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Integrated histological parameters define prognostically relevant groups in atypical endometrial hyperplasia / endometrioid intraepithelial neoplasia

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ABSTRACT

Background: The risk of endometrial cancer (EC) in atypical endometrial hyperplasia/endometrioid intraepithelial neoplasia (AEH/EIN) is highly variable among different series. Recent studies suggested that histological criteria may subdivide AEH/EIN into prognostically relevant groups.

Objective: To assess the risk of EC in AEH/EIN patients stratified according to integrated histological parameters.

Methods: All women with AEH/EIN undergoing hysterectomy within 1 year from diagnosis without undergoing progestin treatment were included. AEH/EINs were subdivided into 3 study groups, based on nuclear atypia and on the presence of focal (<2 mm) stromal disappearance (FSD): low-grade (LG)-AEH/EIN, high-grade (HG)-AEH/EIN, and FSD-AEH/EIN. The rate of EC on the subsequent hysterectomy was assessed in each study group, and differences between study groups were assessed using Fisher's exact test, with a significant p-value<0.05. Reproducibility was assessed by using Cohen's k.

Results: Ninety-six patients were included. Overall, 36/96 patients (37.5%) had EC on the subsequent hysterectomy. The number of EC was 4/42 (9.5%) in LG-AH/EIN, 14/28 (50%) in HG-AH/EIN, and 18/26 (69.2%) in FSD-AEH/EIN. The rate of EC was significantly higher in HG-AH/EIN than in LG-AEH/EIN ($p<0.001$), while it did not significantly differ between HG-AEH/EIN and FSD-AEH/EIN ($p=0.176$). The reproducibility among pathologists was moderate for LG-AEH/EIN *vs* HG-AEH/EIN ($k=0.58$) and substantial for FGD-AEH/EIN *vs* LG-AEH/EIN ($k=0.63$) and HG-AEH/EIN ($k=0.63$).

Conclusion: AEH/EIN can be stratified into prognostically relevant groups based on integrated histological parameters, with a possible major impact on patient management.

BIBLIOGRAPHIC SECTION

ENDOMETRIAL HYPERPLASIA AND ENDOMETRIAL CANCER

EPIDEMIOLOGY

Endometrial hyperplasia (EH) is an hyperproliferative condition characterized by increased gland-to-stroma ratio compared to normal proliferative endometrium[1,2].

The clinical importance of EH is due to its potential to progress to endometrioid endometrial cancer (EC), with “atypical” types of EH considered precancerous lesions[2]. In particular, the estimated progression risk of EH in EC is 8-29%[3].

Incidence of EH is estimated 133/100.000 among women, most commonly observed in women aged 50–54, and rarely in women under 30[4].

Most cases of EH result from high levels of estrogens, not balanced by sufficient levels of progesterone[5]. Continuous estrogen stimulation of the endometrium leads to proliferative changes in the glandular epithelium,

which includes glandular remodeling. This results in glands that vary in shape and are irregularly arranged.

Risk factors for progression to EC include obesity, unopposed estrogen therapy, tamoxifen use, polycystic ovary syndrome (PCOS), and nulliparity[6].

On the other hand, EC is the most common gynecological malignancy in high-income countries[7]. Known risk factors for endometrial cancer are high levels of estrogens without the protective effect of progesterone/progestins (unopposed estrogens), obesity, early age at menarche, nulliparity, late age at menopause, Lynch syndrome (i.e. a hereditary syndrome resulting from germline mutations in DNA mismatch repair genes, such as *MLH1*, *MSH2*, *MSH6*, and *PMS2*, and characterized by familial clustering of colorectal, endometrial and other types of cancer[8]), older age and tamoxifen use[9]. Due to a high prevalence of such risk factors, the incidence of endometrial carcinoma has increased over the past two decades in the US[7,10].

RISK FACTORS

Specific risk factors for EH and EC are associated with a high estrogen environment. Most patients typically present with a characteristic clinical profile that includes a high body mass index (BMI), overweight (BMI: 25-29.9 kg/m²) or obese (BMI ≥ 30 kg/m²), often with other components of the metabolic syndrome. Evidence that a higher body fat (assessed by BMI, waist circumference measurements, and weight gain in adults) is a risk factor for EC is compelling. Glycemic load is likely another risk factor for EH and EC, while evidence suggesting that a sedentary lifestyle and height are causes of endometrial cancer is limited[11].

Obesity is the most common cause of endogenous estrogen overproduction. Excessive adipose tissue increases peripheral aromatization of androstenedione to estrone. In premenopausal women, elevated estrone levels trigger an abnormal feedback loop in the hypothalamic-pituitary-ovarian axis. The clinical findings are oligo- or anovulation. In the absence of ovulation, the endometrium is exposed to virtually continuous estrogen stimulation without subsequent pregestational effect and without menstrual withdrawal bleeding.

According to a meta-analysis involving 6 studies and 3132 cancer cases, the relative risk (RR) for the development of EC in women with metabolic

syndrome is 1.89 [95% confidence interval (CI) 1.34-2.67, P ≤ 0.001]. Considering the individual components of the metabolic syndrome, obesity is associated with the greatest increase in RR of 2.21 (P ≤ 0.001)[12]. The strength of the association between obesity and cancer risk increases with increasing BMI: RR for overweight is 1.32 (95% CI 1.16–1.50) and for obesity 2.54 (95% CI 2.11–3.06)[13]. Other components of metabolic syndrome linked to endometrial cancer include hypertension, with a RR of 1.81 (P = 0.024) or an odds ratio (OR) of 1.77 (1.34–2.34)[14]. Hypertriglyceridemia has a weaker but still significant association (RR 1.17, P < 0.001)[15]. Diabetes mellitus has long been considered an independent risk factor for EH and EC, with an approximate doubling of the risk (OR 2.1; 95% CI 1.40–3.41)[14]. However, the fact that people with type II diabetes mellitus (T2DM) tend to be obese is a confounding factor, and a recent epidemiological study from the United States has questioned the independent role of T2DM as a risk factor for endometrial cancer[16]. Our study showed that, in women diagnosed with EH, diabetes mellitus was significantly associated with the risk of coexistent cancer[17].

Nulliparity and infertility are also classic risk factors for EH and EC. Among the causes of infertility, polycystic ovary syndrome (PCOS) appears to be the most important, with an almost threefold increased risk (OR 2.79–2.89)[18].

Other risk factors for EH and EC include estrogen therapy alone (in the absence of adequate progestin balance), estrogen-producing tumors, and early menarche/late menopause. Estrogen therapy without adequate balance increases the risk of endometrial cancer by 10- to 30-fold if treatment continues for 5 years or more[19]. Estrogen-producing tumors, or ovarian granulosa tumors, and theca tumors carry an increased risk of EC, with up to 20% of women with these tumors having simultaneous endometrial cancer. Both early menarche and late menopause are associated with a 2-fold increased risk for endometrial cancer. The RR is 2.4 for women <12 years versus ≥15 years and is 1.8 for women ≥55 versus <50 years[20].

Family history is another risk factor: endometrial carcinoma is the most common extracolonic manifestation in Lynch syndrome (hereditary nonpolyposis colorectal cancer [HNPCC]). This autosomal dominant syndrome results mainly from mutations in mismatch repair genes. The mismatch repair genes associated with Lynch syndrome are MLH1, MSH2, MSH6 and PMS2[21]. Genetic mutation prevents the repair of base pairing errors, which are commonly produced during DNA replication. Inactivity of this DNA repair system leads to mutations that can promote carcinogenesis. Mutation carriers have a 40 to 60% risk of developing endometrial carcinoma. Among women, the risk of endometrial

carcinoma exceeds that of colorectal carcinoma, and it often develops at a young age. Of the cases of endometrial carcinoma, 2 to 5 percent are attributable to Lynch syndrome[22]. In general, most familial cases develop in premenopausal women[23]. Women carrying mutations in the BRCA1 and BRCA2 genes have an increased risk of breast and ovarian cancer. They may also have a slightly elevated risk of endometrial cancer, but only because breast cancers are often treated with tamoxifen[24].

Tamoxifen causes a two- to three-fold increased risk of developing endometrial cancer because of its modest "unbalanced" estrogenic effect on the endometrium. The increased risk of endometrial cancer almost exclusively affects postmenopausal women, and cancer incidence rates increase linearly with the duration and cumulative dose of tamoxifen therapy. Consequently, women taking tamoxifen should be monitored for endometrial risk and should report any spotting and vaginal bleeding. Routine endometrial surveillance does not increase early diagnosis rates, except in cases where a patient treated with tamoxifen does not present such symptoms or is identified as being at high risk of endometrial cancer[25].

PROTECTIVE FACTORS

The use of combined oral contraceptives (COCs) for at least 1 year leads to 30-50% reduction in the risk of EH and EC, and the risk reduction extends over 10 to 20 years[19]. This most likely results from a preventive chemotherapeutic effect on the endometrium provided by the progestin component. Of course, the progesterone intrauterine device (IUD) also confers long-term protection from EH and EC. Similar protective effects have been found with copper-based IUD types[26].

Smokers have a lower risk of developing EH and EC. The biological mechanism is multifactorial, but is partly related to lower circulating estrogen levels due to reduced body weight, earlier menopause and altered hormone metabolism[27].

CLASSIFICATION

EH can be classified in two different entities according to World Health Organization Classification for Female Genital Tract Tumors of 2020: non-atypical hyperplasia (NAH, benign endometrial hyperplasia) and

atypical endometrial hyperplasia (AEH), also known as endometrioid intraepithelial neoplasia (EIN)[28].

New term	Synonyms	Genetic changes	Coexistent invasive endometrial carcinoma	Progression to invasive carcinoma
Hyperplasia *without* atypia	Benign endometrial hyperplasia; simple non-atypical endometrial hyperplasia; complex non-atypical endometrial hyperplasia; simple endometrial hyperplasia without atypia; complex endometrial hyperplasia without atypia	Low level of somatic mutations in scattered glands with morphology on HE staining showing no changes	< 1 %	RR: 1.01–1.03
Atypical hyperplasia/ endometrioid intraepithelial neoplasia	Complex atypical endometrial hyperplasia; simple atypical endometrial hyperplasia; endometrial intraepithelial neoplasia (EIN)	Many of the genetic changes typical for endometrioid endometrial cancer are present, including: micro satellite instability; *PAX2* inactivation; mutation of *PTEN*, *KRAS* and *CTNNB1* (β-catenin)	25–33 % [5] 59 % [3]	RR: 14–45

Figure 1: WHO 2020 classification of EH[28].

This classification represents an evolution from the previous system proposed in 1994, which identified four categories of EH, based on two characteristics: cytological atypia (loss of polarity, nuclear enlargement and rounding, and presence of nucleoli) and glandular complexity (closely packed glands with irregular profile)[29].

In detail, these categories were:

- simple hyperplasia without atypia (SH);
- simple hyperplasia with atypia (SAH);
- complex hyperplasia without atypia (CH);
- complex hyperplasia with atypia (CAH).

In fact, several studies demonstrated that EH has a dual nature, including benign proliferation reactive to unopposed action of estrogens, as well as precancerous lesions[30].

In order to distinguish between these two entities, alternative classification systems were developed:

- Endometrial Intraepithelial Neoplasia (EIN) system

  The EIN system is based on three histomorphologic parameters, glandular crowding, lesion diameter >1 mm, and cytology different from adjacent endometrium, that allow differentiation between benign hyperplasia and EIN[2,29,31].

- The European Working Group of Experts (EWG) system

  In this classification, Authors combined AEH and well differentiated adenocarcinoma in one category named endometrioid neoplasia (EN), and simple and complex hyperplasia without atypia in another category named benign hyperplasia[32].

With the 2020 revision, the WHO system has conceptually accepted the EIN system, recognizing the presence of only two categories of endometrial hyperplasia: one benign (non-atypical hyperplasia, NAH) and one premalignant (AEH/EIN). In particular, NAH is clearly defined as reactive proliferation and benign hyperplasia is reported as a synonym

of NAH. On the other hand, AH is defined as a premalignant lesion and EIN is reported as a synonym of AH.

However, there is no unanimous consensus on a recommended system. The American College of Obstetricians and Gynecologists (ACOG) and the Society of Gynecologic Oncology (SGO) recommend the use of the EIN system to distinguish premalignant lesions[33,34]. On the other hand, in 2016, joint guidelines from two committees, the Royal College of Obstetricians and Gynecologists (RCOG) and the British Society for Gynecological Endoscopy (BSGE), regarding the management and classification of hyperplasia were published, recommending the WHO classification[35].

However, despite being equated to the EIN system, the WHO classification does not accurately describe the changes adopted to define premalignancy. In fact, some definitions such as “crowded aggregates of cytologically altered glands” refer to the EIN system, while other ones, such as “cytologic atypia superimposed on NAH defines AH”, seem to refer to the previous WHO criteria. Furthermore, SH and CH are listed as synonyms of NAH, while SAH and CAH are listed as synonyms of AH. In this way, cytologic atypia might appear as the only crucial parameter for recognizing precancer; this would define the WHO 2020 system as a collapsed version of the 1994 one.

A meta-analysis on eight studies with 1352 hyperplasias was performed to assess congruence between WHO 1994 and EIN classification systems of endometrial hyperplasia, finding that WHO 1994 system is in fact not congruent with the EIN system and cannot be directly translated into a dual classification [36].

In particular, this difference was partially attributable to the complexity of glandular architecture. Indeed, in the NAH subgroups, the rate of EIN diagnosis was only 6% in SH but over 50% in CH.

Therefore, complexity of glandular architecture appears as a crucial parameter in the risk of progression to EC, as demonstrated by our meta-analysis which showed that, in the absence of cytologic atypia, complexity of glandular architecture significantly increases the risk of progression of EH to cancer, with a risk increase of almost 5-fold[37].

In addition, it was demonstrated that in CH, the risk of occult EC is significantly higher than in SH (12.4% VS 2%)[38].

This means that, by applying EIN criteria, SH is congruently benign, while more than half of CH (which using EIN criteria would have been declared NAH) is premalignant and 1 out of 10 is malignant, highlighting that the presence of a complex glandular architecture may be a crucial premalignant feature, independent from the presence of overt cytologic

atypia. Based on this evidence, complexity of glandular architecture should not be disregarded in the classification of EH.

An application of the WHO system based on only cytologic atypia would cause many precancers to be missed, with a significant risk of progression to malignancy.

In order not to miss premalignant lesions, we have proposed an integration of WHO system with EIN system. Basically, we have suggested using EIN criteria to recognize premalignant EH, substratifying them based on the presence of overt cytologic atypia. According to such approach, three EH category would be identified: benign EH (polyclonal); EIN without overt atypia (premalignant, but with lower risk of cancer); EIN with overt atypia (premalignant, with higher risk of cancer)[30].

In fact, many pathologists are reluctant to make a diagnosis of premalignancy in the absence of evident atypia, especially considering that the adjective “atypical” is included in “AEH/EIN”. In the routine pathology practice, it is not uncommon to encounter foci of crowded glands with no overt atypia, in which distinguishing between non-atypical hyperplasia and AEH/EIN is challenging.

In addition, it was demonstrated a poor correlation between pre-operative endometrial sampling and final diagnosis[39], highlighting that a differential

diagnosis among NAH, AEH/EIN and EC is challenging, especially in the pre-operative setting.

Therefore, identifying additional criteria to predict the risk of EC in AEH/EIN appears clinically relevant.

An attempt in this field was made by Zhang *et al.*, who subdivided AEH/EIN into type A (showing foci of stroma disappearance ≤ 2 mm) and type B (showing intervening stroma among glands). The authors found that type A AEH/EIN had a significantly increased risk of EC on hysterectomy performed within 6 months. However, type B AEH/EIN still showed a relatively high risk of EC (26.2%)[40], comparable to that of previously published series of AEH/EIN[41]. Therefore, a category of low-risk premalignant lesions was not identified.

In 2019, we performed a meta-analysis of the literature regarding the prognostic significance of two different systems for the classification of AEH/EIN, i.e., the 1994 WHO system (which was based on nuclear atypia) and the EIN system (which was based on glandular crowding and cytological demarcation, not requiring overt atypia). We included only patients who underwent hysterectomy within 1 year from index diagnosis. We found that the EIN system was more sensitive in stratifying the risk of EC, while the 1994 WHO system was more specific. Our conclusion was that the integration of the two systems would lead to a more accurate

stratification of the risk of EC in AEH/EIN, with potential benefits in terms of a more tailored management. We proposed to subdivide AEH/EIN into "EIN without overt atypia" (premalignant lesion at lower risk of EC) and "EIN with overt atypia" (premalignant lesion at higher risk of EC). However, we did not assess in detail the objectivity of the criteria to define the presence of overt atypia[30].

In 2021, D'Angelo *et al.* tried to provide objective criteria to grade atypia in AEH/EIN. The authors subdivided 79 cases of AEH/EIN into low-grade AEH and high-grade AEH, the latter characterized by loss of polarity and rounded enlarged nuclei with prominent nucleoli in most cells, with brisk mitotic activity. They found that no case of low-grade AEH showed EC at hysterectomy performed within 1 year from diagnosis, supporting that the grade of atypia provides crucial prognostic information[42].

MOLECULAR FACTORS

Histologic classifications may be affected by several problems, such as low reproducibility, tissue inadequacy, artefact changes, or ambiguous features[43].

In order to improve the reliability of the differential diagnosis, several diagnostic molecular markers have been proposed.

PTEN

Great emphasis has been given to the loss of expression of the tumor suppressor protein phosphatase and tensin homolog (PTEN)[2], since the mutation of PTEN is the most common molecular alteration found in endometrial carcinogenesis[8,9] and occurs in an early phase. In the 2021 ESGO guidelines, the immunohistochemical assessment of PTEN expression is recommended to recognize endometrial precancerous lesion[44].

PTEN gene is located at chromosome 10q23 and encodes a phosphatase which acts as a tumor suppressor. It has a lipid phosphatase activity, which induces cell cycle arrest, upregulates AKT-dependent proapoptotic mechanisms and downregulates Bcl-2-dependent antiapoptotic mechanisms, acting in opposition to PI3K. Moreover, PTEN has also a protein phosphatase activity, which is involved in the inhibition of focal adhesion formation, cell spread and growth-factor-stimulated MAPK signaling[45].

In the four categories of endometrial cancer identified by the Cancer Genome Atlas Research Network (ultramutated, hypermutated, copy

number low, copy number high), PTEN mutations were found in 94%, 88%, 77% of and 15% of cases respectively[46].

Although a loss of PTEN function is involved in endometrial carcinogenesis, we performed a meta-analysis of 18 studies that showed that immunohistochemical evaluation of PTEN expression has a low diagnostic usefulness in the differential diagnosis between benign and premalignant EH[47]. On the other hand, other studies demonstrated that PTEN may have a prognostic value for progression of EH to EC, and for the presence of a coexistent EC after a diagnosis of EH[48,49]. This was recently confirmed by our meta-analysis. However, PTEN evaluation does not appear as a reliable tool in differentiating benign and premalignant EH, demonstrating low sensitivity and specificity[50].

ARID1A

ARID1A/BAF250 is a nuclear protein that participates in forming the SWI/SNF chromatin remodeling complex. The protein is involved in important cellular functions including transcription modulation, DNA damage repair, DNA synthesis and DNA methylation. ARID1A acts as a tumor suppressor. Inactivating mutations of ARID1A result in loss of ARID1A protein expression, a common condition in EC[51].

ARID1A is one of the immunohistochemical marker proposed by the ESGO guidelines to differentiate premalignant EH from benign hyperplasia[44]. However, the diagnostic accuracy of immunohistochemistry for ARID1A is anything but defined.

We found that ARID1A loss was very little sensitive for premalignant EH. This finding resulted in a suboptimal diagnostic accuracy, making ARID1A inadequate as a diagnostic marker. On the other hand, the specificity of ARID1A as a marker of premalignancy was almost perfect (99%). For this reason, ARID1A does not appear adequate as a stand-alone diagnostic marker of premalignant EH, but it may be useful as a "rule-in" test for diagnosis of precancer, due to its excellent specificity[52].

PAX2

PAX2 gene is a member of a paired box gene family consisting of nine components (*PAX1* to *PAX9*), especially expressed during the embryonic development and organogenesis[53]. However, PAX proteins are also involved in several malignancies, acting as proto-oncogenes by transactivating promoters of target genes which regulate cell growth, self-sufficiency, apoptosis and cellular transformation[54]. In particular, PAX2 protein has anti-apoptotic effects binding to the

regulatory region at the 5′end of P53 gene and inhibiting its protein production at the transcriptional level. The expression of PAX2 is upregulated indirectly by the estrogen receptor α pathway[55]. Nevertheless, the role of PAX2 and its changes of expression in endometrial carcinogenesis are still unclear, with the common suggestion that PAX2 expression decreases in endometrial cancer and precancer.

We found that both complete loss and decrease of PAX2 expression were significantly more common in EC and precancerous EH than benign EH[56].

Pathway models for the possible tumor suppressor activity of PAX2 in endometrial carcinogenesis might be suggested by studies about carcinogenesis in other tissues. In particular, several mechanisms have been proposed for tumor suppressor activity of PAX2 in ovarian carcinogenesis. PAX2 knockdown in fallopian tube epithelial cell lines increased expression of the stem cell markers CD44 and SCA1 and reduced the capability of these cells to form differentiated epithelial luminal structures[57]. It has been showed in murine oviductal epithelial cells that wild type p53 improves PAX2 transcription, while mutant p53 decreases[58]. In a fallopian tube model of ovarian cancer with PAX2 and PTEN loss, re-expression of PAX2 repressed the oncogenic

properties of these cells and extended survival[58]. On the other hand, PAX2 expression in a spontaneous ovarian surface epithelium derived model of high-grade serous ovarian carcinoma reduced proliferation and metastasis by increasing COX2 and reducing HTRA1 expression[59]. Altogether, these results suggest PAX2 loss may be an early molecular event in ovarian cancer progression that predisposes cells to further mutations that can drive tumorigenesis, regardless of cell of origin[34]. Such mechanisms might underlie also endometrial carcinogenesis.

BCL2

The anti-apoptotic protein B-cell lymphoma 2 (Bcl-2) is upregulated through the actions of estrogens, and its expression has been observed to increase in proliferative endometrium and in benign EH. Several studies have found a loss of Bcl-2 expression in neoplastic endometrial samples (premalignant EH and EC)[60]. Therefore, Bcl-2 loss has been proposed as a marker to detect intraepithelial neoplasia in endometrial specimens[61].

Our meta-analysis on twenty studies showed that Bcl-2 loss was significantly associated with premalignant features of EH, while no significant differences were found between proliferative endometrium

and benign EH, and between premalignant EH and EC. These findings strongly suggest that Bcl-2 loss may be a marker of endometrial neoplasia, and are compatible with scientific evidence on Bcl-2 physiology[62].

CLINICAL PRESENTATION

The clinical onset of EH and EC is represented, in most cases, by abnormal uterine bleeding, whether it is vaginal blood loss during menopause or unexpected bleeding compared to normal menstrual flow during childbearing age. Abnormal uterine bleeding in the premenopausal population includes a spectrum of disorders that may include menorrhagia, metrorrhagia, and oligomenorrhea[44].

Very rarely, in fact, the neoplasia is asymptomatic and the diagnosis is made accidentally.

Given the increased incidence of endometrial carcinoma and the younger age of onset, it is necessary to subject all patients of childbearing age who present with intermenstrual metrorrhagia to clinical and instrumental examinations. Pain and leucoxanthorrhea are generally due to necrotic colliquative phenomena, typical of the more advanced stages of the

disease. Significant lymph node involvement can cause edema of the lower limbs, pubic area and vagina. Abdominal, pelvic, lumbosacral and gluteal pain, sub-occlusive syndromes, bone pain and dyspnea are late signs of metastatic spread of the disease[63].

SCREENING AND PREVENTION

Currently, mass screening of an asymptomatic premenopausal and postmenopausal population for the early diagnosis of EH and EC is not feasible. Studies conducted on ectocervical sampling have shown a false negative rate of approximately 40-50% because the exfoliated endometrial cells, having undergone the action of the vaginal environment, present alterations that cause them to lose the characteristics that allow the differentiation of the tumor cell from the normal one.

On the other hand, the prognosis of EC is strictly linked to the early diagnosis, in fact, 5-year survival decreases drastically from 78-98% in the case of diagnosis at stage I to 3-10% in the case of diagnosis at stage IV[64].

Screening is recommended only for high-risk groups, such as those with type 2 Lynch syndrome with the desire to preserve fertility, before

prophylactic hysterectomy performed at a later age[65]. In these cases, endometrial surveillance is performed by biopsy and transvaginal ultrasound starting at age 35 (annually until hysterectomy). Prophylactic surgery (hysterectomy and bilateral salpingo-oophorectomy), preferably with a minimally invasive approach, should be considered as an option at age 40 for Lynch syndrome type 2 mutation carriers to prevent endometrial and ovarian cancer[44].

Most cases of endometrial cancer cannot be prevented, but reducing risk factors and introducing protective lifestyle factors, when possible, can reduce the risk of developing the disease. All women should be informed of the risks and symptoms of endometrial cancer and strongly encouraged to engage in regular physical activity and adopt an active lifestyle that can help achieve and maintain an ideal weight, as well as reduce other risk factors for endometrial cancer such as high blood pressure and diabetes. The use of combined oral contraceptives is significantly associated with a reduction in endometrial cancer in all users, the benefit increasing with increasing duration of treatment[66].

DIAGNOSIS

Both EH and EC are most commonly diagnosed following abnormal uterine bleeding.

In any woman complaining of abnormal vaginal bleeding, the first test to be performed is a transvaginal ultrasound (TVUS, Trans-Vaginal Ultrasonography)[67]. This exam allows to evaluate endometrial thickness, which is higher in women at risk of EH or EC. There is no consensus on the cutoff of endometrial thickness to predict the risk of EH or EC, however it should be considered suspicious if >5 mm in postmenopause, while in the fertile period it can vary between 1 and 14 mm, depending on the period of the menstrual cycle[9]. Another suspicious sign is the presence of focal thickening[67]. The addition of color and Power Doppler allows to accurately study the blood perfusion of normal endometrium and the expansive processes affecting it[68].

In cases of TVUS suspicion of EH or EC, the diagnosis of AEH and EC is based primarily on the evaluation of endometrial tissue obtained through targeted biopsy +/- combined with hysteroscopy[9], which represent the diagnostic gold standard[44]. Hysteroscopy is the recommended approach, compared to blinded curettage, as it allows to visualize the uterine cavity and perform a targeted biopsy on areas of altered endometrium[69].

A histopathological diagnosis of AEH/EIN does not require any additional workup aside from the evaluation of perioperative risk.

Pre-operative mandatory work-up includes: family history; general assessment and inventory of co-morbidities; geriatric assessment, if appropriate; clinical examination, including pelvic examination; expert transvaginal or transrectal ultra- sound or pelvic magnetic resonance imaging (MRI)[44].

TREATMENT

Hysterectomy is the preferred treatment for women with AEH/EIN because, over time, the risk of progression to cancer approaches 29%. There is also a high detection rate of concomitant invasive cancers coexisting with atypical hyperplasia[70]. Obstetric and gynecological specialists performing hysterectomy for AEH/EIN should be aware of the possibility of coexistence with invasive cancers and the possible need for surgical staging, for which peritoneal washings would be indicated before performing a hysterectomy.

The standard treatment for atypical hyperplasia associated with the best survival is total hysterectomy, bilateral salpingo-oophorectomy, and optional lymph node staging[44,71].

Two randomized prospective studies comparing minimally invasive with open surgeries showed similar survival with quicker recovery with the minimally invasive approach[72,73], therefore, minimally invasive surgery is the standard approach.

Lymph node assessment has been a part of surgical staging for EC since 1988 [74], but its role in EIN remains debated. Given the clear risk of hidden carcinoma at the time of an EIN diagnosis—with up to 36% presenting as grade 2–3 tumors and up to 47% showing deep myometrial invasion (stage IB[64]), LN assessment during hysterectomy is still under consideration[75].

Sentinel Lymph Node (SLN) biopsy has progressively become the preferred technique for lymph node assessment in EC, offering a reduction in morbidity while still providing essential staging information[44]. Although the likelihood of LN involvement in EIN is low, several factors support including SLN in the staging approach for this condition: 1) SLN analysis aids in making informed decisions about adjuvant therapy, 2) SLN sampling is associated with minimal morbidity, a high negative predictive value, and a short procedure time, 3) a completion surgery in the presence of carcinoma in final pathology is challenging given the post hysterectomy lymphatic disruption[76,77].

This technique might be useful in cases of occult EC in AEH/EIN, since it would guarantee a proper surgical staging and avoid additional surgery

in case of diagnosis of EC on the surgical specimen. However, in a single center retrospective study including 162 patients, the majority of patients (92%) underwent a SLN dissection that did not add value to their care and could be associated with morbidity, such as prolonged surgical time, vessel/nerve injury, lymphocyte formation and lymph-edema, as well as higher costs[78]. Therefore, SLN biopsy in AEH/EIN, although being the standard in early-stage EC, is still debated in literature and further studies are needed.

Fertility-sparing treatment

The diagnosis of AEH/EIN in young women of childbearing age is rare. Although endometrial carcinoma is the most common gynecological neoplasm in developed countries, its incidence in childbearing age is less than 20% and this percentage drops to less than 5% for women under 40 years of age[79]. However, the change in the concept of conceptional age in recent years means that the number of nulliparous women wishing to have children who may be diagnosed with AEH/EIN and who require fertility preservation is no longer unusual, also considering modern assisted

reproduction techniques. Therefore, fertility sparing program should be proposed to these patients.

Patients must be informed that the fertility sparing approach is a non-standard treatment, and that they must be willing to accept close follow-up during and after treatment, that in case of treatment failure or early recurrence, hysterectomy should be considered, which should also be suggested after a possible pregnancy.

Conservative management of AEH/EIN is based on medical treatment with progestins and hysteroscopic resection[80].

The most used and described progestins in the literature are medroxyprogesterone acetate and megestrol acetate; other therapies have also been used such as GnRH analogues, hydroxyprogesterone, letrozole, tamoxifen, oral contraceptives and Levonorgestrel intrauterine device (LNG-IUD).

There are also some works in the literature that propose hysteroscopic resectoscopy of the tumor combined with oral progestins or with the placement of a LNG-IUD[81].

The recent ESGO/ESHRE/ESGE guidelines of 2023 recommend a combined approach consisting of hysteroscopic tumor resection, followed by oral progestins and/or LNG-IUD, as the most effective fertility-sparing treatment both for complete response rate and live birth rate, compared

with other treatment options, while gonadotropin-releasing hormone analogues should not be considered as a first-line treatment[80].

Recommended dose of progestins are[80]:

- Orally administered megestrol acetate at a dose of 160–320 mg/ day or medroxyprogesterone acetate at a dose of 400–600 mg/ day
- A levonorgestrel-intra-uterine device at a dose of 52mg, alone or in combination with oral progestins

It has been shown that the majority of patients respond within 6 months of treatment (about 75%) with only a small additional benefit for extending the treatment up to 12 months[34].

The recommended duration of therapy is 6–12 months, within which a complete response should be achieved. The maximum time to achieve complete response should not exceed 15 months[80].

After the start of treatment, follow-up evaluation should be performed every 3-6 months by hysteroscopy and adequate biopsy until pregnancy is achieved, also through assisted reproduction techniques. Once pregnancy is achieved, total hysterectomy with bilateral salpingectomy and optional bilateral oophorectomy should be suggested[80].

If therapy is ineffective and there is no pathological response, hysterectomy with bilateral salpingectomy should be suggested. Bilateral

oophorectomy may be omitted if the patient is young (<45 years), and without signs of extrauterine disease.

The recurrence rate after conservative therapy for endometrial cancer is reported to be between 30% and 40%, with a median time to manifestation of 12-18 months and a range between 4 and 66 months.

It seems that recurrence increases in relation to time for at least 5 years[82].

Molecular predictors of fertility-sparing treatment response

A considerable percentage of patients do not respond to conservative treatment or show relapses after an initial remission, with the consequent risk of progression to invasive disease. For this reason, in recent years numerous studies have been conducted on clinical, imaging, histological and molecular aspects that could influence the outcome of treatment. Immunohistochemistry has played a major role in this area. Although a large number of immunohistochemical markers have been evaluated, their usefulness is still unclear, and no predictive marker is actually recommended by international guidelines.

The search for predictive markers on pretreatment biopsy has the interesting aim of preventively identifying cases of failure to respond to

conservative treatment, avoiding the risk of disease progression related to ineffective therapy. Despite the large number of markers evaluated, only a few of them have been found to have significant associations.

The most studied predictive markers in the pretreatment phase are progesterone and estrogen receptors. Although the results are variable, a high expression of these receptors is predictive of a good response to treatment in several studies. Furthermore, the isoforms of these receptors, in particular the progesterone receptor B, seem more promising. The loss of PTEN seems to predict a poor response to conservative treatment only if associated with low expression of phospho-AKT; on the contrary, the isolated loss of PTEN is not significant. As regards mismatch repair proteins (MMR), an anomalous expression pattern (including MLH1, MSH2, MSH6, PMS2) strongly predicts a poor response to treatment. MLH1 considered individually is not significant. A high expression of Dusp6 seems associated with a good response to treatment, on the contrary a high expression of GRP78 is predictive of a poor response.

The evaluation of post-treatment markers and their changes during follow-up aims to evaluate the efficacy of the therapy and to investigate the mechanisms of action and resistance. In this regard, predicting individual response early in therapy may allow to adapt the timing of follow-up and, if necessary, modify treatment.

In the follow-up phase, PR, ER and their isoforms seem to show a down regulation in cases of good response, a stable expression in cases of non-response to treatment. In cases of good response, an increased expression of Fas, NCoR, stromal Bcl2 and Dusp6 is observed, and a reduced expression of Bcl2, survivin, Ki67, HE4 and SPAG9. In cases of non-response, an increased expression of GRP78, Nrf2 and AKR1C1, survivin, PAX2 and loss of PTEN associated with high expression of phospho-mTOR is observed.

In conclusion, PR and ER were the most studied predictive markers both in the pre-treatment and follow-up phase, showing conflicting results. The study of PR and ER isoforms may lead to better results; PRB appeared to be the most promising. MMR, Dusp6, GRP78 and PTEN combined with phospho-AKT or phospho-mTOR showed significant results, but further studies are needed to define their accuracy[83].

EXPERIMENTAL SECTION

INTRODUCTION

Atypical hyperplasia/endometrioid intraepithelial neoplasia (AEH/EIN) is regarded as the precursor lesion of endometrial carcinoma (EC) of endometrioid type[28]. On this account, the standard treatment for AEH/EIN is total hysterectomy with bilateral salpingo-oophorectomy and optional sentinel lymph node biopsy; in young patients, a progestin-based conservative treatment can be performed[40]. Histologically, AEH/EIN is characterized by crowded glands and cytological demarcation (i.e., cytological difference from the background endometrium)[28,40]. However, the spectrum of AEH/EIN is broad and includes cases with relatively bland nuclei and low mitotic activity as well as cases with marked nuclear atypia and high mitotic activity. Moreover, the extent of glandular confluence needed to make a diagnosis of EC is still undefined; the most used cutoff is 2 mm. Interestingly, there is a high variability in the percentage of concurrent EC among the several published series of AEH/EIN. In some cases, this percentage is very high (>50%), raising the question whether EC was already present in the index biopsy and misdiagnosed as AEH/EIN based on the adopted criteria[30,40,42].

It is reasonable to hypothesize that refining histological criteria of AEH/EIN may allow identifying prognostically relevant subgroups with different risk of cancer.

In this regard, Zhang *et al.* found that AEH/EIN showing microscopic foci of glandular molding with disappearance of stroma among glands had a higher risk of concurrent EC[40]. In our previous meta-analysis, we suggested to subdivide AEH/EIN based on the presence of nuclear atypia, since cases with overt atypia had a higher risk of EC in the short term[30]. D'Angelo *et al.* proposed a similar stratification and provided objective criteria to grade atypia in AEH/EIN; they found that AEH/EIN with low-grade atypia showed no progression to EC within 1 year[42].

In this study, we assessed the prognostic value of a combined grading system for AEH/EIN, based on the criteria proposed by Zhang *et al.*, D'Angelo *et al.* and our previous study.

METHODS

Study protocol and patients' selection criteria

The study followed an *a priori* defined study protocol and was designed as multicentric observational retrospective cohort study. The study received approval by the Ethical Committee of the University of Naples Federico II (no. 8/20) and was reported following the STrengthening the Reporting of OBservational studies in Epidemiology (STROBE) guidelines and checklist[84].

Electronic databases of the Pathology Unit of the IRCCS Azienda-Ospedaliero Universitaria di Bologna, University of Bologna, Italy, and University Federico II of Naples, Italy, were searched for all patients who received a histological diagnosis of AEH/EIN within the period January 2009 - December 2019. Only patients who underwent hysterectomy within 1 year from the diagnosis of AEH/EIN were included in the study. Patients who were treated conservatively for AEH/EIN were excluded. Patients with AEH/EIN were subdivided into 3 study groups, based on (i) the grade of nuclear atypia and (ii) the presence of foci of confluent glands (FCG): low-grade (LG)-AEH/EIN, high-grade (HG)-AEH/EIN, and FCG-AEH/EIN. The rate of endometrial carcinoma on the subsequent hysterectomy was compared among the study groups, and reproducibility

among pathologists in diagnosing LG-AEH/EIN, HG-AEH/EIN, and FCG-AEH/EIN was assessed.

Histological assessment

Histological slides of the index biopsies and hysterectomy specimens were independently reviewed by two panels of gynecological pathologists (1st panel: A.T., P.C. and L.I.; 2nd panel: D.A., A.S., and G.F.Z.) who were blinded to the outcomes.

Histological assessment was based on the criteria recommended by the World Health Organization (WHO)[1]. A diagnosis of AEH/EIN was made in the presence of an area ≥1 mm of glandular crowding (gland-to-stroma ratio ≥1) with altered cytology[31]. AEH/EIN was further subdivided into three groups: LG-AEH/EIN, HG-AEH/EIN and FCG-AEH/EIN.

LG-AEH/EIN and HG-AEH/EIN were diagnosed when glands were crowded but there still was intervening stroma among glands; they were differentiated based on the presence of high-grade atypia as described by D'Angelo *et al.* (loss of polarity, rounded enlarged nuclei with prominent nucleoli in most cells, brisk mitotic activity)[42].

FCG-AEH/EIN was defined by the presence of at least one focus of confluent glands with no intervening stroma measuring at least 1 mm but not exceeding 2 mm. Confluent glands were defined as back-to-back

glands (with glands occupying ≥95% of the focus), maze-like structures, and/or cribriform structures. In the presence of at least one focus of confluent glands > 2 mm, a diagnosis of endometrial carcinoma was made, in agreement with previously reported criteria[31].

Hysterectomy specimens were subdivided into two groups based on the presence of endometrial carcinoma.

Statistical analysis

The rate of cases with endometrial carcinoma on hysterectomy specimen was calculated in each index diagnosis group. Fisher's exact test was used to assess the difference between groups, with a significant p-value < 0.05. Cohen's κ was used to assess the concordance between pathologists. Statistical analysis was performed using Statistical Package for Social Science (SPSS) 18.0 package (SPSS Inc., Chicago, IL, USA).

RESULTS

A total of 117 patients with a diagnosis of AEH/EIN who underwent hysterectomy within 1 year were identified. Twenty-one cases were excluded at histological review (9 case were non-atypical hyperplasia, 10

were reclassified as endometrial carcinoma, one as serous carcinoma, one as clear cell carcinoma). Finally, 96 cases were included in the study. Forty-two cases (43.7%) were classified as LG-AEH/EIN, 28 cases (29.2%) were classified as HG-AEH/EIN, and 26 cases (27.1%) were classified as FCG-AEH/EIN (Figure 2).

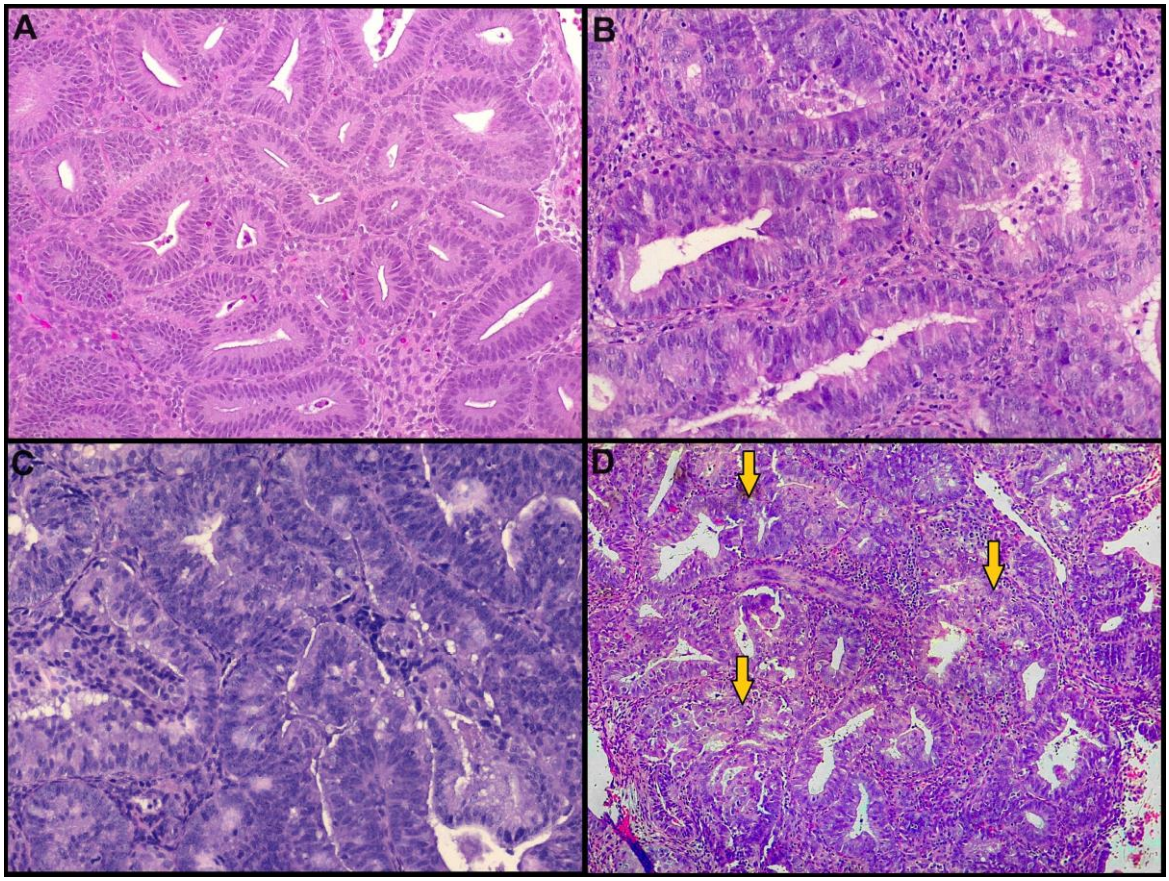

Figure 2: Histological appearance of atypical endometrial hyperplasia/endometrioid intraepithelial neoplasia (AEH/EIN) (hematoxylin-eosin stain). A) low-grade AEH/EIN showing crowded glands separated by intervening stroma, in the absence of overt nuclear atypia (nuclei are ovoidal and polarized) (magnification 200X). B) high-grade AEH/EIN showing crowded glands separated by intervening stroma, with overt nuclear atypia

(nucleomegaly, stratification, nucleolation, loss of polarity) (magnification 200X). C): Confluent glands with loss of intervening stroma (magnification 200X). D) AEH/EIN showing multiple foci of confluent glands < 2 mm (yellow arrows) (magnification 40X).

Overall, 36/96 patients (37.5%) had endometrial carcinoma on the subsequent hysterectomy performed within 1 year from diagnosis. The number of endometrial carcinomas was 4/42 (9.5%) in LG-AH/EIN, 14/28 (50%) in HG-AH/EIN, and 18/26 (69.2%) in FCG-AEH/EIN. The rate of endometrial carcinoma was significantly higher in HG-AH/EIN than in LG-AEH/EIN ($p<0.001$) and in FCG-AEH/EIN than in LG-AEH/EIN ($p<0.001$), while it did not significantly differ between HG-AEH/EIN and FCG-AEH/EIN ($p=0.176$).

Reproducibility among pathologists was moderate in distinguishing between LG-AEH/EIN and HG-AEH/EIN (agreement=80%; k=0.58) and substantial in distinguishing between HG-AEH/EIN and FCG-AEH/EIN (agreement=81.48%; k=0.63) and between LG-AEH/EIN and FCG-AEH/EIN (agreement=82.35%, k=0.63).

Results are summarized in Table 1.

No. of patients	**96**
Age mean (range)	55.7 years (32-93)
Classification	
• **LG-AEH/EIN**	42/96 (43.7%)
• **HG-AEH/EIN**	28/96 (29.2%)
• **FCG-AEH/EIN**	26/96 (27.1%)
EC at hysterectomy	36/96 (37.5%)
• **LG-AEH/EIN**	4/42 (9.5%)
• **HG-AEH/EIN**	14/28 (50%)
• **FCG-AEH/EIN**	18/26 (69.2%)
Difference in EC risk	
• **LG- vs HG-AEH/EIN**	$p<0.001$
• **LG- vs FCG-AEH/EIN**	$p<0.001$
• **LG- vs HG-AEH/EIN**	$p=0.176$
Reproducibility	
• **LG- vs HG-AEH/EIN**	moderate ($k=0.58$)
• **LG- vs FCG-AEH/EIN**	substantial ($k=0.63$)
• **LG- vs HG-AEH/EIN**	substantial ($k=0.63$)

Table 1. Summary of results.

AEH/EIN: atypical endometrial hyperplasia/endometrioid intraepithelial neoplasia; LG: low-grade; HG: high-grade; FCG: foci of confluent glands; EC: endometrial carcinoma.

DISCUSSION

Summary of the main results

This study showed that the 1-year rate of endometrial carcinoma was significantly higher in HG-AEH/EIN (50%) and FCG-AEH/EIN (69.2%) than in LG-AEH/EIN (9.5%), with moderate-to-substantial reproducibility of this combined grading system among pathologists.

Results in the context of published literature

The classification of endometrial hyperplasia has changed over time. The 1994 WHO classification identified four groups: simple, complex, simple atypical, and complex atypical hyperplasia; the presence of nuclear atypia was considered as a crucial feature to predict the risk of endometrial carcinoma[29].

The current classification identifies two groups: NAH, which is a benign proliferation caused by an unopposed action of estrogens, and AEH/EIN,

which is a premalignant lesion. This classification does not require the presence of overt nuclear atypia for a diagnosis of AEH/EIN, but it is sufficient the presence of a crowded area of at least 1 mm exhibiting "cytological demarcation", i.e., different cytological features from background non-crowded endometrium[1,36,40,42].

While this approach may have simplified the approach to endometrial hyperplasia, there are some issues that are still unresolved. In fact, the "cytological demarcation" criterion is based on the assumption that AEH/EIN is a clonal lesion, whereas non-atypical hyperplasia is a functional proliferation. However, clonality in endometrial glands does not necessarily indicate premalignancy and may be found in benign lesions and even in normal endometrium[31,85]. Moreover, "cytological demarcation" might also manifest as increased nuclear polarization compared to the background endometrium[15], which appears in contrast with the premalignant nature of AEH/EIN. In fact, many pathologists are reluctant to make a diagnosis of premalignancy in the absence of evident atypia, especially considering that the adjective "atypical" is included in "AEH/EIN". In the routine pathology practice, it is not uncommon to encounter foci of crowded glands with no overt atypia, in which distinguishing between non-atypical hyperplasia and AEH/EIN is challenging. Such a differential diagnosis will have serious implication on

the patient management; in fact, missing an AEH/EIN may expose the patient to the risk of developing endometrial carcinoma, while an erroneous diagnosis of AEH/EIN may lead to overtreatment with unnecessary hysterectomy. Identifying additional criteria to predict the risk of endometrial carcinoma in AEH/EIN appears therefore clinically relevant.

An attempt in this field was made by Zhang *et al.*, who subdivided AEH/EIN into type A (showing foci of confluent glands with no intervening stroma measuring ≤ 2 mm) and type B (showing glands separated by intervening stroma). The authors found that type A AEH/EIN had a significantly increased risk of endometrial carcinoma on hysterectomy performed within 6 months. However, type B AEH/EIN still showed a relatively high risk of endometrial carcinoma (26.2%)[40], comparable to that of previously published series of AEH/EIN[41]. Therefore, a category of low-risk premalignant lesions was not identified.

In 2019, we performed a meta-analysis of the Literature regarding the prognostic significance of two different systems for the classification of AEH/EIN, i.e., the 1994 WHO system (which was based on nuclear atypia) and the "EIN" system (which was based on glandular crowding and cytological demarcation, not requiring overt atypia). We included only patients who underwent hysterectomy within 1 year from index

diagnosis. We found that the EIN system was more sensitive in predicting the risk of endometrial carcinoma, while the 1994 WHO system was more specific. Our conclusion was that an integration of the two systems would lead to a more accurate stratification of the risk of endometrial carcinoma in AEH/EIN, with potential benefits in terms of a more tailored management. We proposed to subdivide AEH/EIN into "EIN without overt atypia" (premalignant lesion at lower risk of endometrial carcinoma) and "EIN with overt atypia" (premalignant lesion at higher risk of endometrial carcinoma). However, we did not assess in detail the objectivity of the criteria to define the presence of overt atypia[30].

In 2021, D'Angelo *et al.* tried to provide objective criteria to grade atypia in AEH/EIN. The authors subdivided 79 cases of AEH/EIN into low-grade AEH and high-grade AEH, the latter characterized by loss of polarity and rounded enlarged nuclei with prominent nucleoli in most cells, with brisk mitotic activity. They found that no case of low-grade AEH showed endometrial carcinoma at hysterectomy performed within 1 year from diagnosis, supporting that the grade of atypia provides crucial prognostic information[42].

Strengths and Weaknesses

In our study, we assessed a series of 96 AEH/EIN using the criteria proposed by both Zhang *et al.* and D'Angelo *et al.* We defined three categories, named LG-AEH/EIN, HG-AEH/EIN, and FCG-AEH/EIN. In agreement with our previous meta-analysis and the study by D'Angelo *et al.*, we found that patients with HG-AEH/EIN had a significantly increased risk of endometrial carcinoma within 1 year compared to LG-AEH/EIN. In fact, we found that 50% of HG-AEH/EIN cases had endometrial carcinoma on hysterectomy specimen. This percentage is relatively similar to that reported by D'Angelo *et al.* (61%)[42]. These results suggest that the grade of atypia in AEH/EIN is of great importance in predicting the risk of endometrial carcinoma and should therefore be assessed as it is routinary made in other districts such as the uterine cervix. FCG-AEH/EIN cases showed an even higher risk of endometrial carcinoma (69.2%), which appears similar to that found by Zhang *et al.* in type A AEH (75.9%)[40]. Such a high percentage suggests that areas of confluent glands represent small endometrial carcinoma foci even if their diameter does not exceed 2 mm. These foci were identified with substantial reproducibility by pathologists in our study, and it appears clinically relevant to indicate their presence in the pathology report. It

should be remarked that the 2 mm cutoff is not universally accepted among gynecological pathologists[86].

Interestingly, the percentage of endometrial carcinoma was not significantly different between HG-AEH/EIN and FCG-AEH/EIN. Considering the high risk of concurrent endometrial carcinoma in both groups, we suggest that a diagnosis of HG-AEH/EIN or FCG-AEH/EIN on endometrial biopsy may have similar clinical implication to a low-grade endometrial carcinoma diagnosis. It might be reasonable to perform ultrasonography or magnetic resonance imaging in these cases to exclude myometrial/adnexal involvement[44,64].

Regarding LG-AEH/EIN, our result was different from that of D'Angelo *et al.* In fact, no case of LG-AEH/EIN showed endometrial carcinoma in their series, leading them to diminish the clinical significance of this entity[42]. In our series, 9.5% of LG-AEH/EIN cases showed endometrial carcinoma at hysterectomy. Despite being significantly lower than that of HG-AEH/EIN, such percentage is not without clinical significance. In our previous study, we found that complex hyperplasia without atypia had a significant risk of concurrent endometrial carcinoma (11.2%). We postulated that most of those cases diagnosed as complex hyperplasia without atypia according to 1994 WHO criteria would fall in the AEH/EIN category as defined by the EIN criteria, in the absence of overt atypia[37]. In

this view, those cases would overlap with the LG-AEH/EIN category of our current study; this hypothesis can be supported by the similar risk of endometrial carcinoma. While HG-AEH/EIN and FCG-AEH/EIN could be clinically comparable to an early endometrial carcinoma, LG-AEH/EIN appears more in keep with a premalignant condition (as AEH/EIN is supposed to be). Remarkably, all the previous studies and the current one only included patients who underwent hysterectomy in the short term (within 1 year from index diagnosis)[30,40,42]. In the Literature, endometrial carcinomas detected within 1 year from an endometrial hyperplasia diagnosis have usually been regarded as concurrent with the hyperplasia[87]. Therefore, these studies could not assess the risk of progression to endometrial carcinoma in the long term. In our previous meta-analysis on complex hyperplasia without atypia, we found that 13 out of 157 patients (8.3%) with a follow-up > 1 year developed endometrial carcinoma [38]. We might hypothesize that a similar percentage of LG-AEH/EIN will progress to endometrial carcinoma in the long term. Despite being at lower risk compared to HG-AEH/EIN, LG-AEH/EIN still showed a clinically significant percentage of concurrent endometrial carcinoma; a change of treatment compared to the current guidelines for AEH/EIN might not be justified in this category[35].

It should be considered that the amount of tissue might be a factor affecting the accuracy of AEH/EIN stratification. In fact, inadequate sampling may lead to underestimate the risk of endometrial carcinoma. In our series, we did not find evident differences in the amount of tissue among different AEH/EIN groups at a subjective evaluation. However, a computerized morphometric analysis would be necessary for an objective quantification of the amount of tissue. Further studies are necessary in this regard.

Implications for Practice and Future Research

We hope further studies may achieve a more accurate risk stratification of AEH/EIN. In fact, identifying cases of AEH/EIN at low risk of progression may allow patients to be followed carefully to prevent endometrial carcinoma, without undergoing unnecessary hysterectomies (especially in the case of contraindications to surgery). In this field, the combined assessment of histology and immunohistochemistry may be useful, since immunohistochemical markers appeared helpful as both diagnostic and prognostic markers in AEH/EIN[47,50,52,56,88]. The role of molecular analysis in this field has yet to be defined.

CONCLUSION

The assessment of nuclear atypia and of FCG may allow stratifying AEH/EIN into prognostically relevant subgroups. In detail, HG-AEH/EIN shows a high risk of concurrent endometrial carcinoma and might be managed similar to low-grade endometrial carcinoma. On the other hand, LG-AEH/EIN showed a lower but still significant risk of endometrial carcinoma, appearing more in keep with a precursor lesion and with the current guidelines for the management of AEH/EIN. Cases of FCG-AEH/EIN may represent microscopic foci of endometrial carcinoma.

Further studies may further refine the risk stratification of AEH/EIN through an integrated clinicopathological, immunohistochemical and, if necessary, molecular analysis.

REFERENCES

1. Kurman RJ, Carcangiu ML, Herrington CS, et al. WHO Classification of Tumours of Female Reproductive Organs. *World Heal Organ Classif Tumours*. 2014.
2. Sanderson PA, Critchley HOD, Williams ARW, et al. New concepts for an old problem: the diagnosis of endometrial hyperplasia. *Hum Reprod Update*. 2017;23(2):232-254. doi:10.1093/HUMUPD/DMW042
3. Sobczuk K, Sobczuk A. New classification system of endometrial hyperplasia WHO 2014 and its clinical implications. *Przegląd Menopauzalny = Menopause Rev*. 2017;16(3):107. doi:10.5114/PM.2017.70589
4. Reed SD, Newton KM, Clinton WL, et al. INCIDENCE OF ENDOMETRIAL HYPERPLASIA. *Am J Obstet Gynecol*. 2009;200(6):678.e1. doi:10.1016/J.AJOG.2009.02.032
5. Ricci E, Moroni S, Parazzini F, et al. Risk factors for endometrial hyperplasia: results from a case-control study. *Int J Gynecol Cancer*. 2002;12(3):257-260. doi:10.1046/J.1525-1438.2002.01105.X
6. Armstrong AJ, Hurd WW, Elguero S, et al. Diagnosis and

management of endometrial hyperplasia. *J Minim Invasive Gynecol*. 2012;19(5):562-571. doi:10.1016/J.JMIG.2012.05.009

7. Siegel RL, Miller KD, Jemal J. Cancer statistics, 2020. *CA Cancer J Clin*. 70(1):7-30.

8. Ferguson SE, Aronson M, Pollett A, et al. Performance characteristics of screening strategies for Lynch syndrome in unselected women with newly diagnosed endometrial cancer who have undergone universal germline mutation testing. *Cancer*. 120(24):3932-3939.

9. Abu-Rustum N, Yashar C, Arend R, et al. Uterine Neoplasms, Version 1.2023, NCCN Clinical Practice Guidelines in Oncology. *J Natl Compr Canc Netw*. 2023;21(2):181-209. doi:10.6004/JNCCN.2023.0006

10. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2018. *CA Cancer J Clin*. 2018;68(1):7-30. doi:10.3322/caac.21442

11. Clinton SK, Giovannucci EL, Hursting SD. The World Cancer Research Fund/American Institute for Cancer Research Third Expert Report on Diet, Nutrition, Physical Activity, and Cancer: Impact and Future Directions. *J Nutr*. 2020;150(4):663-671. doi:10.1093/JN/NXZ268

12. Esposito K, Chiodini P, Capuano A, et al. Metabolic syndrome and

endometrial cancer: a meta-analysis. *Endocrine*. 2014;45(1):28-36. doi:10.1007/S12020-013-9973-3

13. Zhang Y, Liu H, Yang S, et al. Overweight, obesity and endometrial cancer risk: results from a systematic review and meta-analysis. *Int J Biol Markers*. 2014;29(1). doi:10.5301/JBM.5000047
14. Rosato V, Zucchetto A, Bosetti C, et al. Metabolic syndrome and endometrial cancer risk. *Ann Oncol Off J Eur Soc Med Oncol*. 2011;22(4):884-889. doi:10.1093/ANNONC/MDQ464
15. Hecht JL, Ince TA, Baak JPA, et al. Prediction of endometrial carcinoma by subjective endometrial intraepithelial neoplasia diagnosis. *Mod Pathol*. 2005;18(3):324-330. doi:10.1038/MODPATHOL.3800328
16. Luo J, Beresford S, Chen C, et al. Association between diabetes, diabetes treatment and risk of developing endometrial cancer. *Br J Cancer*. 2014;111(7):1432-1439. doi:10.1038/BJC.2014.407
17. Raffone A, Travaglino A, Saccone G, et al. Diabetes Mellitus Is Associated with Occult Cancer in Endometrial Hyperplasia. *Pathol Oncol Res*. 2020;26(3). doi:10.1007/s12253-019-00684-3
18. Barry JA, Azizia MM, Hardiman PJ. Risk of endometrial, ovarian and breast cancer in women with polycystic ovary syndrome: a systematic review and meta-analysis. *Hum Reprod Update*.

2014;20(5):748-758. doi:10.1093/HUMUPD/DMU012

19. Ali AT. Reproductive factors and the risk of endometrial cancer. *Int J Gynecol Cancer*. 2014;24(3):384-393. doi:10.1097/IGC.0000000000000075

20. Zucchetto A, Serraino D, Polesel J, et al. Hormone-related factors and gynecological conditions in relation to endometrial cancer risk. *Eur J Cancer Prev*. 2009;18(4):316-321. doi:10.1097/CEJ.0B013E328329D830

21. Bansal N, Yendluri V, Wenham RM. The molecular biology of endometrial cancers and the implications for pathogenesis, classification, and targeted therapies. *Cancer Control*. 2009;16(1):8-13. doi:10.1177/107327480901600102

22. Hampel H, Frankel W, Panescu J, et al. Screening for Lynch syndrome (hereditary nonpolyposis colorectal cancer) among endometrial cancer patients. *Cancer Res*. 2006;66(15):7810-7817. doi:10.1158/0008-5472.CAN-06-1114

23. Gruber SB, Thompson WD. A population-based study of endometrial cancer and familial risk in younger women. *Cancer Epidemiol Biomarkers Prev*. 1996;5(6):411-417.

24. Beiner ME, Finch A, Rosen B, et al. The risk of endometrial cancer in women with BRCA1 and BRCA2 mutations. A prospective study.

Gynecol Oncol. 2007;104(1):7-10. doi:10.1016/J.YGYNO.2006.08.004

25. Committee Opinion No. 601: Tamoxifen and uterine cancer. *Obstet Gynecol*. 2014;123(6):1394-1397. doi:10.1097/01.AOG.0000450757.18294.CF

26. Felix AS, Gaudet MM, La Vecchia C, et al. Intrauterine devices and endometrial cancer risk: a pooled analysis of the Epidemiology of Endometrial Cancer Consortium. *Int J Cancer*. 2014;136(5):E410. doi:10.1002/IJC.29229

27. Viswanathan AN, Feskanich D, De Vivo I, et al. Smoking and the risk of endometrial cancer: results from the Nurses' Health Study. *Int J cancer*. 2005;114(6):996-1001. doi:10.1002/IJC.20821

28. Herrington CS, Board WC of TE. *Female Genital Tumours*.; 2020. https://www.research.ed.ac.uk/en/publications/who-classification-of-tumours-female-genital-tumours. Accessed October 27, 2024.

29. Baak JPA, Mutter GL. EIN and WHO94. *J Clin Pathol*. 2005;58(1):1. doi:10.1136/JCP.2004.021071

30. Travaglino A, Raffone A, Saccone G, et al. Endometrial hyperplasia and the risk of coexistent cancer: WHO versus EIN criteria. *Histopathology*. 2019;74(5):676-687. doi:10.1111/HIS.13776

31. Mutter GL. Endometrial intraepithelial neoplasia (EIN): Will it

bring order to chaos? *Gynecol Oncol*. 2000;76(3):287-290. doi:10.1006/gyno.1999.5580

32. Bergeron C, Nogales FF, Masseroli M, et al. A multicentric European study testing the reproducibility of the WHO classification of endometrial hyperplasia with a proposal of a simplified working classification for biopsy and curettage specimens. *Am J Surg Pathol*. 1999;23(9):1102-1108. doi:10.1097/00000478-199909000-00014
33. The American College of Obstetricians and Gynecologists Committee Opinion no. 631. Endometrial intraepithelial neoplasia. *Obstet Gynecol*. 2015;125(5):1272-1278. doi:10.1097/01.AOG.0000465189.50026.20
34. Gallos ID, Yap J, Rajkhowa M, et al. Regression, relapse, and live birth rates with fertility-sparing therapy for endometrial cancer and atypical complex endometrial hyperplasia: a systematic review and metaanalysis. *Am J Obstet Gynecol*. 2012;207(4):266.e1-266.e12. doi:10.1016/J.AJOG.2012.08.011
35. Management of Endometrial Hyperplasia (Green-top Guideline No. 67) | RCOG. https://www.rcog.org.uk/guidance/browse-all-guidance/green-top-guidelines/management-of-endometrial-hyperplasia-green-top-guideline-no-67/. Accessed July 12, 2024.
36. Travaglino A, Raffone A, Saccone G, et al. Congruence between

1994 WHO classification of endometrial hyperplasia and endometrial intraepithelial neoplasia system. *Am J Clin Pathol*. 2020;153(1). doi:10.1093/ajcp/aqz132

37. Travaglino A, Raffone A, Saccone G, et al. Complexity of glandular architecture should be reconsidered in the classification and management of endometrial hyperplasia. *APMIS*. 2019;127(6):427-434. doi:10.1111/APM.12945

38. Travaglino A, Raffone A, Saccone G, et al. Significant risk of occult cancer in complex non-atypical endometrial hyperplasia. *Arch Gynecol Obstet*. 2019;300(5). doi:10.1007/s00404-019-05299-2

39. Piotto MASB, De Azevedo Focchi GR, Marques RM, et al. Assessment of Preoperative Endometrial Histopathological Sampling as a Predictor of Final Surgical Pathology in Endometrial Cancer. *Rev Bras Ginecol Obstet*. 2020;42(10):642-648. doi:10.1055/S-0040-1713802

40. Zhang C, Wang EY, Liu F, et al. Routine histologic features in complex atypical hyperplasia can predict the presence of endometrial carcinoma: a clinicopathological study of 222 cases. *Hum Pathol*. 2018;80:40-46. doi:10.1016/J.HUMPATH.2018.03.009

41. Rakha E, Wong SC, Soomro I, et al. Clinical outcome of atypical

endometrial hyperplasia diagnosed on an endometrial biopsy: institutional experience and review of literature. *Am J Surg Pathol*. 2012;36(11):1683-1690. doi:10.1097/PAS.0B013E31825DD4FF

42. D'Angelo E, Espinosa I, Cipriani V, et al. Atypical Endometrial Hyperplasia, Low-grade: "Much ADO About Nothing." *Am J Surg Pathol*. 2021;45(7):988-996. doi:10.1097/PAS.0000000000001705

43. McCluggage WG. My approach to the interpretation of endometrial biopsies and curettings. *J Clin Pathol*. 2006;59(8):801-812. doi:10.1136/JCP.2005.029702

44. Concin N, Matias-Guiu X, Vergote I, et al. ESGO/ESTRO/ESP guidelines for the management of patients with endometrial carcinoma. *Int J Gynecol Cancer*. 2021;31(1). doi:10.1136/ijgc-2020-002230

45. Okuda T, Sekizawa A, Purwosunu Y, et al. Genetics of endometrial cancers. *Obstet Gynecol Int*. 2010;2010(1). doi:10.1155/2010/984013

46. Getz G, Gabriel SB, Cibulskis K, et al. Integrated genomic characterization of endometrial carcinoma. *Nat 2013 4977447*. 2013;497(7447):67-73. doi:10.1038/nature12113

47. Raffone A, Travaglino A, Saccone G, et al. Loss of PTEN expression as diagnostic marker of endometrial precancer: A

systematic review and meta-analysis. *Acta Obstet Gynecol Scand*. 2019;98(3):275-286. doi:10.1111/AOGS.13513

48. Pavlakis K, Messini I, Vrekoussis T, et al. PTEN-loss and nuclear atypia of EIN in endometrial biopsies can predict the existence of a concurrent endometrial carcinoma. *Gynecol Oncol*. 2010;119(3):516-519. doi:10.1016/J.YGYNO.2010.08.023
49. Baak JPA, Van Diermen B, Steinbakk A, et al. Lack of PTEN expression in endometrial intraepithelial neoplasia is correlated with cancer progression. *Hum Pathol*. 2005;36(5):555-561. doi:10.1016/j.humpath.2005.02.018
50. Raffone A, Travaglino A, Saccone G, et al. PTEN expression in endometrial hyperplasia and risk of cancer: a systematic review and meta-analysis. *Arch Gynecol Obstet*. 2019;299(6):1511-1524. doi:10.1007/S00404-019-05123-X
51. Wu RC, Wang TL, Shih IM. The emerging roles of ARID1A in tumor suppression. *Cancer Biol Ther*. 2014;15(6):655. doi:10.4161/CBT.28411
52. Raffone A, Travaglino A, Saccone G, et al. Diagnostic and prognostic value of ARID1A in endometrial hyperplasia: a novel marker of occult cancer. *APMIS*. 2019;127(9):597-606. doi:10.1111/APM.12977

53. Torres M, Gómez-Pardo E, Dressler GR, et al. Pax-2 controls multiple steps of urogenital development. *Development*. 1995;121(12):4057-4065. doi:10.1242/DEV.121.12.4057

54. Robson EJD, He SJ, Eccles MR. A PANorama of PAX genes in cancer and development. *Nat Rev Cancer*. 2006;6(1):52-62. doi:10.1038/NRC1778

55. Wu H, Chen Y, Liang J, et al. Hypomethylation-linked activation of PAX2 mediates tamoxifen-stimulated endometrial carcinogenesis. *Nat 2005 4387070*. 2005;438(7070):981-987. doi:10.1038/nature04225

56. Raffone A, Travaglino A, Saccone G, et al. PAX2 in endometrial carcinogenesis and in differential diagnosis of endometrial hyperplasia: A systematic review and meta-analysis of diagnostic accuracy. *Acta Obstet Gynecol Scand*. 2019;98(3):287-299. doi:10.1111/AOGS.13512

57. Alwosaibai K, Abedini A, Al-Hujaily EM, et al. PAX2 maintains the differentiation of mouse oviductal epithelium and inhibits the transition to a stem cell-like state. *Oncotarget*. 2017;8(44):76881-76897. doi:10.18632/ONCOTARGET.20173

58. Modi DA, Tagare RD, Karthikeyan S, et al. PAX2 function, regulation and targeting in fallopian tube-derived high-grade serous

ovarian cancer. *Oncogene*. 2017;36(21):3015-3024. doi:10.1038/ONC.2016.455

59. Al-Hujaily EM, Tang Y, Yao DS, et al. Divergent Roles of PAX2 in the Etiology and Progression of Ovarian Cancer. *Cancer Prev Res (Phila)*. 2015;8(12):1163-1173. doi:10.1158/1940-6207.CAPR-15-0121-T

60. Vaskivuo TE, Stenbäck F, Tapanainen JS. Apoptosis and apoptosis-related factors Bcl-2, Bax, tumor necrosis factor-α, and NF-κB in human endometrial hyperplasia and carcinoma. *Cancer*. 2002;95(7):1463-1471. doi:10.1002/cncr.10876

61. Maia H, Maltez A, Athayde CE, et al. Detection of endometrial intraepithelial neoplasia (EIN) in postmenopausal endometrium. *Maturitas*. 2003;46(2):153-161. doi:10.1016/S0378-5122(03)00186-5

62. Travaglino A, Raffone A, Saccone G, et al. Loss of B-cell lymphoma 2 immunohistochemical expression in endometrial hyperplasia: A specific marker of precancer and novel indication for treatment: A systematic review and meta-analysis. *Acta Obstet Gynecol Scand*. 2018;97(12):1415-1426. doi:10.1111/AOGS.13452

63. Colombo N, Creutzberg C, Amant F, et al. ESMO-ESGO-ESTRO Consensus Conference on Endometrial Cancer: diagnosis, treatment

and follow-up. *Ann Oncol Off J Eur Soc Med Oncol*. 2016;27(1):16-41. doi:10.1093/ANNONC/MDV484

64. Berek JS, Matias-Guiu X, Creutzberg C, et al. FIGO staging of endometrial cancer: 2023. *Int J Gynecol Obstet*. 2023;162(2):383-394. doi:10.1002/IJGO.14923

65. Smith RA, Cokkinides V, Brawley OW. Cancer screening in the United States, 2009: a review of current American Cancer Society guidelines and issues in cancer screening. *CA Cancer J Clin*. 2009;59(1):27-41. doi:10.3322/CAAC.20008

66. Fraser IS, Critchley HOD, Munro MG, et al. A process designed to lead to international agreement on terminologies and definitions used to describe abnormalities of menstrual bleeding. *Fertil Steril*. 2007;87(3):466-476. doi:10.1016/J.FERTNSTERT.2007.01.023

67. Savelli L, Ceccarini M, Ludovisi M, et al. Preoperative local staging of endometrial cancer: transvaginal sonography vs. magnetic resonance imaging. *Ultrasound Obstet Gynecol*. 2008;31(5):560-566. doi:10.1002/UOG.5295

68. Epstein E, Van Holsbeke C, Mascilini F, et al. Gray-scale and color Doppler ultrasound characteristics of endometrial cancer in relation to stage, grade and tumor size. *Ultrasound Obstet Gynecol*. 2011;38(5):586-593. doi:10.1002/UOG.9038

69. Garuti G, Angioni S, Mereu L, et al. Hysteroscopic view with targeted biopsy in the assessment of endometrial carcinoma. What is the rate of underestimatated diagnosis? The results of a multicenter Italian trial. *Gynecol Surg*. 2020;17(1):1-7. doi:10.1186/S10397-020-01077-0/TABLES/5

70. Horn LC, Schnurrbusch U, Bilek K, et al. Risk of progression in complex and atypical endometrial hyperplasia: clinicopathologic analysis in cases with and without progestogen treatment. *Int J Gynecol Cancer*. 2004;14(2):348-353. doi:10.1111/J.1048-891X.2004.014220.X

71. Querleu D, Cibula D, Abu-Rustum NR. 2017 Update on the Querleu–Morrow Classification of Radical Hysterectomy. *Ann Surg Oncol*. 2017;24(11):3406. doi:10.1245/S10434-017-6031-Z

72. Janda M, Gebski V, Davies LC, et al. Effect of Total Laparoscopic Hysterectomy vs Total Abdominal Hysterectomy on Disease-Free Survival Among Women With Stage I Endometrial Cancer: A Randomized Clinical Trial. *JAMA*. 2017;317(12):1224-1233. doi:10.1001/JAMA.2017.2068

73. Walker JL, Piedmonte MR, Spirtos NM, et al. Recurrence and survival after random assignment to laparoscopy versus laparotomy for comprehensive surgical staging of uterine cancer: Gynecologic

Oncology Group LAP2 Study. *J Clin Oncol*. 2012;30(7):695-700. doi:10.1200/JCO.2011.38.8645

74. Haltia UM, Bützow R, Leminen A, et al. FIGO 1988 versus 2009 staging for endometrial carcinoma: a comparative study on prediction of survival and stage distribution according to histologic subtype. *J Gynecol Oncol*. 2014;25(1):30-35. doi:10.3802/JGO.2014.25.1.30

75. Costales AB, Schmeler KM, Broaddus R, et al. Clinically significant endometrial cancer risk following a diagnosis of complex atypical hyperplasia. *Gynecol Oncol*. 2014;135(3):451-454. doi:10.1016/J.YGYNO.2014.10.008

76. Rossi EC, Kowalski LD, Scalici J, et al. A comparison of sentinel lymph node biopsy to lymphadenectomy for endometrial cancer staging (FIRES trial): a multicentre, prospective, cohort study. *Lancet Oncol*. 2017;18(3):384-392. doi:10.1016/S1470-2045(17)30068-2

77. Kogan L, Matanes E, Wissing M, et al. The added value of sentinel node mapping in endometrial cancer. *Gynecol Oncol*. 2020;158(1):84-91. doi:10.1016/J.YGYNO.2020.04.687

78. Matanes E, Amajoud Z, Kogan L, et al. Is sentinel lymph node assessment useful in patients with a preoperative diagnosis of

endometrial intraepithelial neoplasia? *Gynecol Oncol*. 2023;168:107-113. doi:10.1016/J.YGYNO.2022.10.023

79. Herrera Cappelletti E, Humann J, Torrejón R, et al. Chances of pregnancy and live birth among women undergoing conservative management of early-stage endometrial cancer: a systematic review and meta-analysis. *Hum Reprod Update*. 2022;28(2):282-295. doi:10.1093/HUMUPD/DMAB041

80. Rodolakis A, Scambia G, Planchamp F, et al. ESGO/ESHRE/ESGE Guidelines for the fertility-sparing treatment of patients with endometrial carcinoma. *Int J Gynecol Cancer*. 2023;33(2):208-222. doi:10.1136/IJGC-2022-004047

81. Casadio P, La Rosa M, Alletto A, et al. Fertility Sparing Treatment of Endometrial Cancer with and without Initial Infiltration of Myometrium: A Single Center Experience. *Cancers (Basel)*. 2020;12(12):1-9. doi:10.3390/CANCERS12123571

82. Park JY, Lee SH, Seong SJ, et al. Progestin re-treatment in patients with recurrent endometrial adenocarcinoma after successful fertility-sparing management using progestin. *Gynecol Oncol*. 2013;129(1):7-11. doi:10.1016/J.YGYNO.2012.12.037

83. Travaglino A, Raffone A, Saccone G, et al. Immunohistochemical predictive markers of response to conservative treatment of

endometrial hyperplasia and early endometrial cancer: A systematic review. *Acta Obstet Gynecol Scand*. 2019;98(9):1086-1099. doi:10.1111/AOGS.13587

84. von Elm E, Altman DG, Egger M, et al. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. *J Clin Epidemiol*. 2008;61(4):344-349. doi:10.1016/J.JCLINEPI.2007.11.008

85. Moore L, Leongamornlert D, Coorens THH, et al. The mutational landscape of normal human endometrial epithelium. *Nature*. 2020;580(7805):640-646. doi:10.1038/S41586-020-2214-Z

86. Rabban JT, Blake Gilks C, Malpica A, et al. Issues in the Differential Diagnosis of Uterine Low-grade Endometrioid Carcinoma, Including Mixed Endometrial Carcinomas: Recommendations from the International Society of Gynecological Pathologists. *Int J Gynecol Pathol*. 2019;38 Suppl 1(Iss 1 Suppl 1):S25-S39. doi:10.1097/PGP.0000000000000512

87. Huang EC, Mutter GL, Crum CP, et al. Clinical outcome in diagnostically ambiguous foci of “gland crowding” in the endometrium. *Mod Pathol*. 2010;23(11):1486-1491. doi:10.1038/MODPATHOL.2010.140

88. Fabbri R, Vicenti R, Magnani V, et al. Ovarian tissue cryopreservation and transplantation: 20 years experience in Bologna University. *Front Endocrinol (Lausanne)*. 2022;13. doi:10.3389/FENDO.2022.1035109