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# Estrogen and Progesterone Receptors in the Normal Female Breast

D. Ricketts, L. Turnbull, G. Ryall, R. Bakhshi, N. S. B. Rawson, J-C. Gazet, C. Nolan, and R. C. Coombes[1]

*Medical Oncology Department, St. George's Hospital Medical School, Cranmer Terrace, London SW17 ORE, United Kingdom [D. R., L. T., G. R., R. B., R. C. C.]; Department of Surgery, St. George's Hospital, Blackshaw Road, London SW17 OQT, United Kingdom [J-C. G.]; Abbott Laboratories, North Chicago, Illinois 60064 [C. N.]; and Department of Epidemiology, Royal Marsden Hospital, Sutton, United Kingdom [N. S. B. R.]*

## ABSTRACT

**We have studied estrogen receptor (ER) and progesterone receptor (PR) in normal breast by immunocytochemistry using tissue biopsies and fine needle aspirates (FNA) and, in the case of ER, by enzyme immunoassay. For ER we found a high degree of reproducibility for biopsies taken from the upper outer quadrant: FNA, $r = 0.56$ ($P < 0.002$); tissue section immunocytochemistry, $r = 0.89$ ($P < 0.0001$); and enzyme immunoassay, $r = 0.76$ ($P < 0.0001$). For PR, FNA ($r = 0.56$, $P < 0.002$) and tissue section ($r = 0.97$, $P < 0.0001$) were also found to be reproducible techniques. Using enzyme immunoassay, we were able to measure ER accurately in normal breast tissue. In 59 samples we found a range of 0-37 fmol/mg cytosol protein (mean, 4 fmol/mg). In an age-matched group of 126 women with breast cancer, we found a significantly higher ER [range, 0-139 fmol/mg; mean, 37 fmol/mg ($P < 0.001$)].**

**We then analyzed the ER and PR content of FNAs obtained from the upper outer quadrant of the normal breast in 143 normal women. We found that in only 23 of 143 samples (16%) were ≥50% epithelial cells stained. There was a relationship between ER and PR ($P = 0.03$) and a higher ER content in European women than in non-European women ($P < 0.03$). The PR content was related to high body mass index ($P < 0.02$) and family history of breast cancer ($P = 0.04$). Samples tended to be more frequently ER positive by FNA if taken in the follicular phase of the menstrual cycle.**

**We conclude that, although the levels of ER and PR are low in normal breast, they can be accurately measured. There is significant variation of ER and PR with several clinical parameters.**

## INTRODUCTION

The study of the ER[2] and PR receptor content of the normal female breast is important for several reasons: (*a*) estrogen is known to play a role in the etiology of breast cancer (1-3), and therefore the ER content of the normal breast may well relate to risk of breast cancer. If so, ER could be used as a marker of breast cancer risk and drugs such as tamoxifen could be used to block its effects (4); (*b*) overexpression of ER, amplification of the gene encoding for ER, or mutations occurring in this gene may be a significant factor in controlling sensitivity to endogenous and exogenous steroid hormones such as oral contraceptives and hormone replacement therapy, both of which have been implicated in the etiology of cancer (5).

Studies of normal female breast ER and PR have been few compared with the many studies of breast cancer. This is partly due to difficulties in obtaining normal breast tissue. Most researchers have used discarded tissue from the periphery of excised benign or malignant breast lesions (*e.g.*, Ref. 6) or reduction mammoplasty specimens from patients with mammary hyperplasia (7-9). In our opinion such tissue is often not macro- or microscopically normal and often demonstrates increased steroid receptor content.[3] Furthermore, until the introduction of monoclonal antibodies to ER and PR, steroid receptor determination with the DCC binding assay required at least 0.5 g of tissue. It was difficult to obtain such large amounts because of ethical and cosmetic considerations. Finally, the DCC method is an insensitive and inaccurate assay at the concentrations of ER and PR levels encountered in normal breast. In our experience, levels in normal breast are so low as to be at the detection limits of DCC.

We have overcome these difficulties by using immunocytochemistry and FNA to determine ER and PR content and by adhering to a strict definition of what constitutes normal breast. In experienced hands (10), FNA is a relatively atraumatic procedure that can be performed as an outpatient procedure without anesthetic, scarring, or complication to reliably provide a sample of breast epithelial cells from a wide area of breast. Our group has previously described the use of FNA to determine the ER and PR status of both breast carcinomas (11) and a preliminary study of normal breast (12).

Few other studies documenting the steroid receptor content of normal breast exist. Using immunohistochemistry, only about 7% of normal breast epithelial cells took up stain (9), compared to a mean of 70% of cells in ER stained sections of breast carcinoma (13) and 41% in ER stained FNA (11). The ER positive cells were found to be scattered singly or in clusters in the ducts or lobules. Staining in lobules exceeds the staining in the ducts, and within lobules the intermediate epithelial layer contained the majority of stained cells (9, 14). Other studies have shown great heterogeneity of staining, with no consistency of staining between adjacent lobules or between different sections from the same specimen (8).

The current study is in two parts. In the first part we attempt to establish the reproducibility of three methods for quantitating steroid receptors in normal breast. In the second part we have correlated the ER and PR of normal breast, using the above tests, to a variety of clinical parameters in premenopausal women, including "at risk" factors for breast cancer and menstrual cycle.

## MATERIALS AND METHODS

### Study Design

**Reproducibility Study.** Forty women undergoing general anesthesia for excision of a benign breast lump were studied. At operation 1 $cm^3$ of normal breast tissue was excised and processed to yield two sequential sections stained for ER (ER-section 1 and ER-section 2) and two sequential sections stained for PR (PR-section 1 and PR-section 2). In all 40 cases enough tissue remained to provide two ER enzyme immunoassay samples (ER-EIA 1 and ER-EIA 2). Of the 40 women, 34 had a normal contralateral breast, and two FNA were obtained from adjacent sites in the upper outer quadrant of the breast (FNA UOQ-1 and FNA UOQ-2) and one FNA from the lower outer quadrant of the same breast (FNA LOQ).

**Relationship between ER and PR Content and Clinical Features.** A total of 143 women attending the breast unit at St. Georges Hospital were included in this study. Prior to inclusion, all women were examined by the author (D. R.) and one consultant in the breast unit to determine whether they fulfilled the criteria of normality as follows: (*a*) no

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[1] To whom requests for reprints should be addressed.

[2] The abbreviations used are: ER, estrogen receptor; PR, progesterone receptor; DCC, dextran coated charcoal; FNA, fine needle aspirate; EIA, enzyme immunoassay; UOQ, upper outer quadrant; LOQ, lower outer quadrant; PBS, phosphate buffered saline.

[3] Unpublished observation.

mastalgia for more than 4 days immediately prior to a period and no midcycle mastalgia; (*b*) no tenderness or nodularity of the breast on palpation; (*c*) the patient had a regular menstrual cycle; (*d*) the patient used no concurrent hormonal treatment for at least 2 months prior to the sample; (*e*) no nipple discharge from either breast; (*f*) no fluid in the fine needle aspirate; (*g*) no previous history of breast cancer in either breast; and (*h*) patients whose subsequent benign biopsies showed atypical hyperplasia. FNA were obtained only from women that fulfilled these criteria. Excision biopsy of normal breast tissue was performed only if the patient concerned was undergoing excision of a benign lump, the benign nature of which was later confirmed histologically, and if the breast tissue surrounding the lump appeared macroscopically and microscopically normal (assessed by D. R. and L. T., respectively).

All patients included in the study gave their informed consent (25% of those approached refused) and notified an investigator (D. R.) of the first day of their next menstrual period after sampling. We also noted the following clinical details of each patient: age; height; weight; racial origin; family history of breast cancer; age at menarche; past use of oral contraceptive pill; number of full term pregnancies; age at first pregnancy; history of breast-feeding; and use of estrogen for suppression of lactation.

On microscopic examination of ER and PR stained slides the following features were accepted as "normal" (15): (*a*) some blunt duct formation; (*b*) microcysts less than 3 mm in diameter; and (*c*) the presence of some apocrine epithelium.

### Methods

**FNA.** FNA were in all cases obtained by one operator (D. R.), using a 10-ml syringe, a 21-gauge needle, and a previously described technique (16). The cellular material obtained was immediately smeared on three poly-L-lysine coated glass slides. All three slides were snap-frozen in dry ice or liquid nitrogen within 30 s. The slides were stored in liquid nitrogen for up to 1 month prior to staining.

For fixation, frozen slides were immersed in 3.6% formol/PBS (pH 7.2) at room temperature for 10 min. They were then washed twice in PBS for a total of 10 min and immersed in absolute methanol for 4 min at −20°C followed by acetone at −20°C for 1 min. Slides were washed again in PBS at room temperature for 10 min.

After fixation slides were immediately processed for ER or PR staining as described previously (17). The antibodies used were H222 for ER and KD 68 for PR. After an indirect peroxidase-anti-peroxidase procedure, receptors were detected by means of the diaminobenzidine hydrochloride/hydrogen peroxide chromagen substrate reaction. Counterstaining was in 2% Harris' type hematoxylin in distilled water for 5 min. Slides were then dehydrated and mounted in a xylene soluble mountant.

After staining, all slides were assessed by L. T. Each slide was assessed for the number of normal breast epithelial cells present and all slides containing less than 50 epithelial cells were excluded from the study. The percentage of breast epithelial cells staining positive was then assessed. A cutoff of 50% was used to determine whether the specimen was "positive." This cutoff was used because in breast cancer patients this percentage correlated closely with ER positivity as defined by response to endocrine therapy (11, 18).

**Sections.** Tissue samples were snap-frozen in liquid nitrogen or dry ice within 1 min of excision; each sample represented at least 1 $cm^3$ of macroscopically normal breast tissue. Samples were stored at −75°C for up to 2 months prior to processing. After histological confirmation of the benign nature of the excised lump, 5 sections (sequential if possible), each 6 $\mu$m thick, were cut from each specimen using a Frigocut cryostat. These sections were fixed and processed in one staining run as described previously; two sequential sections were stained for ER, two were stained for PR, and a fifth was used as control. Methods for immunocytochemical staining for ER (17) and PR (19) have already been published.

Assessment of all slides was performed by L. T., and the result was given as the percentage of breast epithelial cells taking up staining. As for FNA, a cutoff of 50% was used. Any slide containing less than 50 breast epithelial cells or not conforming to the criteria of normality given above was not included in the study.

**Enzyme Immunoassay.** After sections had been cut from samples excised at operation as described above, the remaining tissue was processed for ER-EIA in 59 cases. The specimen was divided in half and each was separately homogenized and assayed. Glass beads coated with D547 anti-ER rat monoclonal antibody were incubated with specimens or the appropriate standards or controls. ER present in the specimen, standard, or control bound to the solid phase and unbound material was removed by washing. Anti-ER antibody (H222 rat monoclonal conjugated with horseradish peroxidase) was incubated with the beads and ER conjugate became bound to the ER on the beads. Unbound conjugate was removed by washing. The beads were next incubated with an enzyme substrate solution (hydrogen peroxide and *o*-phenyldiamine 2-hydrochloride) to develop a color which is a measure of the amount of bound ER conjugate. The enzyme reaction was stopped by the addition of sulfuric acid and the intensity of the color was read by a spectrophotometer at 492 nm. The intensity of the color formed by the enzyme reactions was proportional to the concentration of the ER in the sample within the working range of the assay. A standard curve is obtained by plotting the ER concentration of the standards *versus* absorbance. The ER concentration of the specimens and controls can be determined from the curve, giving a numerical result (20). Cytosol extracts were measured in order to provide comparability to our DCC method.

## RESULTS

**Reproducibility Study.** Six results were obtained for each patient (ER and PR FNA UOQ-1, UOQ-2, and LOQ). Thus 204 FNA results were obtained from 34 patients. Five of 102 (5%) ER-FNA and 9 of 102 (8%) PR-FNA contained less than 50 cells and were not assessed; 190 of 204 (93%) FNA contained more than 50 cells and were assessed as described above. The average total number of breast epithelial cells seen on the 3 slides from each FNA was 2000 (range, 50–3900).

The reliability of ER FNA is illustrated by the comparison of ER FNA UOQ-1 to ER FNA UOQ-2 in 30 patients. The correlation coefficient between the two sets of values is 0.77 ($P < 0.0001$) (Fig. 1). Although the fitted intercept is 4.336, this is not significantly different from 0 at the 5% level of significance. Similarly the slope (1.07) is not significantly different from 1.

Analysis of the results was made difficult by the numbers of FNA ER containing few or no ER positive cells; 17 of 30 (57%) of ER FNA UOQ-1, 15 of 34 (44%) of ER FNA UOQ-2, and 19 of 33 (58%) ER-LOQ contained no ER positive cells. Nine of 30 (30%) patients had no ER positive cells in both ER UOQ-1 and ER-UOQ-2, and 7 of 29 (24%) had no ER positive cells in all 3 ER FNA. Of the 190 assessable FNA, 67 (35%)

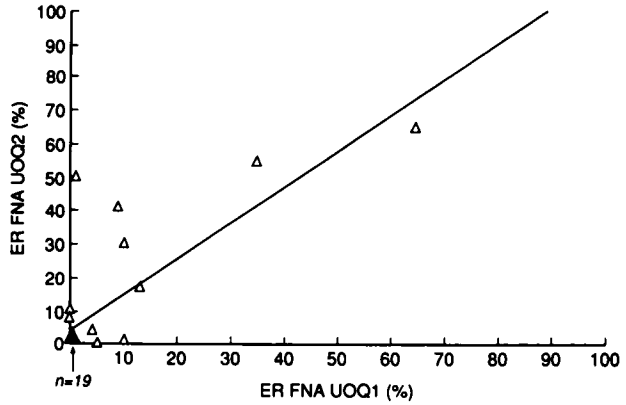


Fig. 1. Relationship between the ER content of two separate aspirates obtained from the upper outer quadrant of the normal breast. There is a highly significant correlation ($P < 0.0001$).

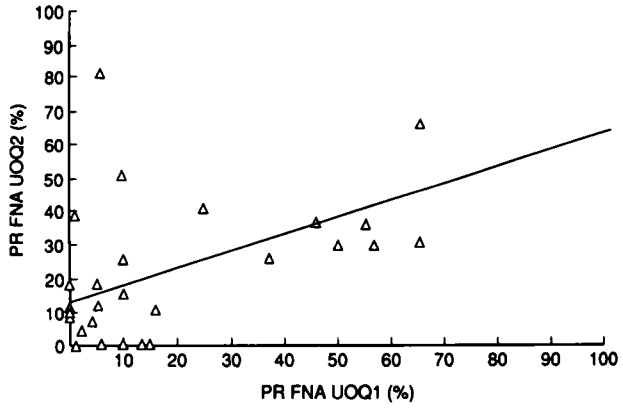


Fig. 2. Relationship between the PR content of two separate aspirates obtained from the upper outer quadrant of the normal breast. There is a significant correlation ($P < 0.002$).

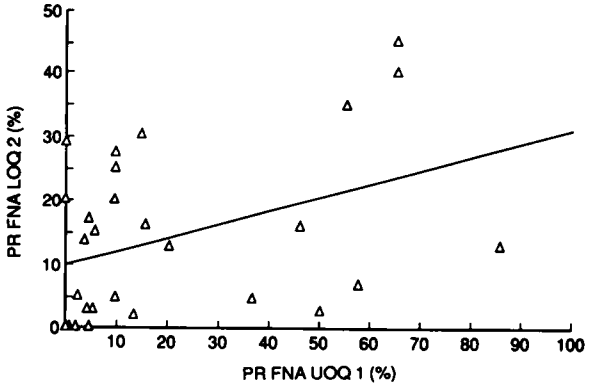


Fig. 3. Relationship between PR content of the aspirates from the lower outer quadrant and the upper outer quadrant. There is a significant relationship ($P < 0.03$).

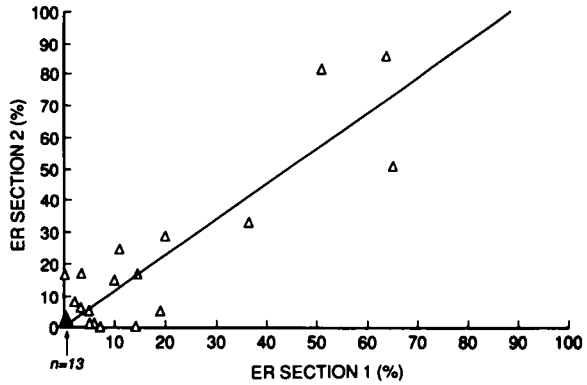


Fig. 4. Correlation between the percentage of cells stained for ER in two separate sections of normal breast from biopsies from the same quadrant. A highly significant correlation exists ($P < 0.0001$).

contained no ER positive or PR positive cells.

Similar results were obtained when comparing PR FNA UOQ-1 to PR FNA UOQ-2 in 28 patients. The correlation coefficient was 0.56 ($P < 0.002$) (Fig. 2). The intercept is 13.05 and the slope is 0.5. Five of 31 (16%) of PR-UOQ-1, 5 of 31 (16%) PR-UOQ-2, and 6 of 31 (19%) of PR-LOQ contained no PR positive cells. All patients had PR positive cells in 1 of 2 assessable PR-UOQ; similarly all patients with 3 assessable PR-FNA ($n = 26$) had PR positive cells in at least 1 PR-FNA.

In 33 patients, ER-UOQ was also compared to ER-LOQ and there was no significant correlation ($P = 0.1173$). In contrast

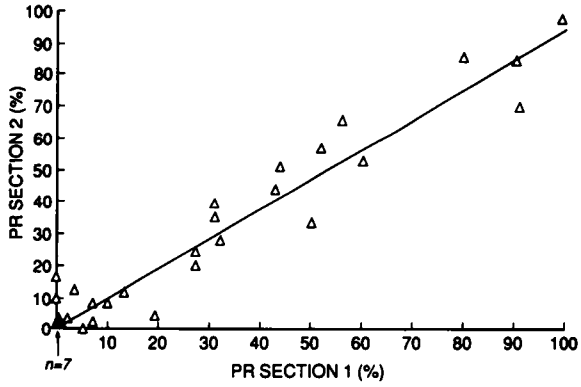


Fig. 5. Correlation between the percentage of cells stained for PR in two separate sections in the same breast. There is a highly significant correlation ($P < 0.0001$).

the correlation coefficient of PR-UOQ-1/2 and PR-LOQ ($n = 31$) was 0.41 ($P < 0.03$) (Fig. 3). The slope is 0.216 and intercept is 9.52.

The reproducibility of ER-section tests is illustrated by the results of ER-section 1 and ER-section 2 in 30 patients. Although 40 specimens were used in this study, 9 of 40 (22.5%) of ER-section 1 and 6 of 40 (15%) of ER-section 2 contained insufficient numbers of breast epithelial cells. Six of 40 (15%) patients had no cells in either ER section. Of the remaining ER sections, 10 of 31 (32%) ER-section 1 and 16 of 34 (47%) ER-section 2 contained no ER positive cells. Nine of 31 (29%) patients had no ER positive cells in either ER-section. There is a clear association between the two sets of results, with $R = 0.89$ ($P < 0.0001$) (Fig. 4). At 95% confidence limits the intercept (0.216) is not significantly different to 0 and the slope (1.12) is not significantly different from 1.

The correlation of 32 pairs of PR section results is 0.97 ($P < 0.0001$) (Fig. 5). The intercept is 1.3, not significantly different from 0. The slope is 0.914, not significantly different from 1. Of 40 pairs of PR-sections, 8 of 40 (20%) of the first section were acellular and 6 of 40 (15%) of the second sections were acellular. Six of 40 (15%) pairs of sections were acellular; 5 of 6 (83%) of these had no cells in both ER-sections either. Of the remaining PR sections, 7 of 32 (22%) section and 6 of 34 (18%) section 2 contained no PR positive cells.

For 39 patients, two ER EIA results were available from the divided specimens of normal breast tissue. The correlation coefficient between these two sets of ER-EIA data is 0.76 ($P < 0.0001$) (Fig. 6). The slope is 0.53 and the intercept is 1.2. Although it was not possible to determine which samples were acellular, 3 of 39 (8%) of the first halves of the sample contained no ER, and 7 of 39 (18%) of the second halves contained no ER. Three of 39 (8%) samples contained no ER in either half of the divided specimen.

**Immunocytochemical Detection of ER and PR in Sections of the Normal Human Breast.** With regard to the localization of ER staining to microscopic structures, we noted considerable heterogeneity. The intensity and frequency of staining varied considerably from patient to patient and in many individual slides from area to area. In individual slides the lobules showed variation of ER staining with speckled staining; one or two cells in each acinus were positive. Although no large ducts were identified, in general small ducts stained more ER positive than lobules. The pattern differed from that seen in acini, and the staining involved linear stretches of epithelium. In all structures the epithelial cells of the ductal epithelium took up stain;

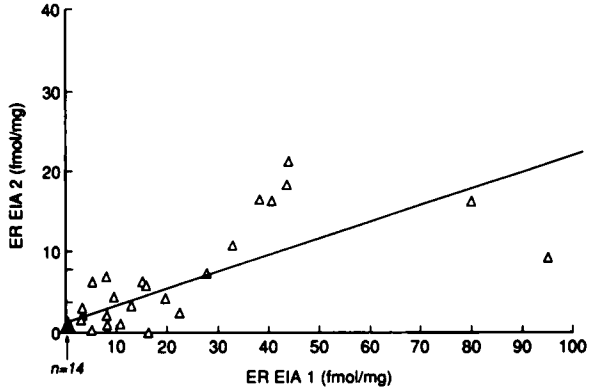


Fig. 6. Correlation between ER measured by enzyme immunoassay of two samples of breast from the same patient. There is a highly significant correlation ($P < 0.0001$).

myoepithelial cells were unstained. PR staining followed a pattern similar to that of ER staining noted above, although in all cases the PR staining was stronger than ER.

**Fine Needle Aspiration Results in Normal Women.** We next analyzed the results of fine needle aspiration cytology in the sample from the upper outer quadrant in 143 normal women. Table 1 gives details and shows the percentage of women in different categories showing more than 50% cells positive for either estrogen receptor or progesterone receptor. Twenty-three of 143 samples contained more than 50% cells stained for ER. Fifty % was chosen as the cutoff because it is used in hormone response studies in breast cancer (13). We found a relationship between the PR and ER positivity with 9 of 17 women who were ER positive being PR positive compared with only 16 of 89 women who were ER negative being PR positive ($P = 0.03$). For the purposes of this study, the results of European (Caucasian) and non-European (Asian, West Indian, and West African) women were analyzed separately. We found a significantly higher proportion of European women with estrogen positive samples; thus 19% of European women have ER positive samples compared with only 4% of non-Europeans ($P = 0.03$). This is not true when considering PR. There was no relationship between the age of the patient (16–55 years) and the percentage of positivity. However, there did appear to be a relationship of PR with body mass index. Thus both Europeans and all (European and non-European) patients with a high body mass index tended to have a higher PR content ($P = 0.02$). In addition, the patients tended to have a higher PR content if they had a family history of breast cancer, with 11 of 26 patients who had a family history having PR positive samples compared with only 20 of 62 without a family history ($P = 0.04$). This is more significant when Europeans alone were considered ($P = 0.01$).

There was no relationship to age at menarche, to oral contraceptive use, or to the number of pregnancies. There was a suggestion that women who had their first pregnancy under the age of 20 years had a higher chance of having higher levels of PR in their normal breast tissue ($P < 0.045$). There is no relationship to breast-feeding.

We also examined the phase of the menstrual cycle during which the sample was obtained. Here we found a significantly higher proportion of samples to be ER positive if taken in the follicular stage of the cycle, both when Europeans were concerned and when all normals were concerned ($P = 0.04$ and $P = 0.03$, respectively). Thus 15 of 60 had ER positive samples in the follicular phase compared with only 6 of 52 in European

Table 1 *Correlation in FNA results of normal breast with clinical details*

| | ER status | | | PR status | | |
|---|---|---|---|---|---|---|
| | ER positive (>50%) | ER negative | *P* | PR positive (>50%) | PR negative | *P* |
| **Race** | | | | | | |
| European | 22 (19) | 94 | 0.03 | 23 (26) | 67 | 0.1 |
| Non-European | 1 (4) | 26 | | 8 (38) | | |
| **PR status (all races)** | | | | | | |
| PR+ | 9 | 16 | 0.03 | | | |
| PR− | 8 | 71 | | | | |
| **Age** | | | | | | |
| European | | | | | | |
| 15–25 | 2 | 10 | | 2 | 4 | |
| 26–35 | 7 | 28 | | 5 | 21 | |
| 36–45 | 8 | 44 | 0.8 | 12 | 33 | 0.69 |
| 46–55 | 4 | 12 | | 5 | 9 | |
| All | | | | | | |
| 15–25 | 2 | 21 | | 5 | 10 | |
| 26–35 | 7 | 37 | | 5 | 26 | |
| 36–45 | 9 | 49 | 0.59 | 17 | 35 | 0.35 |
| 46–55 | 4 | 12 | | 5 | 9 | |
| **Body mass index[a]** | | | | | | |
| European | | | | | | |
| <25 | 17 | 70 | 0.21 | 12 | 58 | 0.047 |
| 25+ | 3 | 19 | | 7 | 12 | |
| All | | | | | | |
| <25 | 17 | 89 | 0.2 | 17 | 67 | 0.02 |
| 25+ | 3 | 25 | | 10 | 14 | |
| **Family history of carcinoma of the breast** | | | | | | |
| European | | | | | | |
| + | 3 | 27 | 0.26 | 11 | 13 | 0.01 |
| − | 16 | 88 | | 13 | 54 | |
| All | | | | | | |
| + | 3 | 28 | 0.2 | 11 | 15 | 0.04 |
| − | 17 | 108 | | 20 | 62 | |
| **Age (yr) at menarche** | | | | | | |
| European | | | | | | |
| 9–12 | 5 | 34 | 0.1 | 12 | 19 | 0.46 |
| 13+ | 15 | 53 | | 12 | 44 | |
| All | | | | | | |
| 9–12 | 6 | 41 | 0.16 | 15 | 22 | 0.39 |
| 13+ | 15 | 70 | | 17 | 53 | |
| **Oral contraceptive use** | | | | | | |
| European | | | | | | |
| Yes | 7 | 62 | 0.24 | 18 | 36 | 0.14 |
| No | 4 | 28 | | 10 | 28 | |
| All normal | | | | | | |
| Yes | 7 | 73 | 0.22 | 21 | 41 | 0.14 |
| **No pregnancies** | | | | | | |
| European | | | | | | |
| 0 | 7 | 28 | | 6 | 20 | |
| 1 | 1 | 12 | 0.62 | 4 | 11 | 0.96 |
| 2 | 8 | 31 | | 8 | 21 | |
| 3+ | 6 | 17 | | 6 | 14 | |
| All normal | | | | | | |
| 0 | 7 | 42 | | 11 | 25 | |
| 1 | 1 | 17 | 0.28 | 4 | 13 | 0.91 |
| 2 | 8 | 35 | | 9 | 24 | |
| 3+ | 7 | 19 | | 8 | 16 | |
| **Age of 1st pregnancy** | | | | | | |
| European | | | | | | |
| 15–19 | 4 | 11 | | 5 | 7 | |
| 20–24 | 6 | 21 | 0.72 | 10 | 14 | 0.055 |
| 25+ | 5 | 25 | | 3 | 22 | |
| All normals | | | | | | |
| 15–19 | 4 | 14 | | 6 | 8 | |
| 20–24 | 7 | 25 | 0.74 | 11 | 16 | 0.045 |
| 25+ | 5 | 28 | | 4 | 29 | |
| **Breast feeding** | | | | | | |
| European | | | | | | |
| Yes | 7 | 36 | 0.2 | 10 | 25 | 0.22 |
| No | 7 | 26 | | 8 | 21 | |
| All normals | | | | | | |
| Yes | 8 | 45 | 0.19 | 13 | 30 | |
| No | 7 | 28 | | 8 | 23 | |
| **Phase of menstrual cycle** | | | | | | |
| European | | | | | | |
| Follicular | 15 | 45 | 0.04 | 11 | 35 | 0.14 |
| Luteal | 6 | 46 | | 13 | 28 | |
| All normals | | | | | | |
| Follicular | 16 | 58 | 0.03 | 14 | 43 | 0.12 |
| Luteal | 6 | 55 | | 15 | 32 | |

[a] Body mass index = $\frac{\text{wt (kg)}}{[\text{ht (m)}]^2}$.

Table 2 *ER of breast tissue measured by enzyme immunoassay in biopsies from 59 normal women*

| | ER positive (>5 fmol/mg cytosol protein) | ER negative | *P* |
|---|---|---|---|
| Race | | | |
| European | 13 | 32 | <0.0146 |
| Non-European | 1 | 11 | |
| Age (yr) | | | |
| 16–25 | 1 | 11 | <0.353 |
| 26–35 | 4 | 13 | |
| 36–45 | 4 | 18 | |
| 46–55 | 5 | 3 | |
| Body mass index[a] | | | |
| 0–24 | 4 | 34 | <0.001 |
| 25+ | 10 | 8 | |
| Menstrual cycle | | | |
| First half | 8 | 21 | <0.227 |
| Second half | 5 | 18 | |
| Family history | | | |
| Positive | 3 | 5 | <0.177 |
| Negative | 10 | 4 | |
| Age at menarche (yr) | | | |
| 0–12 | 6 | 13 | <0.128 |
| 13+ | 6 | 29 | |
| Oral contraceptive use | | | |
| Ever | 9 | 24 | <0.191 |
| Never | 5 | 21 | |
| No. of pregnancies | | | |
| 0 | 5 | 20 | <0.855 |
| 1 | 2 | 6 | |
| 2 | 5 | 11 | |
| 3+ | 2 | 8 | |
| Age of 1st pregnancy | | | |
| 0–25 | 5 | 19 | <0.226 |
| 26+ | 3 | 5 | |
| Breast-feeding | | | |
| Yes | 6 | 15 | <0.319 |
| No | 3 | 8 | |
| Estrogen suppression[b] | | | |
| Yes | 3 | 6 | <0.302 |
| No | 6 | 17 | |

[a] Body mass index = $\frac{\text{wt (kg)}}{[\text{ht (m)}]^2}$.

[b] Estrogen suppression = use of estrogen to suppress lactation postpartum.

women. When all patients were considered together, 16 of 74 had a positive sample compared with only 6 of 61 in the luteal phase. This is confirming previous observations from this unit.

**ER-EIA Results in Normal Women.** Table 2 shows the results of ER-EIA assay of the tissue specimens from 59 normal women. Only 3 of 59 (5%) patients had undetectable levels of ER, and at a cutoff of 5 fmol 13 of 59 (22%) specimens were ER positive. The range of results was 0–37.02 fmol (mean, 4.19 fmol). Significant variation of ER with race was again detected ($P < 0.015$), with only 1 of 12 (8%) non-European women ER positive, compared to 13 of 45 (29%) European women being ER positive. The body mass index was also found to vary significantly with ER ($P < 0.001$). No significant association was detected between ER and any other clinical data, including the menstrual cycle ($P < 0.227$).

We compared these results with an age-matched population of 126 women with breast cancer. In this group we found a significantly higher ER. The range was 0–139 fmol/mg and the mean result was 37 fmol/mg ($P < 0.001$).

## DISCUSSION

The first part of this paper concerns the reproducibility of tests for ER and PR in normal breast tissue. Of the three tests examined, ER- and PR-section appeared to be the most accurate and repeatable. Comparable but decreased accuracy was found with FNA and ER-EIA.

Several factors may have influenced these results. Firstly, in breast cancer cutoffs for ER and PR positivity are determined by the objective response to endocrine therapy. In the study of normal breast and benign breast disease, no such guidelines exist. We thus set a 50% cutoff for the tests utilized because this reflected the best prediction of response to endocrine therapy in breast cancer (13).

Fewer FNAs than sections were inadequately cellular in the study. In our current series the failure rate of FNA was 7% and that of section was 19%. We believe that the poor results of FNA of the normal breast previously reported (21) reflect both the poor cellularity of the normal breast and poor technique. In postmenopausal women whose breasts are very poorly cellular, we obtain adequate numbers of cells in only 50% of slides.[4]

Each patient in the reproducibility study had ER and PR FNA from both the UOQ and LOQ of the breast. The correlation between the UOQ and the LOQ was poor and differed from the correlation between the two UOQ.

ER and PR staining on histological sections of normal breast tissue (ER-section and PR-section) was observed to be markedly heterogeneous within different areas of the same section. In ER-sections only scattered cells within each terminal duct stained positive. ER positive breast epithelial cells were more frequently localized to longitudinal patches of the small ducts. PR staining also followed this pattern, although in nearly all cases PR staining was more pronounced. This is in keeping with current theories that breast cells contain more PR than ER (22). Despite the strong correlation noted between ER-FNA and PR-FNA, in sequential sections there was little correlation between areas of ER positive and PR positive staining. In the normal breast most cellular proliferation occurs at the terminal ducts (23), but no concentration of staining was observed in this region.

All of the tests confirmed that the levels of ER and PR found in noraml breast tissue are lower than the levels found in discrete breast lesions, both benign and malignant. The percentages of normal breast epithelial cells staining ER positive (7%) PR positive (19%) are thus lower than levels in benign mammary dysplasia (16% ER positive and 31% PR positive by DCC), and fibroadenomas (44.5% ER positive, 55% PR positive) (6, 8, 9, 24–30). It may be that overexpression of ER (and perhaps PR) is linked to pathological cell proliferation.

Many tissues of the female genital tract have been shown to undergo menstrual cycle dependent fluctuations in ER and PR content (31–34). Several authors have attempted to determine, using ER-sections (8) and DCC (35), whether normal breast epithelial cells undergo similar changes. The results have been inconclusive. Our previous study using ER-FNA concluded that cyclical variation in ER was present (12). Our current study has again demonstrated statistically significant variation of ER-FNA with the menstrual cycle using a 50% cutoff ($P = 0.034$). In smaller groups of patients, we were unable to reproduce these results using either the ER-section or ER-EIA.

Non-European women tended to have ER negative FNA samples; only 1 of 27 was ER positive at 50% cutoff (although

[4] D. Ricketts, unpublished data.

similar results were not seen with ER-sections). This is interesting in the light of recent studies documenting a higher incidence of ER negative breast tumors in black women (36–43).

In the current study we attempted to correlate the risk factors for breast cancer with the ER and PR content of normal breast. There were few significant correlations, and no clear pattern emerged. A family history of breast cancer correlated only weakly with PR-FNA at the 50% cutoff level in both European ($P = 0.0116$) and "all normals" groups ($P = 0.0432$). ER status was not significantly different in those patients with a family history. The strongest correlation was found with age of menarche and PR-FNA ($P = 0.02$). Weak variation of PR with body mass index and ER with suppression of lactation was also observed.

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