Significance of progesterone receptors (PR-A and PR-B) expression as predictors for relapse after successful therapy of endometrial hyperplasia: A retrospective cohort study

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Abstract

Objective: After successful progestin therapy for endometrial hyperplasia (EH), the risk of relapse remains. We aimed to assess if immunohistochemical (IHC) expression of progesterone receptor isoforms, PR-A and PR-B, in endometrial glands and stroma in pre-treatment endometrial biopsies were related to relapse of EH.

Design and setting: Biopsy material originated from women with low- and medium-risk EH recruited to a recent Norwegian multicentre randomized trial. Participants (n=153) had been treated for six months with three different progestin regimes.

Population: 135 of the 153 women achieved therapy response and underwent follow-up for 24 months after therapy withdrawal. 55 women relapsed during follow-up. Pre-treatment endometrial biopsies from 94 of the 135 responding women were available for IHC staining.

Methods: IHC staining was performed separately for PR-A and PR-B and IHC expression was evaluated in endometrial glands and stroma by a histological score (H-score) using light microscopy.

Main Outcome Measure: IHC expression of PR-A and PR-B in endometrial glands and stroma in women with or without relapse of EH.

Results: Low PR-A in endometrial glands ($p=0.013$) and stroma ($p<0.001$), and high PR-B in endometrial glands ($p=0.001$), in pre-treatment endometrial biopsy have a statistically significant association with relapse of EH. Women with a pre-treatment ratio of PR-A:PR-B≤1 have higher risk of relapse (71%) compared to women with a ratio of PR-A:PR-B>1 (19%) ($p<0.001$).

Conclusion: IHC expression of PR-A and PR-B in pre-treatment endometrial biopsy proves valuable as predictors of relapse in EH.

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Keywords: Endometrial hyperplasia, progestin, relapse, progesterone receptor

Tweetable abstract: Pre-treatment endometrial expression of PR-A and PR-B are valuable predictors of relapse in endometrial hyperplasia

Introduction

Endometrial hyperplasia (EH) represents the preliminary stage of endometrial carcinoma (EC), and one in five cases will proceed to EC if left untreated.[1] The pathogenesis of the disease is not fully understood but it is well known that continuous exposure to endogenous and exogenous estrogen, unopposed by progesterone, is important in development of EH, and eventually EC.[2, 3] Progestin therapy has demonstrated a dose-dependent, curative effect on EH in former publications, the levonorgestrel impregnated intrauterine system (LNG-IUS) being superior to oral administration.[4-10] Nevertheless, when therapy is discontinued, the risk of relapse of EH has proven independent on former progestin therapy regime.[11] In different patient populations, the relapse rate after progestin withdrawal has been shown to vary between 13.7% to 41%.[11, 12]

Progesterone's growth inhibitory effects in the endometrial mucosa are mediated through interaction with nuclear progesterone receptors (PRs), acting as ligand-activated transcriptional factors and being members of the nuclear receptor superfamily.[13] The two most studied isoforms, progesterone receptor A (PR-A) and progesterone receptor B (PR-B), are expressed in endometrial glands and stroma and expression of both are required to ensure normal endometrial differentiation.[14] Alterations in the relative expression levels of PR-A and PR-B can result in aberrant PR signaling with altered gene transcription and such imbalance has been found in early carcinogenesis in hormone-sensitive cancer tissues.[15, 16] Predominant expression of PR-A or PR-B can result from increased expression of one isoform, with or without loss of the other, or isolated loss of one isoform.

The individual role of the PR-A and PR-B isoform in the etiology and prognosis of EH remains unclear, but a deregulation of PR-A and PR-B in either, or both, of the two endometrial tissue compartments (glands and stroma) is likely to be involved in disturbed endometrial proliferation through progression to EH, and EC. However, only a few studies

exist elucidating the prognostic significance of changes in endometrial expression of PR-A and PR-B for progestin responsiveness [17, 18] or relapse [19, 20] in EH.

Our main objective for the present study was to investigate if pre-treatment immunohistochemical (IHC) expression of PR-A and PR-B, in endometrial biopsies from 94 women diagnosed with low- to medium risk EH, are valuable as predictors for early relapse of EH after successful progestin therapy. An identification of reliable and feasible prognostic biomarkers in EH can provide for individualized therapy and follow-up strategies in women affected by this precancerous disease.

Methods

Trial design

Endometrial biopsy material for the present study originated from women between 30 and 70 years with histologically confirmed low- and medium-risk EH recruited to our national multicentre randomized trial.[10] No patient and public involvement (PPI) was included in the design of that study as this process took place during 2002 – 2004, and no formalized requirement for PPI in research existed in our country at that time. Participating women were treated for six months with either LNG-IUS 52 mg (Mirena®, Bayer Pharmaceuticals, Berlin, Germany), oral 10 mg Medroxyprogesterone acetate (MPA) daily or oral 10 mg MPA for 10 days per cycle after written informed consent.[10] The study inclusion period was from 1st of January 2005 to 1st of November 2011 and the treatment period was completed on 1st of May 2012. After six months of treatment, all therapy were withdrawn. To monitor relapse, all patients with primary therapy response (n=135) underwent follow-up with endometrial resampling at six-month intervals for 24 months after therapy discontinuation.[11]

The main outcome measure for the present study was defined as pre-treatment IHC expression of PR-A and PR-B in endometrial glands and stroma in women with or without relapse of EH. Core Outcome Sets (COS) have not yet been developed for EH and could therefore not be applied to our study. Histological material from 94 women with primary therapy response was available for IHC investigation with PR-A and PR-B. Insufficient biopsy material in the paraffin blocks was the reason for excluding 41 of the 135 women for the immunostaining procedure. Patient characteristics, such as age, WHO94 diagnosis, parity, BMI, menopausal status and serum estradiol level, were registered and the IHC expression of PR-A and PR-B in the pre-treatment endometrial biopsies were related to clinical relapse or not.

Endometrial biopsy material

All endometrial biopsy material from each participant, pre-treatment (index), post-treatment (control) and follow-up biopsies, were obtained using an endometrial suction curette (Pipelle®, Laboratoire CCD, Paris, France). The endometrial biopsies were sent to the Department of Pathology at the University Hospital of North Norway for routine assessment. The specimens were fixed in buffered formaldehyde, embedded in paraffin and further processed in the laboratory before standard histological sections were made. A trained gynaecology pathologist (AO) and one additional routine pathologist, both of whom were blinded to each other's diagnosis, performed diagnostic assessment of WHO94 classification by light microscopy. Agreement after discordant results was always obtained after discussion at a two-headed microscope. The index biopsies were classified into one of three groups: simple hyperplasia (SH), complex hyperplasia (CH), or atypical hyperplasia (AH) according to the WHO94 classification, which was considered the gold standard for evaluation of EH at the time the study was performed.[1, 12] Normalized histology in the control biopsies after therapy were defined as ordinary proliferative endometrium or endometrium with progestin effect.[1, 12] All information from the WHO classification of the index and control biopsies were registered and maintained in a separate database and subsequently supplemented by information from hospital records.

Immunohistochemistry

Immunohistochemistry was performed according to customer's advice. Slides (with a thickness of 4-5 µm) were routinely cut from paraffin blocks and placed on Superfrost+glasses. Incubation overnight at 60°C followed. Deparaffinisation, pre-treatment (in Tris-based, slightly alkaline reagent (CC1) for 48 minutes at 95°C) and the staining were performed automatically in a Benchmark Ultra IHC/ISH staining module. Instrument and reagents were provided by Ventana Medical Systems Inc, USA. The first of the two primary antibodies used in the present study was the A-form of Progesterone Receptor, a monoclonal

IgG1 antibody. Clone 16, Novocastra, Leica Biosystems Newcastle Ltd, United Kingdom (PGR A). The initial total protein concentration was 5.1 g/L and the applied dilution 1/150 in Antibody Diluent (Ventana Medical Systems Inc. USA). The other primary antibody was the B-form of Progesterone Receptor, a monoclonal IgG1 antibody. Clone hPRa 2, Thermo Fisher Scientific, USA (PGR B). The initial protein concentration was 0.2 mg/mL and the applied dilution 1/150 in Antibody Diluent (Ventana Medical Systems Inc. USA). After addition of the primary antibody, slides were incubated for 60 minutes at 37°C. Inhibitors were added to prevent nonspecific staining and enhancers were added to reinforce specific staining. Automatic DAB staining in several steps was performed before counterstaining with Hematoxylin. Ventana Medical Systems Inc, USA, provided the detection kits and all ancillaries used in this process. Slides were dehydrated and mounted before assessment.

Interpretation of Immunohistochemistry

Immunostaining for PR-A and PR-B were evaluated semi-quantitatively using an IHC histological score (H-score), which incorporates both the intensity and the distribution of specific staining. The H-score is defined as $HS = \sum(Pi \times i)/100$, where Pi denotes the percentage of stained nuclei, and i denotes the intensity of staining ranging from 1 to 3.[21] Expression in the endometrial glands and stroma were evaluated separately for each specimen. Hot spots (areas with the strongest immunostaining) with a diameter of one cm were examined at 40 X magnification. Both the intensity of staining and the number of stain-positive nuclei were counted. Samples with less than 10% positive nuclei were considered to be receptor-negative and given a score of zero. Samples with more than 10% positive nuclei were considered receptor-positive, and the percentage positive cells was used to compute the H-score. The H-score scale ranged from 0 to 3. A score of zero indicated the absence of staining, while scores of 1, 2 and 3 indicated weak, moderate and strong immunoreactivity, respectively. The H-score was assessed in a two-headed microscope by a trained

gynecological pathologist (AO) and a chief engineer (MA). Both investigators were blinded to the original diagnosis, therapy group and therapy response.

Statistical methods

Descriptive statistics are reported as mean and standard deviation or median and interquartile range for continuous variables based on the distribution of the variable, and as frequencies and percentages for categorical variables. Due to the binary outcome, relapse yes/no, univariable and multivariable logistic regression were performed with PR-A and PR-B in glands and stroma as independent variables. Univariable logistic regression was used to explore unadjusted effects, and multivariable logistic regression was used to adjust for clinical risk factors. PR-A in stroma and PR-A in glands could not be included in the same multivariable analysis due to high correlation. PR-A in stroma was chosen over PR-A in glands due to the lowest p-value in the univariable analysis. In addition, estradiol level is not included in the multivariable analyses due to high correlation with menopausal status. Continuous variables are categorized in the descriptive presentation, but are used as continuous in the regression analyses. Area under the curve (AUC) was calculated on a Receiver Operating Characteristic (ROC) curve. The diagnostic accuracy can generally be categorized as not useful for AUC<0.5, bad for AUC 0.5 – 0.6, sufficient for AUC 0.6 – 0.7, good for AUC 0.7 – 0.8, very good for AUC 0.8 – 0.9 and excellent for AUC 0.9 – 1.0.[22] All statistical analyses were performed using IMB SPSS Statistics Version 24 (IMB Corp., Armonk, NY, USA), and a significance level of 0.05 was considered statistically significant.

Funding

The study has been funded by the University of Tromsø.

Results

Patients

Endometrial biopsies from 94 women with primary therapy response were immunohistochemically stained for PR-A and PR-B (Figure S1). Of these, 37 had been treated by LNG-IUS, 33 by oral 10 mg MPA daily and 24 by oral 10 mg MPA 10 days per cycle. In the present patient material 40 out of 94 women (43%) relapsed during 24 months follow-up, of which 80% were diagnosed with relapse during the first 12 months after therapy withdrawal. Demographic data related to relapse is outlined in Table 1. Women with relapse of EH were generally younger and had a higher median level of serum estradiol than women who did not relapse. The relapsing and non-relapsing women had about similar mean BMI, but a higher proportion of the relapsing women had BMI $\geq$ 26 (58% vs 45%). Atypical hyperplasia was more prevalent in the group of women with relapse compared to women with no relapse (15% vs 6%).

PR-A and PR-B expression in endometrial glands and stroma in index biopsies related to relapse

Mean H-score expression levels for PR-A and PR-B in endometrial glands and stroma for relapsing and non-relapsing women are presented in Figure 1. For PR-A, mean H-score expression was significantly lower in endometrial glands (p-value=0.013) and stroma (p-value <0.001) in women who experienced relapse of EH. Mean H-score expression of PR-B was significantly higher in endometrial glands (p-value=0.001) in relapsing women. The mean H-score expression levels of PR-B in stroma did not differ significantly between relapsing and non-relapsing women (p-value=0.720). Figure S2 demonstrates an example of IHC staining intensity for PR-A and PR-B in endometrial glands and stroma in an index biopsy from one of the participating women.

The results of logistic regression univariable and multivariable analyses are presented in Table 2. Both PR-A in stroma and PR-B in glands remained statistically significant (p-value<0.001, p-value=0.030) when adjusting for clinical risk factors (age, WHO94 diagnosis, BMI and menopausal status). Menopausal status and age were significantly associated to relapse in the univariable analyses, but their significance disappeared in the multivariable analysis.

Subgroup analyses of PR-A and PR-B expression in endometrial glands and stroma in index biopsies related to relapse

We performed subgroup analyses based on the three therapy groups. Due to small number of patients in each group, we only explored unadjusted effects of PR-A and PR-B expression in endometrial glands and stroma in index biopsies related to relapse of EH. In all three therapy groups pre-treatment expression pf PR-A in endometrial stroma was significantly associated to relapse (LNG-IUS: OR 0.21, 95% CI OR 0.07 – 0.63, p-value 0.006, oral 10 mg MPA daily: OR 0.10, 95% CI OR 0.02 – 0.60, p-value 0.012, oral 10 mg MPA 10 days per cycle: OR 0.14, 95% CI OR 0.03 – 0.73, p-value 0.020). Pre-treatment expression of PR-B in endometrial glands was significantly associated to relapse in women treated by LNG-IUS (OR 4.38, 95% CI OR 1.28 – 14.94, p-value 0.018) and oral 10 mg MPA 10 days per cycle (OR 8.30, 95% CI OR 1.47 – 47.00, p-value 0.017).

Ratio of PR-A:PR-B expression in endometrial glands and stroma in index biopsies related to relapse

We evaluated the unadjusted and adjusted effects of pre-treatment ratios of PR-A:PR-B related to relapse of EH (Table 3). A 0.1 unit increase in ratio of PR-A:PR-B in glands led to 19% decreased odds for relapse, but the ratio of PR-A:PR-B in stroma did not have

statistically significant association with relapse. When combining glands+stroma for PR-A and PR-B a 0.1 unit increase in the ratio of PR-A:PR-B led to 17% decreased odds for relapse.

A ROC-curve was calculated for the ratio of PR-A:PR-B (glands+stoma) and demonstrated AUC of 0.771 (p-value 0.000, 95% CI 0.67 – 0.87), indicating moderate diagnostic accuracy for prediction of relapse. A cut-off value of ≤1 gave sensitivity and specificity for prediction of relapse of 75% and 78%, respectively. Likelihood ratio for a positive test result was 3.4 and likelihood ratio for a negative test was 0.32. In the logistic regression analyses, a ratio of PR-A:PR:B≤1 (glands+stoma) showed an 11-fold increased odds for relapse compared to PR-A:PR-B>1 (Table 3). The cumulative relapse rates for women with a ratio of PR-A:PR-B≤1 versus >1 were calculated to 71% and 19%.

Discussion

Main findings

Until date, no reliable prognostic biomarkers have been found to predict relapse of EH to permit individualized long-term progestin therapy and follow-up. Our present results have demonstrated that low PR-A in endometrial glands and stroma, and high PR-B in endometrial glands, in pre-treatment endometrial biopsies are predictors of relapse after successful progestin therapy for EH. These results are in accordance with a recent publication reporting that low PR-A in endometrial stroma and high PR-B in endometrial glands prior to therapy correlated to relapse in EH in a retrospective study population.[20] PR levels prior to therapy were reported only weakly associated with relapse in a study by Gallos and collaborators evaluating expression of estrogen receptor (ER), PR, COX-2, Mlh1 and Bcl-2 as predictors for relapse in women with EH.[19] In contrast to our study, the expression of the two isoforms of PR were not separately reported.[19]

Strengths and limitations

The strength of the current study relates to the origin of the endometrial biopsy material from a recent national multicenter randomized trial with 24 months follow-up. The IHC expression of both isoforms, PR-A and PR-B, were evaluated in individual tissue specimens and for each isoform endometrial glands and stroma were separately assessed. This permits for increased knowledge on isoform specific and tissue specific PR-signaling.

A limitation is that our study participants had underwent progestin therapy in three different treatment arms and it can therefore be questioned if this has influenced our results. In the original publication, upon which the present study is based, relapse rates were shown to be independent on progestin regime. Additionally, our subgroup analyses demonstrated the same trend of pre-treatment expression of PR-A and PR-B in association to relapse as reported for the whole study population. Another limitation is the exclusion of 41 patients due to insufficient material in paraffin blocks. However, about similar number of patients were excluded from each therapy group. Finally, a comparison of our results to results from previous publications is not straight forward as Core Outcome Sets (COS) for literature reporting in EH has not yet been developed. This has led to great variations in e.g. progestin therapy regimes, therapy duration, follow-up duration after successful therapy, therapy withdrawal or not during follow-up, methods for endometrial resampling and histopathological diagnostic methods. However, work has begun to establish COS in EH and hopefully this will improve the use of consensus methodology in EH in the future.[23]

Interpretation

Our present findings indicate opposing actions for PR-A and PR-B in endometrial growth regulation. Potential physiological mechanisms underlying these observations are largely unknown, but different knockout mice models have been established to study individual PR isoform function in endometrial tissue.[3, 24, 25] Data from these studies have revealed that PR-A

is essential for normal function of the endometrial epithelial glands and stroma, while PR-B promotes EH both in response to estrogen alone and to a combination of estrogen and progesterone.[24] Further, in rat uterine cell studies PR-A has been found capable of inhibiting estrogen receptor (ER) activity.[26] Thus, the PR-A isoform seems required to counteract both estrogen- and PR-B-induced proliferation. The applicability of these results to human endometrial tissue can clearly be discussed, but it implies that the relative balance of PR-A and PR-B is critical for the appropriate endometrial response to the hormonal environment.

While PR-A and PR-B is co-expressed equivalent in the epithelial glands in the normal cycling endometrium, some have reported that PR-A is the predominant isoform in the stromal cells.[27] It has recently been reported that the endometrial stroma creates a microenvironment that is decisive for progestin-responsiveness in the endometrial glands.[3] Thus, decreased stromal PR-A is suggested a main determinant to progestin resistance in EC cells.[3] If such interactions between endometrial stromal and glandular cells is of importance in development of EH in humans remains unclear, but low stromal PR-A was the single predictor associated to relapse with lowest p-value (p-value<0.001) in our patient population. However, effective co-culture experiments using transformed human endometrial glandular and stromal cells are lacking to study interaction or mutual influence during carcinogenesis.

The increasing understanding of PR-A and PR-B as distinct, and even contradictory, growth regulators has encouraged the exploration of the role of the relative expression of PR-A and PR-B in cancer types such as EC[28] and breast cancer.[29, 30] Jongen and colleagues found shorter disease free survival and shorter overall survival for EC patients with a ratio of PR-A:PR-B<1. The results of the present study demonstrated an 11-fold increased odds for relapse in patients with pre-treatment PR-A:PR-B≤1 compared to patients with PR-A:PR-B>1. Thus, a pre-treatment ratio of PR-A:PR-B≤1 might represent a useful biomarker in clinical practice to select individuals with the highest risk of relapse.

In current EH management, the risk of relapse after successful progestin therapy has gained increasing attention. Progestin therapy duration for EH has traditionally been 3 – 6 months. Growing evidence has suggested that relapse of EH can be reduced, and probably avoided, as long as progestin therapy is continued. However, no tools to identify women who would benefit from long-term progestin treatment exist. To introduce prolonged progestin therapy for all women with EH is unwanted as such regime will represent over-treatment, and might cause unnecessary side effects, in a substantial number of patients. The present study indicates that the relative expression levels of PR-A and PR-B at the time of EH diagnosis can provide important information regarding probability for relapse. Thus, if our results can be confirmed in a larger population, these biomarkers can get implications for therapy duration, and surveillance frequency after progestin therapy withdrawal, on an individualized basis. IHC analyses of PR-A and PR-B are relatively feasible and low-cost procedures and can easily be implemented in routine EH diagnostics as adjuncts to standard microscopy and image analysis.

Conclusion

We have demonstrated that pre-treatment expression of PR-A in endometrial glands and stroma and PR-B in endometrial glands are valuable as predictors of relapse in EH and that low expression of PR-A to PR-B is associated with higher relapse rates. Increased knowledge of the two progesterone receptor isoforms actions might contribute to new diagnostic and therapeutical strategies in endometrial proliferative diseases.

Disclosure of interest

The authors have no conflicts of interest.

Contribution to authorship

ETS is the main author of the manuscript and has contributed substantially in the planning of the study and interpretation of statistical data. MA has contributed to interpretation of the immunohistochemistry and been responsible for establishing and maintaining the databases of results. LML has performed the immunohistochemical work. ML has been responsible for the statistical work. AØ has been main responsible for planning and accomplishment of the study, microscopy with interpretation of results and manuscript.

Ethical approval

The study was approved by the Regional Committees for Medical and Health Research Ethics on 15th of September (P REK NORD 25/2004) and by the Norwegian Medicines Agency on 13th of May 2005 (ClinicalTrials.gov, NCT01074892). Written informed consent was obtained from all study participants.

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Figure 1. Mean H-score expression levels of progesterone receptor isoforms, PR-A and PR-B, in endometrial glands and stroma in index biopsies. The H-score scale range from 0 to 3. Results are presented as mean value ± Standard error of the mean. Univariable logistic regression analyses were performed to obtain p-values.

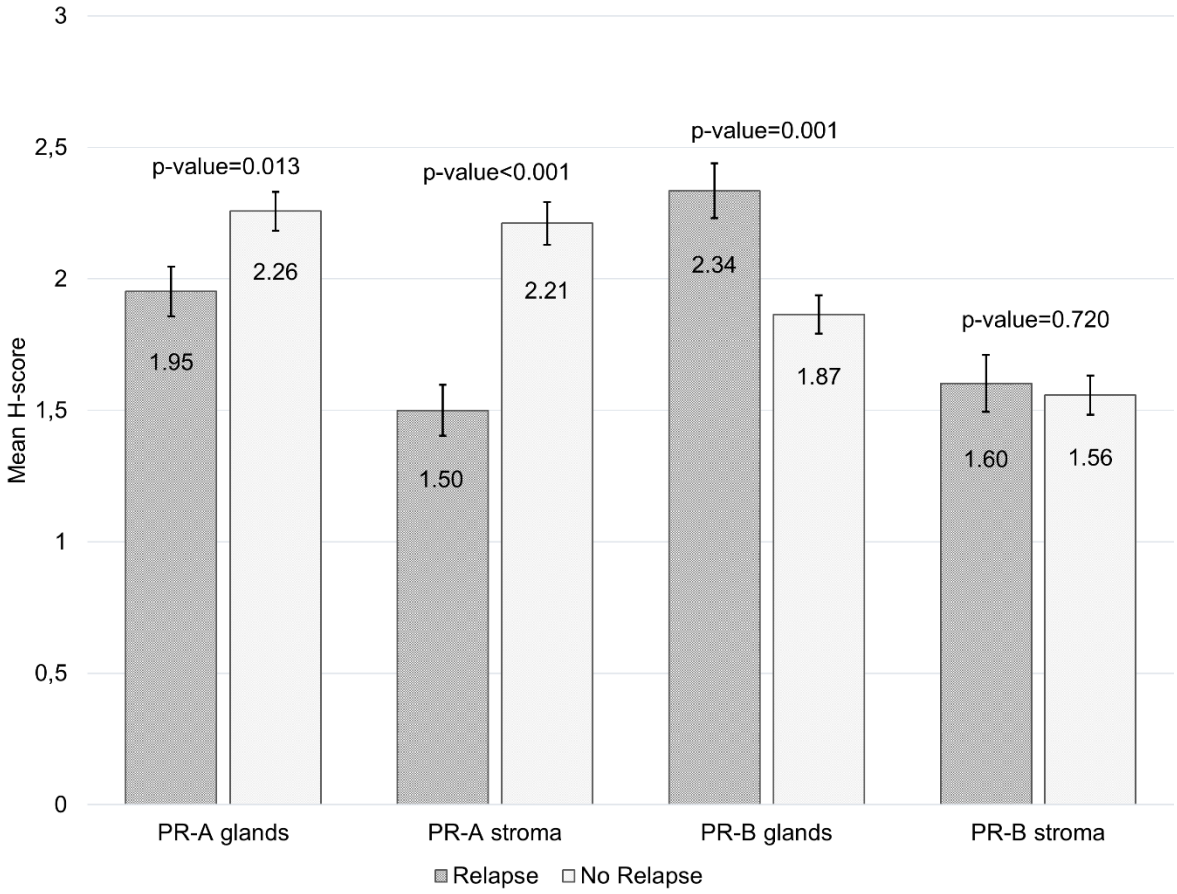

Table 1. Demographic data for the study population related to relapse or no relapse of endometrial hyperplasia during 24 months follow-up.

Characteristics	**n=40, Relapse n (%)**	**n=54, No Relapse n (%)**
Therapy regimen		
LNG-IUS	18 (45.0)	19 (35.2)
MPA continuous	13 (32.5)	20 (37.0)
MPA cyclic	9 (22.5)	15 (27.8)
Age (years), *mean (SD)*	***45.4 (6.0)***	***48.4 (6.8)***
< 43	12 (30.0)	10 (18.5)
43 – 48	12 (30.0)	13 (24.1)
49 – 51	11 (27.5)	12 (22.2)
≥ 52	5 (12.5)	19 (35.2)
WHO94 classification		
SH	2 (5.0)	10 (18.5)
CH	32 (80.0)	41 (75.9)
AH	6 (15.0)	3 (5.6)
D-score		
0-1	7 (17.5)	8 (14.8)
>1	33 (82.5)	46 (85.2)
Parity		
0-1	8 (20.0)	17 (31.5)
2	19 (47.5)	19 (35.2)
3+	13 (32.5)	18 (33.3)
BMI (kg/m^2), *mean (SD)*	***27.5 (5.3)***	***26.7 (5.9)***ǂ
< 23	6 (15.0)	17 (31.5)
23 – 26	11 (27.5)	12 (22.2)
26 - 30	14 (35.0)	9 (16.7)
>30	9 (22.5)	15 (27.8)
Menopausal status#		
Premenopausal	33 (82.5)	26 (48.1)
Perimenopausal	6 (15.0)	22 (40.7)
Postmenopausal	1 (2.5)	6 (11.1)
Estradiol level (nmol/l), *median (IQR)*	***0.34 (0.37)***	***0.16 (0.38)***##
≤0.12	4 (10.0)	20 (37.0)
0.13 - 0.28	9 (22.5)	13 (24.1)
0.29 – 0.54	16 (40.0)	7 (13.0)
≥ 0.55	11 (27.5)	12 (22.2)

Abbreviations: LNG-IUS;Levonorgestrel impregnated system, MPA;Medroksypreogesterone acetate, SH;Simple hyperplasia, CH;Complex hyperplasia, AH;Atypical hyperplasia, SD;Standard deviation, IQR;Interquartile range.

ǂ BMI value missing for 1 women. # Menopausal status was defined according to s-estradiol (nmol/l) and s-FSH (IU/l) assessed before start of therapy. ## Estradiol level missing for 2 women.

Table 2. Unadjusted and adjusted effects of H-score expression levels of PR-A and PR-B related to relapse. PR-A stroma and PR-B glands were both included in the same multivariable analysis and adjusted for age, WHO94, BMI and menopausal status.

	Unadjusted effects			Adjusted effects		
Variable	OR	95% CI OR	p-value	OR	95% CI OR	p-value
PR-A glands	0.39ǂ	0.19 – 0.83	0.013*	-		
PR-A stroma	0.16ǂ	0.07 – 0.37	<0.001**	0.15ǂ	0.05 – 0.39	<0.001**
PR-B glands	3.71ǂ	1.76 – 7.83	0.001**	2.91ǂ	1.11 – 7.62	0.030*
PR-B stroma	1.13ǂ	0.57 – 2.24	0.720	-		
Age (years)	0.932	0.87 – 0.99	0.036*	1.05	0.95 – 1.16	0.336
WHO94			0.088			0.111
SH	Ref.			Ref.		
CH	3.90	0.80 – 19.08	0.093	8.03	1.06 – 60.62	0.043
AH	10.0	1.28 – 78.12	0.028	10.11	0.83 – 123.10	0.070
BMI (kg/m^2)	1.03	0.95 – 1.10	0.507	1.10	0.99 – 1.22	0.090
Menopausal status			0.005**			0.061
Premenopausal	Ref.			Ref.		
Perimenopausal	0.22	0.08 – 0.61	0.004	0.20	0.05 – 0.85	0.030
Postmenopausal	0.13	0.02 – 1.16	0.068	0.08	0.00 – 2.22	0.136
Estradiol level (nmol/l)	1.81	0.77 – 4.28	0.174	-		

Abbreviations: OR;Odds ratio, CI;Confidence interval, SH;Simple hyperplasia, CH;Complex hyperplasia, AH;Atypical hyperplasia.

ǂOdds ratios (OR) are shown for 1 unit increase in H-score levels for PR-A and PR-B. *p<0.05, **p<0.01.

Table 3. Unadjusted and adjusted effects for H-score ratios of PR-A:PR-B related to relapse. Multivariable analyses were performed separately for each ratio with adjustment for age, WHO94, BMI and menopausal status.

	Unadjusted effects			Adjusted effects		
Variable	OR	95% CI OR	p-value	OR	95% CI OR	p-value
Ratio PR-A glands : PR-B glands	0.81ǂ	0.72 – 0.90	<0.001**	0.80ǂ	0.69 – 0.92	0.002**
Ratio PR-A stroma : PR-B stroma	0.97ǂ	0.92 – 1.01	0.143	0.98ǂ	0.93 – 1.02	0.253
Ratio PR-A stroma : PR-B glands	0.78ǂ	0.69 – 0.87	<0.001**	0.76ǂ	0.67 – 0.87	<0.001**
Ratio PR-A total♯ : PR-B total♯	0.83ǂ	0.75 – 0.92	<0.001**	0.83ǂ	0.74 – 0.94	0.004**
Ratio PR-A total♯ : PR-B total♯ ≤ 1	10.5	4.02 – 27.45	<0.001**	11.05	3.41 – 35.80	<0.001**

Abbreviations: OR;Odds ratio, CI;Confidence interval. ǂOdds ratios (OR) are shown for 0.1 unit increase in ratio.

♯Total means glands+stroma. *p<0.05, **p<0.01.

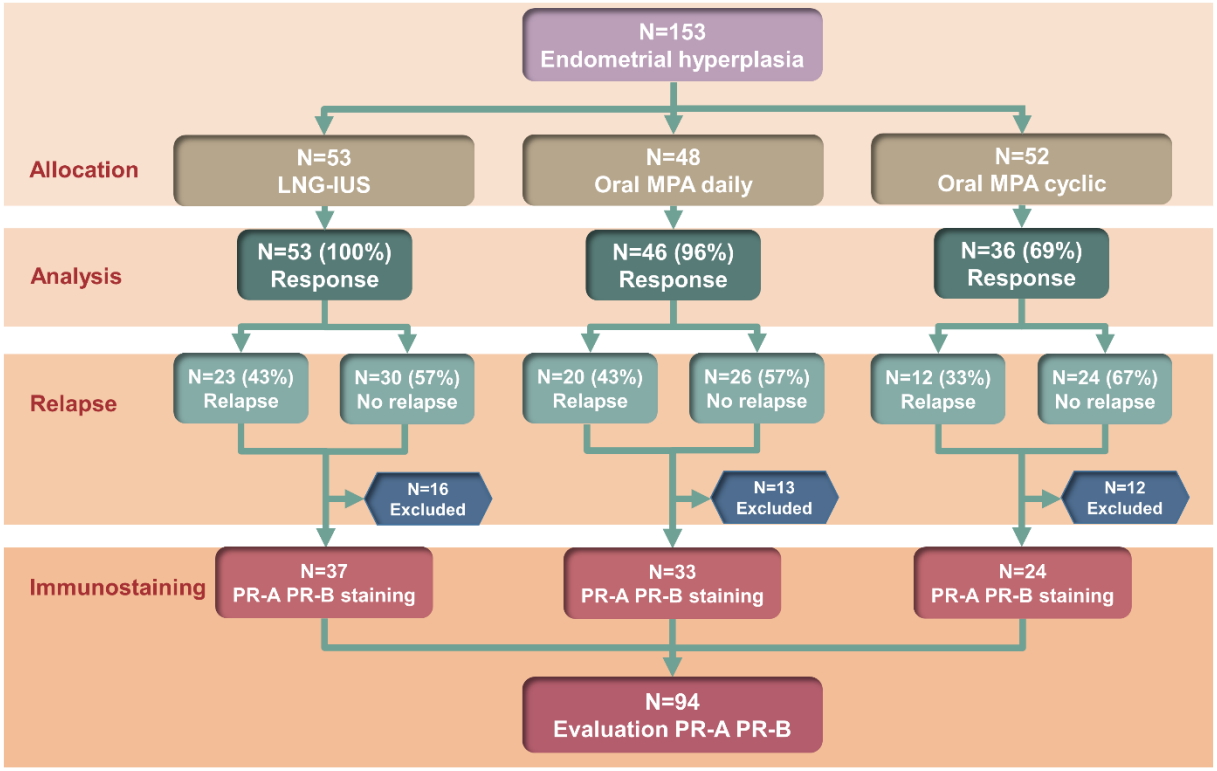

Figure S1. Flowchart showing allocation, therapy response, relapse rates, and number of patients who had biopsies investigated by immunohistochemical staining of progesterone receptor A (PR-A) and progesterone receptor B (PR-B) in endometrial glands and stroma. A total of 41 patients were excluded before evaluation of PR-A and PR-B due to insufficient biopsy material in the paraffin blocks. Abbreviations: LNG-IUS;Levonorgestrel impregnated system, MPA;Medroksyprogesterone acetate

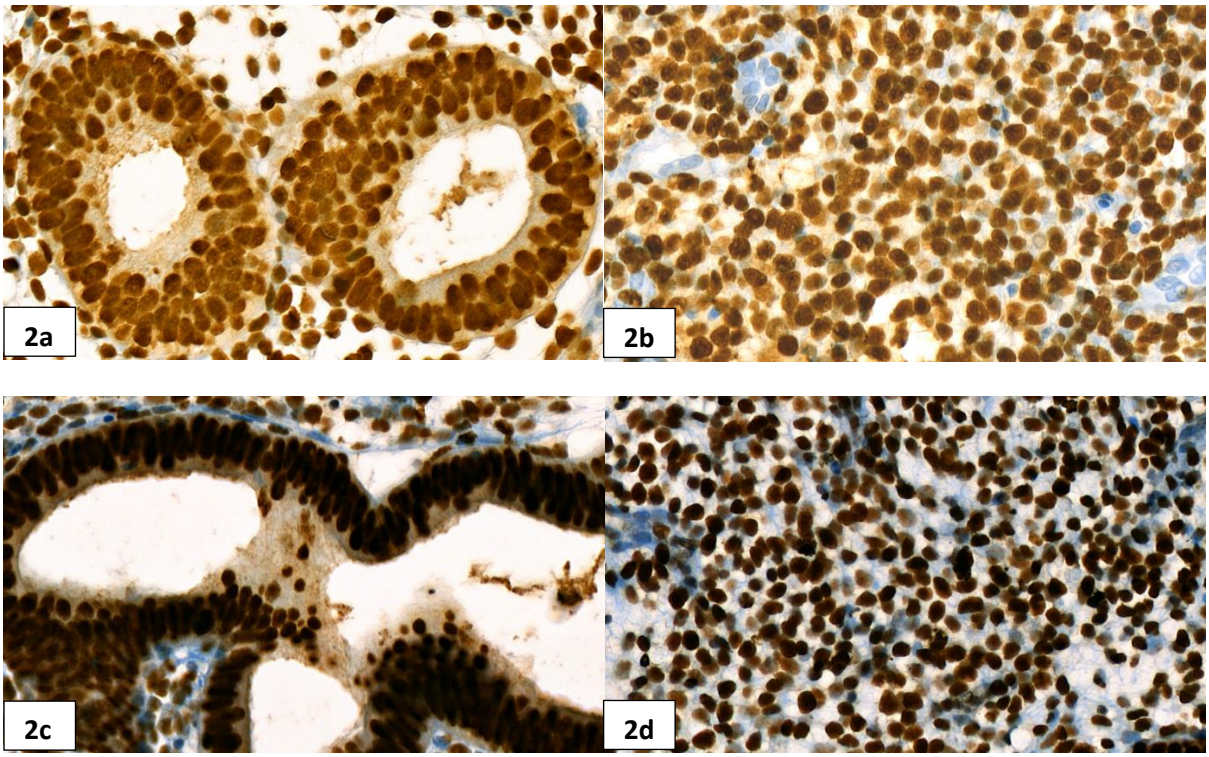

Figure S2. Micrographs demonstrating immunohistochemical staining intensity for progesterone receptor A (PR-A) and progesterone receptor B (PR-B) in an index biopsy form a women diagnosed with CH. She obtained therapy response after six months of progestin therapy with LNG-IUS. She was later diagnosed with relapse 12 months after therapy withdrawal. Figure 2a and 2b represents endometrial glands and stroma, respectively, stained for PR-A. H-score level for PR-A was 1.1 in glands and 1.2 in stroma. Figure 2c and 2d is showing endometrial glands and stroma stained for PR-B. H-score level for PR-B was 2.9 in glands and 2.9 in stroma. Abbreviations: CH;Complex hyperplasia, LNG-IUS;Levonorgestrel impregnated system