5.75)	45.3(21.38)
	t-test			p<0.001	p=0.366	p=0.001	p=0.041
Myoinvasive Cancer	Present		34	56(12.5)	1.2(0.24)	21.2(7.58)	56.8(29.73)
	Absent		199	48(12.5)	1.2(0.17)	19.6(5.61)	46.5(20.16)
	t-test			p=0.001	p=.646	p=0.171	p=.0607
Deep Myoinvasive Cancer	Present		12	56(8.6)	1.3(0.24)	21.6(5.99)	58.4(29.26)
	Absent		221	49(12.9)	1.2(0.17)	19.8(5.94)	47.5(21.52)
	t-test			p=0.078	p=0.044	p=0.295	p=0.095