MANAGEMENT & PROGNOSIS OF ENDOMETRIAL HYPERPLASIA

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Synopsis

This thesis investigates the management and prognosis of endometrial hyperplasia. The literature on conservative therapies for endometrial hyperplasia is systematically reviewed and a meta-analysis is performed to identify the most effective treatment. Further meta-analysis is performed for young women with severe endometrial hyperplasia or cancer to explore the effectiveness of fertility-sparing treatment. A national survey of Gynaecologists is performed to evaluate current and the need for further research. A large cohort study is included that defines the regression and relapse of endometrial hyperplasia with two popular conservative therapies, the Levonorgestrel-releasing intrauterine system (LNG-IUS) and oral progestogens. The LNG-IUS is found to induce regression more often with fewer events of relapse than oral progestogens. A prediction model based on clinical characteristics and biomarkers finds that morbid obesity is an independent predictor for relapse. This research has major implications for clinical practice and a national guideline in process is based on its findings.

Dedication

I dedicate this thesis to my future wife Yasmine for helping me to pursue my research and to my parents, Antonia and Daniil, for teaching me that anything is possible.

Acknowledgments

This work was undertaken while I was MPhil student between August 2008 and August 2009. The remaining work was undertaken while working as a full time specialist trainee. I would like to thank my supervisors, Janesh Gupta, Professor of Obstetrics & Gynaecology, and Raji Ganesan, Consultant Histopathologist, for their expert advice and their patience while completing this work. I am indebted to my friend Arri Coomarasamy, Professor of Gynaecology, for mentoring beyond contracted hours. His help has been instrumental to the completion of this thesis. A special thanks to Wilma Arnold, medical secretary, for her invaluable help with our study and administrative support. A great thank you to my friends and collaborators who contributed to the chapters of this thesis and include Miss Manjeet Shehmar, Dr Thalis Papapostolou, Preeti Krishan, Dr Jason Yap, Professor David Luesley, Dr Madhurima Rajhkowa, Dr Emelie Akesson and Mr Rajesh Varma.

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List of abbreviations

EH Endometrial hyperplasia

EC Endometrial cancer

HRT Hormone replacement therapy

WHO World Health Organisation

SH Simple hyperplasia

CH Complex hyperplasia

SAH Simple atypical hyperplasia

ACH Atypical complex hyperplasia

LNG-IUS Levonorgestrel-releasing intrauterine system

BMI Body Mass Index

CI Confidence interval

RCOG Royal College of Obstetricians and Gynaecologists

IHC Immunohistochemistry

LR Likelihood ratio

COX-2 Cyclooxygenase 2

PTEN Phosphatase and tensin homolog

PGE2 Prostaglandin E2

IQR Interquartile range

SD Standard deviation

Publications from this thesis

Chapter 1.

Varma R, Gallos I, Gupta JK. Endometrial hyperplasia. The Obstetrician and Gynaecologist 2009; 11:77-78.

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Gallos ID, Yap J, Rajhkowa M, Coomarasamy A, Luesley DM, Gupta JK. 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;204:266.e1-266.e12

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Chapter 9.

Gallos ID, Ganesan R, Gupta JK. ER, PR, COX-2, MLH1, and BCL-2 expression predicting regression and relapse of endometrial hyperplasia treated with LNG-IUS (Mirena®): a cohort study. Gynecol. Oncol. 2013;130:58-63.

Chapter 10. A clinical guideline draft has been submitted for review by the Royal College of Obstetricians & Gynaecologists.

Chapter 1: Introduction

Introduction

Endometrial hyperplasia (EH) is the precursor of endometrial carcinoma, which is the commonest gynaecological malignancy in the western world.[1] In 2007 in England and Wales, 7,536 new cases of endometrial cancer (EC) were registered and, although, the incidence of EC is high, the incidence of EH is three times higher and it can progress to cancer if left untreated.[2,3] EH is believed to produce a continuum of lesions that may be precursors to EC of endometrioid histology and require treatment for preventing progression. The EC is thought to be oestrogen-dependant and arise in a background of EH.[4] It arises usually in peri-menopausal or menopausal women when oestrogen, unopposed by progesterone, stimulates endometrial cell growth by binding to oestrogen receptors in the nuclei of endometrial cells. The risk factors for EH are the same as those for EC and include obesity, nulliparity, early menarche, late menopause, anovulatory cycles, Tamoxifen or hormone replacement therapy (HRT) use. There is especially strong evidence that the use oestrogen-only HRT and obesity increase the risk for women developing EH.[5,6] The most appropriate management of EH has been among the most controversial areas in gynaecology since it was known that while EH is not malignant, but a precursor of invasive cancer.[4]

Classification of endometrial hyperplasia

The World Health Organisation (WHO) categorises EH as simple (SH), complex (CH), simple atypical (SAH), or atypical complex (ACH) on the basis of architectural crowding and nuclear atypia.[7] However, in contrast to SAH, most cases of atypical hyperplasia by definition, are characterised by a complex glandular architecture, glandular crowding,

epithelial cells showing the cytological hallmarks of malignancy and lack of endometrial stromal. In a large prospective study, no case of SAH has been found.[7] In accordance with others, we assume that this category, if it does exist, is extremely rare and its existence is disputed.[8,9]

As a result, the WHO classification can be confusing and a simpler classification of SH, CH and ACH has been proposed.[10] SH is often considered a variation of normal endometrium and its risk of progression to cancer is comparable to the normal population (less than 1%).[4] CH has an intermediate risk of progression to cancer (about 3%) and can be treated with hormone therapies.[4] ACH has a high risk of progression to cancer (up to 29%) and the possibility of concomitant cancer (up to 43%) in women undergoing hysterectomy.[4;11]

For the purposes of this thesis we shall classify endometrial hyperplasia as follows:

1. Simple hyperplasia (SH)
2. Complex Hyperplasia
    a) Without Atypia (CH)
    b) With Atypia (ACH)

Molecular pathology

Endometrial hyperplasia is considered to be an oestrogen-dependent benign disease of the endometrium with malignant potential.[4] The key step to this transformation to the majority of the cases appears to be the local oestrogen production from androgens catalysed by the

aromatase enzyme.[12-14] In fact, the aromatase enzyme is detectable in the majority of cases of endometrioid cancer, but not in endometrial hyperplasia.[12,15,16]

PGE2 increases intracellular aromatase activity and stimulates oestrogen biosynthesis, and there is a strong linear association between aromatase and expression of cyclo-oxygenases in uterine and breast cancer specimens, resulting in a complex paracrine and/or autocrine signalling pathway effecting abnormal oestrogen synthesis.[13,17,18] Cyclo-oxygenase (COX) is the rate-limiting enzyme in the prostaglandin biosynthetic pathway that stimulates oestrogen biosynthesis and higher COX-2 expression has been reported in hyperplastic or malignant endometrium than in normal.[12,16,17,19] COX-2 is significantly associated with aromatase expression in endometrial cancer,[12] which suggests that intra-endometrial oestrogen production promotes progression of endometrial hyperplasia to cancer. Recently, the use of aromatase inhibitors has been advocated for endometrial hyperplasia and cancer[20-22] and the beneficial potential of COX-2 inhibitors has been widely described,[23,24] but not applied in clinical practice. In conclusion, the assessment of aromatase/COX-2 activity and steroid receptor status is potentially a key marker for targeted hormonal treatment of endometrial lesions when diagnosed early during cancerogenesis.

The abnormalities in the oestrogen pathway are not the only causative features for endometrial hyperplasia and its malignant potential. The angiogenesis, inhibition of apoptosis and DNA mismatch-repair mechanism or activation of oncogenes are the pathways most commonly described to be involved in endometrial hyperplasia. For example, cyclo-oxygenase plays a major role in endothelial cell migration and is implicated in the production

of pro-angiogenic factors, such as Vascular Endothelial Growth Factor (VEGF) and consequent promotion of endometrial angiogenesis.[19] VEGF production is found to be stimulated by oestrogen concentration and is strongly correlated with the microvessel count.[19,25] It has been shown that the altered expression of proteins i.e. bcl-2, PTEN may play an important role by affecting apoptosis of hyperplastic cells.[26,27] The abnormal methylation of MLH1 is the commonest event in endometrial hyperplasia that generates microsatellite instability (MSI) due to defects of the DNA mismatch-repair mechanism.[25] Oestrogens may increase the rate of mutagenesis of MLH1 through free radical formation as well as its inherent proliferative influence.[25] The combination of these pathways seems to orchestrate the progression of endometrial hyperplasia to cancer with oestrogens to mastermind the process. The expression analysis of the above biomarkers currently helps understand the pathogenesis of endometrial hyperplasia and the pathways involved during this process. However, their utility as predictors for response to progestogen treatment has not been extensively studied yet.

Management of EH

Management of SH

On the management of SH the literature is scarce. The study by Kurman et al is often cited and is unique in the literature because it has followed up 93 women with SH for more than 26 years that did not have any treatment.[4] Only one woman, less than 30 years old, progressed to EC. Interestingly, 81% of the women regressed to normal with no treatment. Hence, the German Working Group of Gynecologic Oncology suggests that this condition can also be managed expectantly. Taking into account that EC lifetime risk is about 3% and this

condition affect women over 50 years of age, it appears that their risk is not much higher than that of the normal population.[28] However, clinicians may opt to treat this condition for symptomatic relief of abnormal uterine bleeding. This is the main reason in Chapter 2 we summarise the literature and also compare the efficacy of available therapies for the different types of hyperplasia.

Management of CH

CH has a low risk of progression to EC, which can take up to 10 years.[4] An evaluation of current practice in treating CH found that most women are managed with hysterectomy.[29] Despite this, medical treatment with progestogens is shown to induce regression in a significant proportion of these women.[29] Traditionally, oral progestogens have been used to treat this condition for inducing regression of CH and reduce the risk of progression to cancer up to 3-fold.[30] Recently, the LNG-IUS has also been used for this purpose.[31] In a previous study the difference in efficacy between LNG-IUS and oral progestogens has been investigated, but the outcomes were not reported using the widely accepted WHO criteria and therefore were difficult to interpret.[32] The progestogen concentrations in the uterine mucosa when delivered through an intrauterine device, directly into the cavity are reported to exceed that of the oral treatment by several-fold.[33] The intrauterine progestogen release is also associated with higher patient satisfaction and, therefore patients are more likely to continue the treatment. This higher chance of patients continuing the LNG-IUS treatment resulting in higher compliance may also explain its better efficacy in treating endometrial hyperplasia compared with oral progestogens.

Management of ACH

Women with ACH are at high risk of progressing to cancer or already have underlying cancer while undergoing hysterectomy.[4;11] In the majority of the cases women are advised to undergo hysterectomy because of the malignancy risk.[29] However, young women with strong fertility desires and women with multiple comorbidities may not be good surgical candidates. There have been some reports of successful therapy with progestogens of ACH and even well-differentiated EC.[34,35] A small percentage of women successfully got pregnant and achieved live births during follow up either with spontaneous conception or with assisted reproductive techniques.[34] However, the feasibility of this treatment option has not been thoroughly investigated and its safety remains a concern.

Prognosis of endometrial hyperplasia

In an important paper, Ferenczy et al reported on 85 women with endometrial hyperplasia who were treated with oral progestogens.[36] The patients who had no evidence of cytological atypia achieved a higher rate of endometrial regression compared to the patients with cellular atypia (86% vs 50%) and recurrence of hyperplasia was less frequent (6% vs 50%).[36] The likelihood of response to hormonal therapy was directly related to the absence of cytological atypia and this is the only marker that is currently used for predicting progestogen response. Patient clinical characteristics such as age, body mass index (BMI), diabetes, hypertension and menopausal status are found to be associated with endometrial hyperplasia. However, currently no studies have evaluated their impact on predicting therapeutic success following progestogen treatment. While one might anticipate that response to progestogens would be predicted by the steroid receptor status of the hyperplastic endometrium this is not proven.

Both oestrogen (ER) and progesterone (PR) receptors are present in high levels in hyperplastic endometria but studies have repeatedly failed to show a relationship between receptor status and response to progestogens.[37-39] This has biological plausibility but many research groups have tried to correlate these biomarkers with outcome in hyperplasia when treated with oral progestogens.[37-39] This is problematic because compliance is an issue with oral progestogens and introduces significant unmeasured confounding. The difficulty in measuring this parameter means that inferences cannot be adjusted for this essential parameter. Other molecular pathways have also been investigated with some recording promising results.[40] These are discussed more in detail in Chapters 7 and 9. The fact is that the accurate stratification of risk will help clinicians follow up adequately these patients at risk of progressive/persistent disease and reassure those with low risk. Consequently, we believe this will reduce unnecessary surgical interventions and NHS costs.

Aims & Objectives of this thesis

My thesis aims to investigate and improve the management and prognosis of women with EH through the following eight objectives:

1. To evaluate the regression rate with oral progestogens and LNG-IUS for women with EH, compare these two main medical therapies and identify the most effective treatment option in the published literature.
2. To evaluate the regression, relapse, and live birth rates of early-stage EC and ACH with fertility-sparing treatment in the published literature.
3. To determine current practice for the management of endometrial hyperplasia through a national survey.

4. To compare the regression rate of the LNG-IUS versus oral progestogens for the treatment of women with EH in a cohort study.

5. To determine the risk of relapse for women with EH treated with LNG-IUS or oral progestogens in a cohort study.

6. To explore the prognostic ability of ER and PR, phosphatase and tensin homolog (PTEN) and aromatase to predict persistent EH when treated with LNG-IUS in a case-control study.

7. To identify clinical predictors for regression and relapse of EH treated with LNG-IUS or oral progestogens.

8. To test the predictive ability of ER, PR, COX-2, Mlh1, and Bcl-2 expression for predicting the outcomes of regression and relapse in women with EH treated with LNG-IUS.

SECTION I MANAGEMENT OF ENDOMETRIAL HYPERPLASIA

Chapter 2: Oral progestogens versus LNG-IUS for EH: a systematic review and meta-analysis.

Abstract

Objective

To conduct a systematic review and meta-analysis of studies evaluating the regression rate of EH with oral progestogens and LNG-IUS.

Methods

Searches were conducted on Medline, Embase, Cochrane Library, and Web of Science, and reference lists of relevant articles were examined. The methodologic index for non-randomised studies was used for quality assessment. Meta-analysis was performed with random effects model.

Results

There were 24 observational studies (1001 women), of low methodologic quality, evaluating the outcome of regression of EH with oral progestogens or LNG-IUS. Meta-analysis showed that oral progestogens achieved a lower pooled regression rate compared with LNG-IUS for CH (pooled rate, 66% vs 92%; *P*=0.01) and ACH (pooled rate, 69% vs 90%; p=0.03). There was no statistical difference in SH (pooled rate, 89% vs 96%; p=0.41).

Conclusion

Oral progestogens appear to induce a lower disease regression rate than LNG-IUS in the treatment of EH.

Introduction

EH is a common diagnosis (5-10%) in women presenting with abnormal uterine bleeding, and can progress to cancer if left untreated.[4] The risk of progression of endometrial hyperplasia to cancer is dependent on the histological diagnosis. The risk of cancer progression is low for women with CH compared to women with ACH.[4] Currently, there are no professional body guidelines for the management of EH. The use of progestogens, which antagonise the oestrogen effect on the endometrium, can induce endometrial regression and prevent progression to cancer.[4] The main oral progestogens used to treat EH are Norethisterone Acetate, Megestrol Acetate and Medroxyprogesterone 17-Acetate. More recently, the LNG-IUS developed primarily as a contraceptive device, has also been used successfully to treat EH.[31] These strategies, if successful could reduce the number of hysterectomies performed for this condition and hence reduce morbidity and healthcare costs. Against this background, we conducted a systematic review of studies evaluating oral and intrauterine progestogens for the treatment of EH and meta-analysed their treatment effects.

Methods

Identification of literature

The population of interest in this systematic review was women with EH, the intervention was treatment with oral progestogens, the comparison was LNG-IUS and the outcome was evidence of disease regression or persistence. The following electronic databases were searched: MEDLINE (1950 to December 2009), EMBASE (1980 to December 2009), Cochrane Central Register of Controlled Trials and Web of Science conference proceedings (ISI Proceedings, 1990 to December 2009). A combination of Medical Subject Headings

(MeSH) and text words were used to generate two subsets of citations, one including studies of EH ("endometr* hyperplas*", "premalignant endometr*", "precancer* endometr*") and the other including studies of progestogens and intrauterine devices or systems ("intrauterine devices medicated", "Levonorgestrel", "mirena", "intrauterine progest*", "LNG-IU*", "progest*", "gestag*"). These subsets were combined with "AND" and limited to "Humans and Female" to generate a subset of citations relevant to our research question. The reference lists of all known primary and review articles were examined to identify cited articles not captured by electronic searches. Language or geographical restrictions were not applied during search or selection. The searches were conducted independently by two reviewers.

Study selection and data extraction

Studies were selected if the participants were women diagnosed histologically with EH, the intervention was treatment with either oral progestogens or LNG-IUS and the outcome was histological disease regression rates, as assessed on endometrial biopsy or hysterectomy specimen. Both controlled and uncontrolled designs were included. Case reports or series with less than five cases were excluded. Studies reporting on women with EH treated with other form of progestogens than oral or LNG-IUS (e.g. injectable, pessaries) were excluded. Studies classifying women with EH in other than the WHO classification[7] were also excluded.

Studies were selected in a two-stage process. First, the titles and abstracts from the electronic searches were scrutinised by two reviewers independently and full manuscripts of all citations that met the predefined selection criteria were obtained. Secondly, final inclusion or

exclusion decisions were made on examination of the full manuscripts. In cases of duplicates, the most recent or the most complete publication was used. Any disagreements about inclusion were resolved by consensus or arbitration by a third reviewer.

Two reviewers completed the quality assessment. The Methodological Index for Non-Randomised Studies (MINORS), which assesses the quality of the included studies, was implemented.[41] Items assessed included selection of cases or cohorts and controls, comparability and information on exposure and outcome. This index was preferred over Newcastle-Ottawa Quality Assessment Scale[42] as we included studies without a control group and the MINORS checklist allows a quality evaluation in studies with and without a control group. From each study, outcome data were extracted in 2x2 tables by the two reviewers. No ethical approval was sought for this study as it was a systematic review and meta-analysis of published manuscripts.

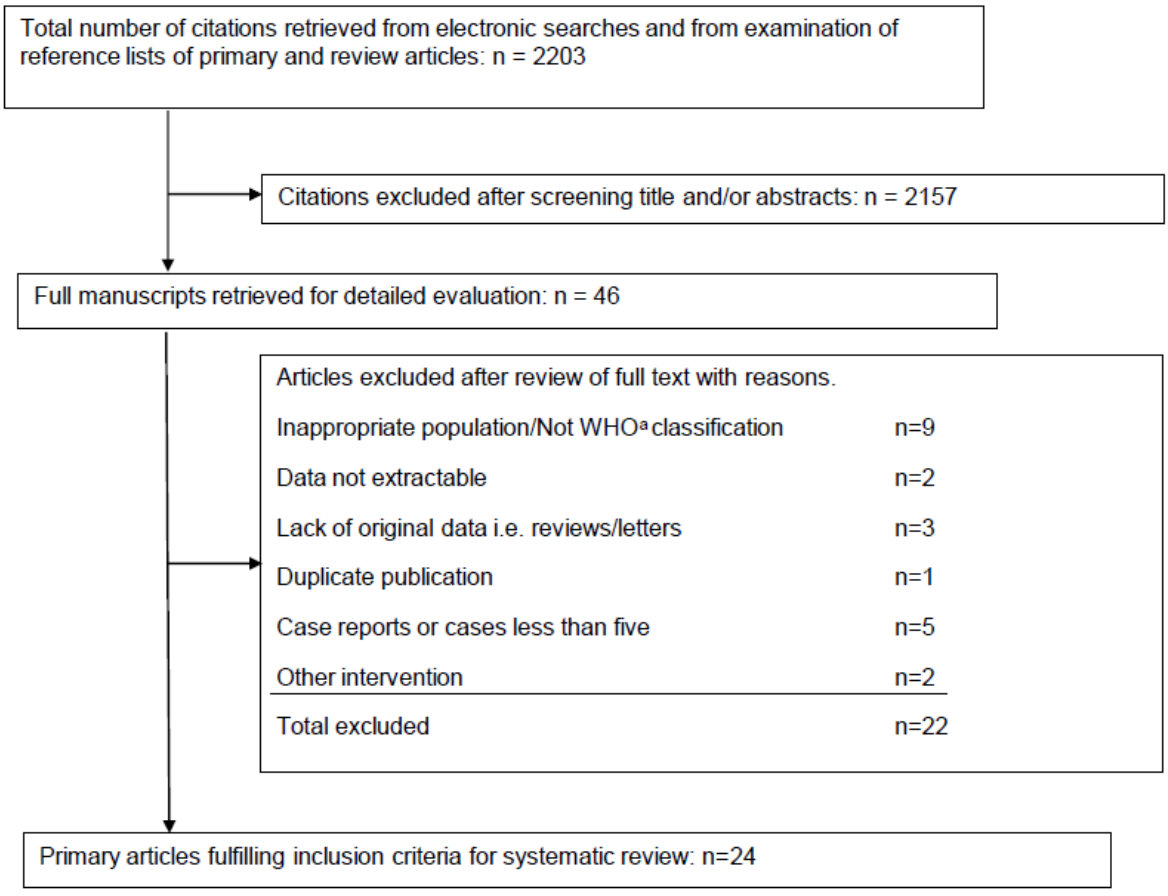
Statistical analysis

Regression rates from individual studies were meta-analysed using random effects model.[43] Heterogeneity of the exposure effects was statistically analysed using the chi-squared test.[44] Exploration of the causes of heterogeneity was planned using variation in features of population, exposure and study quality.[45] The regression rates between the two interventions were compared with the aid of meta-regression. Statistical analyses were performed using Stata 8.0 (Stata Corp, TX, USA).

Results

The search strategy yielded 2203 citations all captured from electronic citations. Of these, 2157 were excluded as it was clear from the title and abstract that they did not fulfil the selection criteria. Examination of the full manuscripts of the remaining 46 articles found that three studies lacked original data (e.g. reviews or letters), one study was a duplicate and 18 studies did not meet the selection criteria. Thus a total of 24 primary studies, including 1001 women with EH were selected for this review[18-31;34;46-64] (Figure 1).

Figure 1 Study selection process for systematic review of oral and intrauterine progestogens for the treatment of EH



The longest follow-up period was eight years. Fifteen studies were case series and nine were controlled studies. The main characteristics of the 24 studies and the MINORS Index are

presented in Table 1. Although all studies included women with either oral progestogens or LNG-IUS, the type, dose, regimen and duration of treatment varied. The type of EH (SH, CH or ACH) treated also varied between the different studies. Most studies were judged to be of poor quality on the MINORS index (Figure 2), with particular low scores for prospective calculation of the study size, prospective recruitment and biased assessment of regression rates.

Figure 2 Quality checklist

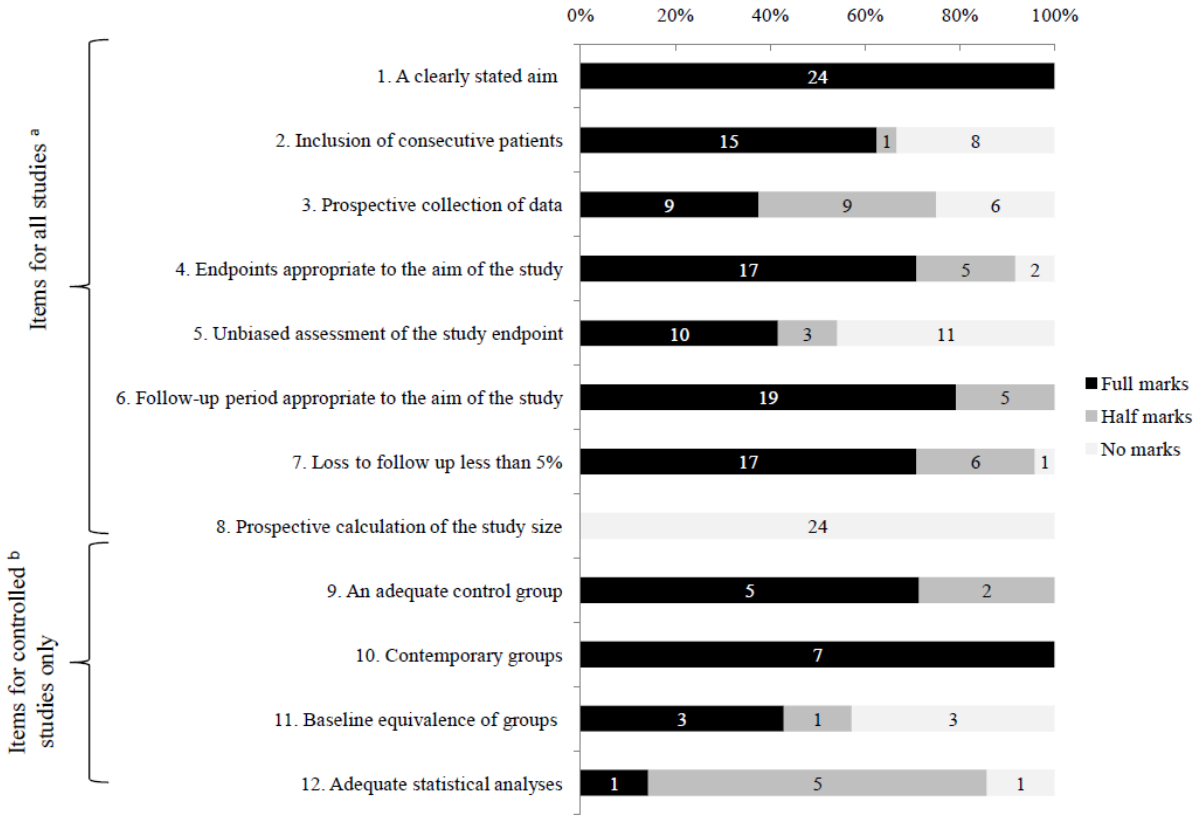


Table 1 Characteristics of the studies included in the systematic review of oral and intrauterine progestogens for the treatment of EH.

Author -Year	Type of study	Study population	Intervention or study groups	Outcome and follow up
Bese-2006 (n=37)	Matched controlled study	Simple hyperplasia (n = 19) and matched controls without hyperplasia (n=18)	Norethisterone 15mg/day for 10 days between the 16th and 25th day for 3 months	Outcome: Histological response at 3 months, proliferative and apoptotic activity Follow up: 3 months
Buttini-2009 (n=57)	Retrospective comparative study	Women with simple (n=33), complex (n=8) or atypical hyperplasia (n=16)	Oral progestogens, usually Medroxyprogesterone 10-20mg/day of unreported duration and regimen (n=10), LNG-IUS[a] followed by hysterectomy (n=7), LNG-IUS[a] alone (n=19) and hysterectomy alone (n=21)	Outcome: Histological response at 6 months and menstrual function Follow up: Variable, range 6-69 months
Clark-2006 (n=281)	Retrospective comparative study	Women with simple (n=55), complex (n=173) or atypical hyperplasia (n=53) **Excluded:** Women with incidental finding of hyperplasia diagnoses on hysterectomy specimens	Oral progestogens of unreported type, dose, duration and regimen (n=77), LNG-IUS[a] (n=29), HRT[b] (n=2), other medical (n=2), endometrial ablation (n=2), hysterectomy (n=109), observation only (n=60)	Outcome: Histological and clinical response Follow up: Mean of 36 months, range 24-48
Guven-2001 (n=27)	Case series study	Women with simple (n=16), complex (n=5) or atypical hyperplasia (n=3)	Megestrol 160-320mg/day for 3 months (n=22), 45 days (n=2), or 60 days (n=3)	Outcome: Histological response every 3-6 months Follow up: Not reported
Haimovich-2008 (n=15)	Prospective case series study	Women with simple hyperplasia (n=15) **Excluded:** Women with uterine hypertrophy or sub-mucosal myomas	LNG-IUS[a] for 24 months	Outcome: Histological response and bleeding pattern at 3, 6, 12 and 24 months Follow up: 24 months

Horn-2004 (n=502)	Retrospective controlled study	Women with complex (n=208) or atypical hyperplasia (n=7) that received progestogens and women treated without progestogens (n=287) **Excluded:** Women with no clinical data regarding follow up, women re-classified into simple hyperplasia after histological re-examination and women with synchronous cancer	Medroxyprogesterone or Norethisterone for 3-5 months. Norethisterone 5mg/day for pre-menopausal women, Medroxyprogesterone 10mg/day for perimenopausal women and 20-50mg/day for postmenopausal women (n=215)	Outcome: Histological response at a median of 4.8 months, range 3-22 Follow up: Not reported
Jarvela-2005 (n=34)	Oral progestogen arm of a randomised controlled trial	Women with simple (n=16) or complex hyperplasia (n=1) that received progestogens and women treated with thermal balloon endometrial ablation (n=17) **Excluded:** Women with previous progestogen use, signs of atypical hyperplasia, pregnancy, desire for fertility, fibroids>3cm or distorting the uterine cavity, genital infections, malignancy or previous endometrial ablation	Group 1) For pre-menopausal women, Medroxyprogesterone 10mg/day from day 15 to 24 for 3 months and for post-menopausal women Medroxyprogesterone, 10mg/day for 3 months (n=17) Group 2) Endometrial ablation (n=17)	Outcome: Histological response, clinical and ultrasound examination at 6 and 12 months Follow up: Not reported

Jobo-2001 (n=53)	Retrospective comparative study	Women with complex aypical hyperplasia (n=53) **Excluded:** Women that refused treatment and follow up	Group 1) Medroxyprogesterone acetate 10mg for 14 days/cycle for 6 months (n=8), Group 2) Medroxyprogesterone acetate 400mg/day for 6 months (n=12) hysterectomy (n=30) and observation only (n=2)	Outcome: Histological response at 10.8 weeks (4-30) for group 1 and 7.3 (3-15) weeks for group 2 Follow up: Mean of 66 months, range 8-281
Kaku-2001 (n=30)	Retrospective case series	Women with atypical hyperplasia (n=18) or endometrial carcinoma (n=12) wishing to preserve their fertility **Excluded:** Women were excluded if found not to have atypical hyperplasia or endometrial carcinoma after pathological review, women were excluded if follow up specimens were not available	Medroxyprogesterone 100-600mg/day for 1-23 months for endometrial hyperplasia and 200-800mg/day for 2-14 months for endometrial cancer	Outcome: Histological response and pregnancy rates every 1-4 months Follow up: Median of 31.5 months, range 10-133
Milam-2008 (n=38)	Retrospective case series study	Women with matched pre-progesterone and post-progesterone treated pairs of endometrial biopsies with endometrial hyperplasia (n=38) **Excluded:** Women with disagreement of diagnosis of endometrial hyperplasia after histological re-evaluation and when there was limited material for immunohistochemical evaluation	Medroxyprogesterone, Megestrol or Norethisterone for a median of 3 months (1-12 months) of unreported dose and regimen	Outcome: Histological and immunohistochemical response Follow up: Not reported

Minagu chi-2007 (n=31)	Case series study	Women with atypical complex hyperplasia (n=12) or Stage IaG1 carcinoma (n=19) who wished to preserve fertility or could not receive surgery due to complications **Excluded:** Women over the age of 40 and those who did not attempt to conceive	Medroxyprogesterone 2.5mg-600mg/day for 3-18 months, mostly 400-600mg/day for 6 months	Outcome: Histological and immunohistochemical response every 2-4 months , pregnancy and hysterectomy rates Follow up: Median of 40.7 months, range 2-109
Randall -1997 (n=67)	Retrospective case series study	Women under age of 40 with atypical hyperplasia (n=32) or well-differentiated carcinoma (n=35) **Excluded:** Women that declined treatment and any follow up and women that declined treatment and endometrial sampling	Oral progestogens Medroxyprogesterone 10-30mg/day for 3-12 months or Megestrol 40-160mg/day for 3-12 months (n=29), ovulation induction (n=2), Bromocriptine (n=1), oral contraceptive (n=1), hysterectomy (n=27)	Outcome: Histological response at 3-6 months, pregnancy and hysterectomy rates Follow up: Mean of 40 months, range 9-79
Rattana chaiyan ont-2005 (n=134)	Prospective case series study	Women with simple (n=116) or complex (n=18) hyperplasia that completed a cycle of progestogens **Excluded:** Women not having progestogen therapy , not having data on endometrial histology, loss to follow up, pregnancy, amenorrhea	Mainly cyclic Medroxyprogesterone 10mg/day and Norethisterone 10mg/day for 12-14 consecutive days per month for 6 months	Outcome: Histological response at 4, 16 and 24 weeks, vaginal bleeding pattern and associated pelvic pathology Follow up: Not reported

Reed-2009 (n=185)	Retrospective case series study	Women older than 18 with complex (n=115) or atypical (n=70) hyperplasia after central pathology review and with an additional pathological specimen 8 weeks to 6 months after index diagnosis **Excluded:** Women with follow-up specimen not available or not diagnostic, dispensed more than 14 days of oestrogen and less than 14 days of progestogen dispensed	Medroxyprogesterone (n=66), Megestrol (n=61) or Norethisterone (n=11) at different doses for 14 days up to 6 months and observation only (n=38)	Outcome: Histological response at 8 weeks up to 6 months Follow up: Mean of 16.4 weeks, range 8-26
Signorelli-2009 (n=21)	Prospective case series study	Women under the age of 40 with atypical hyperplasia (n=10) or endometrial cancer (n=11) wishing fertility potential	Cyclical natural progesterone 200 mg daily from day 14 to day 25 (n=21)	Outcome: Histological response and pregnancy rate every 3 months Follow up: Median of 98 months, range 35-176
Tjalma-2004 (n=8)	Case series study	Women with atypical hyperplasia (n=7) and with endometrial cancer (n=1)	LNG-IUS[a]	Outcome: Histological and immunohistochemical response at 3-6 months Follow up: Mean of 29 months, range 11-51
Varma-2008 (n=105)	Prospective cohort study	Women with simple (n=16), complex (n=80) and atypical hyperplasia (n=9)	LNG-IUS[a]	Outcome: Histological response, hysterectomy and cancer rates at 3, 6, 12, 18, 24 months Follow up: Not reported

Vereide-2006 (n=50)	Prospective cohort study	Women with simple (n=26), complex (n=11) and atypical (n=13) hyperplasia	LNG-IUS[a] (n=21) and oral Medroxyprogesterone 10mg for 10 days per cycle for 3 months (n=29)	Histological and immunohistochemical response at 3 months Follow up: Not reported
Wheeler-2007 (n=44)	Retrospective cohort study	Women with atypical hyperplasia (n=18) or well-differentiated endometrial cancer (n=26)	Oral progestogens of unreported type, dose and duration (n=29) or progesterone-releasing intrauterine device (n=15)	Outcome: Histological response at 1-3, 4-6 and 7-9 months Follow up: Median of 11 months, range was not reported
Wildermeersch-2007 (n=20)	Prospective cohort study	Women with simple (n=12) or atypical hyperplasia (n=8)	LNG-IUS[a] 14 µg releasing (n=7) for 3 years, replaced by a 20µg releasing LNG-IUS[a] (n=13)	Outcome: Histological response and ultrasound endometrial thickness Follow up: Mean of 36 months, range 14-90
Witkiewicz-2010 (n=15)	Retrospective case series	Women with atypical hyperplasia (n=7) or well-differentiated carcinoma (n=8)	Megestrol for a mean of 13.3 months (n=11), Megestrol + IUD[c] for a mean of 31 months (n=2), Megestrol + Medroxyprogesterone for 20 months (n=1), Megestrol + IUD[c] + Depot Medroxyprogesterone for 33 months (n=1). Doses of oral progestogens were not reported.	Outcome: Histological and immunohistochemical response Follow up: Not reported
Yener-1997 (n=30)	Oral progestogens arm of a randomised controlled study	Women with simple hyperplasia (n=30)	Medroxyprogesterone 20mg/day from day 16 to day 25 for 3 months (n=15) and Depot Goserelin subcutaneous implant each 28 days for 3 times (n=15)	Outcome: Histological response at 3 months Follow up: Not reported

Yu-2009 (n=25)	Retrospective cohort study	Women under age of 35 with severe atypical hyperplasia (n=17) or endometrial carcinoma (n=8)	Medroxyprogesterone 250-500mg/day for endometrial carcinoma and 100-500mg/day for atypical hyperplasia (n=22) or Megestrol Acetate or Hydroxyprogesterone Caproate of unreported dose (n=3), all continued for at least 3-6 months after remission	Outcome: Histological response at intervals of 3 months Follow up: Mean of 34.6 months, range 7-114

Regression outcomes for SH

Meta-analysis of the nine studies (213 women) of women with SH treated with oral progestogens showed a pooled regression rate of 89% (95% CI 77-100%) (Figure 3). Pooling the six studies (72 women) of women with SH treated with LNG-IUS found a pooled regression rate of 96% (95% CI 76-100). Meta-regression showed that the pooled regression rates were not statistically significantly different (p=0.41). The p value for the χ^2 test for heterogeneity was 0.95 for oral progestogens and 0.99 for LNG-IUS, indicating little variability in regression rates for these studies.

Regression outcomes for CH

Meta-analysis of the nine studies (389 women) of women with CH treated with oral progestogens showed a pooled regression rate of 66% (95% CI 58-74%). Pooling the four studies (102 women) of women with CH treated with LNG-IUS found a pooled regression rate of 92% (95% CI 65-100%). Meta-regression showed that the pooled regression rates were statistically significantly different (p<0.01). The p value for the χ^2 test for heterogeneity

was 0.86 for the oral progestogens and 0.99 for LNG-IUS, indicating little heterogeneity in the pooled regression rates.

Regression outcomes for ACH

Meta-analysis of the 14 studies (189 women) of women with ACH treated with oral progestogens showed a pooled regression rate of 69% (95% CI 58-83%). Pooling the seven studies (36 women) of women with ACH treated with LNG-IUS found a pooled regression rate of 90% (95% CI 62-100). The pooled regression rates were statistically significantly different (p=0.03). The p value for the χ^2 test for heterogeneity was 0.60 for the oral progestogens and 0.99 for LNG-IUS, indicating little heterogeneity in the pooled regression.

Figure 3 Meta-analysis of studies

Study	Total	Regressed	Rate (%)	95% CI	Regression rates, 95% CI[a]
Simple hyperplasia					
Oral Progestogens					
Bese (2006)	19	19	100	64 – 157	
Buttini (2009)	9	8	89	44 – 178	
Clark (2006)	15	10	67	36 – 124	
Guven (2001)	16	14	88	52 – 148	
Jarvela (2005)	16	10	63	34 – 116	
Milam (2008)	2	2	100	25 – 400	
Rattanachaiyanont (2005)	116	108	93	77 – 112	
Wang (2003)	5	4	80	30 – 213	
Yener (1997)	15	12	80	45 – 141	
Subtotal (95% CI)	**213**		**89**	**77 – 102**	
Test for heterogeneity: p=0.948					
LNG-IUS					
Clark (2006)	3	3	100	32 – 310	
Wildemeersch (2007)	12	12	100	57 – 176	
Buttini (2009)	15	13	87	50 – 149	
Varma (2008)	16	15	94	57 – 156	
Vereide (2006)	11	11	100	55 – 181	
Haimovich (2008)	15	15	100	60 – 166	
Subtotal (95% CI)	**72**		**96**	**76 – 122**	
Test for heterogeneity: p=0.999					
P=0.41 for comparison between oral progestogens and LNG-IUS[b]					**0% 50% 100%**

Complex hyperplasia					
Oral Progestogens					
Buttini (2009)	1	1	100	14 – 710	
Clark (2006)	38	21	55	36 – 85	
Guven (2001)	5	4	80	30 – 213	
Horn (2004)	208	128	62	52 – 73	
Jarvela (2005)	1	1	100	14 – 710	
Milam (2008)	11	6	55	25 – 121	
Rattanachaiyanont (2005)	18	16	89	54 – 145	
Reed (2009)	95	68	72	56 – 91	
Wang (2003)	12	9	75	39 – 144	
Subtotal (95% CI)	**389**		**66**	**58 – 74**	
Test for heterogeneity: p=0.856					
LNG-IUS					
Buttini (2009)	3	3	100	32 – 310	
Clark (2006)	14	9	64	33 – 124	
Varma (2008)	80	73	91	73 – 115	
Vereide (2006)	5	5	100	42 – 240	
Subtotal (95% CI)	**102**		**92**	**65 – 109**	
Test for heterogeneity: p=0.776					
P=0.001 for comparison between oral progestogens and LNG-IUS[b]					**0% 50% 100%**

Atypical Hyperplasia					
Oral Progestogens					
Clark (2006)	5	0	10	1 – 160	
Guven (2001)	3	2	67	17 – 267	
Horn (2004)	7	0	7	0 – 114	
Jobo (2001)	20	15	75	45 – 124	
Kaku (2001)	18	11	61	34 – 110	
Milam (2008)	1	0	50	3 – 799	
Minaguchi (2007)	12	8	67	33 – 133	
Randall (1997)	17	13	76	44 – 132	
Reed (2009)	52	38	73	53 – 100	
Signorelli (2009)	10	1	10	1 – 71	
Wang (2003)	9	5	56	23 – 133	
Wheeler (2007)	13	10	77	41 – 143	
Witkiewicz (2010)	5	1	20	3 – 142	
Yu (2009)	17	14	82	49 – 139	
Subtotal (95% CI)	**189**		**69**	**58 – 83**	
Test for heterogeneity: p=0.604					
LNG-IUS					
Clark (2006)	1	0	50	3 – 799	
Buttini (2009)	1	1	100	14 – 710	
Wheeler (2007)	5	5	100	42 – 240	
Varma (2008)	9	6	67	30 – 148	
Vereide (2006)	5	5	100	42 – 240	
Tjalma (2004)	7	7	100	48 – 210	
Wildemeersch (2007)	8	8	100	50 – 200	
Subtotal (95% CI)	**36**		**90**	**62 – 130**	
Test for heterogeneity: p=0.986					
P=0.03 for comparison between oral progestogens and LNG-IUS[b]					**0% 50% 100%**

Discussion

This review, which included 1001 women with EH, showed that complete regression of EH was achieved in a lower proportion of women treated with oral progestogens compared to women treated with LNG-IUS for complex (66% vs 92%) and atypical hyperplasia (69% vs 90%). There was no significant difference found between the two treatments for simple hyperplasia (89% vs 96%).

Our study provides an overview of the efficacy of oral progestogens and LNG-IUS for the treatment of EH and summarises the current evidence. It has major clinical relevance to the understanding and treatment of EH. We meta-analysed the disease regression rates for both interventions separately for each type of EH. This reduced potential heterogeneity between the studies and enhanced the clinical applicability of our findings. We also assessed the heterogeneity both graphically using forest plots and statistically. We contacted authors of the primary studies for clarification of relevant information. We used a validated tool (MINORS) to rate the quality of the included studies.

However, the strength of these findings is limited by the dearth of primary literature, unreliability of the data due to small numbers and the risk of bias in most of the studies due to their poor quality. Furthermore, the interpretation of these findings should also take into account publication bias, which is likely to result in preferential reporting of cases with good outcomes, leading to possible overestimation of effect. It is plausible that different types and doses of oral progestogens may have a differential effect on disease regression rates, but the large variation in type, dose and regimens of oral progestogens used, prevented us from

performing subgroup analyses to explore the differences in efficacy However, there is no consistent evidence to suggest such a differential effect from the studies included in our review, as well as a large study by Reed et al[30] which found that there are no differences in EH regression rates between the various oral progestogens

We believe that the difference of disease regression rates of oral progestogens and LNG-IUS for the treatment of EH found in our review may be explained by the mode of progestogen delivery. The progestogen concentrations in the uterine mucosa when delivered through an intrauterine device, directly into the cavity are reported to exceed that of the oral treatment by several-fold.[33] The intrauterine progestogen release is also associated with higher patient satisfaction and, therefore patients are more likely to continue the treatment. As discussed before, this higher chance of patients continuing the LNG-IUS treatment resulting in higher compliance may also explain its better efficacy in treating EH compared to oral progestogens. The higher disease regression rate with LNG-IUS can reduce the number of hysterectomies performed for this condition and prevent progression to cancer.

In conclusion, although this review of observational studies found a lower chance of disease regression of EH with oral progestogens compared to LNG-IUS, it should be acknowledged that observational studies are fraught with potential biases and confounders. Our systematic examination of the published literature confirms the scarcity of high-quality evidence to reliably inform clinical practice in this area. Although the differences between oral progestogens and LNG-IUS may be seen as significant, these data should be interpreted with caution. This is because the studies are of observational design with mostly indirect

comparisons between these two methods and small numbers of included women, especially for women with ACH. As a result, the findings may be unreliable and in the absence of randomised studies with at least five years follow-up, (this review only had two studies with over five years follow up data) the efficacy of oral progestogens and LNG-IUS remains in doubt. This review may aid the design of an adequately powered, controlled study to assess the short- and long-term effects of these interventions.

Chapter 3: Regression, relapse, and live birth rates with fertility-sparing therapy for EC and ACH: a systematic review and meta-analysis.

Abstract

Objective

To evaluate the regression, relapse and live birth rates of early-stage EC and ACH with fertility-sparing treatment.

Methods

This study was a meta-analysis of proportions from observational studies with random effects model and meta-regression to explore for heterogeneity.

Results

Thirty-four observational studies, evaluating the regression, relapse and live birth rates of early-stage EC (408 women) and ACH (151 women) with fertility-sparing treatment. Fertility-sparing treatment for EC achieved a pooled regression rate of 76.2%, a relapse rate of 40.6% and a live birth rate of 28%. For ACH the pooled regression rate was 85.6%, a relapse rate of 26% and a live birth rate of 26.3%. Twenty women were diagnosed with ovarian cancer (concurrent or metastatic) during follow-up (3.6%) and 10 progressed to higher than stage I EC (1.9%) from which two women died.

Conclusion

Fertility-sparing treatment of EC and ACH is feasible and selected women can satisfy their reproductive wishes.

Introduction

In 2007, 7,536 women in the UK were diagnosed with EC and 239 of these women were less than 45 years old (3.2%).[2] Often, these women have strong fertility desires as anovulatory infertility is strongly associated with the development of EC and ACH.[65] It is known that these women usually are usually diagnosed with early clinical stage well-differentiated EC, which carries a good prognosis. Traditionally, it is recommended that these women undergo a staging abdominal hysterectomy. However, multiple studies suggest that in selected women with early clinical stage disease this can be managed with fertility-sparing hormonal therapy. The use of progestogens can induce endometrial regression and prevent progression of the disease. Oral progestogens are used to treat EC and ACH but, more recently, the LNG-IUS has also been used successfully to treat ACH.[31] Yet, there is significant uncertainty about the efficacy of these therapies from observational studies with small sample sizes, which makes difficult to counsel the women accordingly. To ascertain the efficacy of these therapies, we conducted a systematic review of observational studies evaluating the regression, relapse and live birth rates for the treatment of EC and ACH and performed a meta-analysis of their treatment effects.

Methods

Identification of literature

The population of interest in this systematic review was women with early clinical stage (FIGO stage I) EC or ACH, the intervention was fertility-sparing therapies and the outcome was evidence of disease regression, relapse and live births. The following electronic databases were searched: MEDLINE (1950 to September 2011), EMBASE (1980 to

September 2011), Cochrane Central Register of Controlled Trials and Web of Science conference proceedings (ISI Proceedings, 1990 to September 2011). A combination of Medical Subject Headings (MeSH) and text words were used to generate two subsets of citations, one including studies of EC ("endometr* cancer*", "malignant endometr*") or EH ("endometr* hyperplas*", "premalignant endometr*", "precancer* endometr*") and the other including studies of fertility-sparing therapies such as progestogens and intrauterine devices or systems ("intrauterine devices medicated", "Levonorgestrel", "mirena", "intrauterine progest*", "LNG-IU*", "progest*", "gestag*", "fertility-sparing therapy", "conservative therapy", "hormone* therapy"). These subsets were combined with "AND" and limited to "Humans and Female" to generate a subset of citations. The reference lists of all known primary and review articles were examined to identify cited articles not captured by electronic searches. Language or geographical restrictions were not applied during search or selection.

Study selection and data extraction

Studies were selected if the participants were women diagnosed histologically with early clinical stage EC or ACH, the intervention was fertility-sparing therapy and the outcomes were histological disease regression, relapse or live birth rates. Case reports or series with less than five cases were excluded. Studies classifying women with EH in other than the WHO classification[7] were also excluded.

Studies were selected in a two-stage process. First, the titles and abstracts from the electronic searches were scrutinised by two reviewers independently and full manuscripts of all

citations that met the predefined selection criteria were obtained. Secondly, final inclusion or exclusion decisions were made on examination of the full manuscripts. In cases of duplicates, the most recent or the most complete publication was used. Any disagreements about inclusion were resolved by consensus or arbitration by a third reviewer. Two reviewers completed the quality assessment. The MINORS tool, which assesses the quality of the included studies, was implemented.[41] From each study, outcome data were extracted in 2x2 tables by the two reviewers.

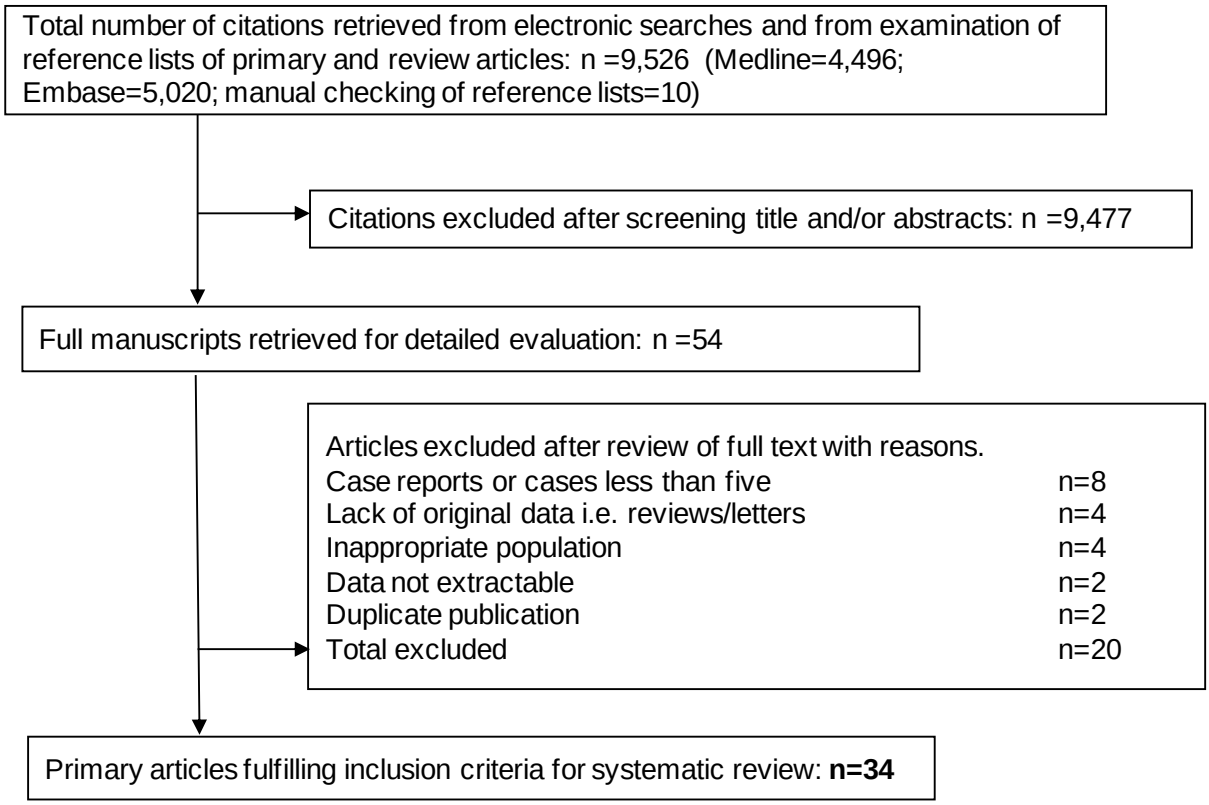
Disease regression was defined as lack of residual EC or CH during follow-up endometrial sampling. Disease relapse was defined EC or CH diagnosis during follow-up endometrial sampling following an endometrial sample that showed disease regression. Live births was defined as the birth of healthy infants during the follow-up period and its rate was calculated as the number of women who had a birth of healthy infants divided by the number of total of women undergoing fertility-sparing therapy. We also counted the number of women that were diagnosed with concurrent or metastatic ovarian cancer or upgraded disease to higher than stage I and deaths from this disease during follow-up.

Statistical analysis

Regression, relapse and live birth rates were extracted from each study and we computed the log of the ratio and its corresponding standard error for each study. We performed the meta-analysis using inverse-variance weighting to calculate the random effects summary estimates.[66] We obtained an estimate of the between-study variance with a random-effects meta-analysis. The square root of this number is the estimated standard deviation of

underlying effects across studies. Since we had relative measures of effect, the confidence intervals were centred on the natural logarithm of the pooled estimate, and the limits exponentiated to obtain an interval on the ratio scale.[67] Forest plots were created for each outcome, showing individual study proportions with confidence intervals (CIs) and the overall DerSimmonian-Laird pooled estimate.[43] Heterogeneity of the treatment effects was assessed graphically with forest plots and statistically analysed using the χ^2 test.[44] Exploration of the causes of heterogeneity for the live birth rate was planned according to the reproductive method and it was assessed with the aid of meta-regression.[45] Statistical analyses were performed using Stata 8.0 (Stata Corp, TX, USA).

Figure 4 Study selection process for systematic review of fertility-sparing treatment for EC and ACH

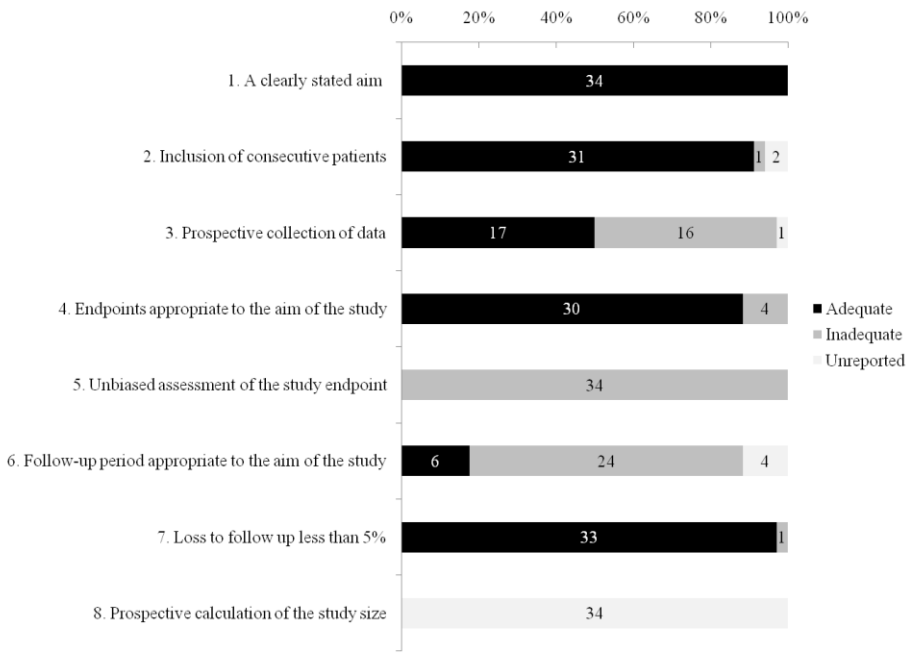


Results

Selection, characteristics and quality of the primary studies

The electronic search strategy yielded 9,516 citations and we retrieved further 10 citations from our manual checking of reference lists of all primary articles. Of these, 9,477 citations were excluded as they did not fulfil the selection criteria. Examination of the full-text of the remaining 54 manuscripts found a total of 34 primary studies, [68-94] including 559 women of which 408 were diagnosed with EC and 151 with ACH, for inclusion in this review (Figure 4). The main characteristics of the 34 studies and the study methodological index are presented in Table 2 and Figure 5.

Figure 5 Quality assessment of the studies for the systematic review of fertility-sparing treatment for EC and ACH



The primary studies included women with well-differentiated EC with 386 women being classified as G1 and 22 women with moderate or poor differentiation (G2 or G3). In 24 studies the women enrolled underwent diagnostic imaging to rule out myometrial invasion or distant disease. In 11 of these 24 studies, serum CA-125 marker was measured to also rule out concurrent ovarian malignancy. The quality of the studies on the MINORS checklist is shown in Figure 5. More in detail, half of the studies were prospective cohorts (17/34) including consecutive patients (31/34) with adequate definition of outcomes (30/34). No studies had a blinded assessment of the outcomes or performed a prospective calculation of the study size. We defined appropriate follow-up to be at least five years and we found that only in 6/34 studies follow-up was more than five years.

Figure 6 Forest plot of metaanalysis of regression rates for fertility-sparing treatment of EC

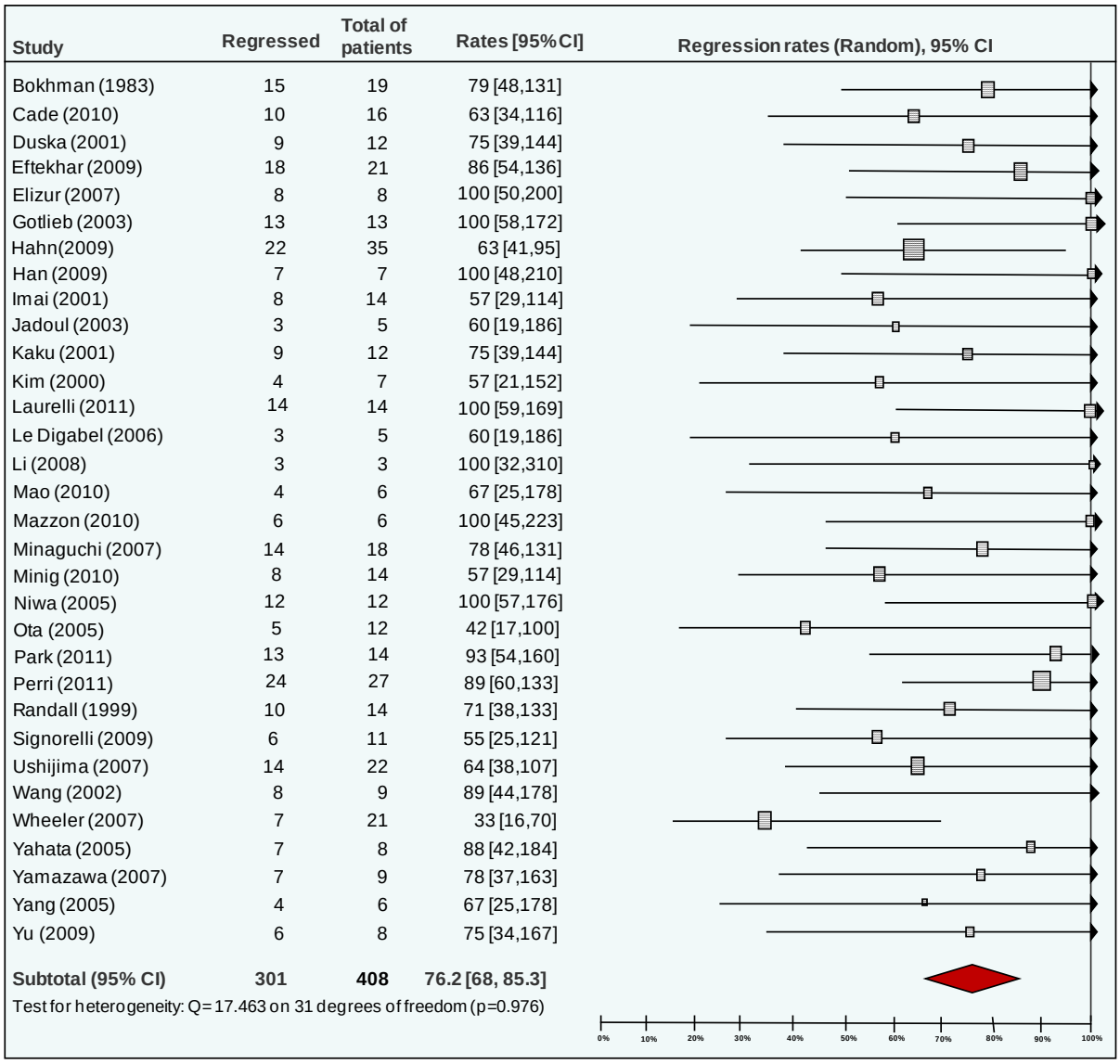


Figure 7 Forest plot of metaanalysis of relapse rates for fertility-sparing treatment of EC

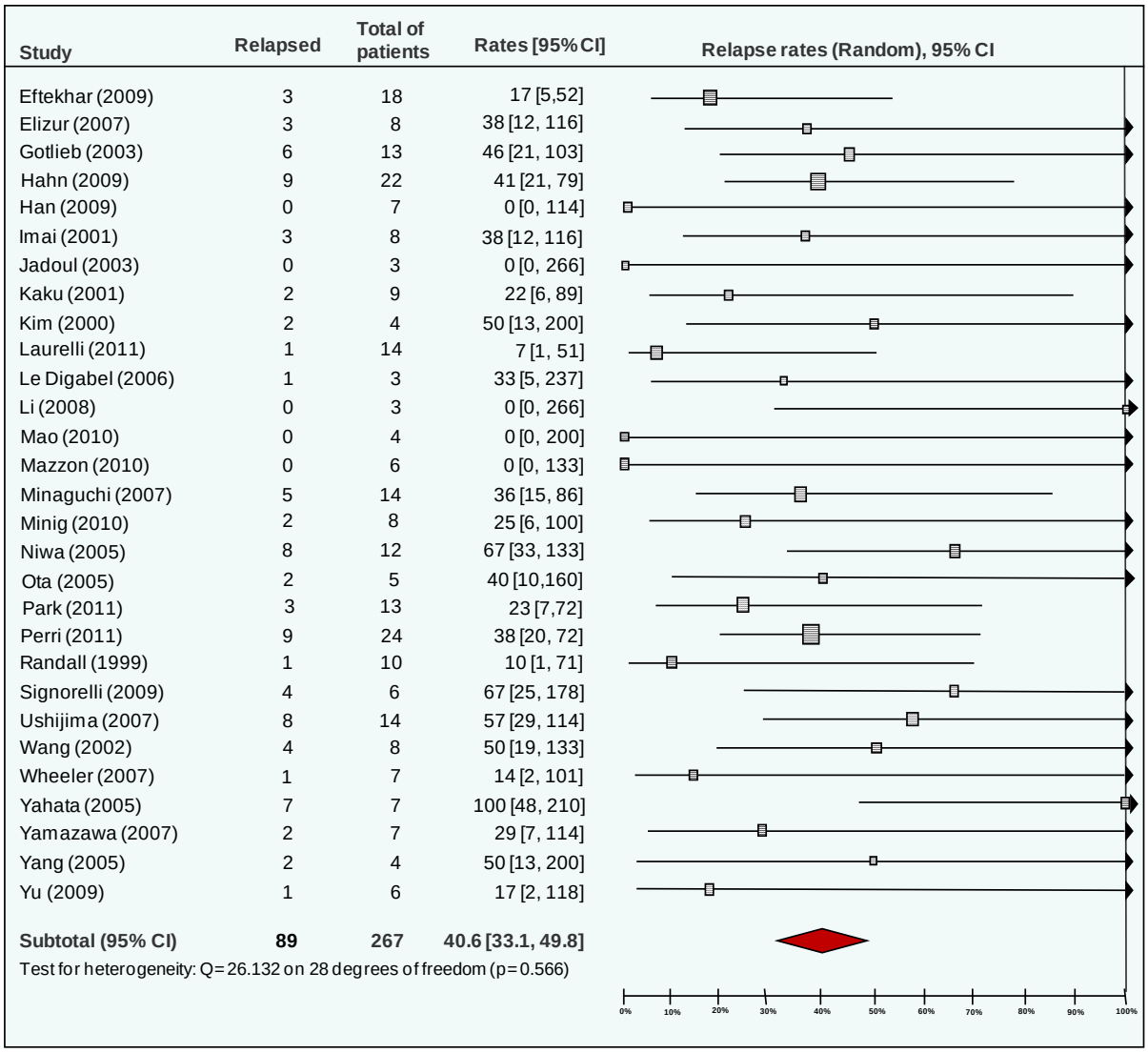


Figure 8 Forest plot of metaanalysis of live birth rates for fertility-sparing treatment of EC

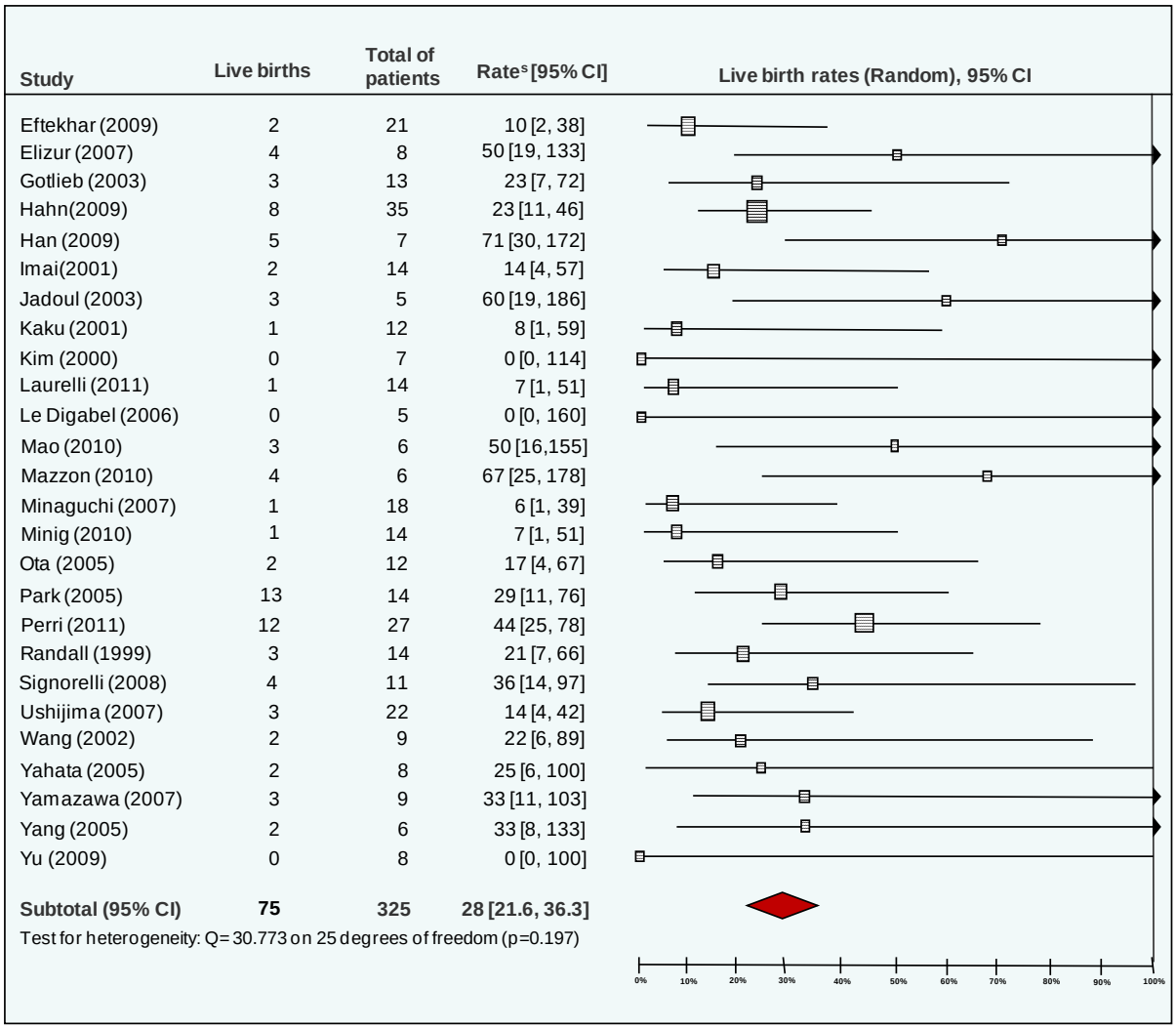


Figure 9 Forest plot of metaanalysis of regression rates for fertility-sparing treatment of ACH

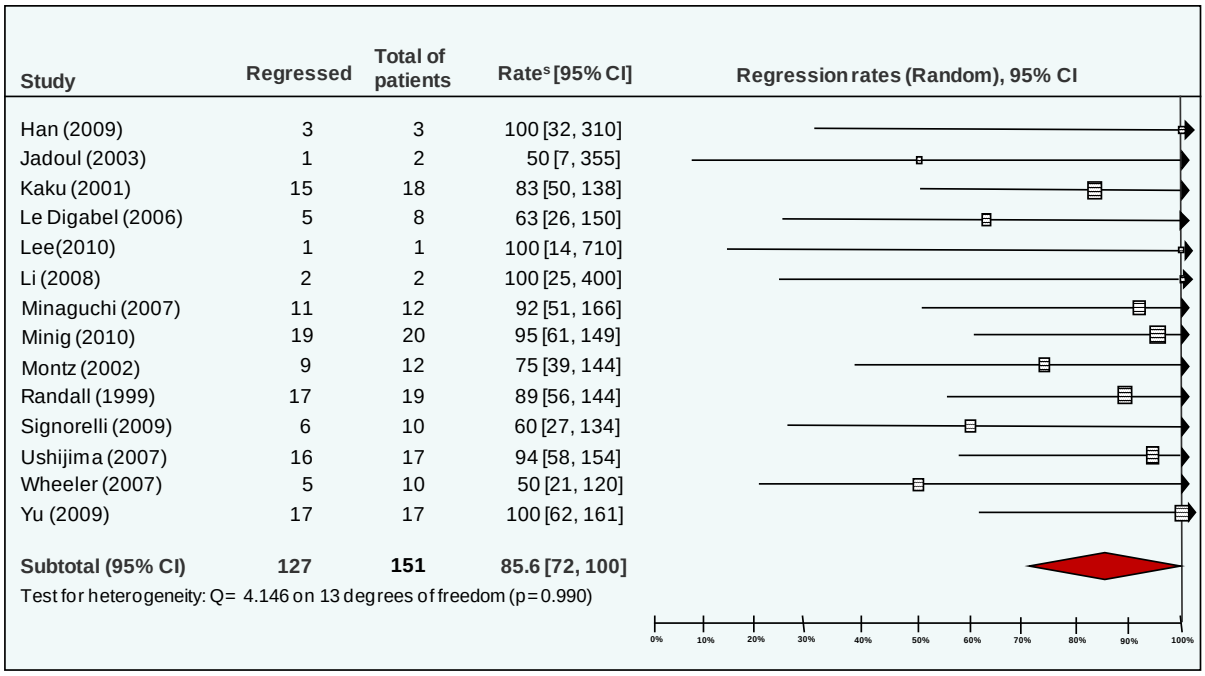


Figure 10 Forest plot of metaanalysis of relapse rates for fertility-sparing treatment of ACH

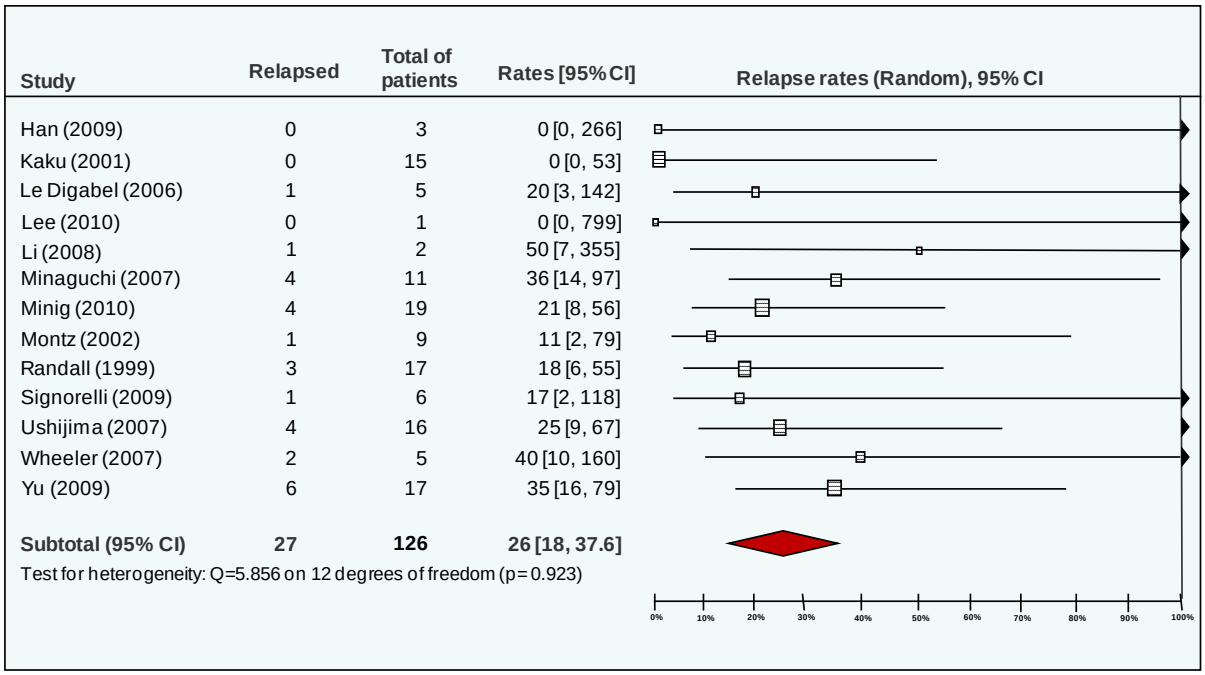


Figure 11 Forest plot of metaanalysis of live birth rates for fertility-sparing treatment of ACH

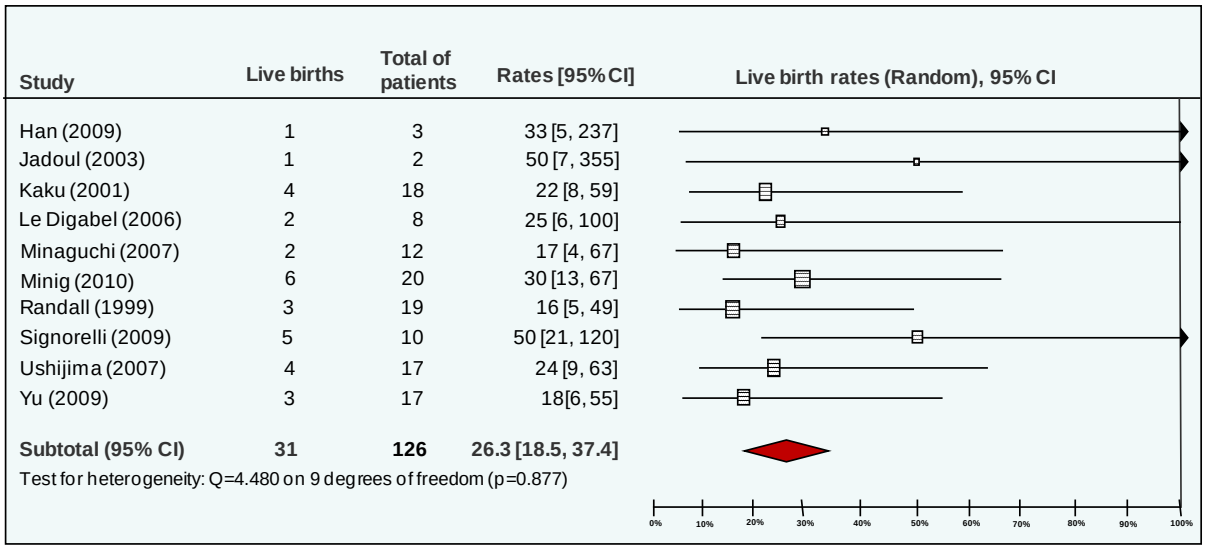


Table 2 Characteristics of the studies.

Author-Year	Recruitment	Study population: Women treated	Investigations prior to treatment to rule out invasion: Imaging	Investigations prior to treatment to rule out invasion: Tumour markers	Intervention or study groups	Outcomes (rates)	Follow-up (median, Range in months)
Bokhman-1985 (n=19)	Prospective	G1 (n=11) or G2 (n=8) EC	No	No	Hydroxyprogesterone 500mg/day for at least 3 months	Regression	n/a
Cade-2010 (n=16)	Retrospective	G1 EC	MRI	No	MPA only (n=4) 60-400mg/day, MPA 200-400mg/day with LNG-IUS (n=9) or LNG-IUS (n=3)	Regression, relapse and live birth	27, 3-134
Duska-2001 (n=12)	Retrospective	G1 EC	No	No	Progestogens at various doses	Regression, relapse, and live birth	82, 6-358
Eftekhar-2009 (n=21)	Prospective	G1 EC	MRI, CT and USD	CA125	MA 160 mg/day	Regression, relapse, and live birth	39, 5-108
Elizur-2007 (n=8)	Prospective cohort study	G1 EC	MRI	CA125	MA 160 mg/day (n=6), MPA 200mg/day (n=1) or 600mg/day (n=1) for at least 3 months	Regression, relapse and live birth	51, 38-75
Gotlieb-2003 (n=13)	Retrospective	G1 (n=11) or G2-3 (n=2) EC	MRI, CT	CA125	MA 160 mg/day (n=8), Hydroxyprogesterone 8-12g/day (n=2), NET 5mg/day (n=1), MPA 200-600mg/day (n=2) for at least 3	Regression, relapse and live birth	35, 10-146

					months		
Hahn-2009 (n=35)	Retrospective	G1 (n=31) or G1 and focal G2 (n=4) EC	MRI, CT and USD	CA125	MA 160 mg/day (n=8) or MPA 250-1500mg/day (n=20) or in combination (n=7)	Regression, relapse and live birth	23, 2-72
Han-2009 (n=10)	Retrospective	G1 (n=5) or G2 (n=2) EC or ACH (n=3)	MRI and USD	CA125	MA 80-160 mg/day (n=7), MPA 20-1000mg/day (n=3) for at least 3 months	Regression, relapse and live birth	31.5, 10-133
Imai-2001 (n=14)	Retrospective	stage I G1 (n=5) or G2 (n=1) and stage II G1 (n=7) or G2 (n=1) EC	No	No	MPA 400-800mg/day	Regression, relapse and live birth	12.9, 7-46
Jadoul-2003 (n=7)	Retrospective	G1 EC (n=5) or ACH (n=2)	No	No	Endometrial resection followed by GnRH-analogues	Regression, relapse and live birth	40, 26-40
Kaku-2001 (n=30)	Retrospective	G1 (n=10) or G2 (n=2) EC or ACH (n=18)	MRI, CT and USD	No	MPA 200-800mg/day for EC (n=12) and 100-600mg/day for ACH (n=18) for 3-6 months	Regression, relapse and live birth	38.7, 17-84
Kim-2000 (n=7)	Retrospective	G1 EC	No	No	MA 160 mg/day for at least 3 months	Regression, relapse and live birth	11.7, 3-30
Laurelli-2011 (n=14)	Prospective	Stage IA G1 EC	MRI and USD	No	Hysteroscopic resection of the tumour followed by MA 160 mg/day for 6 months (n=6) or LNG-IUS (52 mg/day) (n=8) for 12 months	Regression, relapse and live birth	n/a
Le-Digabel-2006 (n=13)	Retrospective	Stage IA G1-2 (n=3) or Stage IB G2-3 (n=2) EC or ACH (n=8)	No	No	Progestogens at various doses (n=6) or LHRH-analogues (n=3) or combination of the two (n=2) or endometrial curettage (n=2)	Regression, relapse and live birth	50.5, 32-77
Lee-2010 (n=12)	Prospective	ACH (n=1), other hyperplasia (n=11)	No	No	Progesterone-releasing IUD system (20µg/day)	Regression and relapse	50.5, 21-82
Li-2008 (n=5)	Prospective	ACH (n=3), other hyperplasia (n=2)	No	No	Letrozole 2.5mg/day for 3 months	Regression, relapse and live birth	40.7, 2-109
Mao-2010 (n=6)	Prospective	G1 EC	MRI, CT and USD	CA125	MA 160 mg/day (n=2), MPA 250-500mg/day (n=4)	Regression, relapse and live birth	29, 4-102
Mazzon-2010 (n=6)	Prospective	Stage IA G1 EC	MRI	CA125	Hysteroscopic resection of the tumour followed by MA 160 mg/day for 6 months	Regression, relapse and live birth	43, 3-75
Minaguchi-2007 (n=31)	Prospective	Stage IaG1 EC (n=19) or ACH (n=12)	MRI, CT and USD	No	MPA 2.5mg-600mg/day, mostly 400-600mg/day for 6 months	Regression, relapse and live birth	55.8, 24-138
Minig-2010 (n=34)	Prospective	Stage IaG1 EC (n=14) or ACH (n=20)	MRI and USD	CA125	LNG-IUS (20µg/day) for 12 months and GnRH analogue (3.75mg depot) for 6 months	Regression, relapse and live birth	43.5, 13-127
Montz-2002 (n=12)	Prospective	Stage IaG1 EC (n=12)	MRI and USD	No	Progesterone-releasing IUD (65µg/day)	Regression and relapse	47.3, 18-135
Niwa-2005 (n=12)	Prospective	Stage IaG1 EC	MRI and USD	CA125	MPA 400mg-600mg/day for at least 6 months	Regression, relapse and	60.2, 8-412

						live birth	
Ota-2005 (n=12)	Retrospective	Stage IaG1 EC	MRI, CT and USD	No	MPA 600mg/day	Regression, relapse and live birth	40, 9-79
Park-2011 (n=14)	Retrospective	Stage IaG1 EC	MRI	No	MPA 250-500mg/day (n=10) or Provera 30mg/day (n=2) or MA 16-240mg/day (n=2)	Regression, relapse and live birth	98, 35-176
Perri-2011 (n=27)	Retrospective	Stage I EC	MRI, CT and USD	CA125	MA 160-320 mg/day (n=21), NET 5 mg/day (n=1), Hydroxyprogesterone 2-3 g/week (n=2), and MPA 100-600 mg/day (n=3)	Regression, relapse and live birth	47.9, 25-73
Randall-1997 (n=33)	Retrospective	G1 EC (n=14) or ACH (n=19)	No	No	MPA 10-30mg/day or MA 40-160mg/day (n=29), ovulation induction (n=2), Bromocriptine (n=1), oral contraceptive (n=1) for 3-12 months	Regression, relapse and live birth	69, 25-113
Signorelli-2009 (n=21)	Prospective	Stage IaG1 EC (n=11) or ACH (n=10)	MRI, CT and USD	CA125, CA19.9	Natural progesterone 200mg/day D14-25	Regression, relapse and live birth	11, n/a
Ushijima-2007 (n=45)	Prospective	Stage IaG1 EC (n=28) or ACH (n=17)	MRI	CA125	MPA 600mg/day with low dose (81mg) aspirin	Regression, relapse and live birth	76.5, 21-118
Wang-2002 (n=9)	Prospective	Stage IaG1 EC	MRI and USD	CA125	MA 160 mg/day and tamoxifen 30 mg/day for 6 months	Regression, relapse and live birth	39, 24-69
Wheeler-2007 (n=44)	Retrospective	G1 EC (n=26) or ACH (n=18)	No	No	Oral progestogens (n=29) or progesterone-releasing IUD (n=15)	Outcome: Regression and relapse	48.8, 14-132
Yahata-2005 (n=8)	Prospective	Stage IaG1 EC	MRI and USD	No	MPA 1800mg/day for at least 3 months	Regression, relapse and live birth	34.6, 7-114
Yamazawa-2007 (n=9)	Prospective	Stage IaG1 EC	MRI and CT	CA125	MPA 400mg/day for at least 6 months	Regression, relapse and live birth	82, 6-358
Yang-2005 (n=6)	Prospective	Stage IaG1 EC	MRI, CT and USD	No	MA 160mg/day for at least 6 months	Regression, relapse and live birth	39, 5-108
Yu-2009 (n=25)	Retrospective	Stage IaG1 EC (n=8) or ACH (n=17)	MRI, CT and USD	CA125	MPA 250-500mg/day for EC and 100-500mg/day for ACH (n=22) or MA or Hydroxyprogesterone (n=3)	Regression, relapse and live birth	51, 38-75

Abbreviations EC: endometrial cancer, ACH: atypical complex hyperplasia, LNG-IUS: Levonorgestrel-releasing Intrauterine System, HRT: Hormone Replacement Therapy, IUD: Intrauterine Device, MPA: Medroxyprogesterone acetate, MA: Megestrol acetate, NET: Norethisterone

Regression, relapse, and live birth rates of fertility-sparing treatment for EC

Meta-analysis of the 32 studies (408 women) of women with EC managed with fertility-sparing treatment found that 301 women regressed with a pooled regression rate of 76.2% (95% CI 68-85.3, Figure 6). The p value for the χ^2 test for heterogeneity was 0.976 indicating insignificant variability in regression rates between the studies. In 29 of these studies (267 women) women were followed up over time with the median ranging from 11 to 76.5 months and the relapse rates were reported. We found that 89 women after an initial regression of the EC they relapsed during follow-up which amounts to a pooled relapse rate of 40.6% (95% CI 33.1-49.8) without significant variability (p=0.566, Figure 7). Meta-analysis of the 26 studies reporting pregnancy outcomes showed that from 325 women undergoing fertility-sparing treatment for EC, 75 women achieved at least one live birth with a pooled live birth rate was 28% (95% CI 21.6-36.3) with minimal heterogeneity (p=0.197, Figure 8).

Regression, relapse, and live birth rates of fertility-sparing treatment for ACH

For ACH, meta-analysis of the 14 studies (151 women) found that 127 women regressed with a pooled regression rate of 85.6% (95% CI 72-100%, Figure 9). The p value for the χ^2 test for heterogeneity was 0.99 indicating no variability in regression rates between the studies. In 13 of these studies (126 women) women were followed up over time with the median ranging from 11 to 76.5 months and the relapse rates were reported. We found that 27 women after an initial regression of the ACH they relapsed during follow-up which amounts to a pooled relapse rate of 26% (95% CI 18-37.6) again without any observed variability (p=0.923, Figure 10). For ACH, meta-analysis of the 10 studies reporting pregnancy

outcomes showed that from 126 women, 31 women achieved at least one live birth with a pooled live birth rate was 26.3% (95% CI 18.5-37.4%) with insignificant heterogeneity (p=0.877, Figure 11).

Assisted reproduction versus spontaneous pregnancy

From the 451 women that had fertility-sparing treatment for EC or ACH, 142 had assisted reproduction treatment to achieve pregnancy and 56 of them achieved at least one live birth. This amounts to a 39.4% live birth rate. The remaining 309 women are presumed to have tried to spontaneously conceive and 46 women achieved at least one live birth with a rate of 14.9%. This difference between assisted reproduction and spontaneous conception in achieving a live birth was statistically significant (p=0.001) in meta-regression analysis.

Safety of fertility-sparing treatment

There were 20 diagnoses of ovarian malignancy during follow-up (20/559, 3.6%) and it was not always clear from the primary studies whether they represented concurrent ovarian malignancies or metastatic ovarian involvement from the endometrial primary neoplasm. The type of ovarian cancer and staging was poorly reported, but 10 women were diagnosed with endometrioid adenocarcinoma of the ovary (10/559, 1.8%). The pre-operative imaging or tumour marker investigations did not appear to reduce this incidence as in 11 studies that carried out these investigations, ovarian malignancy was diagnosed during follow-up in 8 women (8/200, 4%) comparing to 13 studies where only imaging was used and there were 5 ovarian malignancies diagnosed (5/217, 2.3%) and in 10 studies with no such investigations there were 7 ovarian malignancies diagnosed (7/142, 4.9%). There were also 10 women

(10/559, 1.8%) diagnosed with stage II EC or higher after failing treatment. In one case there was a distal lymphatic metastasis involving the obturator lymphatic node.[34] There were two deaths from fertility-sparing treatment for EC (2/559, 0.36%), one from a diagnosis of a synchronous endometrial, ovarian and peritoneal malignancy[83] and one from an ovarian malignancy on a patient who on recurrence only underwent total hysterectomy without salpingo-oophorectomy as did not wish to have menopausal symptoms.[87]

Discussion

This meta-analysis, which included 408 women with EC and 151 with ACH, found that the regression rates with fertility-sparing treatment are very encouraging (76% for EC and 86% for ACH). An also encouraging proportion of women choosing this treatment for preserving their fertility managed to achieve live births (28% of women with EC and 26% of women with ACH). Women choosing assisted reproductive treatment had significantly better results regardless of the initial diagnosis. However, the relapse rates during follow-up are worrying (41% for EC and 26% for ACH). The incidence of ovarian malignancies in 20 women during follow-up is also worrying (3.6%) and the pre-operative imaging or CA-125 testing, even though essential, did not lower this incidence. The upgrade of disease in further 10 cases along with distant metastases in 2 of these cases also represents a considerable risk of this treatment. There were two deaths reported.

Our study provides an overview of the efficacy of fertility-sparing treatment for early-stage EC and ACH and summarises the current evidence. It has major clinical relevance for young women that wish to preserve their fertility. We reduced potential publication bias by

excluding case-reports and cases-series of less than five cases. We contacted authors of the primary studies for clarification of relevant information. Finally, we calculated the events of disease upgrade during follow-up and adverse outcomes with fertility-sparing therapy. Other systematic reviews produced a mean of the observed rates which does not take into account the specific weight of the studies and their variability.[93] The use of a random effects model to combine the data across studies helps to control for differences between the studies. However, since the studies included in this meta-analysis are all observational studies there is bias that is introduced and the strength of the findings in this review including 34 studies is limited by the dearth of primary literature. The unstable study estimates and wide confidence intervals due to small numbers along with the risk of bias in most of the studies due to their study design and short-term follow-up reduce the strength of our inferences. Specifically, the relapse and live birth rates may prove to be higher if women were followed up for at least five years following their diagnosis.[95] It is reported that relapse may be more likely for obese women,[95] but the primary studies included in our analysis did not report the treatment effects taking into account obesity. It is plausible also that different types and doses of hormones may have a differential effect on disease regression rates, but the large variation in type, dose and regimens of oral progestogens used, prevented us from performing subgroup analyses to explore the differences in efficacy. In addition, there were only two studies that used the LNG-IUS and the majority of the evidence is from oral progestogens hence, our findings may not be generalisable to women treated with LNG-IUS and further research is encouraged. The variability across the studies was found to be statistically low, but this test may not be a reliable evaluation of the clinical variation in the studies because of small sample sizes.

We believe that even though the diagnosis of EC or ACH in women that wish to preserve fertility is uncommon, it is a management dilemma for clinicians. Fertility-sparing treatment does represent an option for these women with encouraging results, but also important risks. Women wishing to pursue this treatment would need to be counselled thoroughly about the benefits and the potential risks and informed of paucity of good quality evidence to guide the clinical decision-making. From the available data we can make suggestions for clinical practice and management, but caution is advised as the evidence backing these suggestions is poor. We suggest that pre-treatment investigations, should aim to rule out myometrial invasion and concurrent ovarian cancer, even though there are no reliable tests for this purpose. These should include imaging, such as transvaginal ultrasound and CT or MRI, along with tumour serum markers, but the limitations of these investigations should be taken into account. In the primary studies, these tests did not lower the incidence of ovarian cancer diagnosis during follow-up, but as this is a rare outcome this review may be underpowered to draw strong conclusions on this and we also cannot rule out a different case mix across the studies. We should also point out that there is uncertainty about the treatment regimen and the follow-up, which is reflected in our studies where various therapies were employed. The studies included in this review suggest that when a diagnosis of EC or ACH has been made this should be treated for at least three and up to 12 months. A repeat biopsy should confirm regression before women attempt to get pregnant. Considering the high relapse rate of the disease once the treatment is stopped and the potential of disease progression, it is sensible to recommend to these women to undergo staging hysterectomy with bilateral salpingo-oophorectomy. This should be recommended to women once their family is complete or if fertility-sparing treatment fails, either because of failure in regressing their disease or relapse.

If regression is achieved we would also recommend that these women are encouraged to undertake assisted reproduction treatment in order to maximise their chances of a live birth and minimize time before a hysterectomy, which could prevent them from relapse. Immediate assisted reproduction treatment avoids prolonged unopposed oestrogen stimulation, which could cause women to relapse. Finally, clinicians should consider following women that decline hysterectomy for at least five years or even longer and not to underestimate the risk of relapse.

In conclusion, this review of observational studies found a high chance of disease regression and encouraging live birth rates of early-stage EC and ACH with fertility-sparing treatment followed by assisted reproduction. The risk of disease relapse and upgrade during follow up is considerable. Our systematic examination of the published literature confirms that there is only limited quality observational evidence to inform clinical practice and results should be interpreted with caution. Our review may aid the design of a cohort study to assess the short- and long-term effects of the fertility-sparing treatment.

Chapter 4: Current management of endometrial hyperplasia—a survey of United Kingdom consultant gynaecologists.

Abstract

Objective

To determine current clinical practice for the management of EH.

Methods

We carried out a web-based survey of all UK consultant gynaecologists, from the Royal College of Obstetricians and Gynaecologists (RCOG) database, to evaluate the current practice and to enquire whether a trial between oral progestogens and LNG-IUS for EH is required.

Results

We sent 1090 email invitations and 411 (37.7%) responded to this survey. In total, 338 consultant gynaecologists, who manage patients with EH, responded to all items of the survey. The oral progestogens (33.2%) and the LNG-IUS (52.1%) were the most popular choices for managing CH. The majority of the gynaecologists would explore two conservative choices before embarking into performing a hysterectomy for this condition (130, 52.6%). However, for ACH, the majority of the gynaecologists would perform a hysterectomy (273, 83.2%) and would only consider LNG-IUS or oral progestogens as a second or third option. Two hundred forty-four (72.2%) responded that an RCT for oral progestogens versus LNG-IUS for the management of EH is required. There were 171 (50.6%) gynaecologists that would be willing to randomise in such an RCT.

Conclusion Our survey shows that CH is managed conservatively in UK, with oral progestogens or LNG-IUS, and ACH is managed with hysterectomy. An RCT, between oral progestogens and LNG-IUS for EH, is required to identify the optimum therapy.

Introduction

Non-surgical therapeutic strategies in EH aim to induce disease regression and prevent progression to cancer. These strategies, if successful could reduce the number of hysterectomies performed for this condition and hence reduce morbidity and healthcare costs. Currently, there are no professional body guidelines for the management of EH. The use of progestogens, which antagonise the oestrogen effect on the endometrium, can induce endometrial regression and prevent progression to cancer[4] and the LNG-IUS developed primarily as a contraceptive device, has also been used successfully to treat EH.[31] This system has been proven to achieve higher regression rates than the oral progestogens in our systematic review and meta-analysis of observational studies.[95] However, this systematic examination of the published literature confirms that the quality of the published data was poor with short term follow up and small sample sizes.[95] We conducted a RCOG-based survey to identify the current practice for the treatment of EH and whether there is need for further research in terms of a randomised controlled trial.

Methods

The survey population consisted of all 1268 consultants in UK holding membership of the Royal College of Obstetricians and Gynaecologists (RCOG). They were identified through

the RCOG database that includes consultants that have consented for the College to share their contact details with third parties. The database contained the contact details for 1268 consultants. Of these, 178 email addresses were not valid at the time of the survey. As a result, our population for this survey consisted of 1090 consultants receiving an email invitation. The email invitation contained a link to a website access to the survey. The survey questionnaire was designed to explore the current management of EH. It contained separate questions for CH and ACH. The gynaecologists could rank three different choices out of a list which contained observation only, medical management (i.e. LNG-IUS, oral progestogens) or surgical management (i.e. hysterectomy) or observation only. The gynaecologists were made aware of the current observational evidence quoting the regression rates with oral progestogens (about 70%) and LNG-IUS (about 90%)[96] and on this basis, they were asked whether they believed further research in the form of a randomised controlled trial was necessary. We also enquired about interest for recruiting in such a trial and we invited comments about any serious concerns. The questionnaire also contained two filter questions. There were used for selecting only gynaecologists that manage women with EH for completing this survey. The questionnaire was piloted on 39 consultants for obtaining a user-friendly structured format.

Results

We sent 1090 email invitations and 411 (37.7%) responded to this survey (Table 3). Table 4 shows the preferred choice for managing complex EH.

Table 3 Responses to the survey on the current management of EH in the UK

	N (%)
Number of consultants on the RCOG database	1268
Number of emails to valid addresses sent for the survey (% of those on the RCOG database)	1090 (86)
Responders	
– Obstetricians (excluded from the survey)	26 (6.3)
– Gynaecologists only	134 (32.6)
– Obstetricians and gynaecologists	251 (61.1)
Total number of consultants responding to the survey (% of those sent an invitation)	411 (37.7)
Not managing endometrial hyperplasia and excluded from the survey	47 (12.2)
Number of consultants responding to the survey who manage endometrial hyperplasia	338 (87.8)
Number of consultants that believe an RCT is required (% of those answering the question)	245 (75.2)
Number of consultants willing to randomise (% of those answering the question)	171 (52.6)

The oral progestogens (33.2%) and the LNG-IUS (52.1%) were the most popular choices for managing this condition. The majority of the gynaecologists would explore two conservative choices before embarking into performing a hysterectomy (130, 52.6%). However, for ACH, the majority of the gynaecologists would preferably perform a hysterectomy (273, 83.2%) and would only consider LNG-IUS or oral progestogens as a second or third option (Table 5).

Table 4 Current management of CH in the UK

	1st choice N (%)	2nd choice N (%)	3rd choice N (%)
Observation	22 (6.7)	11 (3.5)	45 (18.2)
Oral progestogens	109 (33.2)	147 (47.1)	36 (14.6)
Mirena (LNG-IUS)	171 (52.1)	128 (41)	12 (4.9)
GnRH analogues	1 (0.3)	3 (1)	9 (3.6)
Aromatase inhibitors	0	1 (0.3)	4 (1.6)
Hysterectomy	17 (5.2)	20 (6.4)	130 (52.6)
Other interventions	8 (2.4)	2 0.6)	11 (4.5)
Responses (% of those responding to the survey	328 (97)	312 (92.3)	247 (73.1)

The main reason for the surgical intervention in these patients is the fear of progression or co-existent EC. From the 338 gynaecologists that we asked if further research is required, 244 (72.2%) responded that an RCT for LNG-IUS versus oral progestogens for the management of EH is required, 81 (23.9%) thought that an RCT was not required and 13 (3.9%) did not respond to this question. There were 171 (50.6%) gynaecologists that would be willing to randomise in such an RCT, 62 (18.3%) that would not randomise, 92 (27.2%) were not sure if they would participate and 13 (3.9%) did not respond to this question. Gynaecologists were reluctant to randomise in such an RCT, either because they manage small numbers of patients with hyperplasia (14/62, 22.6%) and because they did not want or they preferred for gynaecology oncology colleagues to manage ACH patients (13/62, 21%). Interestingly, only 3 (4.8%) gynaecologists had a strong preference for a type of treatment and did not want to randomise to this study for this reason.

Table 5 Current management of ACH in the UK

	1st choice N (%)	2nd choice N (%)	3rd choice N (%)
Observation	0	0	5 (2.4)
Oral progestogens	16 (4.9)	67 (24.9)	110 (52.1)
Mirena (LNG-IUS)	33 (10.1)	155 (57.6)	47 (22.3)
GnRH analogues	1 (0.3)	5 (1.9)	1 (0.5)
Aromatase inhibitors	0	1 (0.4)	7 (3.3)
Hysterectomy	273 (83.2)	35 (13)	31 (14.7)
Other interventions	5 (1.5)	6 (2.2)	10 (4.7)
Responses (% of those responding to the survey	328 (97)	269 (79.6)	211 (62.4)

Discussion

The vast majority of the gynaecologists in UK treat CH with LNG-IUS or oral progestogens. For ACH, the consensus is to perform hysterectomy and only if this is not possible, oral progestogens or LNG-IUS are used for this purpose. The reason for this intervention is the high probability of progression or co-existent EC. More than three out of four gynaecologists in this survey believes more research is required for the management of EH and two out of three of those would be willing to randomise in an RCT comparing LNG-IUS versus oral progestogens for EH. In our knowledge, this is the only published survey evaluating the management of EH. There is much debate around what is the best way to treat this condition, but only low quality observational studies are available to inform the clinical practice.[95] These observational studies showed a higher regression rate of the condition with the LNG-IUS compared to the oral progestogens (about 90% versus 70%).[95] We made this information available to the gynaecologists we surveyed and, despite that, over three out of four of the gynaecologists believed further research was required. However, gynaecologists often tailor their treatment according to age, comorbidities and fertility desire and many

commented in our survey that it influences their clinical decision-making. The diagnostic method (endometrial suction biopsy or curettage) was very rarely mentioned to influence treatment choice. Clinicians believe that a randomised controlled trial can overcome the pitfalls of the low methodological quality observational studies and help them decide if LNG-IUS or oral progestogens are more effective in treating EH. The response rate to this survey was low (37.7%). This introduces selection bias and threatens the internal validity and precision of the results.[96] The reason is that non- responders may have answered differently altering significantly the results of this survey. However, we limited our survey only to consultants that manage this condition, which is almost exclusively of general gynaecological interest. We expect that about 30–40% of the clinicians included in our invitation to complete the survey were obstetricians. This survey may have been of low interest to them, therefore, explaining the poor response from this group. A high number of specialist gynaecologists (i.e. assisted conception specialists or urogynaecologists) would also not be interested in this condition as they would rarely have to manage patients with EH. The design of this study does not allow us to address potential response and recall bias, but we attempted to minimise this with specific and direct questions. Specifically we believe that those clinicians routinely managing these patients on a current day to day basis were more likely to have participated to this survey. Our data from this survey show a strong trend towards conservative management of CH, with LNG-IUS or oral progestogens, and a surgical management of ACH with hysterectomy. It is unlikely that this trend is biased or that it would have changed with a higher response rate. Even though the observational studies are favouring the LNG-IUS, the gynaecologists believe that further research, and specifically an RCT between the LNG-IUS and oral progestogens for EH, is required. The high risk of EC

with the ACH makes the option of hysterectomy almost mandatory. However, the majority of women are diagnosed with CH (>80%) and most of the clinicians would be willing to treat this with hormonal therapy.[29] As shown in this survey, this is done in over 80% of the cases with LNG-IUS or oral progestogens. Even though the observational studies are favouring the LNG-IUS, the clinicians believe that further research, and specifically an RCT between the LNG-IUS and oral progestogens for EH is required.

Chapter 5: LNG-IUS versus oral progestogen treatment for EH: A long-term comparative cohort study.

Abstract

Objective

Compare the regression rate of the LNG-IUS versus oral progestogens for the treatment of women with EH.

Methods This was a comparative observational study with long term follow up in a tertiary care university hospital. Three hundred forty four women with CH or ACH participated in the study. Women were treated with LNG-IUS (n=250) or oral progestogens (n=94). We evaluated the proportion of women that regressed or had hysterectomy after treatment with LNG-IUS compared to oral progestogens by logistic regression adjusting for confounding.

Results

Follow up rate was 95.3%. The mean length of follow up in the two groups was 66.9 ± SD 35.1 months for the LNG-IUS versus 87.2 ± SD 45.5 months for the oral progestogen group. Regression of hyperplasia was achieved in 94.8% (237/250) of patients with LNG-IUS compared to 84% (79/94) of patients treated with oral progestogens (OR=3.46, 95% CI 1.58–7.19). Hysterectomy rates were lower in the LNG-IUS group during follow up (22.1%, 55/250 vs. 37.2%, 35/94, OR=0.48, 95% CI 0.29–0.8). EC was diagnosed in 8 (33%) women that had hysterectomy (n=24) because of failure to regress to normal histology during follow up.

Conclusion

LNG-IUS achieved higher regression rate in treating EH with lower hysterectomy rates than the oral progestogens. Failure to achieve regression of EH carries a high risk of underlying EC.

Introduction

EH is the precursor of EC and without intervention, the risk of progression to carcinoma is significant.[4] ACH has also been associated with up to 43% rate of concomitant carcinoma in women undergoing hysterectomy.[11] As the rate of progression to carcinoma for SH is low and often regresses spontaneously, the treatment interventions are therefore aimed to treat mostly CH and ACH patients.[4] The treatment modality selected is dependent upon the woman's desire to retain fertility, medical fitness for surgical intervention and histological diagnosis. In women in whom cytological atypia is present, the recommended and undisputed definitive treatment remains hysterectomy. Traditionally hysterectomy was recommended for CH cases but it is not possible for all given its potential risks, especially for older or obese patients and those with significant co-morbidities. Medical management of EH is therefore advocated in such cases. In our national survey we found that more than 85% of gynaecologists treat CH with LNG-IUS or oral progestogens.[96] Oral progestogens have been used in various dosages and regimens to treat hyperplasia since 1959, with the commonest treatment time interval of six months and then stopping treatment after regression is confirmed.[95;97] However, our meta-analysis already showed that oral treatment is inferior in treating EH compared to LNG-IUS.[95] This meta-analysis also highlighted the scarcity of high quality comparative studies with long term follow up for assessing the efficacy of these two treatment options and called for further evidence to help decide which one is the treatment of choice.[95] Our objective was to conduct a large comparative cohort study with a long term follow up comparing the regression and hysterectomy rates of treatment with LNG-IUS and oral progestogens in patients diagnosed with CH or ACH.

Methods

This was a comparative cohort study. We included all women diagnosed with CH or ACH that underwent treatment with LNG-IUS or oral progestogens in a tertiary referral Hospital in Birmingham, UK. For women treated with LNG-IUS we obtained demographic and follow up data at the time of the diagnosis from August 1998. All women treated after August 2008 were recruited prospectively in our study with written consent (Appendix 1 and 2). Women with CH and ACH treated with oral progestogens from August 1998 until August 2008 were invited for long term follow up in our clinic and continue to be followed up ever since. These women were identified through a central electronic histopathology database, which includes all patients diagnosed with EH in our hospital for the study with no missing patients. The histopathological diagnoses were undertaken by two experienced gynaecological pathologists working independently; referral to the other pathologist for a second opinion was made in cases where there was diagnostic doubt, and a mutual consensus was then achieved. Women were reviewed in our gynecology outpatient clinic following diagnosis and were offered LNG-IUS (Mirena®, Bayer Healthcare Inc.), oral progestogens or hysterectomy as part of our routine clinical practice. Women diagnosed with ACH were counselled and offered a hysterectomy. Women who declined surgery or who were medically unfit to undergo surgery were offered LNG-IUS or oral progestogens. Women underwent regular outpatient clinic review and endometrial histological surveillance by outpatient endometrial sampling. Our practice was to perform histological surveillance on a six-monthly basis for the first two years and yearly thereafter until 5 years and then the patients were given a choice to have continued yearly surveillance. Women that did not adhere to this strategy were invited for clinic review in order to obtain long term follow up outcome. Ethical approval

from the Coventry & Warwickshire Research and Ethics Committee was obtained for this study (LREC 09/H1211/30).

For all women in the study (n = 344), baseline data were recorded for: histological subtype, age, ethnic background, body mass index, parity, menopausal status, medical history of hypertension or diabetes, use of exogenous hormones (e.g. HRT, tamoxifen) and ultrasound measurement of endometrial thickness for post-menopausal women. For women on HRT we advised to stop it until endometrial regression was achieved and then it was restarted as necessary. Tamoxifen treatment was normally continued. Menopause was defined as a minimum of 12 consecutive months of amenorrhea, for which there was no other obvious pathological or physiological cause. Missing data were sought also from primary care clinicians. The time from baseline histology until the last follow up was also recorded for all patients.

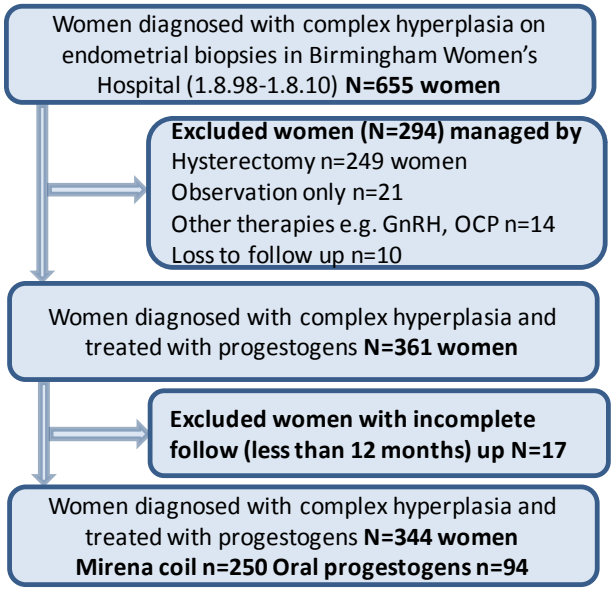
The primary outcome for this study was to determine the proportion of women with CH or ACH showing histological regression after treatment with LNG-IUS compared to oral progestogens. For this assessment, the results of follow-up histological examinations were classified as 1) *Complete Regression* – atrophy of glands, oedematous fibrotic stroma or pseudodecidualisation, with no evidence of hyperplasia. 2) *Persistence or Progression* – failure to completely regress with evidence of CH, ACH or carcinoma. The secondary outcomes we studied were the hysterectomy rate for each treatment, the time interval from treatment initiation to complete regression and the proportion of patients in both groups

diagnosed with EC during follow-up. All outcomes were evaluated with an intention to treat basis.

The baseline characteristics and outcomes for the LNG-IUS and oral progestogen groups were analysed using Mann-Whitney *U* tests for non-parametric data and Pearson χ^2 tests for categorical data. Analysis of outcomes between both treatment groups was performed by logistic regression to compute odds ratios (OR) with their 95% CI adjusting for potential confounding factors. We adjusted for correlated confounding factors ($p<0.1$) with both treatment modality and outcome and these were incorporated into the final model.[83] We constructed our survival analysis using the Cox proportional hazards model as it accounts for variable duration of follow-up, censoring of subjects, proportionality of event occurrence, and time-to-event.[99] To convert the results of the Cox model into absolute risk estimates, we calculated survival within our population by using Kaplan-Meier estimates.[100,101] Missing data were handled by complete case analysis for our exposure (treatment modality) and outcomes (regression and hysterectomy) and by multiple imputation for confounding variables.[102,103] All analyses were performed using STATA Version 12.1 (Stata Corp, College station, TX, USA).

Results

Figure 12 Schematic representation of patients included in study analysis



Of the 655 women diagnosed with CH or ACH over the 12 year study period, 361 women were treated with progestogens (Figure 12). We had incomplete data on follow up for 17 women and these were excluded. Our follow up rate was therefore 95.3% (344/361). The final study group consisted of 250 women in the LNG-IUS group and 94 women in the oral progestogen group. The mean length of follow up in the two groups was 66.9 ± SD 35.1 months for the LNG-IUS versus 87.2 ± SD 45.5 for the oral progestogen group. The duration of treatment with oral progestogens consisted of three (29.8%, 28/94), six (63.8%, 60/94) or twelve months (6.4%, 6/94) and then the treatment was stopped. The most common type of progestogen therapy given was norethisterone (50%), followed by medroxyprogesterone acetate (43%), dydrogesterone (2%), megestrol acetate (1%) and a combination of therapies (4%). Progestogen therapy was given either cyclically (32%) or continuously (68%). Baseline characteristics between both treatment groups were compared and found to be similar for all variables with the exception of age and menopause (Table 6).

Table 6 Baseline characteristics

		LNG-IUS (n=250)	Oral Progestogens (n=94)	*P* value
		n (%)	n (%)	
Age (years)		Mean 52.7 ± SD 10.6	Mean 48.5 ± SD 11.6	0.001
Parity		Mean 2.1 ± SD 1.5	Mean 1.7 ± SD 1.9	0.095
BMI (kg/m^2)		Mean 33 ± SD 9.5	Mean 32.2 ± SD 8	0.493
Endometrial thickness on USS (mm) for menopausal women		Mean 9.9 ± SD 5.6	Mean 10.9 ± SD 6.4	0.245
Ethnic Group	Caucasian	196 (78.4)	72 (76.6)	
	Asian	29 (11.6)	11 (11.7)	
	Other	10 (4)	9 (9.6)	
	Unknown	15 (6)	2 (2.1)	0.78
Menopausal Status	Premenopausal	119 (47.6)	63 (67)	
	Postmenopausal	131 (52.4)	31 (33)	0.001
Hypertensive		91 (36.4)	26 (27.7)	0.139
Diabetic		41 (16.4)	13 (13.8)	0.551
HRT / Tamoxifen use in last 5 years	None	199 (79.6)	81 (86.2)	
	HRT	42 (16.8)	10 (10.6)	
	Tamoxifen	9 (3.6)	3 (3.2)	0.165
Endometrial Histology	Atypical hyperplasia	21 (8.4)	13 (13.8)	
	Complex hyperplasia	229 (91.6)	81 (86.2)	0.137

The women in the LNG-IUS group were older (mean 52.7 years ± SD 10.6 versus 48.5 ± 11.6, p=0.001) and more often menopausal compared to the oral progestogen group (52.4%, 131/250 versus 33%, 31/94, p≤0.001). The body mass index was not available for 27/344 (7.8%) patients and also the endometrial thickness was not measurable in 9/162 (5.6%) of post-menopausal women.

Regression of hyperplasia was achieved in 94.8% (237/250) of patients with LNG-IUS compared to 84% (79/94) of patients treated with oral progestogens (Table 7) and this difference was found to be statistically significant (OR=3.46, 95% CI 1.58–7.19, p=0.001).

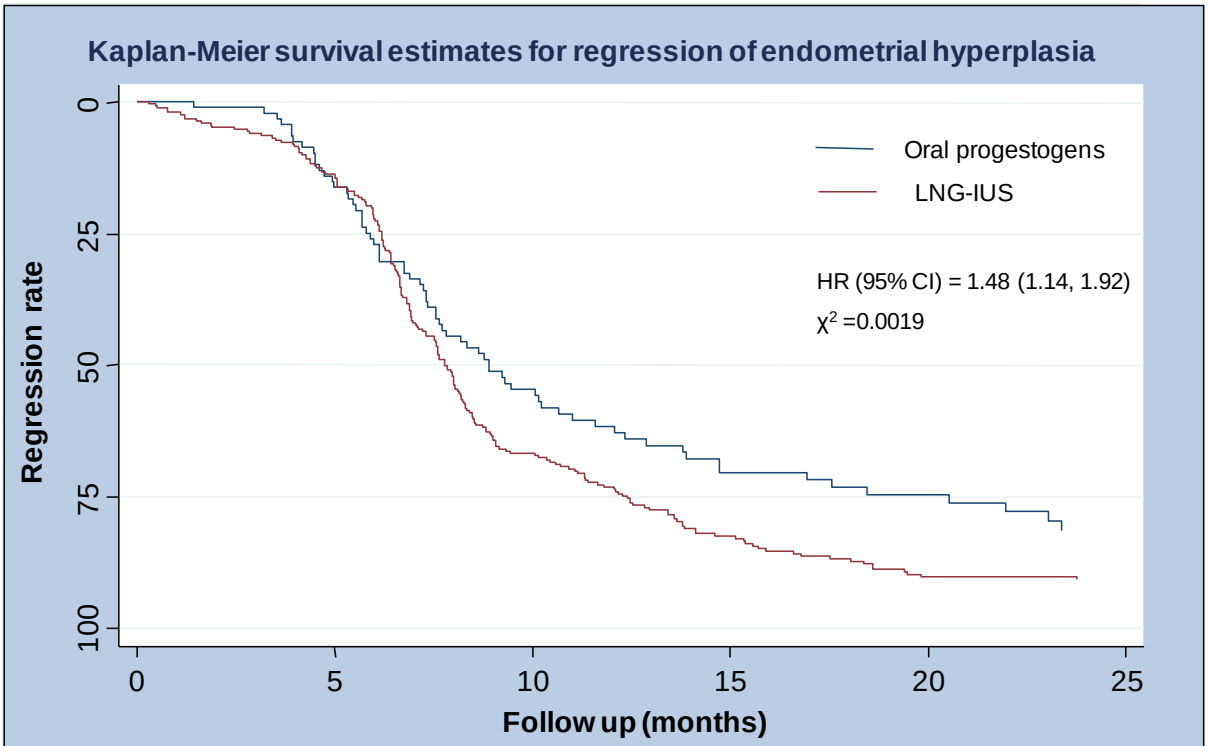
Table 7 Outcomes of patients treated with LNG-IUS compared oral progestogens

	LNG-IUS (n=250)	**Oral Progestogens** (n=94)	*P* value	Odds ratio	Adjusted Odds ratio
	n (%)	n (%)			
Time from diagnosis to last histological follow up (months)	Mean 66.9 ± SD 35.1	Mean 87.2 ± SD 45.5	<0.001		
Regression of hyperplasia	237/250 (94.8)	79/94 (84)	0.001	3.46 (1.58-7.59)	3.04 (1.36-6.79)
- Complex hyperplasia	221/229 (96.5)	73/81 (90.1)			
- Atypical hyperplasia	16/21 (76.2)	6/13 (46.2)			
Hysterectomy Performed	55/250 (22.1)	35/94 (37.2)	0.004	0.48 (0.29-0.8)	0.48 (0.28-0.81)
Cancer diagnosed	6/250 (2.4)	4/94 (4.3)	0.361		

Regression rates were higher for CH compared to ACH for both LNG-IUS (96.5%, 221/229 vs. 76.2%, 16/21; p≤0.001) and oral progestogens (90.1%, 73/81 vs. 46.2%, 6/13; p≤0.001). Hysterectomy rates were also significantly lower in the LNG-IUS group compared to the oral group during follow up (22.1%, 55/250 vs. 37.2%, 35/94, OR=0.48, 95% CI 0.29–0.8, p<0.004). From the total of 10 women (4 CH, 6 ACH) diagnosed with EC during follow up, 7 were originally treated with LNG-IUS (6/250, 2.4%) and 4 with oral progestogens (4/94, 4.3%; p=0.361). They were all found to be at early stage EC (Stage Ia, G1 for 5 women and Ib G1 for 4 women) apart from one woman who was diagnosed with endometrioid cancer of the ovary (Stage Ib) according to the latest FIGO classification.[104] The 28 women that did not achieve regression were strongly recommended to undergo hysterectomy from which 24

eventually underwent this procedure in a median time of 12.9 months from diagnosis (IQR 10.1 to 16.4 months) and 8 were diagnosed with EC on the hysterectomy specimens (33.3%, 8/24). From the remaining four women, one is well and undergoing assisted reproduction treatment, two declined further biopsies and are currently undergoing long term clinical follow up only and one was lost to follow up after 18 months. On logistic regression, age was found to be independently correlated with both treatment modality and the regression outcome. As a potential confounder we adjusted the odds ratio for outcomes of EH regression and hysterectomy (Table 7).

Figure 13 Kaplan-Meier survival curves for all events of EH regression in women treated either with LNG-IUS or oral progestogens. CI = confidence interval; HR= hazard ratio.

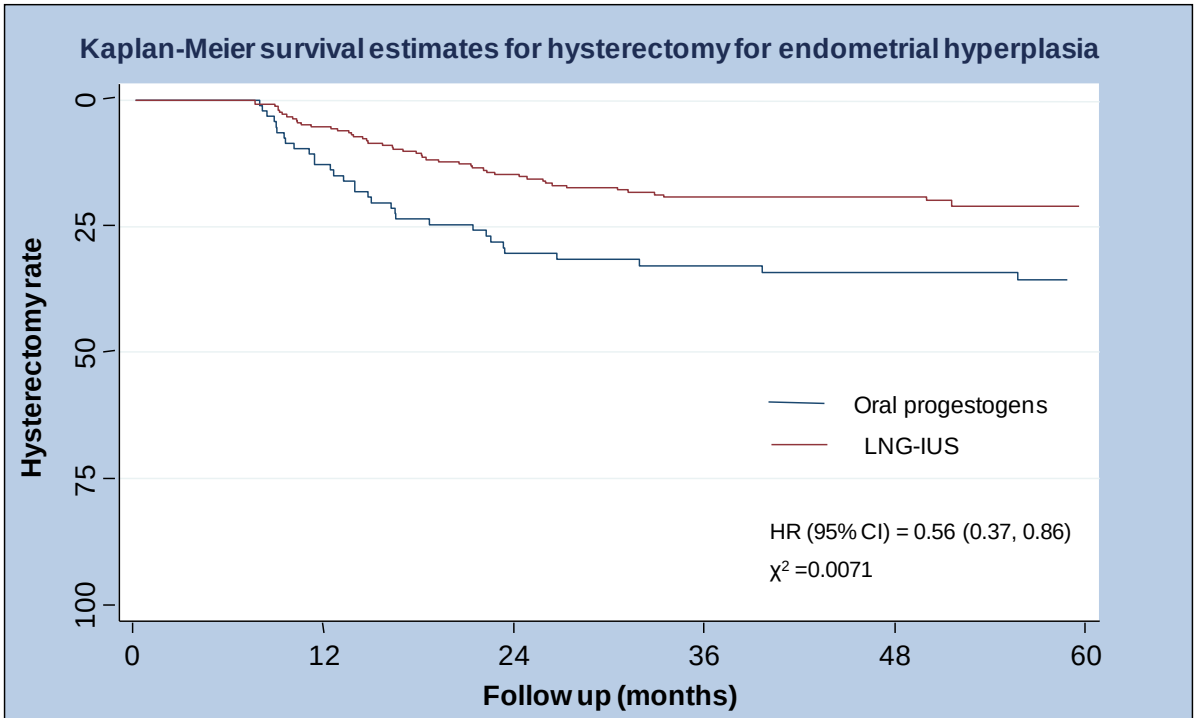


Women with hyperplasia by therapy	0 months t with hyperplasia	12 months n regressed/t (%)	18 months n regressed/t (%)	24 months n regressed/t (%)
LNG-IUS	250	182/250 (72.8)	213/250 (85.2)	221/250 (88.4)
Oral progestogens	94	58/94 (61.7)	67/94 (71.3)	72/94 (76.6)
Total	344	240/344 (69.8)	280/344 (81.4)	293/344 (85.2)

The survival analysis indicates that regression was higher with LNG-IUS at 12, 18 and 24 months of follow up (Hazard ratio 1.48, 95% CI 1.14-1.92, p=0.002; Figure 13). The majority of the women achieved regression by 24 months and specifically it was achieved in 93.6% (221/236) of women treated with LNG-IUS and 91.1% (72/79) of women treated with oral progestogens by this time point. The survival analysis for hysterectomy indicates that hysterectomy was less likely to happen in women treated with LNG-IUS from 12 up to 60

months of follow up (Hazard ratio 0.56, 95% CI 0.37-0.86, p=0.007; Figure 14). Specifically, by 60 months, 22% of women (55/250) treated with LNG-IUS underwent hysterectomy compared to 37.2% of women (35/94) treated with oral progestogens.

Figure 14 Kaplan-Meier survival curves for all events of hysterectomy for EH in women treated either with LNG-IUS or oral progestogens. CI = confidence interval; HR= hazard ratio; hyst.=hysterectomy.



Therapy	0 months Total	12 months n hyst./t (%)	24 months n hyst./t (%)	36 months n hyst./t (%)	48 months n hyst./t (%)	60 months n hyst./t (%)
LNG-IUS	250	13/250 (5.2)	36/250 (14.4)	46/250 (18.4)	46/250 (18.4)	55/250 (22)
Oral progestogens	94	12/94 (12.8)	28/94 (29.8)	30/94 (31.9)	31/94 (33)	35/94 (37.2)
Total	344	25/344 (7.3)	64/344 (18.6)	76/344 (22.1)	77/344 (22.4)	90/344 (26.2)

Discussion

To our knowledge, this is the largest study with the longest follow up period examining the efficacy of LNG-IUS in the treatment of EH and comparing it with the current standard treatment of oral progestogens. The results of our study have shown that complete regression

of EH was achieved in a higher proportion of women treated with LNG-IUS (95%) compared to women treated with oral progestogens (84%) with, consequently, lower rates of hysterectomy (22% versus 38%). Women failing to regress to normal histology had a high risk of cancer diagnosis at the time of the hysterectomy, which was up to 33%.

The long term follow up provides valuable information about the efficacy of treatment modalities for CH and ACH. The inclusion of the vast majority of eligible women and the size of this study eliminates potential selection bias. We achieved a very high percentage of follow up (>95%) for our primary outcome of endometrial regression at 12 months and we reduced potential follow-up bias. We also measured and adjusted for a large number of potential confounding factors. The observational design, though, cannot exclude residual confounding from unmeasured variables. Follow-up differed in the two groups and the retrospective inclusion of women treated with oral progestogens has introduced performance and verification bias. Despite the retrospective recruitment of women treated with oral progestogens, women were recalled for follow up by contacting them through their primary care clinicians. This reduced the amount of missing data and increased our follow up rate up to 95%. Our follow up strategy with endometrial sampling on a six-monthly basis for the first two years and yearly thereafter ensured robust surveillance. Pragmatic follow up visits were arranged on a patient-to-patient basis at variable time intervals, but the majority of women were followed up at least yearly.

The efficacy of LNG-IUS has been assessed in a few former studies, and is consistent with our findings. These studies have all reported a regression rate above 90%.[15,16] A study

previously published from our centre showed that the regression rate of 109 patients treated with LNG-IUS was 92%.[31] Ultimately, we found that despite a larger cohort, our results for the regression rate with LNG-IUS are still consistent with those previously published from our centre. In terms of comparing LNG-IUS efficacy with other therapies, there is only one other observational study which has examined the efficacy of LNG-IUS versus oral progestogens[32]. Orbo et al studied the regression rate of oral progestogens (54%) and LNG-IUS (100%).[32] This study used a different classification system to assess the degree of hyperplasia, which makes it difficult to correlate their outcomes with the WHO classification criteria.[7] This study also used low dosages of oral progestogens (medroxyprogesterone 10mg/day cyclical - 10 day use/cycle), which may account for the lower rates of regression observed.

We believe that the difference of regression and relapse rates of LNG-IUS over oral progestogens for the treatment of EH found in our study can be explained by the mode of progestogen delivery as explained before. Additional issues of compliance (100% with LNG-IUS) and adverse effects such as nausea, weight gain, headaches, thrombophlebitis and hypertension, also limit the overall efficacy of oral progestogens. The LNG-IUS is associated with higher patient satisfaction and, therefore patients are more likely to continue the treatment.[105] This higher chance of patients continuing the LNG-IUS treatment may also explain its better efficacy in treating EH compared to oral progestogens. In addition, the duration of the treatment appears also to be an important factor for achieving disease regression and avoiding hysterectomy. The LNG-IUS provides a standard daily dose of progestogens for five years, where the oral progestogen treatment is likely to be discontinued

by clinicians following evidence of disease regression. Interestingly, the duration of treatment with oral progestogens was usually six months (64%) and occasionally extended up to 12 months (6%), but regression continued during follow up and in some cases, when the treatment was actually stopped. Spontaneous regression of SH and CH, even with no treatment, has been described before,[4] and a study observed a spontaneous regression rate up to 50% during follow up.[32] This is likely to occur in perimenopausal women as they become menopausal and cease their anovulatory cycles causing oestrogen decline.

Overall, the use of LNG-IUS for EH is found to be associated with higher regression and lower hysterectomy rates compared to oral progestogens. This study suggests that LNG-IUS should be offered as initial treatment and only patients that decline it should be offered oral progestogens as an alternative. However, we advise caution on the interpretation of this finding as the follow-up differed in the two groups and we cannot rule out unmeasured residual confounding. In view of the relatively high underlying EC rates in non-regression cases (up to 33%), we recommend that women undergo hysterectomy if six-monthly histological surveillance within 24 months from diagnosis fails to indicate regression. The women with ACH are particularly at high risk and should be monitored more carefully. The excellent efficacy of LNG-IUS also makes it less justifiable to offer hysterectomy as a first line treatment for patients with CH. Further research should be directed in identifying prognostic factors that could help recognise patients that are less likely to respond to LNG-IUS treatment. This would facilitate careful patient selection for the LNG-IUS and will reduce the proportion of hysterectomies performed unnecessarily.

Chapter 6: Relapse of EH after conservative treatment: A cohort study with long term follow up.

Abstract

Objective

The LNG-IUS and oral progestogens are used to treat women with endometrial hyperplasia and achieve regression. There is uncertainty on further surveillance for those women as the risk for relapse is unknown. Our objective in this study was to determine the risk of relapse for women with EH treated with LNG-IUS or oral progestogens?

Methods

A cohort study of 219 women with CH or ACH that were treated and achieved initial regression with LNG-IUS (n=153) or oral progestogens (n=66) from August 1998 until December 2007 and followed up for more than five years. Mean length of follow up in the two groups was 74.7 ± SD 31.8 months for the LNG-IUS versus 87.6 ± SD 42.2 months for the oral progestogen group. We evaluated the proportion of women that relapsed or had hysterectomy after initial regression with LNG-IUS compared to oral progestogens by logistic regression adjusting for confounding. The time from regression to relapse was explored through a survival analysis.

Results

Relapse of EH occurred in 13.7% (21/153) of women treated with LNG-IUS compared to 30.3% (20/66) of women treated with oral progestogens (OR=0.37, 95% CI 0.18–0.73, p=0.005). Relapse rates over long term follow up were lower for CH compared to ACH for both LNG-IUS (12.7%, 18/142 vs. 27.3%, 3/11; p=<0.001) and oral progestogens (28.3%,

17/60 vs. 50%, 3/6; p≤0.001). The survival analysis indicates that relapse occurred less often with LNG-IUS at 12, 24, 36, 48, 60 and more than 60 months of follow up (Hazard ratio 0.37, 95% CI 0.2-0.7, p=0.001). There were no events of relapse after 48 months from regression with oral progestogens, but some women treated with LNG-IUS relapsed after 60 months when treatment was discontinued. Hysterectomy rates were lower in the LNG-IUS group during follow up (19.6%, 30/153 vs. 31.8%, 21/66, OR=0.52, 95% CI 0.27–1, p=0.05). EC was diagnosed in 2 (11.8%) women that had hysterectomy (n=17) because of relapse. We were unable to accurately estimate the cancer risk in women who relapse during follow up as only 17 out of 41 who relapsed underwent hysterectomy.

Conclusion

Relapse of endometrial hyperplasia after initial regression occurs often and long term follow up is advised.

Introduction

In our meta-analysis we found that the LNG-IUS achieves regression in up to 92% of women.[95] This meta-analysis finds that the regression with LNG-IUS is higher than with oral progestogens and as a result it has the potential to reduce the number of hysterectomies performed for this condition.[95] In our own comparative cohort study we have discovered that the regression with LNG-IUS is more likely and this is the reason for fewer hysterectomies for women treated with LNG-IUS.[8] The mainstay treatment for ACH is hysterectomy as explained before because of the high risk of progression to cancer and the possibility of concomitant cancer in women undergoing hysterectomy. Hormonal therapies have also been used to treat ACH in young women that wish to preserve their fertility and it may be the only option for women with severe comorbidities.

Despite the initial regression of EH with hormonal therapies, we advised caution because of the possibility of relapse.[106] In a meta-analysis for young women with ACH treated with hormones the summary estimate of the relapse rate was about 26%.[106] Considering that the majority of the studies in the literature have short durations of follow up the risk of relapse of ACH following an initial regression may even be higher. On the other hand, the majority of clinicians treat women with CH with hormonal therapies, but the risk of relapse for these women remains unknown.[106] This prevents many clinicians from embarking on long term follow up. Even though, it is known from a case-control study that women diagnosed with CH are at higher risk of progression to cancer than healthy women, the risk of relapse and a strategy for following up these women remains to be defined.[107] In this study, we have

conducted a cohort study with more than five years follow up for defining the relapse risk for women with CH and ACH treated with LNG-IUS or oral progestogens.

Methods

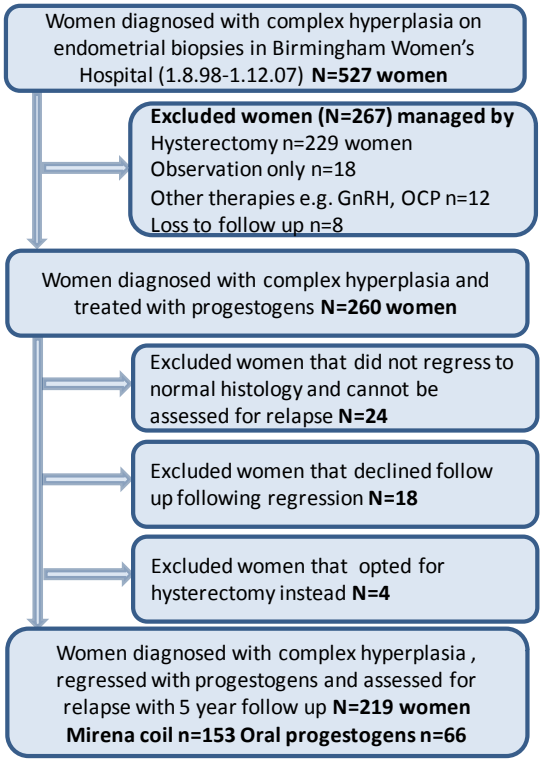
This was a comparative observational study. We included all women diagnosed with CH or ACH that underwent treatment with LNG-IUS or oral progestogens from August 1998 until December 2007 in a tertiary referral Hospital in Birmingham, UK. Patient selection has been described in detail in Chapter 5. We opted to include women until December 2007 to ensure at least five year follow up for all participants. Following initial regression, women were followed yearly thereafter for five years to ascertain if relapse occurred and then the patients were given a choice to have continued yearly surveillance.

The primary outcome for this study was to determine the proportion of women with CH or ACH that had a relapse of EH or cancer after showing histological regression following treatment with LNG-IUS compared to oral progestogens. Our follow up policy aimed to ensure a maximum rate involving also primary care clinicians. For this assessment, the results of follow-up histological examinations following the initial regression were classified as 1) *Complete Regression* – atrophy of glands, oedematous fibrotic stroma or pseudodecidualisation, with no evidence of hyperplasia. 2) *Relapse* – failure to remain in regression with evidence of CH, ACH or carcinoma. The secondary outcomes we studied were the hysterectomy rate for each treatment, the time interval from regression to relapse and the proportion of patients in both groups diagnosed with endometrial cancer during

follow-up. All outcomes were evaluated with an intention to treat basis. The statistical section is already described in Chapter 5.

Results

Figure 15 Schematic representation of patients included in study analysis



During the study period, 527 women were diagnosed with CH or ACH and 260 were treated with progestogens (Figure 15). We have excluded women that failed to achieve regression after progestogen treatment (n=24). We have also excluded women that did not accept long term follow up following their initial regression (n=18) or opted for hysterectomy (n=4). As a result, we have included 219 women in our study from which, 66 were treated with oral progestogens and 153 with LNG-IUS. Table 8 shows the baseline characteristics of the women according to the type of treatment. Women treated with oral progestogens were older

and more likely to be menopausal. The mean follow up in the two groups was 74.7 ± SD 31.8 months for the LNG-IUS group and 87.6 ± SD 42.2 months for the oral progestogen group.

Table 8 Baseline characteristics of women regressed with LNG-IUS or oral progestogens and assessed for relapse

		LNG-IUS (n=153)	**Oral Progestogens** (n=66)	*P* value
		n (%)	n (%)	
Age (years)		Mean 53 ± SD 10.1	Mean 50.4 ± SD 11.5	0.091
Parity		Mean 2.2 ± SD 1.4	Mean 2 ± SD 2.2	0.533
BMI (kg/m^2)		Mean 33.3 ± SD 10.2	Mean 32.5 ± SD 9	0.629
Endometrial thickness on USS (mm) for menopausal women		Mean 10.2 ± SD 6.1	Mean 11.2 ± SD 7.7	0.377
Ethnic Group	Caucasian	116 (75.7)	49 (74.3)	
	Asian	19 (12.5)	9 (13.6)	
	Other	10 (6.6)	7 (10.6)	
	Unknown	8 (5.2)	1 (1.5)	0.75
Menopausal Status	Premenopausal	75 (49)	46 (69.7)	
	Postmenopausal	78 (51)	20 (30.3)	0.005
Hypertensive		58 (37.9)	19 (28.8)	0.195
Diabetic		23 (15)	9 (13.6)	0.788
HRT / Tamoxifen use in last 5 years	None	121 (79.1)	56 (84.9)	
	HRT	30 (19.6)	8 (12.1)	
	Tamoxifen	2 (1.3)	2 (3)	0.36
Endometrial Histology	Atypical hyperplasia	11 (7.2)	6 (9.1)	
	Complex hyperplasia	142 (92.8)	60 (90.9)	0.629

The relapse rate following regression with LNG-IUS treatment was 13.7% (21/153) and it was higher for ACH (27.3%, 3/11) than for CH (12.7%, 18/142). The relapse rate following regression with oral progestogens was 30.3% (20/66) and similarly it was higher for ACH (50%, 3/6) than for CH (28.3%, 17/60). The difference in relapse rates was significant

between LNG-IUS and oral progestogens (p=0.004) and this was confirmed when adjusted for menopause (adjusted OR= 0.34, 95% CI 0.17-0.7, Table 9). As a result, there were less hysterectomies performed with LNG-IUS treatment (19.6%, 30/153) compared to oral progestogens (31.8%, 21/66, p=0.05). Overall, 41 women relapsed during follow up and were offered hysterectomy. Only 17 women underwent hysterectomy and two were diagnosed with endometrial cancer (11.8%). One woman initially diagnosed with CH and treated with LNG-IUS progressed to endometrioid cancer with concomitant granulosa cell tumour of the ovary after 36 months from initial regression. Another woman initially diagnosed with ACH and treated with oral progestogens progressed to Stage Ia endometrioid cancer after six months from initial regression.

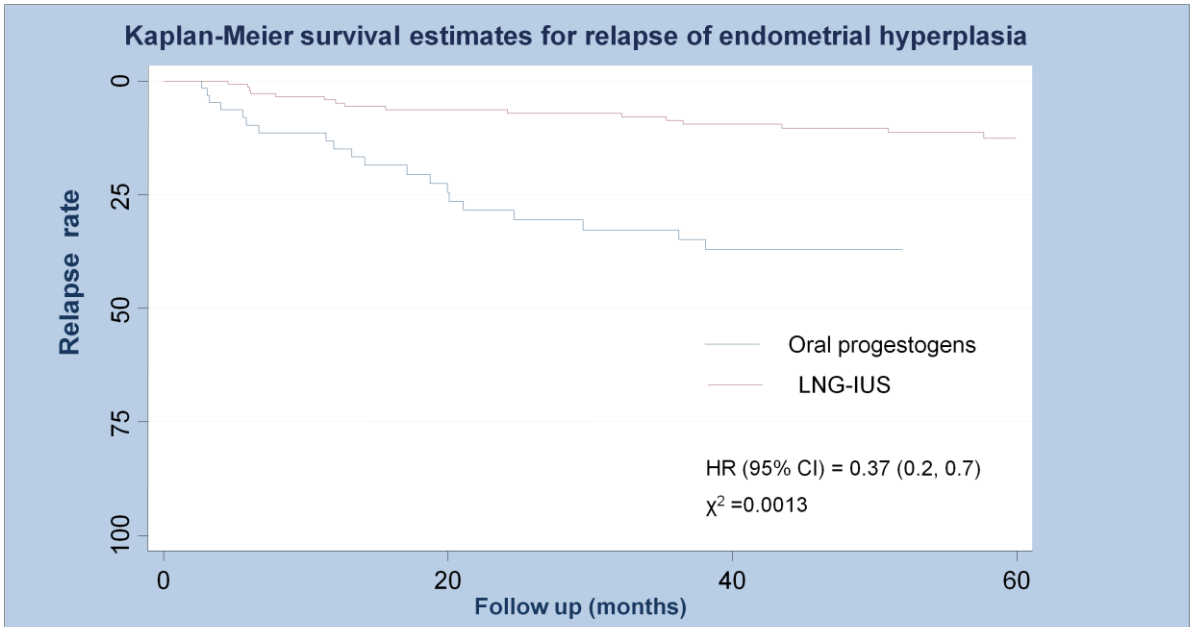
Table 9 Outcomes of patients assessed for relapse following initial regression after treatment with LNG-IUS or oral progestogens

	LNG-IUS (n=153)	**Oral Progestogens** (n=66)	*P* value	Odds ratio (95% CI)	Adjusted Odds ratio (95% CI)
	n (%)	n (%)			
Time from diagnosis to last histological follow up (months)	Mean 74.7 ± SD 31.8	Mean 87.6 ± SD 42.2	0.066		
Relapse of hyperplasia	21/153 (13.7)	20/66 (30.3)	0.005	0.37 (0.18-0.73)	0.34 (0.17-0.7)
- Complex hyperplasia	18/142 (12.7)	17/60 (28.3)			
- Atypical hyperplasia	3/11 (27.3)	3/6 (50)			
Hysterectomy Performed	30/153 (19.6)	21/66 (31.8)	0.05	0.52 (0.27-1)	0.49 (0.25-0.97)
Cancer diagnosed	1/153 (0.65)	1/65 (1.5)	0.539		

The survival analysis on Figure 16 indicates that relapse occurs less frequently after initial regression with LNG-IUS treatment than with oral progestogens over the five year follow up (HR=0.37, 95% CI 0.2 to 0.7, p=0.001). Relapse occurs also sooner with oral progestogens. Relapse occurred at a median time of 32.2 months ± IQR 11.3 to 57.7 months following

LNG-IUS treatment compared to 13.7 ± IQR 5.7 to 20.6 months following oral progestogens. No relapse was observed after 48 months from initial regression with oral progestogens compared to LNG-IUS where five women relapsed after the five year period when this treatment was discontinued.

Figure 16 Kaplan-Meier survival curves for all events of EH relapse in women initially regressed after treatment with LNG-IUS or oral progestogens. CI = confidence interval; HR= hazard ratio.



Cumulative event rates of relapse by fixed time points

Therapy	12 months n relap./t (%)	24 months n relap./t (%)	36 months n relap./t (%)	48 months n relap./t (%)	60 months n relap./t (%)	>60 months n relap./t (%)
LNG-IUS	6/153 (3.9)	9/153 (5.9)	12/153 (7.8)	14/153 (9.2)	16/153 (10.4)	21/153 (13.7)
Oral progestogens	9/66 (13.6)	16/66 (24.2)	18/66 (27.3)	20/66 (30.3)	20/66 (30.3)	20/66 (30.3)
Total	15/219 (6.8)	25/219 (11.4)	30/219 (13.7)	34/219 (15.5)	36/219 (16.4)	41/219 (18.7)

Discussion

Management of EH with progestogens is aimed to induce endometrial regression and prevent women from undergoing hysterectomy. This may be particularly appealing to young women wishing to preserve their fertility or women with multiple comorbidities who are poor surgical candidates. However, relapse of ACH or CH following treatment with progestogens

is common. The risk is higher when women are treated with oral progestogens compared to LNG-IUS and results in more hysterectomies. However, the differences in patient selection and subsequent follow up, similarly to Chapter 5, may have introduced performance and differential verification biases. Despite this limitation, this study is unique in the literature as it follows up women for more than five years and covers the period from their initial regression following progestogen treatment to the time of relapse. Our data suggest that this risk is high and discontinuing follow up after an initial regression is not justified. Women treated with oral progestogens relapse earlier and no further events were recorded after 48 months from the initial regression. Women treated with LNG-IUS may relapse after five years when the LNG-IUS treatment is stopped and therefore we propose that if a replacement LNG-IUS is not carried out then these patients should be followed up for at least a further year. Women that relapsed during follow up were diagnosed with endometrial cancer in up to 12% of cases.

The design of our study and the long term follow up provides valuable information about the risk of relapse and aids the follow up strategy for these women. An important limitation also discussed previously is the differences in inclusion of women in the two groups and subsequent differences in their follow up that may have contributed to the observed differences in relapse rates. However, we minimised missing follow-up data for the women treated with oral progestogens before August 2008 by recalling them for long term surveillance. We achieved a high percentage of follow up for relapse rate at 12, 24, 36, 48 and 60 months and we reduced potential follow-up bias. From our intended sample size we achieved a 98% follow up for the relapse rate at 12 months, 94% for 24 months, 88% for 36

months, 79% for 48 months and 73% for 60 months. We also measured and adjusted for a large number of potential confounding factors. The observational design, though, cannot exclude residual confounding from unmeasured variables, such as change of weight during follow up or new onset of diabetes. We were also unable to accurately estimate the risk of cancer in women who relapse during follow up as only 17 out of 41 underwent hysterectomy.

The LNG-IUS is the treatment of choice for ACH and CH but oral progestogens remain popular among clinicians.[96] It has been accepted practice to treat until an endometrial regression is confirmed histologically. Following this confirmation there was uncertainty whether they warrant further follow up. The literature is scarce on the optimum follow up strategy and guidelines are lacking. Previous studies have concentrated mostly on the time taken for women with ACH or CH to regress to normal endometrium.[31,32] Two small studies did not report any diagnoses of endometrial cancer during follow up but did not specifically report on the risk of relapse during follow up.[32;47] However, a case control study found that women with a previous diagnosis of ACH or CH were at higher risk of developing endometrial carcinoma over the long term, which may be up to 21 times higher than the average population risk.[107] This may be justified from the high risk of relapse of endometrial hyperplasia following initial treatment.

The difference of relapse rates of LNG-IUS over oral progestogens for the treatment of EH found in our study can be explained by the duration of treatment. The LNG-IUS provides a standard daily dose of progestogens for five years, whereas the oral progestogen treatment is likely to be discontinued by clinicians following evidence of disease regression. In our

cohort this was commonly at six months. Despite stopping the progestogen treatment we did not observe any cases of relapse after 48 months. This is in contrast to a few relapse events after discontinuing LNG-IUS treatment at five years. We are unable to explain this difference between the oral and LNG-IUS groups but this should highlight to the clinicians that relapse may occur after stopping LNG-IUS treatment after a five year period. It is envisaged that if the precipitating cause for EH cease to exist during follow up, such as HRT, it is unlikely that EH will reoccur. In other cases, if the cause is not abolished as it is often the case with obese women, the high oestrogen concentrations may be causal to the relapse of EH. Further research should focus on predictors to identify women at high risk of relapse and prioritise their long term follow up.

To conclude, this study indicates that relapse for women with ACH or CH treated with progestogens is common. Discontinuing follow up following an initial regression is not justified and should be continued for at least five years and particularly so after LNG-IUS treatment is stopped. Women who relapse during follow up should be subjected to hysterectomy as there may be underlying undiagnosed cancer.

SECTION II PROGNOSIS OF ENDOMETRIAL HYPERPLASIA

Chapter 7: Prognostic significance of ER and PR expression in LNG-IUS treatment of EH: an immunohistochemical study.

Abstract

Objective

To identify immunohistochemical (IHC) predictors of persistent EH when treated with LNG-IUS.

Methods

We performed IHC of ER, PR, PTEN and aromatase in EH treated with LNG-IUS and explored their prognostic significance. The baseline pre-treatment EH of a selected prospective cohort was analysed [CH (n=29) and ACH (n=5)]. Study participants were categorised into those that showed endometrial regression (responders, n=28) and those that showed non-regression or histological progression to atypia or malignancy (non-responders, n=6). IHC expression was expressed as a histological score (HS).

Results

Responders compared to non-responders showed significantly higher histological scores for ER and PR. Absence of ER and PR predicted non-responder status with likelihood ratios of 9.33 (95% CI 2.19-39.81) and 2.92 (95% CI 1.47-5.79), respectively. Neither PTEN nor aromatase expression were associated with LNG-IUS therapy responsiveness.

Conclusion

Responsiveness of EH to LNG-IUS therapy may be determined through analysis of baseline oestrogen and progesterone receptors but these exploratory findings require confirmation in a larger dataset.

Introduction

The principal aims of medical treatment of CH are to prevent histological progression to cancer, induce endometrial regression, and minimise the frequency and amount of any abnormal uterine bleeding. From our study we have shown that LNG-IUS achieves such outcomes in more than 90% of the cases.[8] Given EH is an oestrogen-dependent proliferation, there is widespread consensus, supported by experimental data, that aberrant progesterone or oestrogen metabolism of the endometrium may be causal to the initiation, progression and malignant transformation of EH.[37] Differential expression of ER and PR could also contribute to the variation of efficacy in progestogen therapy of EH. Other molecular pathways have been implicated in the generation of benign and malignant uterine pathology and these include the involvement of aromatase and PTEN.[15,26] However, no studies have explored their association to the treatment or malignant progression of EH. We assessed the prognostic accuracy of ER, PR, aromatase and PTEN receptor expression for predicting the efficacy of LNG-IUS treatment of EH over long term follow up.

Methods

Thirty-four cases of CH and ACH were selected from a prospectively collected dataset of EH treated by LNG-IUS. The criteria for selection were: at least six months of endometrial follow up (either by endometrial biopsy or hysterectomy) following LNG-IUS commencement; availability of all pre-treatment baseline and interval treatment endometrial specimens; for each subject, all samples should have demonstrated, in a consistent manner without histological reversion, either endometrial regression or persistent hyperplasia or histological upgrading. Due to the overall small number of non-responders and cases with

atypia these cases were actively included in the study population as we felt they provided valuable information. All other cases were selected at random, regardless of demographics or other clinical information.

The prospective dataset has been previously reported[31] and includes consecutively prospectively recruited women with EH treated by LNG-IUS whose treatment commenced from 1999 until 2004. Endometrial sampling was performed on a three to six-month basis for the first year and yearly thereafter for five years. The interpretation of baseline pre-LNG-IUS treatment and interval treated hyperplasia histologies were made by two experienced gynecological pathologists according to WHO criteria.[7]

The selected study women (n=34) were classified as either responders or non-responders. Responders were defined as subjects showing endometrial regression with no evidence of continuing hyperplasia. Endometrial regression has been previously defined and denotes progestogen effects on the endometrium which includes: gland atrophy, glands separated by plump polygonal pseudodecidualised stromal cells and epithelial metaplasia. Non-responders were defined as subjects showing persisting hyperplasia and/or histological upgrading to ACH or EC.

Six 10 µm thick sections were cut from paraffin-embedded tissue blocks from baseline before LNG-IUS therapy EH tissue specimens for each selected study participant. Immunostaining was performed upon five sections and the remaining section underwent standard haematoxylin and eosin staining. Immunostaining was performed using Dako Autostainer

and Dako detection kit K5007 (Dako, Glostrup, Denmark) according to the manufacturer's protocol. De-paraffinisation and antigen retrieval was achieved with W-CAP (Surgipath Europe Ltd) pH 8.0 in water bath at 98°C, apart from PTEN, which was immersed in Dako Low pH (pH 6.0) antigen retrieval fluid and heated to 98°C in water bath. Slides were incubated for one hour with primary antibodies. Mouse monoclonal anti-progesterone receptors (Form-A and Form-B, diluted 1:50) were purchased from Novocastra, Newcastle upon Tyne, UK. Mouse monoclonal anti-oestrogen receptor –α (diluted 1:150) was purchased from Dako, Glostrup, Denmark. Mouse monoclonal anti-aromatase (diluted 1:50) and rabbit monoclonal anti-PTEN were purchased from Serotec, Oxford, UK and Abcam, Cambridge, UK, respectively. All antibodies were diluted using Dako Universal Antibody Diluent. Endometrial controls for each antibody from the same patient were processed and analysed in parallel with immunostained endometrial samples. Positive normal proliferative endometrium controls were utilized for ER, PR, and PTEN and as a negative control for aromatase expression.

For ER, PR, glandular and stromal staining was recorded using a semi-quantitative histological score (HS). Aromatase expression in glandular cytoplasm was scored as absent or present. Staining with PTEN was assessed for the absence or presence of PTEN-null glands. The HS incorporates both the intensity and the distribution of specific staining and its methodology has been validated by another group.[108] The HS equates to Σ (Pi x i)/100, where Pi denotes the percentage of stained cells and i denotes the intensity of the staining ranging. We used a modified version of the HS, which we developed, such that the intensity of staining (i) was scored in an ordinal manner 0–3: the group assigned 3 displayed strong

staining intensity compared to the corresponding normal endometrium; the group assigned 2 displayed moderate staining intensity, equivalent to normal endometrium; the group assigned 1 displayed weak staining intensity; and the group assigned 0 was negative for staining. To interpret the results in an easier manner we subdivided the percentage staining (Pi) into four ordinal groups: 0 (<5% of cells), 1 (5-25% of cells, very focal), 2 (25%-75% of cells, focal) and 3 (>75% of cells; diffuse). Local ethics approval was obtained from the South Birmingham Research and Ethics Committee prior to the commencement of this study (LREC 2002/057).

The level of intra-observer agreement was calculated using Cohen's kappa index ± Standard Error (k ± SE). The strength of association for biomarker expression and responder/non-responder status to LNG-IUS therapy was analysed using non-parametric tests (Mann Whitney U and chi-squared tests). Corresponding likelihood ratios (LR) were generated based on the presence or absence of histological biomarker expression. All statistical tests were two-sided at the 5% level of significance and were performed using SPSS Version 16.0 for Windows (Release 16.0.1, 15 Nov 2007, SPSS Inc.).

Results

The baseline characteristics of the study group [CH (n=29) and ACH (n=5)] are shown in Table 10. The median follow-up for all study participants was 26 months (95% CI 23.1-36.8). The agreement regarding histological scoring between the two histopathologists was 84.5% (K statistic = 0.811 ± 0.026).

Table 10 Baseline characteristics*

Age	Mean 51.9; Standard Deviation 9.1; Range 36-77
BMI (kg/m^2)	Mean 32.6; Standard Deviation 7.6; Range 21-49
Parity	Mean 1.8; Standard Deviation 1.5; Range 0-6
Nulliparous	9 (26.5)
Menopausal	18 (52.9)
Hypertensive	14 (41.2)
Diabetic	5 (14.7)
Non-atypical complex	29 (85.3)
Atypical complex	5 (14.7)

*Values in parentheses are percentages unless stated otherwise

The majority of study participants responded to LNG-IUS therapy (n=28 responders; n=6 non responders). The median time interval for responders was 9.9 months (95% CI 4.8-14.9). Of the six non-responders: four demonstrated persistent hyperplasia, one upgraded from CH to ACH (at five months of LNG-IUS therapy) and one upgraded from ACH to well-differentiated FIGO Stage Ia EC (at 10 months of LNG-IUS therapy). Hysterectomies were performed in all six non-responders.

Table 11 The correlation of the baseline pre-treatment expression of biomarkers to LNG-IUS therapeutic responsiveness

		Response to LNG-IUS		
		Responders (n = 28)	**Non-responders (n = 6)**	***p* value***
Histology	Complex	25 (86.2)	4 (13.8)	0.205
	Atypical	3** (60)	2 (40)	
ERα	Present	26 (92.9)	2 (7.1)	**0.004**
	Absent	2 (33.3)	4 (66.7)	
PR (A or B)	Present	20 (95.2)	1 (4.8)	**0.021**
	Absent	8 (61.5)	5 (38.5)	
Aromatase	Present	6 (100)	0	0.562
	Absent	22 (78.6)	6 (21.4)	
PTEN	Present	17 (81)	4 (19)	0.475
	Absent	11 (84.6)	2 (15.4)	

Statistically significant associations are in bold and values in parentheses are percentages

*p value was calculated by χ^2 test or Fisher's exact test, where appropriate, p value < 0.05 is considered significant,

**Three out of five women with atypical hyperplasia with complete response to LNG-IUS had strong ERα and PR (A, B) expression.

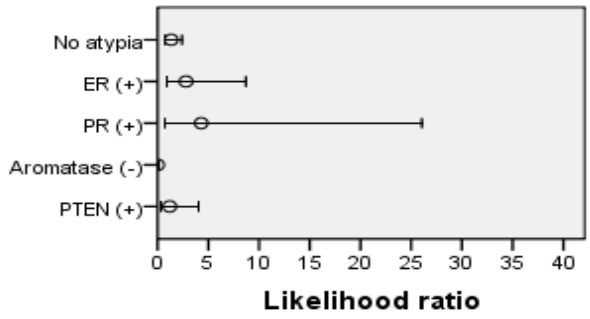
Responders exhibited significantly higher quantitative HS for ER (2.21 ± SD 0.9 vs. 1.04 ± SD 1.05, p=0.026), PRA (2.04 ± SD 0.92 vs. 1.12 ± 0.92, p=0.042) and PRB (1.96 ± SD 0.92 vs. 0.88 ± SD 0.89, p=0.011) compared to the non-responders. The absence or presence of atypia, ER, PR (either A or B), aromatase, PTEN expression in relation to responder or non-responder status is depicted in Table 11. Responders exhibit significantly higher proportions of ER and PR expression than non-responders. Histological atypia (compared to non-atypia),

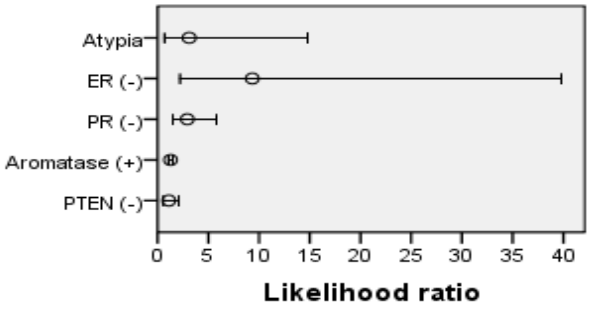
presence of aromatase, or presence of PTEN, were not significantly associated with LNG-IUS responsiveness (Table 12). The absence of ER and PR expression predicted non-responder status with likelihood ratios of 9.33 (95% CI 2.19-39.81,) and 2.92 (95% CI 1.47-5.79), respectively (Figure 12).

Table 12 Prediction of LNG-IUS therapeutic response

Markers for responders to LNG-IUS*	LR	Lo CI 95%	Up CI 95%
No atypia	1.34	0.75	2.39
ERα (+)	2.79	0.89	8.68
PR (+)	4.29	0.71	26.04
Aromatase (-)	-	-	-
PTEN (+)	1.18	0.35	3.99
Markers for non-responders to LNG-IUS*	**LR**	**Lo CI 95%**	**Up CI 95%**
Atypia	3.11	0.66	14.76
ERα (-)	**9.33**	**2.19**	**39.81**
PR (-)	**2.92**	**1.47**	**5.79**
Aromatase (+)	1.27	1.05	1.54
PTEN (-)	1.1	0.58	2.08

Statistically significant associations are in bold
Numbers are likelihood ratios with the respective confidence intervals
* (+) and (-) intend the presence or absence of the biomarker respectively





Discussion

This study demonstrates that responsiveness of EH to LNG-IUS therapy may be determined through analysis of baseline pre-treatment ER and PR status of the EH. The ER and PR status was found to be of a higher predictive value than the presence or absence of cytological atypia. Neither PTEN nor aromatase expression were associated with LNG-IUS therapy responsiveness. However, the conclusions are only applicable in the context of the

patient population described in this paper since the sample was not representative of the general population of EH patients. It should also be noted that the higher 18% non-response rate reported in this paper should not be assumed to be epidemiologically representative because of the selected sample and small number of cases.

Our study is original as we believe it to be the first study to explore prognostic biomarkers for EH treated by LNG-IUS. Selecting participants that possessed a mean duration of nearly two years of endometrial histology follow up, with consistent trends in their histological sampling analysis, helped to ensure greater validity and biological discrimination between responder and non-responder status with LNG-IUS therapy. The association of ER and PR expression to LNG-IUS responsiveness was observed in both semi-quantitative (histological scoring) and binary presence/ absence histological descriptor systems. This implies the observation is likely to be reliable. Furthermore, the study design incorporates the analysis of the pre-treatment EH specimen. This ensures the study design is pragmatic and clinically useful, as it intends to recreate the prognostic analysis that may be applied in future clinical management approaches for women with CH.

We accept there may be limitations in our study that lessens the reliability of our conclusions. Our study sample size is small and our estimate is likely to be unstable and also may be underpowered to detect statistically significant differences in biomarker expression. In particular, our absence of showing a prognostic role for aromatase or PTEN should not preclude exploration of their roles in future hyperplasia research. The small study sample size also precluded our ability to perform multivariate regression analysis to correct for

known confounding influences from socio-demographic (e.g. BMI, parity) factors and inter-relationships between biomarkers.

Our discovery of a prognostic association for ER and PR expression with EH is not unexpected and has biological plausibility. The unopposed action of oestrogen is causal to EH and the progestogens induce regression of EH by antagonising the oestrogen effect on the endometrium.[97] Ferenczy et al found the likelihood of response to progestogens to be directly related to the absence of cytological atypia.[36] Our study suggests that a molecular receptor-based classification for EH may have greater clinical prognostic value than that based on traditionally used cyto-architectural histological description (i.e. presence of atypia, complex architecture). Our study contrasts with previous studies that have failed to show a relationship between sex steroid receptor status and response to oral progestogens.[39] It is possible that the progestogen type, dosage, and method of drug delivery for these studies were inappropriate to achieve optimum endometrial regression, and the LNG-IUS used in our study may have superior therapeutic efficacy. Nilsson et al compared the levonorgestrel concentrations in the uterine mucosa by different therapeutic regimens.[33] The dose of levonorgestrel provided by the intrauterine device was reported to exceed that of the traditional systemic treatment by several-fold. In combination with presumed lower efficacy, the small sample sizes of the previously reported oral progestogen studies meant that, overall, they were unable to detect a significant relationship between sex steroid receptor expression and EH treatment responsiveness.

We accept there is a need for further confirmatory research to validate the findings of our exploratory study. It is important to identify the proportion of women who fail to respond to LNG-IUS and improve patient selection for this therapy. As a result, women at risk of treatment failure or malignant transformation may be offered closer endometrial surveillance or hysterectomy. Nonetheless, we believe this study may serve as an impetus for future research on the identification and evaluation of a clinical prognostic model for EH in order to improve health outcomes associated with this pre-malignant condition. Such a model may incorporate and integrate biodemographic parameters (e.g. age, BMI, parity), histological cyto-architecture classification and molecular phenotyping. Furthermore, identifying a particular 'molecular signature' may provide a better understanding of the aetiopathology of EH and help design novel treatment strategies (e.g. combination therapies) for both hyperplasia and uterine cancer.

Chapter 8: Prediction of regression and relapse of EH treated with LNG-IUS or oral progestogens: A cohort study.

Abstract

Objective To identify predictors and to estimate their prognostic accuracy for regression and relapse of endometrial hyperplasia treated with levonorgestrel-releasing intrauterine system or oral progestogens.

Methods This was a cohort study of women treated with levonorgestrel-releasing intrauterine system or oral progestogens for complex hyperplasia or atypical complex hyperplasia for women wishing to preserve their fertility or those who were unfit for surgery. Hazard ratios with the Cox proportional hazards model and Kaplan-Meier survival estimates for independent predictors were calculated.

Results

Regression was evaluated in 344 women over a 12-year period with a median follow-up of 58.8 months (interquartile range [IQR] 38.4–96.4, range 12–148.2) for levonorgestrel-releasing intrauterine system compared with 95.1 months (IQR 41.6–124.6, range 13.2–162) for oral progestogens. In women treated with levonorgestrel-releasing intrauterine system for complex hyperplasia, we found that 221 women regressed (96.5%, 221/229) and body mass index (BMI) 35 or higher was associated with failure to regress (hazard ratio [HR] 5.51, 95% confidence interval [CI] 1.05–28.87, p=0.043). Relapse was evaluated in 219 women over a 9-year period with median follow-up of 67 months (IQR 50.4–103.5, range 14.5–146.4) for levonorgestrel-releasing intrauterine system and 96.8 months (IQR 62.3–122, range 6–151.5) for oral progestogens. In women treated with levonorgestrel-releasing intrauterine system for

complex hyperplasia, we found that 124 women relapsed (87.3%, 124/142) and BMI 35 or higher was found to be a strong independent predictor of relapsed endometrial hyperplasia (HR 18.93, 95% CI 3.93–91.15, p<0.001). Only 3.3% of women with complex hyperplasia treated with levonorgestrel-releasing intrauterine system and with BMI less than 35 relapsed during long term follow-up compared with 32.6% of women with BMI 35 or higher.

Conclusion

BMI 35 or higher is strongly associated with failure to regress and relapse of complex hyperplasia treated with levonorgestrel-releasing intrauterine system.

Introduction

In our previous study, we found that women with EH treated with LGN-IUS or oral progestogens often relapse following their initial regression and this occurs more often with oral progestogens than with LNG-IUS.[109] In 1989, Ferenczy et al found that women with cytological atypia were less likely to achieve endometrial regression and were also more likely to relapse during follow up.[36] BMI, age, menopause and diabetes are associated with EH[111] and could also represent prognostic markers for the outcomes of endometrial regression or relapse of EH treated conservatively, but these have not yet been investigated. In this study, our objective is to investigate the predictive ability of clinical characteristics for regression and relapse of EH treated with LNG-IUS or oral progestogens.

Methods

This was a cohort study. We included all women diagnosed with CH or ACH that underwent treatment with LNG-IUS or oral progestogens from August 1998 until December 2010 for the outcome of regression (n=344) as described in Chapter 5. For the outcome of relapse we opted to include women from August 1998 until December 2007 to ensure at least five year follow up for all participants (n=219) and the methodology is described in detail in Chapter 6. The primary outcome for this study was to determine the prognostic value of baseline clinical characteristics for women with CH or ACH treated with LNG-IUS or oral progestogens to predict regression and relapse. For this assessment, the results of follow-up histological examinations were classified as described in Chapters 5 and 6. The secondary outcomes we studied were the time interval from treatment initiation to complete regression and from regression to relapse during follow-up. For the outcome of regression we included all

women diagnosed with CH or ACH who underwent treatment with levonorgestrel-releasing intrauterine system or oral progestogens from August 1998 to December 2010 (n=344), and for the outcome of relapse until December 2007 to allow for at least five years of follow-up (n=219). All outcomes were evaluated with an intention to treat basis.

The baseline characteristics and outcomes for the LNG-IUS and oral progestogen groups were analysed using Mann-Whitney *U* tests for non-parametric data and Pearson χ^2 tests for categorical data. For variables with a Gaussian distribution we report means and SDs and for skewed data medians and IQR. We performed survival analysis using the Cox proportional hazards model as it accounts for variable duration of follow-up, censoring of subjects, proportionality of event occurrence, and time-to-event.[99] We computed the proportional changes in hazard for predicting variables and converted the results of the Cox model into absolute risk estimates. We calculated survival within our population by using Kaplan-Meier estimates for independent predictor variables.[100,101] Missing data were handled by complete case analysis for our outcomes (regression and relapse) and by multiple imputation for predicting variables.[102,103] All analyses were performed using STATA Version 12.1 (Release January 2012, STATA Corporation).

Results

Predicting regression of EH

Patient inclusion, follow up, baseline characteristics and regression rates are described in Chapter 5. Regression was achieved more often for women with CH (96.5%, 95 CI 93.3-

98.2, 221/229 for levonorgestrel-releasing intrauterine system and 90.1%, 95% CI 81.7-94.9, 73/81 for oral progestogens) than for ACH (76.2%, 95% CI 54.9-89.4, 16/21 for levonorgestrel-releasing intrauterine system and 46.2%, 95% CI 23.2-70.9, 6/13 for oral progestogens). Women with CH treated with levonorgestrel-releasing intrauterine system more often had a BMI of 35 or higher (HR 5.51, 95% CI 1.05-28.87, p=0.043, Table 13). We did not identify significant predictors for women treated with oral progestogens or for women with ACH.

Table 13 Univariate analysis for the prediction of regression of CH when treated with LNG-IUS or oral progestogens.

Prognostic Variable		Levonorgestrel-Releasing Intrauterine System (n=250)				Oral Progestogens (n=94)			
		Persisted Hyperplasia (n=8)	**Regressed Hyperplasia (n=221)**	**Hazard Ratio (95% CI)**	***P***	**Persisted Hyperplasia (n=8)**	**Regressed Hyperplasia (n=73)**	**Hazard Ratio (95% CI)**	***P***
Age	Younger than 40	2 (25)	13 (5.9)	1		2 (25)	17 (23.3)	1	
	40–60	6 (75)	166 (75.1)	0.54 (0.11-2.79)	0.464	5 (62.5)	46 (60.3)	0.68 (0.13-3.66)	0.657
	Older than 60	0	42 (19)	NA	NA	1 (12.5)	14 (16.4)	0.7 (0.63-7.8)	0.773
Parity	Nulliparous	3 (37.5)	40 (18.1)	1		3 (37.5)	28 (34.3)	1	
	1-2 children	3 (37.5)	104 (47.1)	0.36 (0.07-1.82)	0.218	2 (25)	25 (36.2)	0.88 (0.14-5.38)	0.892
	3 or more children	2 (25)	77 (34.8)	0.41 (0.06-2.58)	0.342	3 (37.5)	28 (38.4)	1.32 (0.26-6.68)	0.74
Ethnicity	White	5 (62.5)	174 (78.7)	1		6 (75)	54 (74)	1	
	Asian	0	27 (12.2)	NA	NA	1 (12.5)	9 (12.3)	1.04 (0.12-8.79)	0.971
	Other	3 (37.5)	20 (9.1)	0.87 (0.16-4.63)	0.868	1 (12.5)	10 (13.7)	0.73 (0.09-6.11)	0.772
Diabetes		1 (12.5)	34 (15.4)	1.13 (0.14-9.39)	0.912	1 (12.5)	11 (15.1)	0.48 (0.06-4.02)	0.502
Hypertension		1 (12.5)	77 (34.8)	0.58 (0.07-4.87)	0.62	3 (37.5)	20 (27.4)	1.83 (0.43-7.75)	0.411

Menopause	4 (50)	112 (50.7)	2.08 (0.46-9.43)	0.34	2 (25)	25 (34.3)	0.69 (0.14-3.43)	0.647
Hormone therapy or tamoxifen use	2 (25)	44 (19.9)	2.5 (0.48-13)	0.277	0	11 (15.1)	NA	NA
Body mass index 35 or higher	5 (62.5)	65 (31)	5.51 (1.05-28.87)	0.043	4 (57.1)	15 (24.6)	2.4 (0.54-10.79)	0.252
Endometrial thickness greater than 9mm	1 (20)	88 (46.8)	0.25 (0.03-2.36)	0.227	4 (57.1)	28 (48.3)	1.24 (0.28-5.57)	0.779

* Data are n (%) unless otherwise specified. CI, confidence interval.

Predicting relapse of EH

Patient inclusion, follow up, baseline characteristics and regression rates are described in Chapter 6. Women with relapse of CH in the levonorgestrel-releasing intrauterine system group were more often diabetic (33.3% compared with 11.3%, HR 2.91, 95% CI 1.09-7.76, p=0.033; Table 14), had an endometrial thickness greater than 9mm (75% compared with 45.7%, HR 3.35, 95% CI 1.1-10.4, p=0.037), and more often had a BMI 35 or higher (82.4% compared with 25%, HR 13.37 95% CI 3.8-46.7, p<0.001). In multivariate analysis of women with CH treated with levonorgestrel-releasing intrauterine system, BMI 35 or higher was found to be a strong independent predictor of relapsed endometrial hyperplasia (HR 18.93, 95% CI 3.93-91.15, p<0.001; Table 15). The cumulative event rates in Figure 17 show that only 3.3% of those women with BMI less than 35 will relapse during long term follow-up compared with 32.6% of women with BMI 35 or higher. One woman in the former group that relapsed by 52 months had a BMI of 34.2 and in this dataset, after 24 months from diagnosis, no woman with BMI less than 34 relapsed after initial regression with levonorgestrel-releasing intrauterine system.

Table 14 Univariate analysis of Hazard ratios for the prediction of relapse of CEH when treated with LNG-IUS or oral progestogens

Prognostic Variable		Levonorgestrel-Releasing Intrauterine System (n=250)				Oral Progestogens (n=94)			
		Relapsed Hyperplasia (n=18)	**Regressed Hyperplasia (n=124)**	**Hazard Ratio (95% CI)**	***P***	**Relapsed Hyperplasia (n=17)**	**Regressed Hyperplasia (n=43)**	**Hazard Ratio (95% CI)**	***P***
Age	Younger than 40	0	8 (6.5)	1		2 (11.8)	10 (23.3)	1	
	40–60	14 (77.8)	97 (78.2)	NA	NA	11 (64.7)	27 (62.8)	2.47 (0.55-11.18)	0.239
	Older than 60	4 (22.2)	19 (15.3)	NA	NA	4 (23.5)	6 (13.9)	3.18 (0.58-17.4)	0.182
Parity	Nulliparous	3 (16.7)	20 (16.1)	1		6 (35.3)	15 (34.9)	1	
	1–2 children	10 (55.6)	56 (45.2)	0.94 (0.26-3.45)	0.931	7 (41.2)	15 (30.2)	1.19 (0.4-3.56)	0.749
	3 or more children	5 (27.8)	48 (38.7)	0.68 (0.16-2.86)	0.601	4 (23.5)	15 (34.9)	0.62 (0.18-2.21)	0.464
Ethnicity	White	18 (85.7)	109 (83.2)	1		9 (47.4)	40 (83.3)	1	
	Asian	2 (9.5)	13 (9.9)	1 (0.23-4.37)	0.995	6 (31.6)	6 (6.3)	4.36 (1.54-12.36)	0.006
	Other	1 (4.8)	9 (6.9)	0.59 (0.08-4.44)	0.61	4 (21)	5 (10.4)	2.29 (0.7-7.45)	0.169
Diabetes		6 (33.3)	14 (11.3)	2.91 (1.09-7.76)	0.033	4 (23.5)	4 (9.3)	1.96 (0.64-6.01)	0.24
Hypertension		9 (50)	41 (33.1)	2.33 (0.92-5.9)	0.075	5 (29.4)	13 (30.2)	1.43 (0.5-4.07)	0.508
Menopause		9 (50)	61 (49.2)	1.1 (0.43-2.77)	0.847	7 (41.2)	10 (23.3)	1.98 (0.75-5.21)	0.165
Hormone therapy or tamoxifen use		1 (5.6)	29 (23.4)	0.16 (0.02-1.22)	0.078	3 (17.7)	6 (14)	1.19 (0.34-4.16)	0.781
Body mass index 35 or higher		14 (82.4)	29 (25)	13.37 (3.83-46.69)	<0.001	5 (41.7)	9 (25.7)	1.67 (0.53-5.27)	0.381
Endometrial thickness	greater than 9mm	12 (75)	48 (45.7)	3.35 (1.08-10.4)	0.037	6 (40)	16 (48.5)	0.9 (0.32-2.54)	0.844

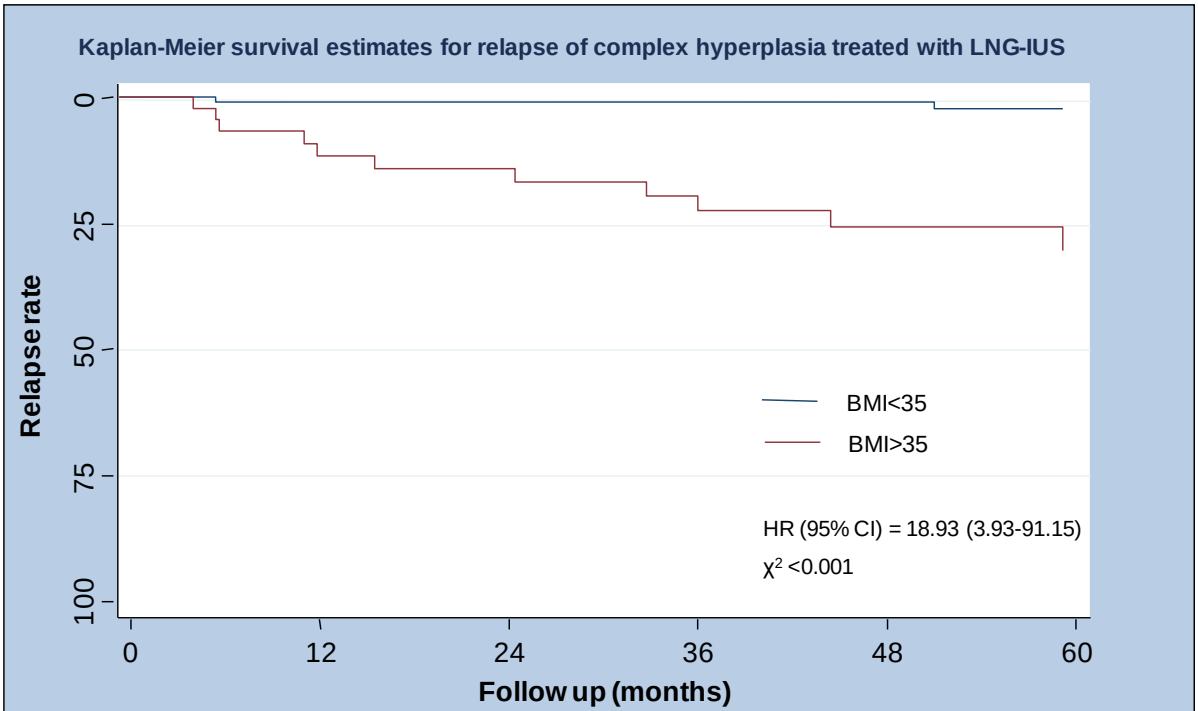
* Data are n (%) unless otherwise specified. CI, confidence interval.

Table 15 Multivariate analysis of Hazard ratios for the prediction of relapse of CH when treated with LNG-IUS or oral progestogens.

Prognostic Variable	Levonorgestrel-Releasing Intrauterine System (n=250)	
	Hazard Ratio (95% CI)	***P***
Diabetes	0.99 (0.32-3.11)	0.991
BMI 35 or higher	18.93 (3.93-91.15)	<0.001
Endometrial thickness greater than 9mm	2.73 (0.82-9.16)	0.103

CI, confidence interval.

Figure 17 **Kaplan-Meier survival curves for all events of CEH relapse in women treated either with LNG-IUS. CI = confidence interval; HR= hazard ratio.**



Cumulative event rates of relapse by fixed time points

Therapy	12 months n relap./t (%)	24 months n relap./t (%)	36 months n relap./t (%)	48 months n relap./t (%)	60 months n relap./t (%)	60 months n relap./t (%)
BMI<35 (n=90)	1/90 (1.1)	1/90 (1.1)	1/90 (1.1)	1/90 (1.1)	2/90 (2.2)	3/90 (3.3)
BMI≥35 (n=43)	4/43 (9.3)	6/43 (14)	9/43 (20.9)	10/43 (23.3)	11/43 (25.6)	14/43 (32.6)
Total	5/133 (3.8)	7/133 (5.3)	10/133 (7.5)	11/133 (8.3)	13/133 (9.8)	17/133 (12.8)

Discussion

In this study, we found that morbid obesity (BMI>35) is strongly associated with relapse of CH after initial regression with LNG-IUS treatment. This is independent of the presence of diabetes or endometrial thickness in these women. This study also finds a weak association with BMI 35 or higher and failure to regress CH when treated with levonorgestrel-releasing intrauterine system. No predictors for regression or relapse for women treated with oral

progestogens and for women initially diagnosed with atypical complex hyperplasia were identified.

We have previously described that the LNG-IUS is more successful treatment for women with EH than oral progestogens.[8;95] It induces initial regression more often than oral progestogens and women are less likely to relapse during follow up compared to oral progestogens.[8;109] However, relapse is common with both therapies and long term follow up is suggested.[109] From this study we can conclude that not all women need long term follow up when treated with LNG-IUS. The majority of women can be safely reassured that relapse is rare. The women that would benefit from long term follow up are women with raised BMI over 35, as almost one out of three will relapse. The reason appears to be that the excess of endogenous oestrogens persists over time and takes the toll in the antagonism with the Levonorgestrel of the LNG-IUS. The hypothesis of excess body weight causing endometrial proliferation through oestrogen excess and chronic hyperinsulinemia is not new and has biological coherence.[111] This suggests that this modifiable risk factor for EH may require further intervention to prevent relapse.

The cohort design for women treated with LNG-IUS with the long term follow up allows the accurate estimation of the predictive ability of clinical characteristics to predict regression or relapse. We involved primary care clinicians in the data collection and follow up and this resulted in our high follow up rate and our dataset with very few missing data. We have measured many variables that may confound our results and we have adjusted our estimates where necessary. Unfortunately, we did not engage in repeated measures of variables during

follow up that may differ from the baseline. For example, we did not monitor the BMI during the follow up and only values at the baseline were used for our analysis. The majority of the predictors reported in our study are not found to be associated with regression or relapse. However, our study has less than 80% power for avoiding type II error and there is a high likelihood that the predictors we have investigated may represent false negatives. Specifically, for women treated with oral progestogens or initial diagnosis of atypical complex hyperplasia our sample size is particularly small to draw conclusions about the predictive ability of the exposures investigated. This study has implications in clinical practice as it aids prognosis and helps decide a strategy for surveillance of women with CH. We have suggested that all women with CH should be followed up for at least 24 months to establish if regression occurs. Following initial regression after 24 months, we suggest long term surveillance for women with levonorgestrel-releasing intrauterine system and BMI 35 or higher for further 60 months (five years), resulting in a total of seven years of follow-up. Women treated with oral progestogens should be followed up for further 48 months as relapse is more common, but no woman relapsed after this cut-off, of a total of six years.[109] We cannot make conclusions on the follow-up for ACH from this study, but the risk for failure to regress and relapse is likely to be higher and long term follow-up is advised. Our experience requires external validation in other institutions to ensure our findings can be generalised and applied. Our next study is focusing on biomarkers that could aid the prognostic ability of predictors such as BMI and improve its accuracy.

Chapter 9: Predictive ability of Oestrogen (ER), progesterone (PR), COX-2, Mlh1, and Bcl-2 expression for regression and relapse of endometrial hyperplasia treated with LNG-IUS: a cohort study.

Abstract

Objective

To test the predictive ability of immunohistochemical oestrogen (ER), progesterone (PR), COX-2, Mlh1, and Bcl-2 expression for predicting the outcomes of regression and relapse in women with endometrial hyperplasia treated with the Levonorgestrel-releasing intrauterine system (LNG-IUS).

Methods

We included all women diagnosed with CH or ACH that underwent treatment with LNG-IUS from August 1998 until September 2008. Immunohistochemistry was performed with conventional methods and recorded using a semi-quantitative score (Q score) by two blinded assessors. Women were followed with endometrial biopsies to record regression and relapse. The biomarker predictive ability was analyzed using the Cox proportional hazards model.

Results

The median follow-up was 72.1 months (IQR 59.1-89.8). The Q score agreement between assessors was 82.6% (K statistic = 0.801 ± 0.036). The majority of study participants initially regressed to normal endometrium following LNG-IUS therapy (n=164 regressed; n=10 persisted). From the 164 women that regressed with LNG-IUS we were able to assess 152 women for relapse from which 18 relapsed. We found a weak association for persisted endometrial hyperplasia with ER and PR expression with Q score on the 5^{th} and 10^{th} centile.

No associations were found for COX-2, Mlh1 and Bcl-2 protein expression for regression and for any of the biomarkers for relapse.

Conclusion

We found that poor expression of ER and PR is weakly associated with persisting endometrial hyperplasia and COX-2, Mlh1, and Bcl-2 expression are not predictive. None of the biomarkers is predictive for relapse in women with endometrial hyperplasia treated with LNG-IUS.

Introduction

EH is considered to be an oestrogen-dependent benign disease of the endometrium.[112] Aberrant progesterone or oestrogen metabolism of the endometrium may be causal to the initiation, progression and malignant transformation of EH.[112] We have already generated a hypothesis that the lack of ER and PR can predict poor response to treatment.[113] However, the key step to this transformation to the majority of the cases appears to be local oestrogen production from androgens catalysed by the aromatase enzyme.[12,15] There is a strong linear association between aromatase and expression of COX-2 in uterine and breast cancer specimens, resulting in a complex paracrine and/or autocrine signalling pathway effecting abnormal oestrogen synthesis.[12,15] COX-2 is the rate-limiting enzyme in the prostaglandin biosynthetic pathway that stimulates oestrogen biosynthesis and higher COX-2 expression has been reported in hyperplastic or malignant endometrium than in normal.[13,16,17] COX-2 is significantly associated with aromatase expression in EC, which suggests that intra-endometrial oestrogen production promotes progression of EH to cancer.[18] There is a strong linear association between aromatase and cyclo-oxygenases in breast cancers and combinations of aromatase and COX-2 inhibitors are now being used in therapeutic trials for breast cancer.[17] Hence, the assessment of aromatase/COX-2 activity and steroid receptor status is potentially a key marker for targeted hormonal treatment of endometrial lesions when diagnosed early during cancerogenesis.

The abnormalities in the oestrogen pathway are not the only causative features for EH and its malignant potential. The angiogenesis, inhibition of apoptosis and DNA mismatch-repair mechanism or activation of oncogenes are the pathways most commonly described to be

involved in EH. It has been shown that the altered expression of proteins, such as Bcl-2, may play an important role by affecting apoptosis of hyperplastic cells.[19] The abnormal methylation of Mlh1 is the commonest event in EH that generates microsatellite instability (MSI) due to defects of the DNA mismatch-repair mechanism.[25] Oestrogens may increase the rate of mutagenesis of Mlh1 through free radical formation as well as its inherent proliferative influence.[25] The combination of these pathways seems to orchestrate the progression of EH to cancer with oestrogens masterminding the process. The expression analysis of the above biomarkers currently helps understand the pathogenesis of EH and the pathways involved during this process. However, the evidence on their predictive ability for response to progestogen treatment has been limited.[114] In this study, we wish to test the hypothesis that the differential expression of IHC markers for ER, PR, COX-2, Mlh1, and Bcl-2 may predict regression or relapse of EH with LNG-IUS treatment over long term follow up.

Methods

Study population

This was a cohort study. We included all women diagnosed with CH or ACH that underwent treatment with LNG-IUS from August 1998 until December 2007 in a tertiary referral Hospital in Birmingham, UK. We have excluded women with no follow up histology, insufficient tissue for IHC and inadequate IHC for scoring. The histopathological diagnoses were undertaken by two experienced gynaecological pathologists working independently; referral to the other pathologist for a second opinion was made in cases where there was diagnostic doubt, and a mutual consensus was then achieved. Women were reviewed

following diagnosis and were offered LNG-IUS (Mirena®, Bayer Healthcare Inc.), oral progestogens or hysterectomy as part of our routine clinical practice. Women diagnosed with ACH were counselled and offered a hysterectomy. Women who declined surgery or who were medically unfit to undergo surgery were offered LNG-IUS or oral progestogens. We included in this study only women opting for treatment with LNG-IUS. Study participants underwent endometrial histological surveillance by outpatient endometrial sampling. Histological surveillance was performed on a six-monthly basis for the first two years and yearly thereafter for five years and then the patients were given a choice to have continued yearly surveillance. Women that did not adhere to this strategy were invited for clinic review in order to obtain long term follow up outcome. Ethical approval from the Coventry & Warwickshire Research and Ethics Committee was obtained for this study (LREC 09/H1211/30).

For all women in the study, baseline data were recorded as described in Chapter 5. The primary outcome for this study is to determine the prognostic value of ER, PR, COX-2, Mlh1, and Bcl-2 expression for women with CH or ACH treated with LNG-IUS to predict regression and relapse. For this assessment, the results of follow-up histological examinations were classified as described in Chapters 5 and 6.

The biomarker predictive ability was analysed using descriptive statistics and Pearson χ^2 test for categorical data. For variables with a Gaussian distribution we report means and SDs and for skewed data medians and IQRs. We performed survival analysis using the Cox proportional hazards model to estimate the proportional changes in hazard for predicting

variables, as it accounts for variable duration of follow-up, censoring of subjects, proportionality of event occurrence, and time-to-event.[99] Missing data were handled by complete case analysis for our predicting markers and outcomes (regression and relapse). The level of intra-observer agreement was calculated using Cohen's kappa index ± Standard Error (k ± SE). All analyses were performed using STATA Version 12.1 (Release January 2012, STATA Corporation).

IHC

Six 5 µm thick sections were serially cut from formalin fixed paraffin-embedded tissue blocks from baseline pre-LNG-IUS treated EH tissue specimens for each selected study participant. Immunostaining was performed upon five sections and the remaining section underwent standard haematoxylin and eosin staining. Immunostaining was performed using Dako Autostainer and Dako detection kit K5007 (Dako, Glostrup, Denmark) according to the manufacturer's protocol. De-paraffinisation and antigen retrieval was achieved with W-CAP (Surgipath Europe Ltd) pH 8.0 in water bath at 98°C. Slides were incubated for one hour with primary antibodies. All antibodies were diluted using Dako Universal Antibody Diluent. Primary antibodies used were mouse monoclonal anti-estrogen receptor–α , clone 1D5 (Dako, Glostrup, Denmark; 1:150 dilution), mouse monoclonal anti-progesterone receptor-A and B, clone 1A6 (NovoCastra, Newcastle, UK; 1:50 dilution), mouse monoclonal anti-human COX-2, clone 4H12 (NovoCastra, Newcastle, UK; 1:50 dilution), mouse monoclonal anti-Mlh1, clone G168-728 (Cell Marque, Rocklin, California, USA; 1:100 dilution) and mouse monoclonal anti-human Bcl-2 oncoprotein, clone 124 (Dako, Glostrup, Denmark; 1:100), For IHC markers, glandular and stromal staining was recorded

using a semi-quantitative score (Q score). The Q score is a validated scoring method that incorporates both the intensity and the distribution of specific staining and hence was preferred over H score.[108] Intensity and proportion of stained cells were added for the Q score, which had a range of 0 to 8. Two assessors carried out the Q scoring independently, blinded to the outcome. The total proportion of cells staining positively at any intensity was scored as 0 (no cells staining), 1 (when <1% cells stained), 2 (when 1-10% cells stained), 3 (when 10-33% cells stained), 4 (when 34-66% cells stained), or 5 (when 67-100% cells stained). The intensity was scored according to the overall appearance as 0, none (no staining); 1, weak (only visible at high power magnification); 2, moderate (visible at low power magnification); 3, strong (striking even at low power magnification). Two assessors carried out the Q scoring independently, blinded to index diagnosis and outcome. Q score cut-offs were explored for all centiles and each of the biomarkers and two were reported with the lowest p value.

Results

During the study period we treated 196 women with LNG-IUS for CH or ACH and excluded women where material was not available for IHC (n=19) or was inadequate for scoring (n=3). The baseline characteristics of the 174 women included in our study are shown in Table 16. The median follow-up for all study participants was 72.1 months (IQR 59.1-89.8). The agreement regarding histological scoring between the two histopathologists was 82.6% (K statistic = 0.801 ± 0.036).

Table 16 Baseline characteristics

		Persisted hyperplasia (n=10)	Regressed hyperplasia (n=164)
Age	- <40	2 (20)	9 (5.5)
	- 40-60	8 (80)	116 (70.7)
	- >60	0	39 (22.4)
Parity	Nulliparous	3 (30)	33 (20.1)
	1-2 children	2 (20)	74 (45.1)
	>3 children	5 (50)	57 (34.8)
Ethnicity	White	6 (60)	136 (82.9)
	Asian	1 (10)	16 (9.8)
	Other	3 (30)	12 (7.3)
Diabetes		2 (20)	30 (18.3)
Hypertension		4 (30.8)	87 (36.7)
Menopause		8 (80)	93 (56.7)
HRT or Tamoxifen use		3 (30)	34 (24.7)
Body Mass Index >35*		6 (60)	53 (33.8)
Endometrial thickness>9mm*		3 (33.3)	69 (47.9)
Cytological atypia		5 (50)	14 (8.5)

* Endometrial thickness was not measured in 21 women and BMI was not available in 7 women.

The majority of study participants initially regressed to normal endometrium following LNG-IUS therapy (n=164 regressed; n=10 persisted). The women who had persisted EH: five demonstrated persistent hyperplasia of the same type, one upgraded from CH to ACH, four upgraded to well-differentiated FIGO Stage IA (n=2) or IB (n=2) EC. From the 164 women that initially regressed with LNG-IUS we were able to assess 152 women for relapse as 11 women declined long term follow up and one opted for hysterectomy. During follow up 18 (11.8%) women relapsed for which 9 women had hysterectomy and one woman initially

diagnosed with CH progressed to endometrioid cancer with concomitant granulosa cell tumour of the ovary after 36 months from initial regression. Relapse occurred in a median of 32.2 months (IQR 11.3- 57.7).

Table 17 Univariate analysis of Hazard ratios for the prediction of regression of endometrial hyperplasia when treated with LNG-IUS. (a) Centile cut-off with lowest p value (b) Centile cut-off with second lowest p value.

(a)

IHC markers	Persisted hyperplasia (n=10)	Regressed hyperplasia (n=164)	Hazard ratio (95% CI)	P value
ER≥2 (5^{th} centile)	7 (70)	160 (97.6)	0.09 (0.01-0.39)	0.001
PR≥2 (5^{th} centile)	8 (80)	159 (97)	0.32 (0.09-0.88)	0.02
COX-2≥7 (75^{th} centile)	4 (40)	53 (32.3)	0.88 (0.72-1.38)	0.142
MLH1≥6 (90^{th} centile)	5 (50)	69 (42.1)	1.52 (0.89-3.21)	0.276
BCL-2≥6 (25^{th} centile)	8 (80)	129 (78.7)	1.33 (0.56-3.36)	0.162

(b)

IHC markers	Persisted hyperplasia (n=10)	Regressed hyperplasia (n=164)	Hazard ratio (95% CI)	P value
ER≥4 (10^{th} centile)	6 (60)	151 (92.1)	0.19 (0.05-0.47)	0.008
PR≥4 (10^{th} centile)	7 (70)	136 (82.9)	0.46 (0.17-0.91)	0.02
COX-2≥3 (10^{th} centile)	10 (100)	154 (93.9)	1.49 (0.82-2.68)	0.189
MLH1≥4 (75^{th} centile)	6 (60)	71 (43.3)	1.77 (0.89-3.45)	0.107
BCL-2≥5 (10^{th} centile)	9 (90)	150 (91.4)	0.96 (0.82-1.23)	0.978

The correlation of the baseline pre-treatment Q score and histological regression with LNG-IUS therapy is depicted in Table 17. Among women with CH or ACH at index biopsy, weak

associations were found for ER and PR expression on the 5th and 10th centile of the Q score and histological regression. COX-2, Mlh1 and Bcl-2 protein expression were not predictive of regression of EH. The correlation of the baseline pre-treatment Q score and evidence of relapse with LNG-IUS therapy is depicted in Table 18. Among women with CH or ACH at index biopsy, no associations were found between ER, PR, COX-2, Mlh1 and Bcl-2 protein expression and relapse of EH.

Table 18 Univariate analysis of Hazard ratios for the prediction of relapse of endometrial hyperplasia when treated with LNG-IUS. (a) Centile cut-off with lowest p value (b) Centile cut-off with second lowest p value.

(a)

IHC markers	Relapsed hyperplasia (n=18)	Regressed hyperplasia (n=134)	Hazard ratio (95% CI)	P value
ER≥2 (5th centile)	14 (77.7)	115 (85.8)	0.38 (0.05-2.92)	0.354
PR≥5 (25th centile)	13 (72.2)	91 (67.9)	1.6 (0.36-7.1)	0.538
COX-2≥5 (25th centile)	15 (83.3)	95 (70.9)	2.89 (0.62-15.72)	0.157
MLH1≥5 (50th centile)	5 (27.7)	53 (39.6)	0.39 (0.19-1.27)	0.117
BCL-2≥6 (25th centile)	16 (88.9)	96 (71.6)	4.33 (0.56-33.45)	0.159

(b)

IHC markers	Relapsed hyperplasia (n=18)	Regressed hyperplasia (n=134)	Hazard ratio (95% CI)	P value
ER≥4 (10th centile)	14 (77.7)	110 (82.1)	0.56 (0.08-4.26)	0.572
PR≥6 (50th centile)	12 (66.7)	91 (67.9)	1.02 (0.35-2.98)	0.974
COX-2≥6 (50th centile)	15 (83.3)	97 (72.4)	2.37 (0.51-10.96)	0.269
MLH1≥6 (75th centile)	4 (22.2)	47 (35.1)	0.53 (0.17-1.81)	0.282
BCL-2≥7 (50th centile)	12 (66.7)	74 (55.2)	1.93 (0.25-14.87)	0.528

Figure 18 ER expression (Q score=8) for a woman that regressed with LNG-IUS treatment for complex endometrial hyperplasia.

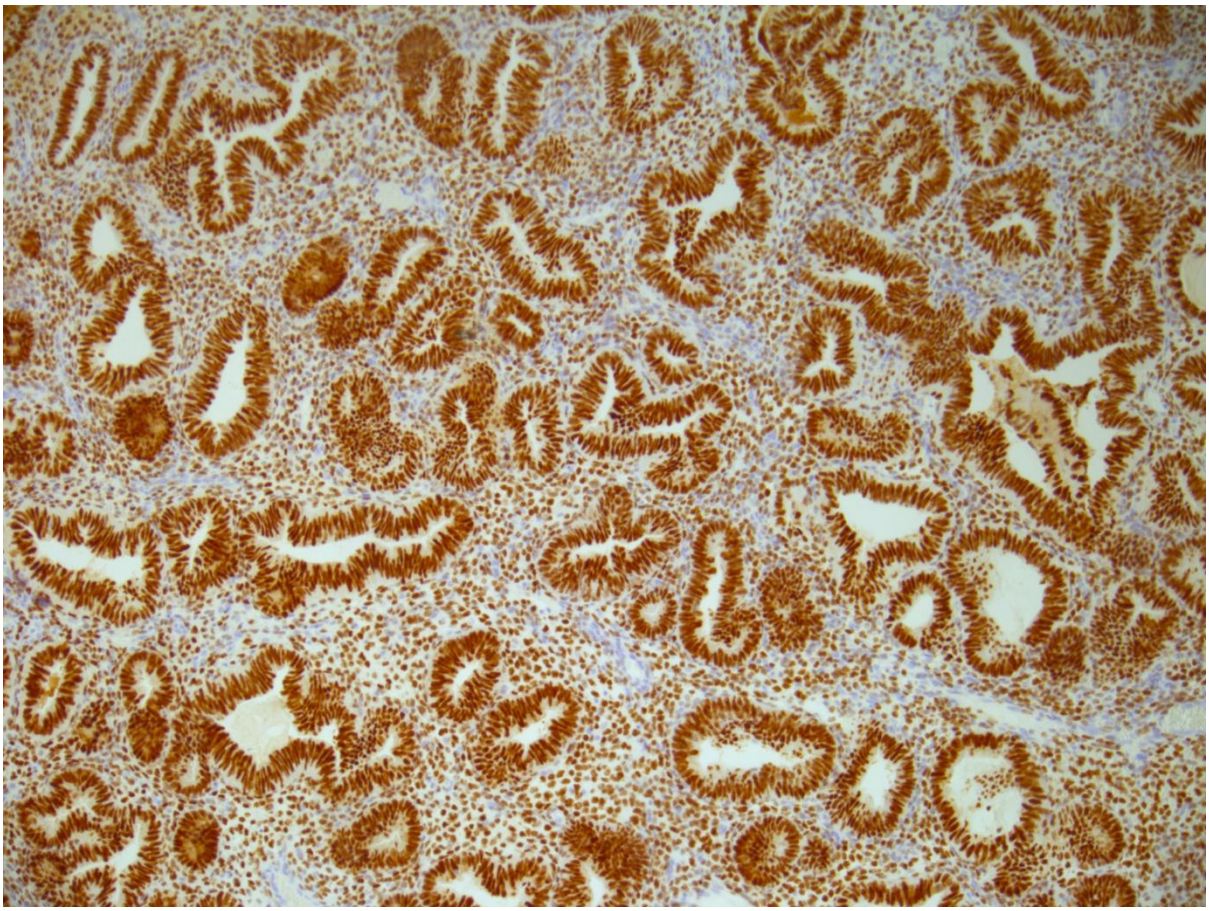


Figure 19 PR expression (Q score=7) for a woman that regressed with LNG-IUS treatment for complex endometrial hyperplasia.

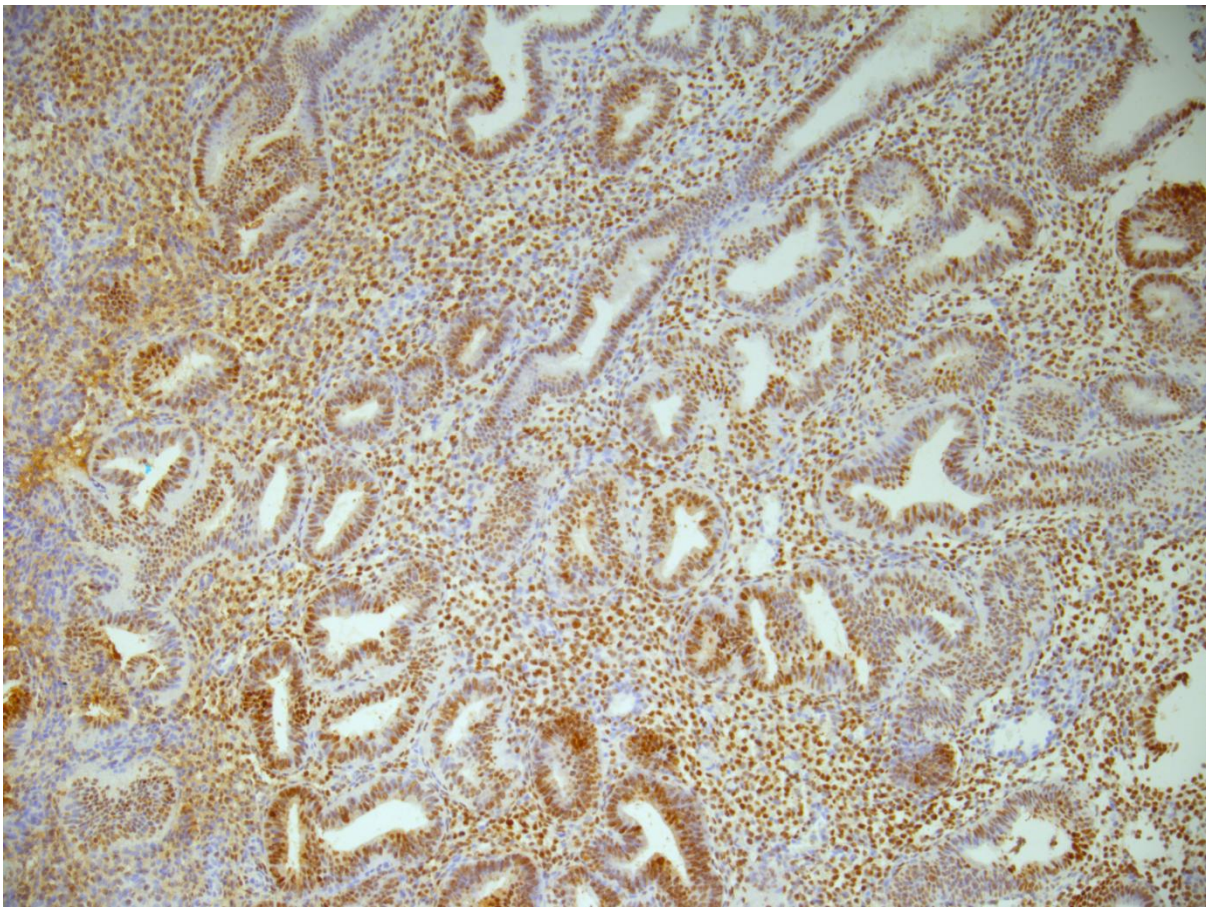


Figure 20 MLH1 expression (Q score=7) for a woman that regressed with LNG-IUS treatment for complex endometrial hyperplasia.

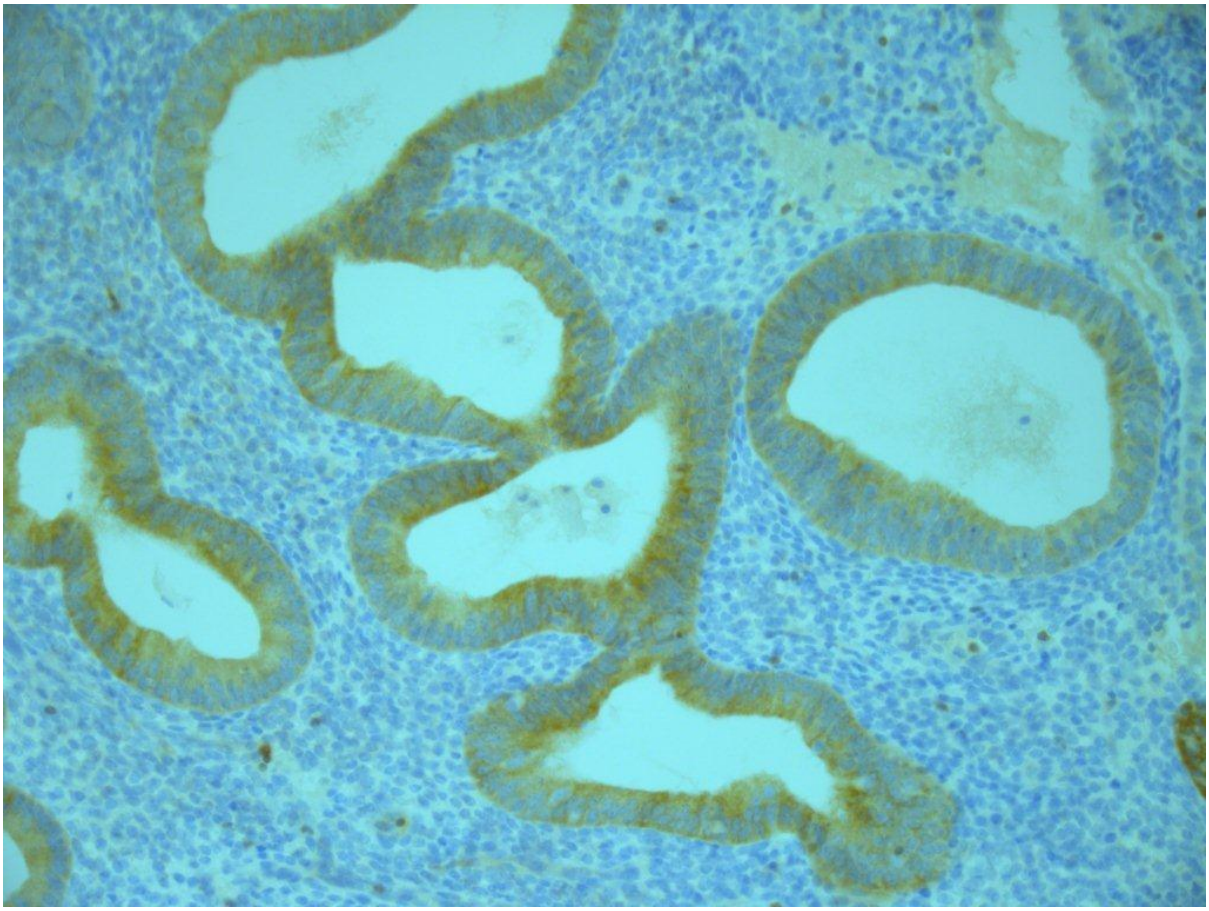


Figure 21 BCL2 expression (Q score=8) for a woman that regressed with LNG-IUS treatment for complex endometrial hyperplasia.

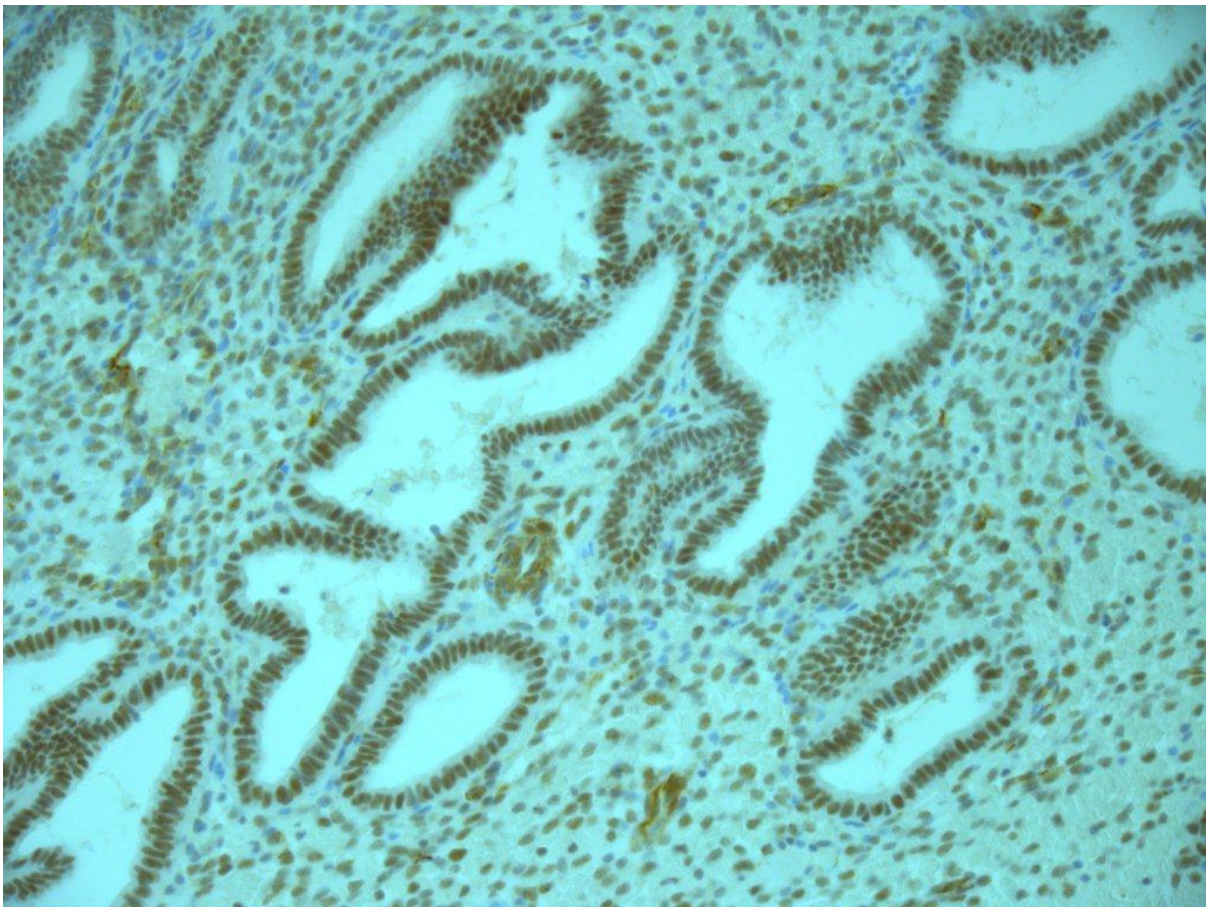
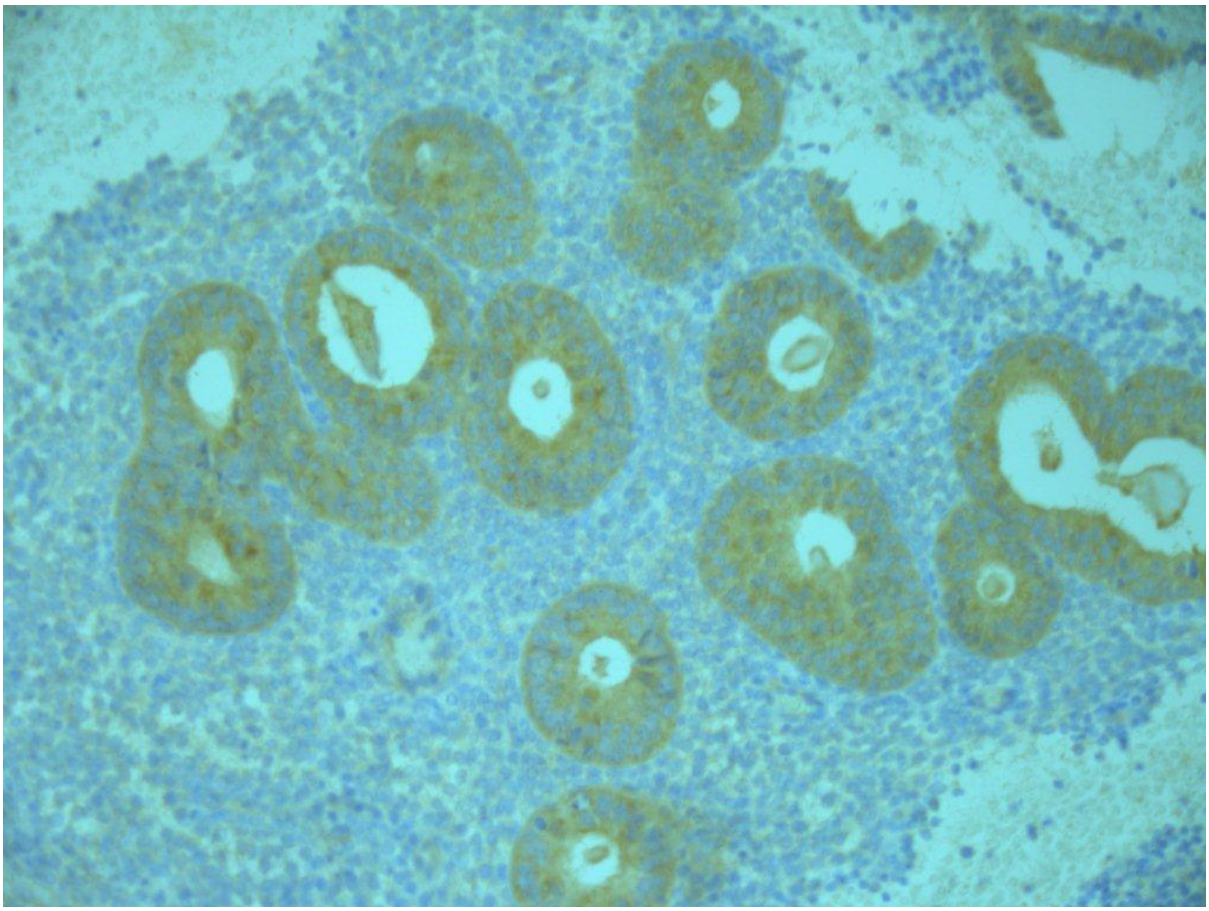


Figure 22 COX2 expression (Q score=8) for a woman that regressed with LNG-IUS treatment for complex endometrial hyperplasia.



Discussion

We publish the first large cohort study of women treated with LNG-IUS for CH or ACH assessing IHC predictors of regression or relapse. This study demonstrates that EH regression and relapse during follow up with LNG-IUS therapy is weakly associated with poor expression of ER and PR. COX-2, Mlh1 and Bcl-2 protein expression are not predictive. None of the biomarkers is predictive for relapse.

The results of this study are confirming our exploratory case-control study which found that the lack of ER and PR may predict women with persisting EH, but the predictive ability is attenuated .[117] We have described that the case-control study design of our exploratory study has the risk of overestimating accuracy up to 3-fold by introducing spectrum bias.[116,117] The cohort design of this study is the gold standard design to estimate the predictive ability of those markers and we believe our finding that these are poor predictors of important clinical outcomes in this context is likely to be reliable. Our study is large but there were few events recorded as only 10 women had persisted hyperplasia and 18 relapsed. The majority of the predictors reported in our study are not found to be associated with regression or relapse. However, our study is likely to be underpowered for avoiding type II error and there is a high likelihood that the predictors we have investigated may represent false negatives.[117,118] We have also employed two assessors for scoring the IHC blinded to diagnoses and outcomes minimizing misclassification and observer bias. It is worth mentioning that in this study we used the outpatient endometrial sample as a monitoring tool. This is more acceptable to women inpatient and likely to be more accurate than ultrasound estimation of endometrial thickness, but there is no evidence assessing the monitoring value of these tools. However, the LNG-IUS induces morphologic changes to the endometrium including glandular atrophy, stromal pseudodecidualization, and leucocyte infiltration. Glandular metaplastic changes, nuclear atypia and stromal myxoid change are also present and can pose significant diagnostic challenges during the histological surveillance.[119]

We have described previously the rationale for differential expression of ER and PR that could also contribute to the variation of efficacy in progestogen therapy of EH. In the

literature, there are several studies investigating the ER and PR protein expression in EH treated with oral progestogens.[37-39] These studies find conflicting and weak evidence that ER and PR protein expression may be predictive of outcomes in women treated with oral progestogens for EH.[37-39] We have suggested that compliance with this therapy is an important confounding factor that is difficult to adjust for as it is difficult to measure.[116] With LNG-IUS therapy compliance is no longer an issue. Despite abolishing this potential confounding the predictive ability of our IHC markers did not improve. There are other molecular pathways (such as COX-2, Mlh1 or Bcl-2) that have been implicated in the generation of benign and malignant uterine pathology, but we did not find those to be useful predictors of regression or relapse of EH.

Identifying women at higher risk of persistent or relapsed EH with progestogen treatment may have important implications for improving health outcomes for women with EH by improving patient selection for LNG-IUS therapy and ensuring closer endometrial surveillance for women at risk of treatment failure or malignant transformation. We have found that obesity may be a strong predictor of women that relapse during follow up when treated with LNG-IUS. This is important because relapse is common and women at low risk for relapse can be discharged from further follow up and women at high risk can have close surveillance to ensure they remain in regression.[8] Possibly, because persistent hyperplasia is less common (about 10%) with LNG-IUS it may be unlikely that we find an IHC predictor that will raise the probability of persistent hyperplasia so high that clinicians would not opt to try LNG-IUS treatment. On the other side, if a predictor can ensure that these women almost certainly are going to respond to treatment this will not change clinical practice. Clinicians

would wish to have histological evidence of regression of EH and would follow up women until regression is proven.

Further research from other groups is needed to validate our findings from this study. Nonetheless, we believe this study may shape future research on the identification and evaluation of a clinical prognostic model for EH in order to improve health outcomes associated with this pre-malignant condition. Such a model may incorporate and integrate biodemographic parameters (e.g. age, BMI, parity), histological cyto-architecture classification and molecular phenotyping. Furthermore, this model is likely to be more clinically useful if it identifies accurately women likely to regress and not relapse during follow up who can be safely reassured.

Chapter 10: Conclusions & Recommendations

Summary of findings

We have made a significant contribution to improve the management and prognosis of EH through the findings from this thesis. Our findings, but also the important limitations are summarised per Chapter below:

Chapter 1

In this Chapter we have discussed the definition, risk factors, classification and prior knowledge for the management and prognosis of EH. This was important to identify gaps of knowledge and make a significant contribution where is required. We have proposed a simplified version of the WHO classification and disseminated the advantages of omitting SAH, which is of disputed existence and created confusion among clinicians because of the uncertainty in its management.

Management of EH

Chapter 2

In Chapter 2 we searched and meta-analysed the regression rates recorded in observational studies with the most popular conservative therapies for EH. We compared the regression rates, defined as conversion of CH or ACH to simple hyperplasia or proliferative endometrium and proliferative endometrium from SH, achieved by the LNG-IUS and oral progestogens for each type of EH. We found that for CH and ACH the regression rates for LNG-IUS were high and significantly higher compared to oral progestogens. There was no statistical difference in simple hyperplasia. We found only small case-series of poor quality

predominantly for evaluating the regression rates, which we summarised and indirectly compared reducing the accuracy of the estimated regression rates.

Chapter 3

In this Chapter we investigated the regression, relapse and live birth rates with fertility-sparing treatment for ACH and EC. Fertility-sparing treatment for EC achieved a high regression rate with an encouraging live birth rate. For ACH the pooled regression and live birth rate was even higher. However, for both conditions we observed a high relapse rate of the disease, defined as relapse of ACH or EC during follow-up. We also found that diagnosis of ovarian cancer (concurrent or metastatic) during follow-up, progression of the disease and even death are both risks of this approach. Similarly to Chapter 2, we found only small case-series with short follow-up predominantly for evaluating the regression, relapse and live birth rates, which we summarised and indirectly compared reducing the accuracy of the estimated rates.

Chapter 4

From this Chapter we summarised the current clinical practice of managing EH. We presented to Gynaecologists nationally the observational findings and enquired whether future research would improve the management of this condition. We found that most clinicians treat CH with LNG-IUS or oral progestogens and ACH with hysterectomy. Despite LNG-IUS appearing to be more effective in treating EH clinicians used equally LNG-IUS and oral progestogens and would wish more research on the effectiveness of these

therapies. The response rate to this survey was low and selection bias threatens the internal validity and precision of the results.

Chapter 5

In this Chapter we analysed our findings from the largest cohort study in the literature with long-term follow up. We found that women treated for CH and ACH with LNG-IUS and compared their regression and hysterectomy rates to oral progestogens. We found that regression, as defined before, is more likely with LNG-IUS and hysterectomies are less when compared to oral progestogens. We defined the time to regression and defined the EC risk for women that fail to regress. However, the observational design cannot exclude residual confounding from unmeasured variables and follow-up differed in the two groups with the retrospective inclusion of women treated with oral progestogens introducing performance and verification bias.

Chapter 6

Following up our cohort from Chapter 5 we defined the relapse risk for women that initially regressed with LNG-IUS or oral progestogens. We obtained more than five year follow up for all women. We found that relapse of CH and ACH after initial regression, defined as relapse of CH during follow-up, occurs often but it occurs less often in women treated with LNG-IUS than with oral progestogens. We also calculated the time to relapse and the EC risk for women that relapsed during follow up. The follow-up was adequate reducing attrition bias, but differed in the two compared groups with the retrospective inclusion of women treated with oral progestogens introducing performance and verification bias.

Prognosis of EH

Chapter 7

Our first study on the prognosis included in this Chapter generated the hypothesis that women who fail to regress after treatment with LNG-IUS may be predicted from ER and PR status on the index endometrial biopsy. Neither PTEN nor aromatase expression were associated with LNG-IUS therapy responsiveness. These results were encouraging but needed further testing on prospective cohort study. However, the size was small, the estimate was likely to be unstable and the case-control design overestimated the accuracy of the biomarkers.

Chapter 8

In this Chapter we investigated the prognostic ability of clinical characteristics to predict regression and relapse for women treated for EH with LNG-IUS or oral progestogens. We found that BMI is a strong independent predictor of relapse but no predictors were found to be independent for the outcome of regression. This study was larger than Chapter 7 and likely more accurate because of the cohort design, but still had less than 80% power for avoiding type II error.

Chapter 9

From this Chapter we wished to complement our prediction model from the previous Chapter and improve its accuracy for women treated with LNG-IUS. We found that ER, PR, COX-2, Mlh1, and Bcl-2 expression is not predictive of regression or relapse in women with

endometrial hyperplasia treated with LNG-IUS. Likewise, this study was larger than Chapter 7 and likely more accurate because of the cohort design, but still had less than 80% power for avoiding type II error.

Chapter 10

In our last Chapter we summarise our findings and make recommendations for clinical practice. Our recommendations become an essential part of a national guideline endorsed by the RCOG for the management and prognosis of EH, which is still in process. Our recommendations are summarised in the next section.

Implications for clinical and research practice

In this section we summarise the implications for clinical practice from the findings discussed previously. Those are summarised per chapter below:

Chapter 1

From this Chapter our simplified version of the WHO classification and disseminated through our guideline group and publications will improve the management of EH by omitting a dubious group of uncertain significance.

Management of EH

Chapter 2

From this research by defining the regression rates for LNG-IUS and oral progestogens we are empowering the counselling of women with EH by providing the effectiveness information and improve clinician knowledge. Improving clinician knowledge and confidence in these therapies can reduce unnecessary hysterectomies.

Chapter 3

From this Chapter we offer clinicians excellent information for counselling these women with an early clinical stage of ACH and EC and an evidence-based approach in their management. The editors of the American Journal of Obstetrics & Gynecology also highlighted this contribution in their commentary on our article, which was their choice for the respective issue.

Chapter 4

This Chapter is likely to have indirect implications in practice as clinicians may chose to amend their practice by reproducing the practice of the majority of the clinicians.

Chapter 5

From this Chapter we have contributed the largest cohort enforcing the efficacy of LNG-IUS treatment over oral progestogens. By defining the time to regression and the EC risk in women that fail to regress we recommend a clear window in which LNG-IUS or oral

progestogen treatment is expected to have the desired effect of endometrial regression maintaining women safe from disease progression.

Chapter 6

From this research in Chapter 6 we expect a major change in current practice. Clinicians discharge women from further follow up following initial regression with LNG-IUS or oral progestogens but are likely to change this practice because of the relapse risk. Relapse is very common and the progression to EC is significant warranting further surveillance. We have made clear recommendations from our research how long women should be followed up based on our findings, while appreciating the limitations of differences in follow-up between the two treatment groups.

Prognosis of EH

Chapter 7

Our study generated the hypothesis ER and PR need further evaluation as they constitute good predictors of persistent EH with LNG-IUS and encouraged research for testing this hypothesis.

Chapter 8

This Chapter has major implications for clinical practice as Chapter 6. BMI is found to be a strong independent predictor of relapse and we have recommended that women without this exposure can be safely discharged from further follow up and women with this exposure

should be followed up over the long term as almost one out of three is likely to relapse. We have made recommendations on the duration of follow up based on our findings.

Chapter 9

This Chapter has great implications for future research. The predictors we have studied were investigated from several research groups with conflicting findings. We found them to be poor predictors in the largest cohort study described in the literature and our results are likely to be reliable even though not excluding completely a type II error. Researchers were recommended to investigate novel markers for outcomes of EH and especially for relapse, which has major implications on following up women over the long term and it can complement the prognostic accuracy of BMI.

Chapter 10

Our recommendations through a national guideline endorsed by the RCOG for the management and prognosis of EH, will have a great impact on the clinical practice because of widespread dissemination and improved reliability.

Recommendations for further research

- The difference in regression and relapse rates for LNG-IUS and oral progestogens is almost undisputable for many clinicians. A recent application for funding of a randomised trial was rejected because of concerns over recruitment and we believe that equipoise for a randomised trial between the two may not be there anymore and prospective cohort studies from other

centres are advised to prove the reproducibility of our findings. An update for this systematic review is required now to integrate our studies.

- Similarly, our findings for women with ACH and EC need to become more robust by integrating more studies. This will improve reliability and an update of the systematic review is recommended in 1-2 years.
- With our research we may have contributed to a change in practice of many clinicians who will now prefer LNG-IUS over oral progestogens for EH. A further national survey showing this shift in practice will convince researchers that a randomised trial between those may not be longer feasible.
- Our cohort study needs to continue the follow up and our database interrogated again at 10 year follow up. This will provide unique data in the literature for the effectiveness of LNG-IUS and oral progestogens along with improving our understanding of the natural history of the disease.
- Further research is needed to indentify novel predictors of relapse to improve the accuracy of BMI. This will result in less women being followed over the long term and reduce NHS costs.

Contributions to the chapters of the thesis

Chapter 1

Ioannis D Gallos

Chapter 2

Ioannis D Gallos: Development of protocol and search strategy; Execution of literature searches; Literature selection; Data extraction; Data entry; Analysis; Drafting and Revision of the manuscript

Manjeet Shehmar: Quality appraisal and data extraction

Shakila Thangaratinam: Development of protocol and critical revision of the manuscript

Thalis Papapostolou: Literature selection and Data extraction

Arri Coomarasamy and Janesh Gupta: Critical revision of the manuscript

Chapter 3

Ioannis D Gallos: Development of protocol and search strategy; Execution of literature searches; Literature selection; Data extraction; Data entry; Analysis; Drafting and Revision of the manuscript

Jason Yap: Literature selection; Quality appraisal; Data extraction

Madhurima Rajhkowa, David Luesley, Arri Coomarasamy and Janesh Gupta: Critical revision of the manuscript

Chapter 4

Ioannis D Gallos: Inception, Development of questionnaire and Design of the study; Data extraction; Data entry; Analysis; Drafting and Revision of the manuscript

Olumide Ofinran and Manjeet Shehmar: Development of questionnaire

Arri Coomarasamy and Janesh Gupta: Critical revision of the manuscript

Chapter 5

Ioannis D Gallos: Inception and design of the study; Patient recruitment; Data entry; Analysis; Drafting and Revision of the manuscript

Preeti Krishan: Data entry and drafting the manuscript

Manjeet Shehmar: Data entry and drafting the manuscript

Raji Ganesan and Janesh Gupta: Critical revision of the manuscript

Chapter 6

Ioannis D Gallos: Inception and design of the study; Patient recruitment; Data entry; Analysis; Drafting and Revision of the manuscript

Preeti Krishan: Data entry and drafting the manuscript

Manjeet Shehmar: Data entry and drafting the manuscript

Raji Ganesan and Janesh Gupta: Critical revision of the manuscript

Chapter 7

Ioannis D Gallos: Analysis; Drafting and Revision of the manuscript

Emelie Akesson: Design of the study; Patient selection; Data entry

Raji Ganesan, Rajesh Varma and Janesh Gupta: Critical revision of the manuscript

Chapter 8

Ioannis D Gallos: Inception and design of the study; Patient recruitment; Data entry; Analysis; Drafting and Revision of the manuscript

Raji Ganesan and Janesh Gupta: Critical revision of the manuscript

Chapter 9

Ioannis D Gallos: Inception and design of the study; Patient recruitment; Data entry; Analysis; Drafting and Revision of the manuscript

Raji Ganesan and Janesh Gupta: Critical revision of the manuscript

Chapter 10

Ioannis D Gallos

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Appendix

1. **Patient information leaflet [Version 2, 9th April 2009]**

2. **Patient consent form [Version 2, 9th April 2009]**