Histopathology and Molecular Biology of EIN

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INTRODUCTION

The diagnostic strategies outlined here are based upon an integrated picture of endometrial carcinogenesis from diffuse hormonal changes (benign hyperplasia sequence) to the earliest recognizable premalignant lesions (Endometrial Intraepithelial Neoplasia, EIN) [1]. In the past, both generalized hormonal responses and localized premalignant lesions were lumped under the term "endometrial hyperplasia," with various modifiers such as "adenomatous", "mild, moderate, and severe", and "atypical" that had no uniform meaning. The WHO 1994 classification system subdivided hyperplasias by architectural complexity and cytologic atypia [2]. Although this practice has been widespread, and has had a benefit of unifying terminology, it fails to optimally stratify patients according to those pathologic mechanisms and cancer risks necessary for appropriate therapeutic triaging. Diagnoses are poorly reproducible [3].

The EIN diagnostic schema (Table I) is a practically oriented disease classification incorporating a greatly expanded scientific evidence base that was unavailable in 1994 at the time the 4-class WHO hyperplasia system was formulated [1;4-14]. Two major disease classes are recognized: hormonal effects of unopposed estrogens (benign hyperplasia) and emergent neoplastic precancerous lesions (endometrial intraepithelial neoplasia (EIN)) [15]. The diagnosis is highly reproducible, averaging kappa=0.72 in a recent study of 20 community pathologists compared to an expert panel [14].

Table I: Endometrial Diagnostic Schema and ICD9 Codes

Nomenclature	Topography	Functional Category	Treatment	ICD9 Code
Benign Endometrial Hyperplasia	Diffuse	Prolonged Estrogen Effect	Hormonal therapy, Symptomatic	621.34
EIN, Endometrial Intraepithelial Neoplasia	Focal progressing to diffuse	Precancerous	Hormonal or surgical	621.35
Endometrial adenocarcinoma, endometrioid type, well differentiated	Focal progressing to diffuse	Malignant	Surgical stage-based	182.0

Geometry of
Benign, Premalignant, and Malignant lesions

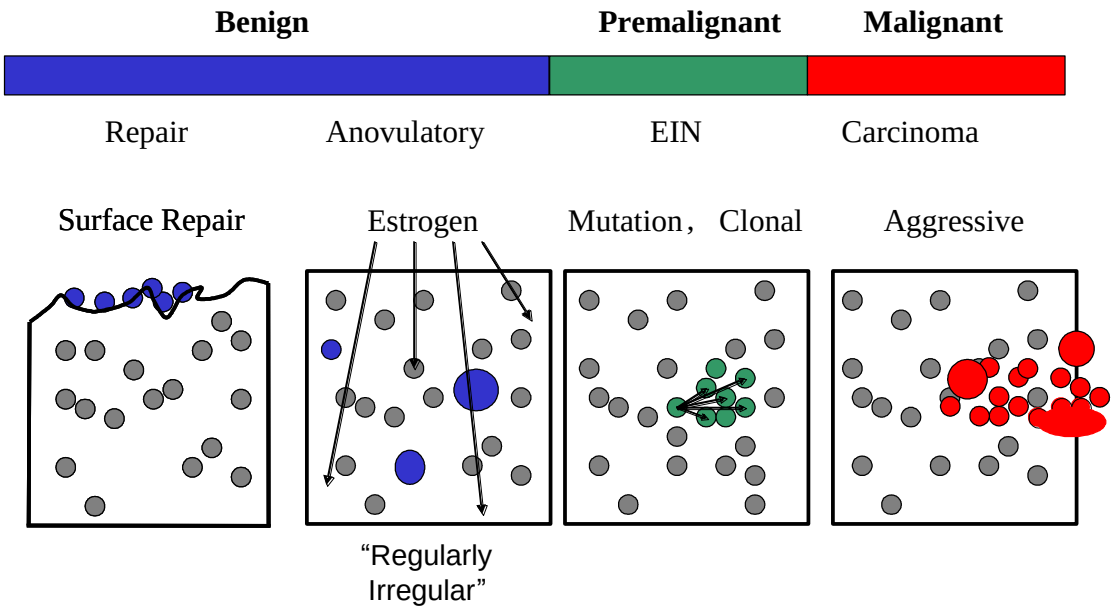

Figure: Topography of EIN Large scale topography has been greatly underestimated as a useful feature in resolving the reactive, premalignant, or malignant characteristics of lesions. It is the geographic aggregation of similarly altered endometrial glands, a reflection of clonal growth, which is seen in EIN and adenocarcinoma but not in hormonally induced changes. Careful examination of tissue integrity, including stromal breakdown and presence or absence of inflammation, will assist in identification of localizing reactive processes.

Estrogen Related Lesions

Diagnostic Features

Abundant curettings with characteristically diffuse and widespread morphologic features typify endometria altered by unopposed estrogens. The histologic changes of disordered proliferative and benign endometrial hyperplasia are conceptually and morphologically well represented as a unified disease spectrum, separate and discontinuous from EIN. The histologic hallmark of the benign hyperplasias is a generalized but non-uniform proliferation of architecturally variably shaped glands that equal or exceed the quantity of the stroma.

Disordered proliferative endometrium.

Disordered proliferative endometrium is an exaggeration of the normal proliferative phase without significant increase in the overall ratio of glands to stroma. The changes involve the entire endometrial compartment, and are evident at low magnification as sacculated dilations (microcysts) randomly scattered amongst tubular glands lined by mitotically active epithelial cells. Characteristically, glands affected by tubal differentiation are randomly interspersed amongst proliferative glands, and they also may demonstrate tubular, branching, or cystic architecture.

Benign endometrial hyperplasia.

Benign endometrial hyperplasia develops from disordered proliferative endometrium under the continued influence of unopposed estrogens. The entire endometrial compartment

contains variable gland densities caused by remodeling of stroma and glands to the extent that in some areas the gland to stroma ratio exceeds 1:1. It is the increased gland density that distinguishes benign hyperplasia from disordered proliferative endometrium. Individual glands may be tubular, cystic, or branching, and these forms are commingled throughout. On a large scale the endometrium appears uniformly affected, however, at medium magnification local admixtures of individually variable glands present quite differing appearances among separate microscopic fields. This combination of low magnification uniformity, made up of variable medium magnification fields, can be described as "regularly irregular".

A critical feature of benign hyperplasia is that the cytology does not change between architecturally crowded and uncrowded areas. This reflects the systemic hormonal etiology of the process that similarly exposes the entire endometrium, and allows its distinction from EIN.

Estrogen production from persistent follicles or by peripheral conversion following the menopause is inconstant. When the estrogen level declines slowly, massive breakdown does not occur and the glands lose mitotic activity. These endometria retain the architectural features of a bulky endometrium with altered gland architecture, but the glands demonstrate a mitotically inactive and non-stratified appearance and may be karyorrhectic. With waning estrogen levels, endometrial bulk declines towards an atrophic pattern, sometimes with cysts.

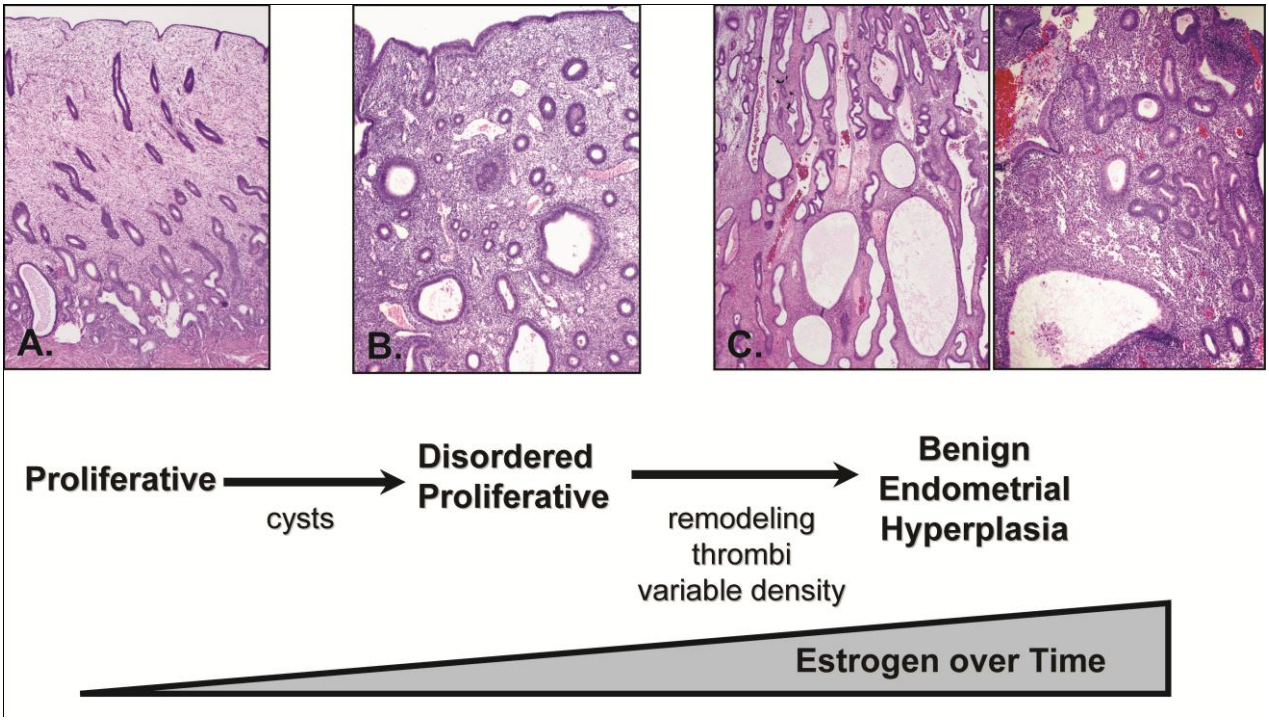

Figure 1: Progressive Effects of Unopposed Estrogens.
Early effects of unopposed estrogen are scattered cysts in an otherwise normal appearing proliferative endometrium, known as disordered proliferative endometrium. Continued exposure causes a progressive spectrum of histopathologic change (left to right) including increasing irregularity of gland density and shape, scattered alterations of cytologic appearance known as benign hyperplasia. Established benign hyperplasias demonstrate a high degree of remodeling between glands and stroma of the expanded, hyperplastic, endometrial compartment, in which the ratio of glands to stroma exceeds 1.0 in most or all of the endometrial compartment. Fibrin thrombi, stromal breakdown and associated reactive epithelial changes commonly develop, and must be carefully distinguished from neoplastic processes.

Endometrial Intraepithelial Neoplasia [1]

Endometria Intraepithelial Neoplasia (EIN) is a clonal proliferation of architecturally and cytologically altered premalignant endometrial glands which are prone to malignant transformation to endometrioid (Type I) endometrial adenocarcinoma. EIN lesions are non-invasive genetically altered neoplasms which arise focally, and may convert to malignant phenotype upon acquisition of additional genetic damage. Diagnostic criteria for EIN have been developed by histopathologic correlation with clinical outcomes, molecular changes, and objective computerized histomorphometry.

EIN should not be confused with unrelated serous intraepithelial carcinoma (serous EIC), which is an early phase of (Type II) papillary serous adenocarcinomas of the endometrium.

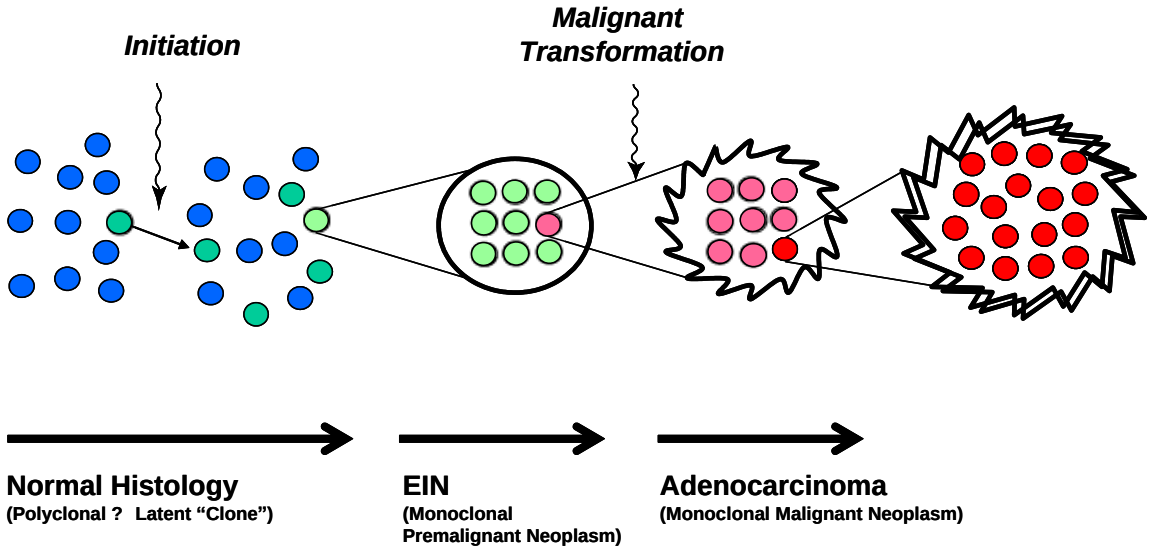

Figure 1: Clonal Origin of EIN. The first genetic changes (such as PTEN inactivation) which initiate endometrial carcinogenesis are unaccompanied by any phenotypic alterations at the light microscopic level. This "latent", phase of cytologically and architecturally normal but genetically altered cells may persist for years in a normally menstruating woman. Low cancer risk, combined with lack of a rational therapeutic response, are reasons that systematic screening and treatment of these "latent" phase lesions is unwarranted at present. As additional genetic damage accumulates, higher risk morphologically altered mutant clones declare themselves by demonstrating those architectural and cytologic alterations that distinguish EIN. Malignant transformation of EIN lesions, which occurs at least 46-times more frequently than non-EIN tissues, warrants careful diagnosis and treatment. Endocrine modifiers of endometrial cancer risk act upon the latent and EIN phases of this sequence by tipping the balance of clonal expansion vs. involution.

Biomarkers for EIN.

The clonal nature of EIN is well illustrated by in situ demonstration of acquired genetic lesions shared amongst all cells of the neoplastic clone. This was originally accomplished with investigational-only methods that require targeted isolation and analysis of DNA from lesional and background tissues, including non-random X chromosome inactivation, and presence of unique clone-specific mutations in genes such as (PTEN [16], PAX2 [17]) and microsatellites [6].

There are several important limitations that prevent these markers from being applicable to routine diagnostic use. First, at least a third of EIN occurrences lack changes in these genes, so a requirement for loss of PAX2 or PTEN is insensitive in detection of EIN.

Second, loss of function of these genes occurs before an EIN lesion develops, so marker loss alone is nonspecific to EIN lesions.

Table II: Proportion of Endometrial Tissue Samples Showing Loss of PAX2 and PTEN Protein Expression, by Diagnosis.

	EIN (n=52)	Cancer (n=62)
PAX2 null	71.2%	77.4%
PTEN null	44.2%	67.7%
Joint PAX2 and PTEN Null	30.8%	54.8%
Joint PAX2 and PTEN Expression	15.4%	9.7%

Diagnosis of EIN

EIN is diagnosed by a pathologist using routine (hematoxylin and eosin stained) sections prepared from a representative endometrial sample [1;18]. It should be noted that EIN is a precursor of endometrioid endometrial adenocarcinomas and is unrelated to "serous Endometrial Intraepithelial Carcinoma," or serous "EIC" which is a manifestation of [19] papillary serous type endometrial adenocarcinomas.

1.EIN Diagnostic Criteria

All of the diagnostic criteria of must be met in order to make an EIN diagnosis. Size, architecture, and cytology features are easy EIN diagnostic criteria. Much more difficult are exclusion of benign mimics and adenocarcinoma from the differential diagnosis.

Table III: EIN Diagnostic Criteria. All must be met in one fragment Modified after [20].

EIN Criterion	Comments
Architecture	Area of Glands greater than Stroma
Cytology	Cytology differs between architecturally crowded focus and background, or clearly abnormal.
Size >1 mm	Maximum linear dimension exceeds 1mm.
Exclude mimics	Benign conditions with overlapping criteria: Basalis, secretory, polyps, repair, etc..
Exclude Cancer	Carcinoma if mazelike glands, solid areas, polygonal "mosaic-like" glands, myoinvasion, or significant cribriforming

a.Architecture: Gland area exceeds stromal area:

A cardinal architectural feature of endometrial precancers is glandular crowding, with a threshold quantitative cutoff for EIN lesions of gland area greater than stromal area. Areas with large dominant cysts should always be avoided in making this assessment.

b.Cytology of architecturally crowded area is different than background, or clearly abnormal:

There is no absolute standard for cytologic features of EIN lesions, but the cytology of EIN is usually clearly demarcated as divergent from that of co-existing benign endometrial tissues in the same patient. The manner of cytologic change in EIN varies considerably from patient to patient, and can include but not be limited to, increased variation in nuclear size and

contour, clumped or granular chromatin texture, change in nucleoli, change in nuclear/cytoplasmic ratio, and altered cytoplasmic differentiation. Stereotypical static descriptions of cytologic atypia, such as nuclear rounding and appearance of nucleoli are met in many but not all EIN lesions.

Cytologic changes in some EIN lesions are manifest as a change in differentiation state to a tubal, mucinous, micropapillary, or eosinophilic phenotype. These must be distinguished from the scattered random pattern of hormonally, or surface located repair-induced "metaplasias." Further details of how to interpret non-endometrioid EIN lesions are presented in the "Pitfalls" section below.

In those cases with no normal glands for internal reference, it is necessary to assess the freestanding cytology of relevant fragments in the context of their architectural features. Some EIN lesions occupy the entire tissue sample, and should not be underdiagnosed for lack of a convenient benign gland in the area.

c.Size >1mm in maximum dimension:

Accurate EIN diagnosis requires a contiguous field of glands sufficiently large to enable reliable assessment of architecture. A minimum lesion size of 1 mm maximum dimension was required in the previous clinical outcome studies [21-24] for an EIN lesion to achieve elevated cancer risk. That area of an EIN lesion which meets architectural (gland area) and cytologic (changed) criteria for diagnosis must measure a minimum of 1mm in maximum dimension, a scale which usually encompasses more than 5-10 glands. There is no formal evidence that once beyond the minimum 1mm, EIN lesions should be stratified by size.

d.Exclusion of Benign Mimics

Patients with one of the conditions listed below may still have an EIN, but this diagnosis should be made with careful consideration into how the coexisting factor(s) may modify the criteria for EIN diagnosis. If a specimen is refractory to confident diagnosis, a comment as to the nature of the problem may be useful in directing management.

1. **Reactive changes** caused by infection, physical disruption, recent pregnancy, or recent instrumentation. These can cause piling up of the epithelium, and loss of nuclear polarity..
2. **Artifactual gland displacement**. Beware diagnosing an EIN lesion if the cytology is identical between areas with crowded compared to uncrowded glands! Many of these are artifactual disruptions where the stroma is sheared and glands pushed in apposition .
3. **Persistent Estrogen Effect**: As described above. Notably, increases in gland density are not coordinated with a change in cytology.
4. **Endometrial polyps** contain irregularly spaced glands in which scattered glands may differ from native endometrium due to their tendency to have reduced hormonal responsiveness.
5. **Endometrial breakdown** is one of the most common settings for overdiagnosis of a benign endometrium as a precancer or cancer. Breakdown may follow an ovulatory or anovulatory cycle and persist into the transitional period between late menses and early proliferative endometrium. Altered cytology is due to piling up of epithelial cells unsupported by stroma, and associated nuclear change.

e.Exclusion of Carcinoma

Cancer may coexist with EIN in an individual patient, but should be always be separately diagnosed because current management of carcinoma differs from that for EIN.

EIN is composed of individual glands lined by an epithelium one cell layer thick. The epithelium may be pseudostratified, but should not be cribriform or composed of solid areas of epithelial cells. Presence of any of the following features involving neoplastic glands is inconsistent with EIN, and a diagnosis of carcinoma should be entertained. Unfortunately, myometrium is rarely available for evaluation in a biopsy or curettage specimen.

1. Meandering or “mazelike” lumens
2. Solid epithelium
3. Cribriform architecture.
4. “Mosaic” gland pattern of distorted polygonal glands with threadlike intervening stroma

Common EIN Diagnostic Problems

Uncommon presentations of common diseases, and suboptimal specimens are two of the many sources of diagnostic difficulty in endometrial pathology. Combined with a "normal" reference point which changes dynamically throughout the month, and during the life cycle, the very definition of "abnormal" depends on the clinical setting. This section will serve as an introduction to some of the more common problems, with suggestions for coping strategies that will not compromise management of the patient.

Table IV: Pitfalls in EIN Diagnosis.

Problem	Response
Fragmented or Distorted	Get levels and ask for a rebiopsy soon (within 3 months) if still worried
Suspicious for EIN but <1mm	Section deeper and evaluate context 1)If extends to edge of fragment <1mm, likely sampling error. recommend rebiopsy soon (within 3 months) 2)If small area in larger fragment, likely a subdiagnostic “pre-EIN”. make descriptive diagnosis and recommend followup biopsy in 6 months
Suspicious for EIN but >50% VPS	Descriptive diagnosis and followup in 6 months
EIN in Polyp	Apply usual EIN criteria, using polyp itself as the background for cytologic comparison. EIN in polyps are usually discrete.
Non-Endometrioid Differentiation	If glandular, can use EIN criteria but must rule out specific cancer.
Squamous Morules	Make diagnosis based upon gland component, mentally subtracting morules. Do not consider cribriform if morule separates peripheral lumens
Progestin Effect	Withdraw hormones and rebiopsy 2-4 weeks after cessation of withdrawal bleed

Clinical Outcomes in Women with EIN

Dichomatous classification of endometrial lesions as low risk (benign hyperplasia) vs. high risk (EIN) facilitates clinical management, as these entities represent discrete clinicopathologic processes with quite different clinical outcomes. For this reason, a 2-class schema such as EIN is effective in communicating intended management based on underlying disease [25].

The negative cancer predictive value of a representative endometrial biopsy which lacks EIN is very high, at 99% [15]. If the clinician is confident of sampling adequacy, and the pathologist has not indicated some particular problem in interpretation of the specimen, observational follow-up with symptom management can be justified. Not all cases are so straightforward, however, as there will be individual cases where there is lingering concern of sampling adequacy, discordance between the clinical presentation and pathologic diagnosis rendered, or interpretive uncertainty by the pathologist. In these instances the diagnostic process may be considered incomplete, and repeat sampling indicated.

Co-existing occult adenocarcinoma: 37%

39% of EIN lesions coexist with well differentiated adenocarcinoma that may not be evident on the initial biopsy [12;15]. 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. Women with abnormally configured luminal cavities, or extensive intrauterine adhesions can be difficult to sample adequately.

Long term progression to adenocarcinoma: 42-fold increased risk.

Long term endometrial cancer risk can be defined as development of endometrial carcinoma more than one year after initial EIN diagnosis. Calculated in this fashion, an EIN diagnosis confers a 42-fold increased risk for endometrial adenocarcinoma. This level of risk is the basis for current standard of care being a hysterectomy.

Reference List

1. Mutter GL, Zaino RJ, Baak JPA, Bentley RC, Robboy SJ. The Benign Endometrial Hyperplasia Sequence and Endometrial Intraepithelial Neoplasia. Int J Gynecol Pathol 2007; 26:103-114.
2. Scully RE, Bonfiglio TA, Kurman RJ, Silverberg SG, Wilkinson EJ. Uterine corpus. Histological Typing of Female Genital Tract Tumors. New York: Springer-Verlag, 1994: 13-31.
3. Zaino RJ. Endometrial hyperplasia: is it time for a quantum leap to a new classification? Int J Gynecol Pathol 2000; 19(4):314-321.
4. Mutter GL, Chaponot M, Fletcher J. A PCR assay for non-random X chromosome inactivation identifies monoclonal endometrial cancers and precancers. Am J Pathol 1995; 146:501-508.
5. Jovanovic AS, Boynton KA, Mutter GL. Uteri of women with endometrial carcinoma contain a histopathologic spectrum of monoclonal putative precancers, some with microsatellite instability. Cancer Res 1996; 56:1917-1921.
6. Mutter GL, Boynton KA, Faquin WC, Ruiz RE, Jovanovic AS. Allelotype mapping of unstable microsatellites establishes direct lineage continuity between endometrial precancers and cancer. Cancer Res 1996; 56:4483-4486.
7. Mutter GL. Histopathology of genetically defined endometrial precancers. Int J Gynecol Pathol 2000; 19:301-309.

8. Mutter GL, The Endometrial Collaborative Group. Endometrial intraepithelial neoplasia (EIN): Will it bring order to chaos? Gynecol Oncol 2000; 76:287-290.
9. Mutter GL, Baak JPA, Crum CP, Richart RM, Ferenczy A, Faquin WC. Endometrial precancer diagnosis by histopathology, clonal analysis, and computerized morphometry. J Pathol 2000; 190:462-469.
10. Hecht JL, Ince TA, Baak JP, Baker HE, Ogden MW, Mutter GL. Prediction of endometrial carcinoma by subjective endometrial intraepithelial neoplasia diagnosis. Mod Pathol 2005; 18:324-330.
11. Carlson JW, Mutter GL. Endometrial Intraepithelial Neoplasia Is Associated With Polyps And Frequently Has Metaplastic Change. Histopathology 2008; 53:325-332.
12. Mutter GL, Kauderer J, Baak JPA, Alberts DA. Biopsy histomorphometry predicts uterine myoinvasion by endometrial carcinoma: A Gynecologic Oncology Group Study. Hum Pathol 2008; 39:866-874.
13. Semere LG, Ko E, Johnson NR et al. Endometrial intraepithelial neoplasia clinical correlates and outcomes: A practice-based experience. Obstet Gynecol 2011; 118 (1):21-28.
14. Usubutun A, Mutter GL, Saglam A et al. Reproducibility of endometrial intraepithelial neoplasia diagnosis is good, but influenced by the diagnostic style of pathologists. Mod Pathol 2012; 25(6):877-884.
15. Baak JP, Mutter GL, Robboy S et al. The molecular genetics and morphometry-based endometrial intraepithelial neoplasia classification system predicts disease progression in endometrial hyperplasia more accurately than the 1994 World Health Organization classification system. Cancer 2005; 103(11):2304-2312.
16. Mutter GL, Lin MC, Fitzgerald JT et al. Altered PTEN expression as a diagnostic marker for the earliest endometrial precancers. J Natl Cancer Inst 2000; 92:924-930.
17. Monte NM, Webster KA, Neuberg D, Dressler GR, Mutter GL. Joint loss of PAX2 and PTEN expression in endometrial precancers and cancer. Cancer Res 2010; 70:6225-6232.
18. Mutter GL, Zaino RJ, Baak JPA, Bentley RC, Robboy SJ. Benign endometrial hyperplasia and EIN. In: Robboy SJ, Mutter GL, Prat J, Bentley R, Russell P, Anderson MC, editors. Robboy's Pathology of the Female Reproductive Tract. New York, New York: Elsevier, 2009: 367-391.
19. Ambros RA, Sherman ME, Zahn CM, Bitterman P, Kurman RJ. Endometrial intraepithelial carcinoma: A distinctive lesion specifically associated with tumors displaying serous differentiation. Hum Pathol 1995; 26:1260-1267.
20. Silverberg SG, Mutter GL, Kurman RJ, Kubik-Huch RA, Nogales F, Tavassoli FA. Tumors of the uterine corpus: epithelial tumors and related lesions. In: Tavassoli FA, Stratton MR, editors. WHO Classification of Tumors: Pathology and Genetics of Tumors of the Breast and Female Genital Organs. Lyon, France: IARC Press, 2003: 221-232.
21. Baak JPA, Mutter GL, Robboy S et al. In endometrial hyperplasias, the molecular-genetics and morphometry-based EIN classification more accurately predicts cancer-progression than the WHO94. Cancer 2005; 103(11):2304-2312.
22. Baak JP, Orbo A, van Diest PJ et al. Prospective multicenter evaluation of the morphometric D-score for prediction of the outcome of endometrial hyperplasias. Am J Surg Pathol 2001; 25(7):930-935.
23. Dunton C, Baak J, Palazzo J, van Diest P, McHugh M, Widra E. Use of computerized morphometric analyses of endometrial hyperplasias in the prediction of coexistent cancer. Am J Obstet Gynecol 1996; 174:1518-1521.
24. Baak JPA, Nauta J, Wisse-Brekelmans E, Bezemer P. Architectural and nuclear morphometrical features together are more important prognosticators in endometrial hyperplasias than nuclear morphometrical features alone. J Pathol 1988; 154:335-341.
25. Trimble CL, Method M, Leitao M et al. Management of Endometrial Precancers. Obstet Gynecol 2012; 120(5):1160-1175.