ORIGINAL ARTICLE

The effects of tamoxifen on proliferation and steroid receptor expression in postmenopausal endometrium

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Aim: To study the effects of tamoxifen on the proliferation index and oestrogen receptor (ER) and progesterone receptor (PR) expression in postmenopausal endometrium.
Methods: A total of 125 endometrial specimens of postmenopausal women, comprising benign endometria from tamoxifen users (n = 35) and non-users (n = 24), and endometrial cancer from tamoxifen users (n = 15) and non-users (n = 51), were immunohistochemically examined using MIB-1, anti-ER, and anti-PR antibodies in endometrial epithelium and stroma.
Results: In benign endometrium the mean MIB-1 index in the epithelium was higher in tamoxifen users than in non-users (mean, 13% (SD, 13%) *v* mean, 2% (SD, 2%); $p < 0.05$), whereas in endometrial cancer the MIB-1 index was higher, but similar in tamoxifen users and non-users (mean, 32% (SD, 24%) and mean, 35% (SD, 18%)). The expression of ER was comparably high in benign epithelium from tamoxifen users and non-users (97% and 92%, respectively), but in endometrial cancer it was lower in tamoxifen users (60% and 88%; $p < 0.05$). The expression of PR in stromal cells was higher in tamoxifen users, both in benign (84% *v* 54%) and in malignant endometrium (33% *v* 10%; $p < 0.05$).
Conclusion: The proliferation index (as measured by MIB-1) in benign endometrial epithelium is higher in tamoxifen users than in non-users, and this might play a role in the reported higher incidence of endometrial cancer in postmenopausal tamoxifen users. The increased expression of PR in stroma from tamoxifen users with both benign and malignant endometrium demonstrates an additional oestrogenic effect of tamoxifen on the endometrial stroma.

Tamoxifen is a triphenylethylene derivative with predominantly antioestrogenic properties and it is the endocrine treatment of choice in all stages of breast cancer. Tamoxifen has oestrogen agonistic and antagonistic effects in different tissues, depending on the ambient oestradiol concentration. Since the publication of the paper by Fornander *et al* in 1989,[1] an increased incidence of endometrial carcinoma has been observed in postmenopausal patients with breast cancer using tamoxifen. In larger epidemiological studies, the relative risk of endometrial carcinoma is estimated to be two to threefold, the risk increasing with the duration and the cumulative dose of tamoxifen treatment.[2–7] In postmenopausal women tamoxifen exerts a proliferative effect on the endometrium, as measured by the hyperdiploid fraction,[8] and clinical studies have reported an increased incidence of endometrial polyps and hyperplastic endometrial changes, mostly interpreted as evidence of the agonistic effect of tamoxifen.[9–14] Histology of tamoxifen exposed postmenopausal endometrium in general shows a characteristic picture of a condensated, hypercellular, collagen rich stroma, whereas the glandular epithelium stays thin and atrophic or metaplastic, the flattened epithelium lining cystically dilated glands or polyps.[8 13 15] The incidence of benign, tamoxifen induced changes by far exceeds the incidence of endometrial cancer,[14] suggesting that most of these endometrial changes do not progress to cancer. Endometrial surveillance with transvaginal ultrasonography, as suggested by many clinicians, offers a high false positive rate in tamoxifen users, and is not warranted in asymptomatic women.[14 16] Nevertheless, the process by which tamoxifen affects proliferation of the endometrium and the contribution of tamoxifen to the carcinogenesis of endometrial cancer is still unknown. The nuclear antigen Ki67 is a proliferation associated protein, which is present in proliferating (G1, S, G2, and M phases) but absent in resting (G0) cells.[17] In the endometrium of healthy premenopausal women a rise in oestradiol values coincides with a rise in the percentage of epithelial nuclei expressing Ki67,[18] the oestrogen receptor (ER), and the progesterone receptor (PR).[19] On paraffin wax embedded tissue sections, the monoclonal antibody MIB-1 reacts with the Ki67 antigen and gives an immunohistochemical staining pattern that is identical to that seen with anti-Ki67 antibody in frozen sections.[20]

> "The process by which tamoxifen affects proliferation of the endometrium and the contribution of tamoxifen to the carcinogenesis of endometrial cancer is still unknown"

The aim of our present study was to analyse the effects of tamoxifen on proliferation and ER and PR expression in postmenopausal endometrium. We hypothesised that Ki67 expression in tamoxifen users is increased in glandular epithelium and that this is related to ER receptor status. Because oestrogen agonists can also induce the expression of PR, PR may also be upregulated in tamoxifen users. Because tamoxifen could potentially act differently on epithelial and stromal cells, the MIB-1 index and the expression of ER and PR were scored separately in the endometrial epithelial and stromal cells. To compare these parameters, four patient groups were selected, with benign and malignant endometrium both from tamoxifen users and non-users.

METHODS

Patients

Four postmenopausal patient groups were selected. The first group (group I) consisted of patients with breast cancer using

Abbreviations: ER, oestrogen receptor; PR, progesterone receptor

Table 1 Patient characteristics

	N	Median age (range)	Median duration of tamoxifen in months (range)
Benign endometrium			
(I) Tamoxifen	35	57 (45–83)	27 (5–84)
(II) No tamoxifen	24	62 (50–78)	–
Endometrial cancer			
(III) Tamoxifen	15	66 (50–85)	18 (3–90)
(IV) No tamoxifen	51	68 (51–89)	–

Table 2 Histopathology of the endometrium

Groups	Histopathology	Number	Grade (number)
Benign endometrium			
(I) Tamoxifen	Endometrial polyps	18	
	Adenomatous hyperplasia	1	
	Cystic atrophy	16	
(II) No tamoxifen	Endometrial polyps	3	
	Simple hyperplasia	1	
	Proliferative endometrium	3	
	Atrophic endometrium	17	
Endometrial cancer			
(III) Tamoxifen	Papillary serous carcinoma	2	
	Endometrioid adenocarcinoma	13	
			Grade 1 (8)
			Grade 2 (2)
			Grade 3 (3)
(IV) No tamoxifen	Papillary serous carcinoma	2	
	Endometrioid adenocarcinoma	49	
			Grade 1 (29)
			Grade 2 (16)
			Grade 3 (4)

tamoxifen 20–40 mg daily as adjuvant treatment. Menopause was defined as no menstrual periods in the preceding 12 months and oestradiol concentrations of < 0.10 nmol/litre. These patients attended the gynaecology outpatient clinic of the University Hospital Groningen from January 1995 until December 1998, as part of a gynaecological screening programme (n = 91). Outpatient hysteroscopy and uterine curettings or biopsies were performed in patients with an endometrial thickness of > 5 mm on ultrasound, which occurred in 62 cases. Endometrium was obtained in 32 patients (22 asymptomatic patients, 10 patients with vaginal bleeding), whereas in 27 patients insufficient tissue was obtained for assessment. In three patients with thickened endometrium on ultrasound, a hysterectomy was performed for other reasons. A total of 35 benign endometrial samples was included in group I. Group II comprised non-malignant endometrium from the uteri of 24 postmenopausal women with stage I cervical cancer, who had never used tamoxifen and were currently not using hormonal treatment. All cases were retrieved from the database files of the department of pathology, University Hospital Groningen, from 1995 until 1998. Group III consisted of 15 patients with breast cancer who developed endometrial carcinoma during or after tamoxifen use. Cases were obtained from the database of the Northern Dutch Cancer Registry from 1989 until 1996. In group IV, cases were retrieved from the database of the department of gynaecology, University Hospital Groningen, from 1991 until 1998; the group comprised patients with endometrial carcinoma who had never used tamoxifen and were not using hormonal treatment at time of diagnosis (n = 51).

Methods

Sections (4 μm thick) of formalin fixed, paraffin wax embedded tissue were stained with standard haematoxylin and eosin. For immunohistochemistry, sections were mounted on 3′3′-aminopropylethoxysilane (Sigma-Aldrich, Diesenhofen, Germany) coated glass slides. After antigen retrieval by an autoclave method,[21] the heat stable monoclonal antibody MIB-1 (Immunotech, Marseille, France), directed against an epitope of the Ki67 antigen, was used as a marker for proliferation.[17] For the detection of ER and PR, primary monoclonal antibodies (Immunotech) were incubated overnight at 80°C in 0.01M Tris/HCl (buffer pH 9.0). All slides were then incubated with a second rabbit antimouse peroxidase labelled polyclonal antibody, using diaminobenzidine as chromogen, and counterstained with haematoxylin. Normal colonic mucosa was used as positive control for MIB-1. Positive controls for ER and PR were breast cancer specimens with known steroid receptor status. Negative controls underwent the same procedure omitting the primary antibodies.

Scoring

Histopathology was reviewed by an experienced gynaecopathologist (HH). Mitoses were counted in 10 fields of vision at ×40 magnification in epithelium and stroma, and the mitotic index was defined as the percentage of positive cells of the cells counted. The expression of MIB-1, ER, and PR was evaluated both in glandular epithelium and stroma. For counting MIB-1 positive epithelial cells, we used an eyepiece grid at ×40 magnification and counted the epithelial cells crossing 10 consecutive horizontal lines. In cases of normal endometrium, 10 consecutive fields were counted. In cases of tamoxifen induced endometrial pathology, counting was performed in the field with the highest epithelial density. In the carcinoma cases, we selected well fixed fields of tumour, whereas in cases of apparent tumour heterogeneity we counted 10 fields in the area with the lowest MIB-1 index and 10 fields in areas with the highest MIB-1 index. The overall score is given as the mean of these two indices. Fields with

Table 3 MIB-1 index and the expression of ER and PR in benign and malignant endometrial epithelium according to histological diagnosis and tamoxifen use

	Mean (SD) MIB-1 index	ER positive n (%)	PR positive n (%)
Benign endometrium			
(I) Tamoxifen	12.7 (13.2)*†	34 (97)†	34 (97)
(II) No tamoxifen	1.7 (1.8)‡	22 (92)	21 (88)
Endometrial carcinoma			
(III) Tamoxifen	31.8 (23.8)	9 (60)*	11 (73)
(IV) No tamoxifen	34.8 (17.5)	44 (88)	40 (80)

*Tamoxifen *v* no tamoxifen: p<0.05; †benign *v* malignant epithelium in tamoxifen users: p<0.05; ‡benign *v* malignant epithelium in non-users: p<0.05.
ER, oestrogen receptor; PR, progesterone receptor.

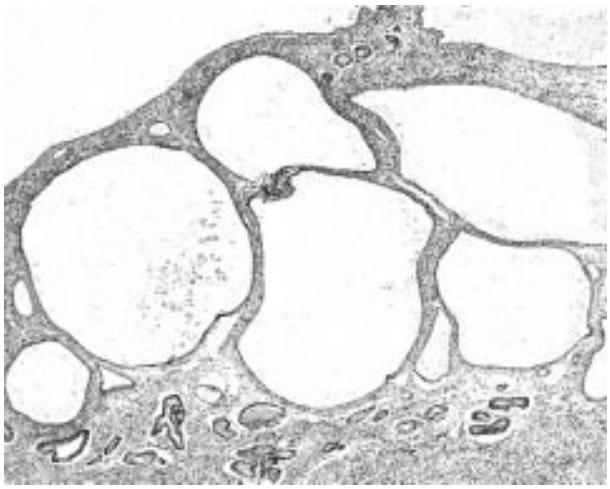

Figure 1 Haematoxylin and eosin staining of endometrial epithelium in a tamoxifen user. Note the flattened monolayered epithelium lining cystically dilated glands and the cell rich stroma, responsible for the irregular ultrasonographic thickening of the endometrium.

Figure 2 MIB-1 staining in the nuclei of the glandular epithelium of benign endometrium in a tamoxifen user.

squamous metaplasia were excluded from counting. An average of 707 epithelial cells (207–1585) was counted for each sample. An average of 820 stromal cells (408–1364) was counted for each sample. Endometrial epithelial and stromal cells were considered to be positive for MIB-1, ER, or PR if nucleoplasm or nucleoli were unequivocally stained. The MIB-1 index was defined as the percentage of positive cell nuclei. A specimen was considered positive for ER or PR if staining of > 5% of nuclei of epithelial or stromal cells was seen. All samples were scored without knowledge of the clinical data, by one investigator (KtH). At random, 10% of the samples were read by two other investigators (MJM, HH). The paired differences between the different investigators in the MIB-1 index were small: mean, 0.7%; SD, 4.9%; 95% confidence interval, 1.3% to 2.8%.

Statistics

Data were analysed using the SPSS software for Windows (8.0) (SPSS Inc, Chicago, Illinois, USA). For statistical analysis of the Ki67 data the Mann-Whitney U test was used. For ER and PR analysis a χ^2 test was performed, corrected for continuity. Only p values < 0.05 were considered significant.

RESULTS

Patient characteristics

Table 1 summarises the patient characteristics. All patients were postmenopausal and the median ages in the four patient groups were similar. In group I, all patients had been using tamoxifen at the time of tissue sampling, with a median duration of 27 months (range, 5–84 months). In group III, the median interval between the start of tamoxifen and the detection of endometrial carcinoma was 18 months (range, 3–90 months).

Histopathology

Table 2 shows the histopathology results. The 35 endometrial samples in group I comprised 18 endometrial polyps, one sample with complex non-atypical hyperplasia, and 16 with cystic atrophy, including the three hysterectomy specimens. The histology of the polyps showed a striking resemblance to the cystic atrophy of the endometrium (fig 1).[15] Both were composed of cystically dilated glands, covered with monolayered or pseudostratified metaplastic epithelium. The nuclei of the epithelial cells were small, round to oval, with small nucleoli and few (if any) mitotic figures. In the stroma, no mitotic activity was found. The stroma was cellular, especially around the glands, with focal oedema and formation of collagen.[13 15] The best descriptive diagnosis of the polyps was "adenofibroma". Mitotic figures were seldom found (< 0.5%) in both the stroma and the epithelium of tamoxifen users and non-users, although no statistical analysis was performed on these data. Group II showed atrophic endometrium in 17 patients, endometrial polyps in three, simple hyperplasia in one, and proliferative endometrium without atypia in three patients. In groups III and IV, two of the 15 tumours and two of the 51 tumours were papillary serous carcinomas, respectively; the other tumours were all endometrioid adenocarcinomas, with a tendency for higher grade tumours in tamoxifen users (group III) (table 2).

Table 4 ER and PR expression in endometrial epithelial and stromal cells according to histological diagnosis and tamoxifen use

	ER positive		PR positive	
	Epithelium n (%)	Stroma n (%)	Epithelium n (%)	Stroma n (%)
Benign endometrium				
(I) Tamoxifen	34 (97)*	33 (94)*	34 (97)	27 (84)†‡
(II) No tamoxifen	22 (92)	18 (75)§	21 (88)	13 (54)§
Endometrial cancer				
(III) Tamoxifen	9 (60)†	4 (27)	11 (73)	5 (33)
(IV) No tamoxifen	44 (88)	16 (32)	40 (80)	5 (10)

*Benign *v* malignant epithelium in tamoxifen users: p<0.05; †tamoxifen *v* no tamoxifen: p<0.05; ‡benign *v* malignant stroma in tamoxifen users: p<0.05; §benign *v* malignant stroma in non-users: p<0.05.

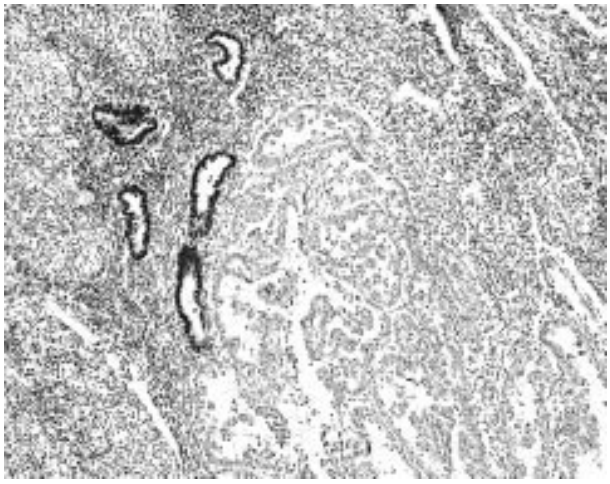

Figure 3 Oestrogen receptor staining in an endometrioid adenocarcinoma of the endometrium in a tamoxifen user. Note the positive staining in the basal glands and negative staining in the tumour cells.

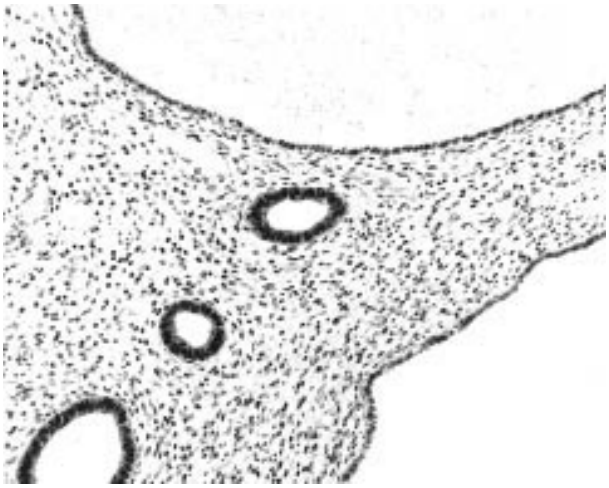

Figure 4 Progesterone receptor staining in the epithelial and stromal cells of benign endometrium in a tamoxifen user.

MIB-1, ER, and PR expression in epithelial cells

Table 3 shows the MIB-1 index, and the expression of ER and PR in epithelium. The mean MIB-1 index in benign endometrial epithelium was higher in tamoxifen users (mean, 13% (SD, 13%) *v* mean 2% (SD, 2%); $p < 0.05$). In endometrial cancer, the mean MIB-1 index was higher, but did not differ between tamoxifen treated and untreated patients. However, in endometrial stroma, the MIB-1 index in all groups was very low (< 0.5%). Figure 2 shows MIB-1 staining in cystic atrophy in a tamoxifen user.

The expression of ER in benign epithelium was high and did not differ between tamoxifen users and non-users, whereas in tamoxifen users the expression of ER was significantly different in benign and malignant endometrium ($p = 0.005$). The expression of ER in endometrial cancer was lower in tamoxifen treated than in untreated patients (60% *v* 88%; $p = 0.05$; fig 3). Scoring of endometrial stroma revealed a high expression of ER in benign stroma, and a lower expression of ER in the malignant counterpart (both $p < 0.05$), although there was no significant difference between those patients exposed to tamoxifen and those who were not (table 4).

The expression of PR in the epithelium was similarly high in all groups. However, in stroma, the expression of PR was upregulated in tamoxifen users compared with non-users in benign (84% *v* 54%; $p < 0.05$) but not in malignant endometrium (table 4; fig 4). The expression of PR in stroma was higher in benign endometrium, both in tamoxifen users and non-users (both $p < 0.05$).

DISCUSSION

Our study shows a higher proliferative state, as determined by MIB-1 index, of benign endometrial epithelium in postmenopausal tamoxifen users when compared with untreated women. However, this was not reflected by the histomorphology of the epithelium or by mitotic figures in standard haematoxylin and eosin staining. On the contrary, the epithelium in tamoxifen users remained thin whereas the stroma became cell rich. However, the MIB-1 index in the stroma was also very low. The increased MIB-1 index in the endometrial epithelium might be indicative of an increased proliferative inclination in this subgroup of postmenopausal tamoxifen users. The fact that the control groups (II and IV) comprise endometrial tissue from women without breast cancer could be a source of bias, because breast and endometrial cancer share several risk factors. Another potential source of bias is the fact that there were more symptomatic patients in the tamoxifen users with benign endometrium (group I) than in non-users with cervical cancer (group II), because abnormal bleeding is usually associated with increased proliferation. The endometrial cancer risk in tamoxifen users is increased two to threefold and therefore it is very likely that this higher proliferative state has played a role in its development.[4 7 22] Recently, two studies on the modulation of Ki67 expression by tamoxifen in endometria of premenopausal and postmenopausal women have been published.[23 24] These studies show results similar to ours. They are both small studies and in one study controls were hormone replacement users[24] and there were only six postmenopausal women in the other.[23] Our study is the first in which the MIB-1 index was studied in a larger group of postmenopausal women, and the first in which the effects of tamoxifen are described both in endometrial epithelium and stroma. Because tamoxifen has an agonistic effect on postmenopausal endometrium and ER and PR expression may be upregulated, we have related the MIB-1 index to ER and PR expression. In the benign endometrium most of the samples

Take home messages

- In benign glandular endometrial epithelium, the proliferation index was higher in tamoxifen users than in non-users, using the MIB-1 index as a measure of proliferation
- This increased proliferation might play a role in the observed higher incidence of endometrial cancer in tamoxifen users
- In stromal cells, tamoxifen was associated with a higher expression of the progesterone receptor, suggesting an oestrogenic effect on the stroma that is independent of histological diagnosis
- These data support the theory of an oestrogenic effect of tamoxifen on postmenopausal endometrial epithelium and stroma

were ER positive, irrespective of tamoxifen use, consistent with the findings of others.[22 25] However, in benign endometrium, we observed an increased expression of PR in stromal cells of tamoxifen users compared with non-users. In malignant endometrium this difference was not significant. This higher stromal PR expression in the endometrium is consistent with an agonistic effect of tamoxifen on the stroma, mostly independent of the histological diagnosis. Whether or not the upregulation of PR in stroma by tamoxifen is related to the relatively higher incidence of stromal tumours[22 26] and malignant mixed Müllerian tumours[24] in tamoxifen induced endometrial cancer still is not clear. However, our findings differ from those of Schwartz *et al* in tamoxifen users, who found increased expression of PR only in glandular epithelium and decreased expression of ER in the stroma.[27] An explanation for this discrepancy could be the small size of their study.

Progestagens have an antimitotic effect on endometrial epithelial cells. Despite the upregulation of the expression of PR in tamoxifen exposed endometrium, clinical studies have not yet shown a beneficial effect of systemic progestins on the prevention of tamoxifen induced endometrial proliferation,[11 28 29] although withdrawal bleeding has been described.[29] In a recent randomised trial, a levonorgestrel releasing intrauterine device resulted in an 85% stromal decidualisation of tamoxifen primed endometrium, although endometrial ultrasonographic appearance and thickness remained unchanged and an excess of bleeding appeared in the treatment group.[30] Only data from a large prospective trial can answer the question of the efficacy of progestin treatment in the prevention of endometrial cancers and polyps in longterm tamoxifen users.

> "In benign endometrium, we observed an increased expression of the progesterone receptor in stromal cells of tamoxifen users compared with non-users"

In the group of tamoxifen users with endometrial cancer, we found that the percentage of ER positive tumours was lower (60%) than in non-users with sporadic cancer (88%), as has been described previously.[31] The presence of ER and PR was linked to well differentiated endometrial carcinomas with a good prognosis, whereas the absence of receptors was considered to predict invasive growth and a poor prognosis.[32] Some studies describe more adverse histology for tamoxifen induced endometrial carcinomas,[7 22 33] and a lower expression of ER compared with non-users,[22] although there is some controversy over this subject.[3 34 35] In our study, the histology of the endometrial carcinomas of tamoxifen users (group III) and non-users (group IV) showed only two papillary serous tumours in both groups and, as a consequence, in group III the adverse histology constitutes a greater part of the carcinomas than in group IV. Moreover, there is a tendency for higher grade tumours in the tamoxifen group (table 2).

In conclusion, using the MIB-1 index as a measure of proliferation, we found a tamoxifen associated higher proliferative state in the glandular epithelium, which might play a role in the observed higher incidence of endometrial cancer in tamoxifen users. In stromal cells, we found a tamoxifen associated higher expression of PR, suggesting an oestrogenic effect on the stroma that is independent of histological diagnosis. Therefore, our data support the theory of an oestrogenic effect of tamoxifen on postmenopausal endometrial epithelium in addition to stroma.

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REFERENCES

1 **Fornander T**, Rutqvist LE, Cedermark B, *et al*. Adjuvant tamoxifen in early breast cancer: occurrence of new primary cancers. *Lancet* 1989;**1**:117–20.
2 **Sasco AJ**, Chaplain G, Amoros E, *et al*. Endometrial cancer following breast cancer: effect of tamoxifen and castration by radiotherapy. *Epidemiology* 1996;**7**:9–13.
3 **Fisher B**, Constantino JP, Redmond CK, *et al*. Endometrial cancer in tamoxifen-treated breast cancer patients: findings from the National Surgical Adjuvant Breast and Bowel Project (NSABP) B-14. *J Natl Cancer Inst* 1994;**86**:527–37.
4 **Van Leeuwen FE**, Benraadt J, Coeberg JW, *et al*. Risk of endometrial cancer after tamoxifen treatment of breast cancer. *Lancet* 1994;**343**:448–52.
5 **Rutqvist LE**, Johansson H, Signomklao T, *et al*. Adjuvant tamoxifen therapy for early stage breast cancer and second primary malignancies. Stockholm Breast Cancer Study Group. *J Natl Cancer Inst* 1995;**87**:645–51.
6 **Curtis RE**, Boice JD, Shriner DA, *et al*. Second cancers after adjuvant tamoxifen therapy for breast cancer. *J Natl Cancer Inst* 1996;**88**:332–4.
7 **Mignotte H**, Lasset C, Bonadonna V, *et al*. Iatrogenic risks of endometrial carcinoma after treatment for breast cancer in a large French case-control study. Federation Nationale des Centres de Lutte Contre le Cancer (FNCLCC). *Int J Cancer* 1998;**76**:325–30.
8 **Decensi A**, Fontana V, Bruno S, *et al*. Effect of tamoxifen on endometrial proliferation. *J Clin Oncol* 1996;**14**:434–40.
9 **Lahti E**, Blanco G, Kauppilla A, *et al*. Endometrial changes in postmenopausal breast cancer patients receiving tamoxifen. *Obstet Gynecol* 1993;**81**:660–4.
10 **Kedar RP**, Bourne TH, Powles TJ, *et al*. Effects of tamoxifen on uterus and ovaries of postmenopausal women in a randomised breast cancer prevention trial. *Lancet* 1994;**343**:1318–21.
11 **De Muylder X**, Neven P, De Somer M, *et al*. Endometrial lesions in patients undergoing tamoxifen therapy. *Int J Gynaecol Obstet* 1991;**36**:127–30.
12 **Love CD**, Muir BB, Scrimgeour JB, *et al*. Investigation of endometrial abnormalities in asymptomatic women treated with tamoxifen and an evaluation of the role of endometrial screening. *J Clin Oncol* 1999;**17**:2050–4.
13 **Ismail SM**. Pathology of endometrium treated with tamoxifen. *J Clin Pathol* 1994;**47**:827–33.
14 **Cohen I**, Perel E, Flex D, *et al*. Endometrium pathology in postmenopausal tamoxifen treatment: comparison between gynaecologically symptomatic and asymptomatic breast cancer patients. *J Clin Pathol* 1999;**52**:278–82.
15 **Mourits MJ**, van der Zee AG, Willemse PH, *et al*. Discrepancy between ultrasonography and hysteroscopy and histology of endometrium in postmenopausal breast cancer patients using tamoxifen. *Gynecol Oncol* 1999;**73**:21–6.
16 **Gerber B**, Krause A, Müller H, *et al*. Effect of adjuvant tamoxifen on the endometrium in postmenopausal women with breast cancer: a prospective long-term study using transvaginal ultrasound. *J Clin Oncol* 2000;**18**:3464–70.
17 **Gerdes J**, Schwab U, Lemke H, *et al*. Production of a mouse monoclonal antibody reactive with a human nuclear antigen associated with cell proliferation. *Int J Cancer* 1983;**31**:13–20.
18 **Tabibzadeh S**. Immunoreactivity of human endometrium: correlation with endometrial dating. *Fertil Steril* 1990;**54**:624–31.
19 **Snijders MP**, de Goeij AF, Debets-Te Baerts MJ, *et al*. Immunocytochemical analysis of oestrogen receptors and progesterone receptors in the human uterus throughout the menstrual cycle and after the menopause. *J Reprod Fertil* 1992;**94**:363–71.
20 **Cattoretti G**, Becker MH, Key G, *et al*. Monoclonal antibodies against recombinant parts of the Ki-67 antigen (MIB 1 and MIB 3) detect proliferating cells in microwave-processed formalin-fixed paraffin sections. *J Pathol* 1992;**168**:357–63.

21 **Emanuels A**, Hollema H, Koudstaal J. Autoclave heating: an alternative method for microwaving? *Eur J Morphol* 1994;**32**(suppl 2–4):337–40.
22 **Bergman L**, Beelen ML, Gallee MP, *et al*. Risk and prognosis of endometrial cancer after tamoxifen for breast cancer. Comprehensive Cancer Centres' ALERT Group. *Lancet* 2000;**356**:881–7.
23 **Hachisuga T**, Hideshima T, Kawarabayashi T, *et al*. Expression of steroid receptors, Ki-67, and epidermal growth factor receptor in tamoxifen-treated endometrium. *Int J Gynecol Pathol* 1999;**18**:297–303.
24 **Elkas J**, Armstrong A, Pohl J, *et al*. Modulation of endometrial steroid receptors and growth regulatory genes by tamoxifen. *Obstet Gynecol* 2000;**95**:697–703.
25 **Kommoss F**, Karck U, Prompeler H, *et al*. Steroid receptor expression in endometria from women treated with tamoxifen. *Gynecol Oncol* 1998;**70**:188–91.
26 **Mourits MJ**, Hollema H, Willemse PH, *et al*. Adenosarcoma of the uterus following tamoxifen treatment for breast cancer. *Int J Gynecol Cancer* 1998;**8**:168–71.
27 **Schwartz LB**, Krey L, Demopoulos R, *et al*. Alterations in steroid hormone receptors in the tamoxifen-treated endometrium. *Am J Obstet Gynecol* 1997;**176**:129–37.
28 **Cohen I**, Figer A, Altaras MM, *et al*. Common endometrial decidual reaction in postmenopausal breast cancer patients treated with tamoxifen and progestogens. *Int J Gynecol Pathol* 1996;**15**:17–22.
29 **Powles TJ**, Bourne T, Athanasiou S, *et al*. The effects of norethisterone on endometrial abnormalities identified by transvaginal ultrasound screening on healthy post-menopausal women on tamoxifen or placebo. *Br J Cancer* 1998;**78**:272–5.
30 **Gardner FJE**, Konje JC, Abrahams KR, *et al*. Endometrial protection from tamoxifen-stimulated changes by a levonorgestrel-releasing intrauterine system: a randomised controlled trial. *Lancet* 2000;**356**:1711–17.
31 **Cohen I**, Beyth Y, Altaras MM, *et al*. Estrogen and progesterone receptor expression in postmenopausal tamoxifen-exposed endometrial pathologies. *Gynecol Oncol* 1997;**67**:8–15.
32 **Punnonen R**, Mattila J, Kuoppala T, *et al*. DNA ploidy, cell proliferation and steroid hormone receptors in endometrial hyperplasia and early adenocarcinoma. *J Cancer Res Clin Oncol* 1993;**119**:426–9.
33 **Magriples U**, Naftolin F, Schwartz PE, *et al*. High-grade endometrial carcinoma in tamoxifen-treated breast cancer patients. *J Clin Oncol* 1993;**11**:485–90.
34 **Barakat RR**, Wong G, Curtin JP, *et al*. Tamoxifen use in breast cancer patients who subsequently develop corpus cancer is not associated with a higher incidence of adverse histologic features. *Gynecol Oncol* 1994;**55**:164–8.
35 **Fisher B**, Constantino JP, Wickerham DL, *et al*. Tamoxifen for prevention of breast cancer: report of the National Surgical Adjuvant Breast and Bowel Project P-1 study. *J Natl Cancer Inst* 1998;**90**:1371–88.
36 **Ismail SM**. Gynaecological effects of tamoxifen. *J Clin Pathol* 1999;**52**:83–8.

ECHO

Economy of scale

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Babies in hospital may be spared discomfort and even blood transfusions with a device that measures haemoglobin concentration in only 20 μl of blood. The HemoCue requires just a fraction of the 500 μl samples routinely requested for haemoglobin estimation and has already been tested for estimations at the bedside in children and fetuses. Now a study in a district general hospital has established its suitability for neonates, in whom it would help to reduce the impact of frequent blood sampling in intensive care.

Blood samples tested directly by the HemoCue and the hospital's standard STKS Coulter method showed a mean difference in haemoglobin concentration of only 2.5 g/l. Virtually all (95%) of the 82 samples tested fell within a 7.5 g/l difference in concentration, below that judged to be clinically significant.

Two sets of samples were tested. One set comprised 32 samples taken into EDTA from babies in the intensive care or low dependency units, from which 2×10 μl aliquots were placed into HemoCue cuvettes containing dried reactants and measured for their absorbance. Within one minute a comparison of the readings is converted into a displayed concentration. Then the original samples were analysed by the haematology laboratory by the standard method. The other set comprised 50 random neonatal samples already held in the haematology laboratory, which were tested by each method.

▲ *Archives of Disease in Childhood:Fetal and Neonatal Edition* 2002:**86**:00–00.