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Predictive ability of estrogen receptor (ER), progesterone receptor (PR), COX-2, Mlh1, and Bcl-2 expressions for regression and relapse of endometrial hyperplasia treated with LNG-IUS: A prospective cohort study

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HIGHLIGHTS

- Weak expression of ER and PR is associated with failure of regression for endometrial hyperplasia treated with LNG-IUS.
- COX-2, Mlh1 and Bcl-2 cannot predict regression and none of the biomarkers predicts relapse of endometrial hyperplasia treated with LNG-IUS.
- There were few events of regression and relapse and a type II error cannot be excluded.

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ABSTRACT

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

Methods. We recruited prospectively all women diagnosed with complex or atypical complex hyperplasia 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 analysed 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 expressions with Q score on the 5th and 10th centiles. No associations were found for COX-2, Mlh1 and Bcl-2 protein expressions 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 expressions are not predictive. None of the biomarkers is predictive for relapse in women with endometrial hyperplasia treated with LNG-IUS.

Introduction

Endometrial hyperplasia (EH) is diagnosed three times more commonly than endometrial cancer and it can progress to cancer if left untreated [1,2]. A survey found that clinicians prefer to treat complex hyperplasia (CH) with the Levonorgestrel-releasing intrauterine system (LNG-IUS) [3]. In observational studies, it is also found that EH regression occurs more often with LNG-IUS than with oral progestogens [4,5]. In a recent study, we described 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 [6]. For women with atypical complex hyperplasia (ACH), hysterectomy is the indicated treatment since 29% progress to cancer and up to 43% of women already have concomitant carcinoma [2,7]. However, it may not be possible for all given its potential risks, especially for older or obese patients and those with significant comorbidities. Medical management is therefore advocated in such cases and the therapy of choice

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is also the LNG-IUS as it induces regression and prevents relapse in most women [3].

EH is considered to be an oestrogen-dependent benign disease of the endometrium [8]. Aberrant progesterone or oestrogen metabolism of the endometrium may be causal to the initiation, progression and malignant transformation of EH [8]. We have already generated a hypothesis that the lack of estrogen receptor (ER) and progesterone receptor (PR) can predict poor response to treatment [9]. 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 [9]. There is a strong linear association between aromatase and expression of cyclo-oxygenases (COX-2) in uterine and breast cancer specimens, resulting in a complex paracrine and/or autocrine signalling pathway effecting abnormal oestrogen synthesis [9–11]. 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 the normal endometrium [12–14]. COX-2 is significantly associated with aromatase expression in endometrial cancer, which suggests that intra-endometrial oestrogen production promotes progression of EH to cancer [15]. There is also 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 [14]. 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 [16]. 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 [17]. Oestrogens may increase the rate of mutagenesis of Mlh1 through free radical formation as well as its inherent proliferative influence [17]. 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 [18]. In this study, we wish to test the hypothesis that the differential expression of immunohistochemical (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.

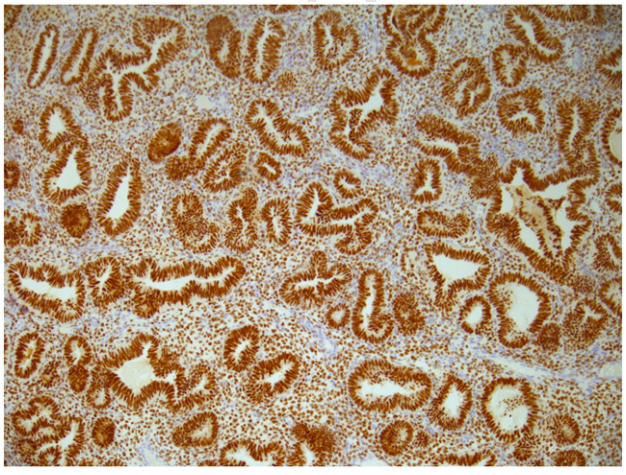

Fig. 1. ER expression (Q score = 8) for a woman that regressed with LNG-IUS treatment for complex endometrial hyperplasia.

Table 1
Baseline characteristics.

Baseline data		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[a]		6 (60)	53 (33.8)
Endometrial thickness > 9mm[a]		3 (33.3)	69 (47.9)
Cytological atypia		5 (50)	14 (8.5)

[a] Endometrial thickness was not measured in 21 women and BMI was not available in 7 women.

Material and methods

Study population

This was a prospective 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. Women were reviewed in our gynaecology 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. Study participants underwent diagnosis and endometrial histological surveillance by office endometrial sampling on a six-monthly basis for the first two years and yearly thereafter for five years. When this was not possible or inadequate sample was collected, hysteroscopic curettage was performed. 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).

The primary outcome for this study is to determine the prognostic value of ER, PR, COX-2, Mlh1, and Bcl-2 expressions 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 1) *Complete Regression* — atrophy of glands, edematous fibrotic stroma or pseudodecidualisation, with no evidence of hyperplasia. 2) *Persistence or Progression* — failure to completely regress with evidence of CH, ACH or carcinoma. 3) *Relapse* — failure to remain in regression with evidence of CH, ACH or carcinoma. All outcomes were evaluated with an intention to treat basis.

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 standard deviations (SD) and for skewed data medians and interquartile ranges (IQR). 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 [20]. Missing