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Steroid receptors and proliferative activity in non-neoplastic and neoplastic endometria*

H. Pickartz[2], R. Beckmann[1], B. Fleige[1], W. Düe[1], J. Gerdes[1], and H. Stein[1]

Institutes of Pathology, [1] Universitätsklinikum Steglitz, Free University, [2] Krankenhaus Spandau, D-1000 Berlin

Summary. In the glands of cyclic endometria, proliferative activity (PA), as revealed by expression of the Ki-67 antigen, is highest in the proliferative phase (P) and early secretory phase (S1). The PA decreases in the middle secretory phase (S2). In the stroma the PA is low during the whole cycle. In P and S1, the oestrogen receptor (ER) and the progesterone receptor (PR) are strongly expressed in glands and stroma. The number of positive cells and the staining intensity decreases in S2, particularly in the glands. In atrophic endometria, fibro-glandular polyps and in endometria with arrested secretion the PA is low in both glands and stroma. ER and PR can be detected in glands and stroma. The PA in atypical hyperplasias is only slightly higher than in cyclic endometria and endometria with simple hyperplasia. The ER and PR levels are comaparable to those in proliferative endometria. The PA of endometrial adenocarcinomas is positively and the ER and PR negatively correlated with the degree of de-differentiation. No ER-negative carcinoma displays the PR. Immunohistologically, non-neoplastic receptor positive tissue can be seen in many ER- and PR-negative carcinomas. These structures may falsify the biochemical receptor analysis.

Key words: Endometrium – Neoplastic/non-neoplastic – Proliferative activity – Steroid hormone receptor expression – Immunohistology

Introduction

The development of monoclonal antibodies (mAb) for the immunohistological assessment of proliferative activity (PA), the oestrogen receptor (ER) and the progesterone receptor (PR) provides a new tool for functional pathomorphology. We took this chance to investigate the relationship between the PA and the ER/PR content in cyclic, non-cyclic, hyperplastic and neoplastic endometria.

Biochemical receptor assays may be falsified by receptor-positive tissue components in a receptor-negative tumour sample. The synoptical analysis of the histological structure and the receptor content by immunohistology avoids this problem.

Offprint requests to: H. Pickartz, Institute of Pathology, Krankenhaus Spandau, Lynarstrasse 12, D-1000 Berlin 20

* Prof. Dr. Curt Froboese dedicated to his 99th birthday

Material and methods

Endometrial tissue (Table 1) was obtained from 130 women between the ages of 23 and 85 years treated at the Department of Gynaecology, Klinikum Steglitz, Free University, Berlin. The patients had been admitted for acyclic bleeding, post-menopausal bleeding, leiomyomas, abnormal ecto-cervix, sterilization, infertility or uterine prolapse.

Endometria influenced by exogenous hormones ($n=18$) at the time of curettage or hysterectomy were diagnosed as cyclic endometria (3), irregular shedding (1), arrested secretion (1), atrophic (2), irregular proliferation (5), simple hyperplasia (1), atypical hyperplasias (3) and adenocarcinoma grade 3 (1). Of the atypical hyperplasias, 2 showed nuclear atypia grade 2. The content of the hormone drugs was variable: oestrogen/gestagen combinations ($n=7$), oestrogens ($n=6$), gestagens ($n=1$), oestrogen/androgen combinations ($n=1$); in 2 cases the hormone drug was not elucidated.

Immediately after a curettage or hysterectomy the specimens (curettings in 0.9% NaCl) were transferred packed in ice to the Institute of Pathology. One part of the tissue was snap-frozen in

Table 1. Composition of the specimens examined

130 Endometria	82 Curettings
	48 Hysterectomy specimens
Diagnoses	41 Normal, cyclic
	5 Atrophy
	4 Polyps
	2 Irregular shedding
	1 Arrested secretion
	28 Irregular proliferation
	10 Simple hyperplasia
	11 Atypical hyperplasia
	28 Adenocarcinomas

liquid nitrogen and stored at −90° C. The other part of the tissue was fixed in formaldehyde, embedded in paraffin and further processed routinely. Apart from the immunohistological staining, H&E-sections were taken from the deep frozen tissue.

For conventional histological diagnosis endometria with functional disturbances were classified in accordance with Dallenbach-Hellweg (1987), precancerous lesions and carcinomas by following the criteria worked out by the International Society of Gynaecological Pathologists (Kurman and Norris 1987). In order to elucidate how architectural and nulcear atypia are correlated with PA and ER/PR expression, in cases of endometrial hyperplasias these parameters were evaluated separately (low, and moderate nuclear atypia and low, moderate and severe architectural atypia).

For the same reason, in carcinomas three different nuclear and architectural grades of atypia were differentiated. The grading numbers were counted; carcinomas with 5 or 6 points were assessed as grade 3 carcinomas, those with 4 points as grade 2 and those with 2–3 points as grade 1 carcinomas. Twenty-three carcinomas were of endometrioid type; 5 were papillary carcinomas. The conventional light microscopical assessment of the lesions was performed on paraffin sections.

The mAb against the ER (Greeene and Jensen 1982; Press and Greene 1984) was obtained from Abbott Diagnostics (Wiesbaden, FRG), the PR antibody (Logeat et al. 1983; Perrot-Applanat et al. 1985) from Transbio-Sarl (Paris, France). The mAb against ER was produced in the rat, the PR antibody in the mouse. The rabbit anti-mouse-Ig serum was obtained from Dakopatts, (Copenhagen, Denmark) the mouse anti-rat serum from Dianova (Hamburg, FRG). The APAAP (alkaline phosphatase-anti-alkaline phosphatase) complexes were produced according to the method of Cordell et al. (1984).

Calf intestinal alkaline phosphatase (10 mg) (Sigma, Munich, FRG) was incubated with 10 ml of the alkaline-phosphatase-hybridoma supernatant. The production of the mAb Ki-67 was described by Gerdes et al. (1983, 1984). The bridging antibodies were preincubated with human serum proteins. They showed no cross-reactivity with human tissue components.

Immunohistological staining was carried out on cryostat sections (about 7 µm) using the APAAP method (Cordell et al. 1984).

For the detection of the antigen Ki-67 the sections were allowed to dry overnight. Immediately prior to the staining they were fixed in acetone and chloroform (30 min each). For the detection of ER and PR the sections were fixed in Zamboni's solution for 10 min (Stefanini et al. 1967). The sections for ER and PR staining were stored overnight at 4° C in phosphate-buffered saline (PBS) which further contained 0.25 M sucrose, 3 mM $MgCl_2$ and glycerine (59%). Prior to staining the sections were washed in PBS. They were then incubated with the primary antibody against ER (ER-ICA-Kit, Abbott; dilution 1:5) and against PR (dilution 1:30). The sections for the detection of Ki-67 were incubated with the primary antibody in optimal dilution tested before. After washing in PBS the sections for the ER labelling were incubated (30 min) with mouse anti-rat-Ig serum (diluted 1:80 with 1:8 prediluted human serum). After washing all the sections in PBS they were incubated with rabbit anti-mouse serum (1:20 with 1:8 prediluted human serum). After another washing in PBS the sections were incubated with the APAAP complex (dilution 1:30 to 1:90). The incubation steps with all secondary antibodies were repeated twice for ER-ICA and once for Ki-67 and PR-ICA for 10 min. The alkaline phosphatase was visualized as described by Stein et al. (1985) by using new fuchsin. The sections were counterstained with hemalum and then covered in glycerine gelatine. Immunohistochemical staining was controlled by: incubation with the secondary antibodies only; and developing the alkaline phosphatase only (endogenous alkaline phosphatase). The control staining gave consistently negative results.

Immunoreactivity was evaluated by estimating the labelled cell nuclei (in %). For ER-ICA and PR-ICA, staining intensity was also assessed (weak, moderate, strong). The number of labelled cells was estimated in 10% steps; additionally levels of 5 and 95% were estimated.

For the detection of the different antigens only the nuclear labelling was taken into account. Analysing the Ki-67 antigen, squamous epithelia showed a non-specific cytoplasmic staining, which was interpreted as being a cross-reaction with a different antigen and was not evaluated.

For statistical evaluation the U-test and Spearman's rank correlation coefficient were used (Sachs 1968). Significance was assumed for $p \leq 0.05$.

Results

The results for cyclic endometria are shown in Table 2 and Figs. 1a–c, 3a, b and 4. With Ki-67 the glands of the proliferating endometria show an irregular pattern of staining. The labelling of the basalis glands is much lower when compared with the glands of the functionalis. Focal crowding of labelled nuclei is quite frequent. The mean number of labelled gland epithelia (in %) varies considerably in the different endometria. The labelling density increases in S1 and strongly decreases in S2 and S3. In the stroma the labelling density remains at about the same level during the whole cycle; zonal accentuation cannot be seen.

The growth fraction in P3 significantly differs from the growth fraction in S2 and S3 (U-test).

With ER-ICA the glandular epithelia in P and S1 are ER-positive in about 90%, the labelling intensity of the nuclei being strong to moderate. In the functionalis layer the amount of positive epithelia decreases in S2 to 60% and in S3 to 40%; at the same time the staining intensity decreases to a moderate to weak level. The U-test reveals a significant difference in the ER content of the glandular epithelia from P3 to S3. In the basalis layer the ER content remains high during the whole cycle.

The percentage of positive cells in the stroma is reduced from 90% in P to 60% in S2 and slightly increased to 70% in S3. The highest staining intensity is found in P; it is low in S1 and moderate in S2 and S3. Vascular and inflammatory cells are always ER-negative.

With PR-ICA in P, more than 90% of glandular and stromal cells are strongly PR-positive; in S1 and S2 the PR-content remains high in the glands; in S3 it decreases distinctly in the functionalis layer with a weak staining intensity. The difference of the PR content between P3 and S3 is significant. In the stromal layer the PR content remains high; the staining intensity decreases only slightly towards the end of the cycle. Like the ER, the PR in the basalis layer remains strongly expressed in glands and stroma during the whole cycle.

In non-cyclic endometria the findings are summarized in Table 2. With Ki-67 in atrophic endometria only a few cells are labelled in glands and stroma. The fibroglandular polyps show the same results in the stroma; the glandular levels vary between 0–5%. Similar results are gained in the two endometria with irregular shedding.

Endometria with irregular proliferation have a lower PA than cyclic endometria in P. In irregular proliferated endometria without secretory transformation the PA is slightly higher than with secretory transformation.

Table 2. Summary of all immunohistochemistry data (mean, median, range) of cyclic dysregulated, irregularly proliferated, hyperplastic and neoplastic endometria

Type of lesion	Ki-67 glands %	ER glands % StI	PR glands % StI	Ki-67 stroma %	ER stroma % StI	PR stroma % StI
P ($n=18$)						
Mean	19.1	88.9 s	94 s	6.5	84.7 s	95 s
Median	12.5	95 s	95 s	5	90 m	95 s
Range	1–70	20–99 m-s	90–95 w-s	1–20	50–95 w-s	95–96 s-s
P1+P2 ($n=11$)						
Mean	19	91.2 s	95 s	5.5	86.4 s	95 s
Median	10	92.5 s	95 s	3.5	90 s	95 s
Range	1–70	70–99 m-s	95–95 s-s	1–15	60–95 w-s	95–95 s-s
P3 ($n=7$)						
Mean	19.3	85.4 s	92.5 m	8.1	82.1 m	95 s
Median	12.5	95 s	92.5 m	7.5	90 m	95 s
Range	5–45	20–99 m-s	90–95 w-s	1–20	50–95 w-s	95–95 s-s
S1 ($n=9$)						
Mean	24.6	84.2 s	95 s	5.4	72.7 w	95 s
Median	20	90 m	95 s	1	80 w	95 s
Range	1–50	50–95 w-s	95–95 s-s	1–21	30–90 w-m	95–95 s-s
S2 ($n=3$)						
Mean	0.7	60 m	95 s	3.7	60 m	87.5 s
Median	1	70 m	95 s	5	60 m	90 s
Range	0–1	30–80 m-s	95–95 s-s	1–5	50–70 w-m	80–95 m-s
S3 ($n=11$)						
Mean	0.8	42.3 w	16 w	6.8	70 m	89.2 m
Median	1	40 w	10 w	5	70 m	90 m
Range	0–2	5–90 w-s	5–40 w-s	1–20	40–95 w-s	80–95 m-s
Atrophy ($n=5$)						
Mean	1.6	80 m	84 s	1	89 m	87.5 s
Median	1	80 m	84 s	1	90 s	87.5 s
Range	0–5	60–95 m-s	70–98 m-s	1–1	80–95 m-s	85–90 s-s
Irregular shedding ($n=2$)						
Mean/median	1	86.5 m	17.5 m	3	90 m	70 s
Range	1/1	75/98 w/s	5/30 w/s	1/5	90/90 m/s	70/70 m/s
Polyps ($n=4$)						
Mean	2.8	83.8 s	92.5 s	1	80 s	65 m
Median	2.5	80 s	92.5 s	1	87.5 s	65 m
Range	0–5	75–100 m-s	90–95 s-s	1	50–95 w-s	50–80 w-s
Arrested secretion ($n=1$)						
	1	80 m	95 s	5	80 m	95 s
Irregular proliferation ($n=28$)						
Mean	11.7	86.2 s	95 s	5.1	84.5 s	91.3 s
Median	5	90 s	95 s	3	90 s	95 s
Range	0–40	30–99 w-s	90–99 s-s	0–20	60–98 w-s	70–95 m-s
Simple hyperplasia ($n=10$)						
Mean	22.4	84.5 s	96.8 s	8.6	88.2 m	90 m
Median	22.5	90 s	95 s	10	80 s	95 m
Range	1–50	60–95 w-s	95–99 s-s	1–15	65–95 w-s	60–95 w-s
Atypical hyperplasia ($n=11$)						
Mean	27.7	88.2 s	88.4 s	5.8	89 s	86 m
Median	20	95 s	95 s	5	90 s	87.5 s
Range	5–70	50–99 m-s	50–99 s-s	1–20	60–99 w-s	70–99 w-s
Carcinomas (total) ($n=28$)						
Mean	49.6	39.3 s	33.2 s			
Median	60	20 m	0 s			
Range	10–70	0–90 w-s	0–90 m-s			
G1 ($n=8$)						
Mean	38.8	81.3 s	80 s			
Median	35	90 s	80 s			
Range	10–60	30–90 w-s	0–90 m-s			
G2 ($n=8$)						
Mean	48.8	48.8 m	23 m			
Median	55	60 m	0			
Range	10–70	0–90 m-s	0–90 w-s			
G3 ($n=12$)						
Mean	57.5	5 m	1.3 w			
Median	60	0 m	0			
Range	30–70	0–20 m-m	0–10 w-w			

P/S 1, 2, 3: proliferative/secretory phase, early, middle, late; StI: staining intensity; w, weak; m, moderate; s, strong; $n=130$); ER, oestrogen receptor; PR, progesterone receptor

With ER-ICA in atrophic endometria, in fibro-glandular polyps and in endometria with irregular shedding the ER is detected in 80–90% in glands and stroma, with a moderate to strong staining intensity. Irregularly proliferating endometria without secretory transformation contain the ER in about 90% in glandular and stromal cells with a moderate to strong staining intensity; in secretory transformed endometria the ER content is diminished.

With PR-ICA 90% of glandular epithelia are intensively stained in fibro-glandular polyps; the stromal cells express the PR in 65% with a moderate staining intensity. In atrophic endometria more than 80% of the glandular and stromal cells are strongly labelled. In endome-

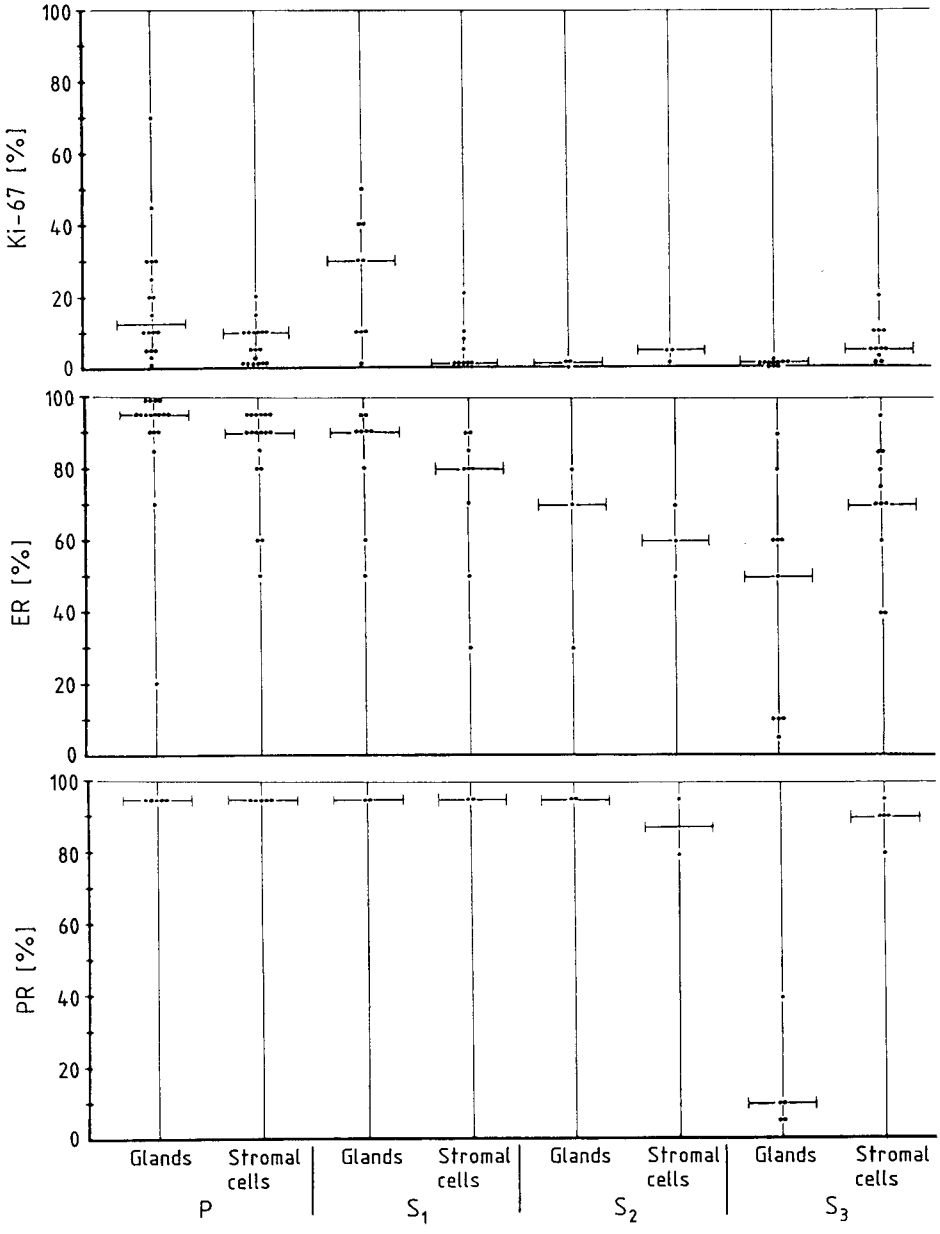

Fig. 1a–c. Cyclic endometria; growth fraction (**a**), oestrogen receptor (ER) content (**b**) and progesterone receptor (PR) content (**c**) % of labelled nuclei of glands and stroma in the proliferative phase (*P*), in the early (*S1*), middle (*S2*) and late (*S3*) secretory phase. *Bar* = median

tria with irregular shedding the PR is found in 17.5% of the glandular cells and in 70% of the stromal cells the staining intensity is moderate. Almost all stromal and glandular cells of endometria with irregular proliferation show the PR with a strong staining intensity. With secretory transformation the PR content is not diminished.

Only a few endometria treated with exogenous hormones showed the same histology in this group and a statistical comparison with the endometria without exogenous hormones is not possible. A single case comparison of the endometria with and without exogenous hormones, regarding the growth fraction, the ER and the PR content respectively, shows no essential differences. An obvious hormonal influence on the immunohistochemical results cannot be observed.

In simple hyperplasia (see Table 2; Figs. 2a–c, 7) Ki-67 showed endometria with simple hyperplasia and a nearly identical growth fraction in glands and stroma. Proliferating endometria showed the same values. In secretory transformed endometria with simple hyperplasia ($n=5$) the growth fraction is slightly lower than in non-secretory endometria ($n=5$). The range is wider in secretory transformed endometria. Endometria with irregular proliferation have a slightly lower growth fraction than those with simple hyperplasia.

For ER-ICA glands and stroma of endometria with simple hyperplasia express the ER in more than 80% with a strong staining intensity in both components. In non-secretory hyperplastic endometria more glandular and stromal cells are ER-positive and the staining intensity is higher than in endometria with secretory activity. There are no significant differences between irregularly proliferated endometria and those with simple hyperplasia.

In simple hyperplasias the PR content is higher than

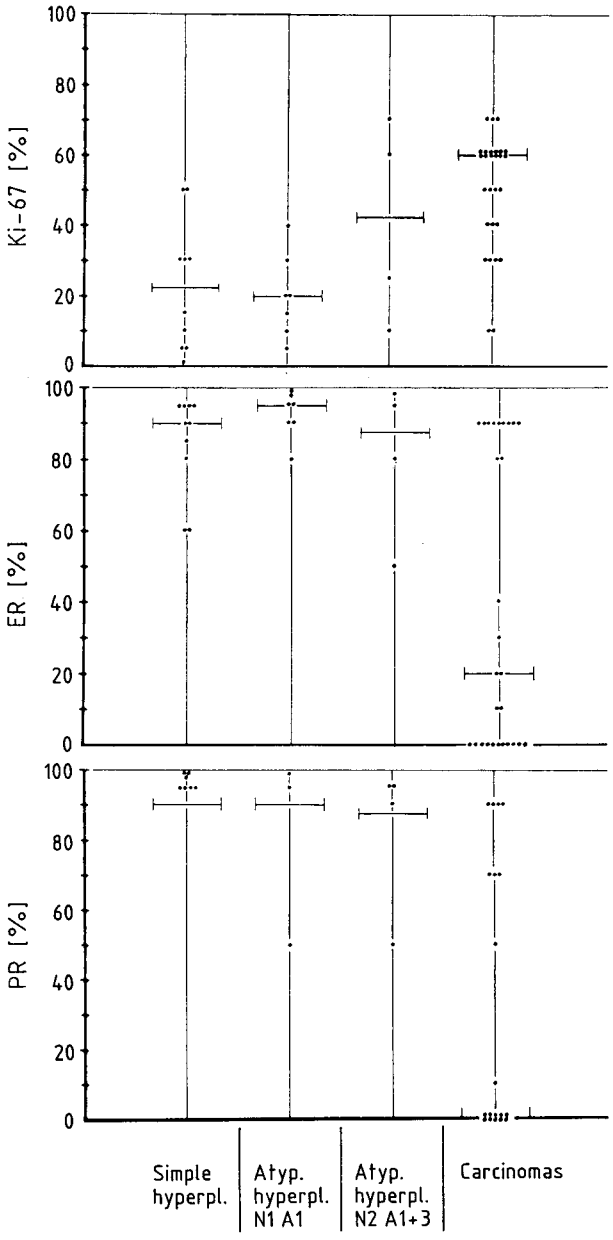

Fig. 2a–c. Endometria with simple and atypical hyperplasia [glands and stroma; *N*, nuclear; *A*, architectural grade of atypia (*1*=low, *2*=moderate, *3*=strong) endometrial carcinomas (glands)]; growth fraction (**a**), ER content (**b**) and PR content (**c**) (% of labelled nuclei). *Bar*=median

90%. The staining intensity in the glands is always strong (in the stroma moderate to strong). The results are similar to irregular proliferation.

In atypical endometrial hyperplasia (see Table 2; Figs. 2a–c, 5) Ki-67 revealed that the growth fraction in these endometria is only slightly higher than the corresponding level in simple hyperplasias. The stromal growth fraction is low. The mean level of the growth fraction in atypical hyperplasias with low nuclear atypia ($n=7$) is 20%; it increases distinctly in atypical hyperplasia with moderate nuclear atypia to 41.3%, but the data vary considerably. The hyperplastic endometria with low architectural atypia ($n=9$) reveal a mean growth fraction of 30 (5–70)%, those with severe ($n=2$) architectural atypia have a growth fraction of 10% and 25%. One endometrium with moderate nuclear and severe architectural atypia shows a very low glandular growth fraction of 10%. All cases of atypical hyperplasia contain the ER in the glandular cells (mean level 88%) with a mostly strong staining intensity. The stroma yields analogous findings. The PR content shows nearly the same staining pattern as the ER.

In the 28 carcinomas examined (see Tables 2 and 3; Figs. 2a–c, 6a–b, 7 and 8) Ki-67 revealed an average growth fraction of about 50% (10–70%), median 60%. The growth fraction in carcinomas increases from G1 to G3; single levels vary considerably. The 17 ER-positive carcinomas have a mean growth fraction of 43%, the 8 ER- and PR-negative carcinomas one of 61.3%. The growth fraction of the 5 papillary carcinomas (mean value 48%) differs only slightly from the growth fraction of non-papillary carcinomas. The G1 carcinomas have a significantly lower growth fraction than the G3 carcinomas (U-test).

In ER-ICA staining 11 of the 17 ER-positive carcinomas show more than 80% positive tumour cells. In 6 further carcinomas the amount of ER-positive tumour cells is 10–40%; the mean staining intensity is moderate.

Of 11 ER-negative carcinomas 8 were G3 tumours; no ER-negative carcinoma belongs to the group of highly differentiated carcinomas. Of 17 ER-positive carcinomas 4 are grade 3 tumours. Only 3 of 10 carcinomas with severe nuclear and architectural atypia are ER-positive; the ER content in these 3 carcinomas varies between 10 and 20%. All G1 carcinomas and 5 of the 8 G2 carcinomas are ER-positive. Carcinomas of different grades contain the ER in a distinctly different percentage of cells, but the staining intensity differs only slightly (G1 and G2: moderate to strong; G3: moderate). Poorly differentiated parts of ER-positive carcinomas are frequently receptor-negative. All ER-negative carcinomas but only 8 out of 17 ER-positive carcinomas show a growth fraction of $\geq 50\%$. The growth fraction and the ER content are inversely correlated.

The tumour tissue often contains myometrium or non-tumourous endometrium with simple or atypical hyperplasia. These structures are always ER-positive with a mostly strong staining intensity.

The PR was investigated in 19 carcinomas; 9 contain the PR. No ER-negative carcinoma displays PR. As with ER staining the samples occasionally contain PR-positive non-tumourous structures apart from the PR-negative carcinoma tissue.

Out of 7 investigated G3 carcinomas, 6 are PR-negative. The PR was investigated in 4 of 5 papillary carcinomas. One tumour has a focal PR content of 10% and 2 tumours show 90% positive tumour cells with a moderate staining intensity. One papillary carcinoma is PR-negative. In PR-positive tumours the mean level of labelled cells is 70%. The staining intensity is strong in 5 cases, moderate in 3 cases and weak in 1 case. Focal PR expression in carcinomas with heterogeneous differentiation is found in the better differentiated parts. PR

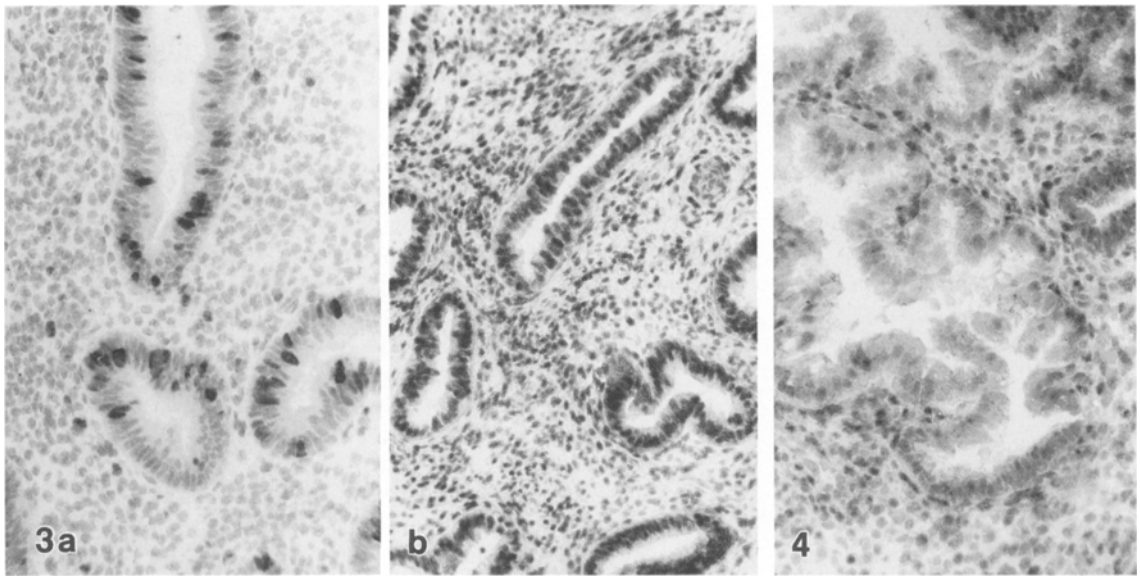

Fig. 3a, b. Cyclic endometrium, late proliferative phase. **a** Ki-67 labelling (glands 20%, stroma 1%) ×296; **b** ER labelling (glands 99%, staining intensity; strong; stroma 95%, staining intensity: strong). ×148

Fig. 4. Cyclic endometrium, late secretory phase. PR content (% of labelled nuclei), glands 10%, staining intensity: weak, stroma 90%, staining intensity: moderate. ×296

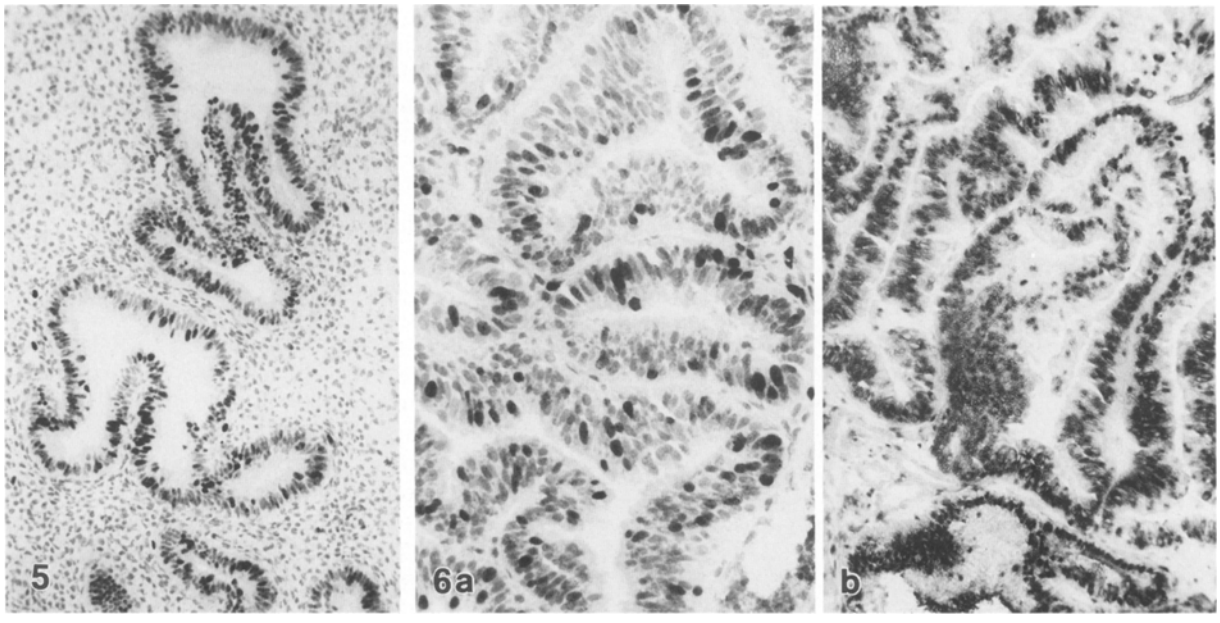

Fig. 5. Ki-67 labelling in atypical endometrial hyperplasia (nuclear atypia grade 2), glands 60%, stroma 1%. ×128

Fig. 6a, b. Endometrioid adenocarcinoma, grade 2. **a** Growth fraction (40%); **b** ER: 90% labelled nuclei, strong staining intensity. ×128

content is positively correlated with the ER (Spearman's rank correlation coefficient). G1 carcinomas reveal a significantly higher PR content than G2 and G3 carcinomas (U-test).

There is no correlation between the patients' ages and the Ki-67, ER and PR content.

Discussion

Initially, the PA in animal and human endometria was investigated by counting mitoses in glands and stroma (Ahrens and Prinz 1957). Tissue autoradiography later provided more precise data on the proliferating endome-

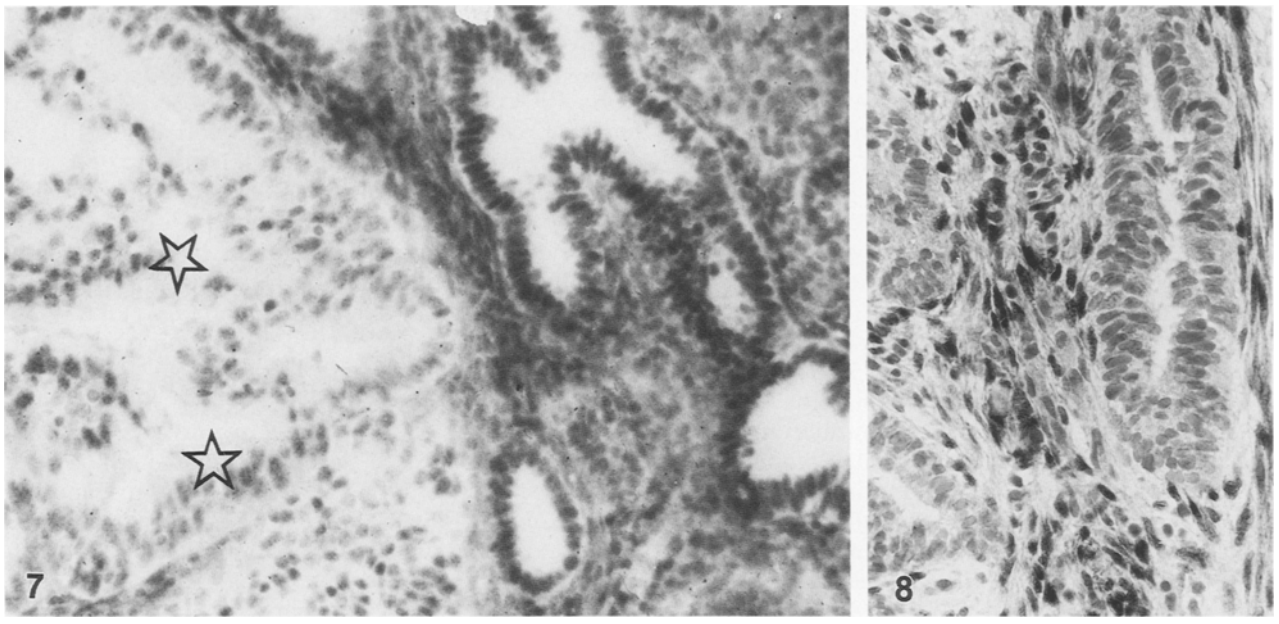

Fig. 7. Endometrioid adenocarcinoma, grade 1 and simple endometrial hyperplasia. ER: moderate to weak staining in the tumour (*asterisks*), strong labelling in the hyperplastic glands. Paraffin section. ×194

Fig. 8. Endometrioid adenocarcinoma, grade 2. ER: no ER expression in the carcinoma, strong ER staining in the infiltrated stroma and myometrium. ×310

Table 3. Growth fraction, ER/PR expression in endometrial adenocarcinomas with different receptor status (mean values, percentage of stained nuclei; s, strong; m, medium; w, weak staining intensity)

	Ki-67	ER	PR
Adenocarcinomas			
ER+PR+ (n=9)	46	65 ms	70 m
ER+PR− (n=2)	55	30 m	–
ER−PR− (n=8)	61	–	–

trial compartments (Fettig 1965; Ferenczy et al. 1979). However, using DNA flow cytometry (Feichter et al. 1982; Tsou et al. 1985) or the flow-cytometric evaluation of the nucleotide-phosphodiesterase (Tsou et al. 1985) is less valuable as the contribution of the different cell and tissue components cannot be separated.

Single cell cytophotometry in Feulgen-stained sections (Wagner et al. 1968; Sachs et al. 1974; Böhm and Sandritter 1975) allows differentiation of the various cell and tissue components. However, the method is time-consuming and can only be carried out in a small number of cells. The mAb Ki-67 labels all cell nuclei which are not in the G0 phase or in the initial G1 phase of the cell cycle (Gerdes et al. 1984). The immunohistological demonstration of the Ki-67 antigen on cryostat sections allows a quick evaluation of the growth fraction and a simultaneous evaluation of the histology.

In agreement with our results in normal cyclic endometria, other investigators have found an increase of the PA during the proliferative phase and a decrease towards the end of the secretory phase. The autoradiographic investigations of Fettig (1965) and Ferenczy et al. (1979) show maximal values for the PA in periovulatory endometria in glands and stroma and a marked decrease towards the late secretory phase in the glands, which are similar to our results. In contrast, however, we found the stroma to have a marked increase of the PA in he late secretory phase. Ferenczy et al. (1979) investigated the PA in the functionalis and basalis layer. Our data are gained by evaluation of the functionalis layer; the PA in the basalis layer remains constantly low throughout the whole cycle and this may account for the discrepancy. The proliferation of all cell compartments in atrophic endometria, in fibro-glandular polyps, in endometria with irregular shedding and in arrested secretion is low. The four polyps did not show focal glandular hyperplasia.

In analysing the PA of hyperplastic endometria, some authors have found a marked increase in atypical (adenomatous) hyperplasia opposite to simple hyperplasia (Ahrens and Prinz 1957; Fettig 1965; Sachs et al. 1974; and Feichter et al. 1982). Thornton et al. (1988) observed a PA in hyperplastic endometria which lies between the level of secretory and proliferative cyclic endometria. Differentiation of the various architectural and nuclear grades has not been undertaken by these authors.

In our investigations the PA in simple hyperplasia remains at the same level as the maximal PA of cyclic endometria. In atypical hyperplasias the PA is only slightly higher. A marked increase can be seen with rising nuclear grade. These results correlate with the increased cancer risk of atypical hyperplasias with nuclear atypia (Kurman and Norris 1987). The mean levels of the PA of endometrial carcinomas are distinctly higher than in atypical hyperplasias, but the levels vary considerably

in both groups. There is remarkable overlapping between G1 carcinomas and atypical hyperplasias. The slight differences of the PA between atypical hyperplasias and grade 1 carcinomas confirm the data of Feichter et al. (1982) and show that the Ki-67 labelling index is not suitable for differentiation between precancerous lesions and carcinomas.

The significantly higher PA of grade 3 carcinomas as opposed to the grade 1 carcinomas in our investigations has also been found by Lindahl et al. (1984) and Geisinger et al. (1986).

Our ER analysis in cyclic endometria shows a maximum in the late proliferative phase and a strong reduction of the ER content in the secretory phase, in accordance with the results of previous biochemical examinations (Bayard et al. 1978; Levy et al. 1980; Vikho et al. 1980). A comparison of biochemical and immunohistochemical methods reveals the same results (Thornton and Wells 1987). Biochemical receptor analyses which differentiate nuclear and cytoplasmic receptors show only slight differences (Bayard et al. 1978; Levy et al. 1980).

Immunohistochemical investigations of the ER expression in cyclic endometria show equivalent results (Press et al. 1984; Charpin et al. 1986; Bergeron et al. 1988). Biochemical PR studies reveal higher levels in the proliferative phase than in the secretory phase (Pollow et al. 1977, 1981; Rodriguez et al. 1979). Bayard et al. (1978) and Levy et al. (1980) investigated the cytosolic and nuclear PR separately and found marked differences between discordant maximal levels of the nuclear (post-ovulatory) and cytosolic (late proliferative phase) compartment. The immunohistochemical PR results presented by Press et al. (1988) showed a constant, marked PR content in glands and stroma in the proliferative phase and early and middle secretory phase which are equivalent to our results. The discordance between the PR content in glands and stroma cannot be evaluated by the biochemical analysis.

The high PR content in precancerous lesions in our study is well correlated with other biochemical studies (Rodriguez et al. 1979; Vikho et al. 1980) and immunohistochemically (Charpin et al. 1986). Bergeron et al. (1988) found a decreasing ER level in precancerous lesions with nuclear atypia which is in contrast with our results.

Biochemical studies of the ER and PR level in carcinomas almost always show a decreasing ER/PR level with increasing tumour grade (McCarty et al. 1979; Spona et al. 1979). Pertschuk et al. (1986) compared immunohistochemistry with biochemical ER assay. All immunohistochemically ER-positive lesions are biochemically ER-positive as well. The falsification of the biochemically assay by tissue components with a markedly different receptor content has been pointed out. Similar observations have been made by Charpin et al. (1986) and Thornton et al. (1987). The inverse correlation between the ER/PR content and the PA in endometrial carcinomas in our study has also been found in a biochemical/autoradiographical study (Lindahl et al. 1984). When evaluating the PA by flow cytometry and the receptors biochemically, Geisinger et al. (1986) found a negative correlation between the PA and PR expression, but not with the PA and the ER.

As PR-rich carcinomas respond better to gestagen therapy (Young et al. 1976; Spona et al. 1979) PR expression is an important factor in therapeutic decisions. Carcinomas with a large growth fraction and a low ER/PR content are more likely to respond to chemotherapy in contrast to carcinomas with a small growth fraction and a high ER/PR content, which are more likely to respond to hormone treatment (Kauppila et al. 1980). Carcinomas with a large growth fraction and a high ER/PR expression may respond better to a combined therapy.

Our study demonstrates the superiority of immunohistochemical analysis of steroid hormones and PA compared with other techniques. It is less expensive and less time-consuming than biochemistry and provides a simultaneous histological and immunohistochemical assessment of the sample. This type of analysis should be preferred to previous methods, particularly in clinical pathology.

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