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Estrogen Receptor Analysis on Fine Needle Aspirates and on Histologic Biopsies from Human Breast Cancer*

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Abstract—*This study provides the results from estrogen receptor analysis of fine needle aspirates from* 115 *cases of primary or recurrent breast cancer. There was a strong correlation between estrogen receptor levels obtained with needle aspirates and with histologic tissue specimens from the same case. If, however, the amount of DNA in the fine needle aspirate was lower than approximately* 10 μg, *a low estrogen receptor value was not reliable. The needle aspiration method for estrogen receptor determination appears to be of great advantage for patients with inoperable carcinoma. Twenty of* 22 *cases of inoperable carcinoma yielded aspirates that could be successfully analyzed.*

INTRODUCTION

THE CONCENTRATION of estrogen receptor protein (ER) in breast cancer is a useful indicator of the likelihood of response to endocrine therapy of mammary carcinoma [1]. The ER-analysis is routinely performed on surgically removed tumor specimens. However, surgical biopsies may not be easily obtainable in many inoperable or recurrent carcinomas and after preoperative irradiation ER-determination is unreliable as the primary tumor often undergoes advanced degenerative changes or totally disappears following irradiation [2]. Recently a pilot study [3] indicated that reproducible ER-levels can be obtained from analyses of fine needle aspirates from breast cancer. In the pilot study fine needle aspirates were collected from tumors immediately after mastectomy and were analysed by the sensitive method of isoelectric focusing in polyacrylamide gel. The present study examines the results of ER-analysis on fine needle aspirates collected from patients with primary or recurrent breast cancers prior to surgery. For comparison the analysis was also performed on histologic tissue material after surgical biopsy or mastectomy.

MATERIALS AND METHODS

Collection of materials

In 96 cases of cytologically diagnosed primary breast cancer and in 19 cases of chest wall or lymph node recurrences of breast cancer, a percutaneous needle aspirate was taken from the tumor using the technique described by Franzén and Zajicek [4, 5]. The needle (o.d. 0.7 mm) used was that routinely used for cytological diagnosis. Double aspirations were performed and the material thus obtained was ejected into a solution of ^{3}H-estradiol as described below. The needles were then rinsed with the same solution. One drop of the diluted aspirate was smeared onto a slide for microscopic control. In operable cases a piece of tumor was as well selected for conventional ER-analysis at the time of operation (Table 1).

Microscopic examination

The cytological smears were stained according to the May–Grünwald–Giemsa method and the amount of tumor cells in the cytological specimens was subjectively estimated as none, sparse, a moderate number of and numerous cells. All cases of operable carcinomas were histologically verified.

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Abbreviation: ER, estrogen receptor.

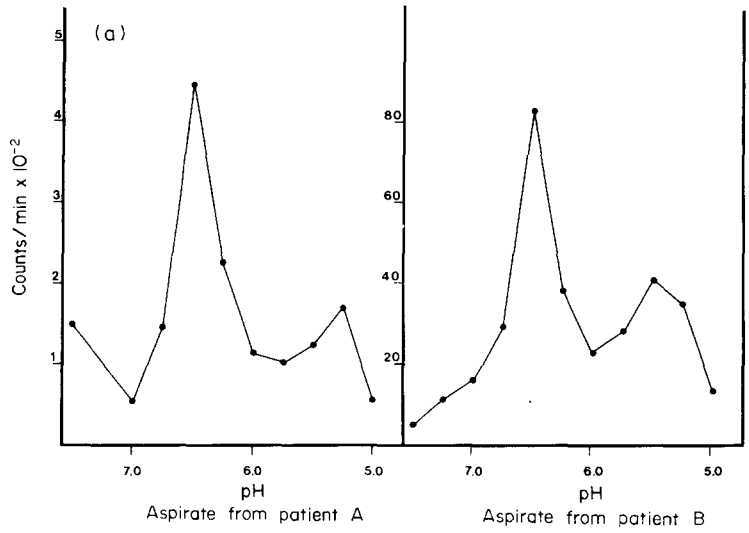

Fig. 1. Isoelectric focusing of the estrogen receptor from needle aspirates from two patients. (a) Needle aspirates were collected from two patients with inoperable primary mammary carcinomas and analyzed for estrogen receptor as described in Materials and Methods. Patient A had little (0.11 fmole/μg DNA) and patient B much (3.8 fmole/μg DNA) receptor.

Biochemical method

Thin needle aspirates were injected into 0.5 ml of 5 nM ^{3}H-estradiol (New England Nuclear, 141 Ci/mmole) in 0.01 M Tris–HCl, 0.001 M EDTA, pH 7.4 and frozen within 15 min. The material was then kept at −70°C for a few weeks. After thawing the aspirates were homogenized in an all glass homogenizer and further incubated at 0°C for 45 min. The aspirates were then centrifuged at 15,000 ***g*** for 20 min. An aliquot of the supernatant solution was removed and the protein content was estimated by measuring the difference in absorbance at 280 and 310 nm which is given as optical density units (ODU) at 280–310 nm. Sediments after centrifugation of the homogenate contained the cell nuclei and were taken for measurement of DNA according to Burton [6] as reference for quantitation of specific binding. Burton's method was micromodified; the sediments were incubated once in 300 μl of 0.5 M perchloric acid at 80°C for 30 min to hydrolyse DNA. The hydrolysate was then used incubated with 400 μl of Burton's reagents [6] and the absorbance at 600 nm was read in microcuvettes.

Five μg crystallized trypsin (Worthington) was added per ODU of cytosol and after incubation for 30 min at 10°C the cytosol was shaken with one-fifth volume of dextran-coated charcoal (dextran:charcoal 1:10 w/w). After centrifugation at 800 ***g*** for 10 min the sample was placed in 300 μl sample frames on polyacrylamide gels (LKB, pH range 3.5–10) near the cathode at approximately pH 8 and subjected to isoelectric focusing for 2 hr as previously described [7]. After pH measurement the gel was cut into 5 mm slices and placed in 5 ml Istagel (Packard). After incubation overnight at 20°C the radioactivity was measured in a Packard liquid scintillation counter with 36% counting efficiency. For each experiment a diagram was drawn and the baseline radioactivity was subtracted from the single peak of radioactivity in the pH 6.5 region which represented the receptor bound steroid. Figure 1 illustrates two diagrams obtained with two tumors containing different levels of ER. Figure 1(a) was selected since in this analysis an unusually larger peak of radioactivity was observed in the pH 5 region where the sex hormone binding globulin has been shown to focus [7, 8]. ER-determinations were only performed on material obtained through aspiration if the microscopic control demonstrated the presence of tumor cells (Fig. 1).

Statistical methods

ER-values are not normally distributed, but, as has been shown earlier [9], their logarithms are closer to the normal distribution. The logarithms of the ER-values have therefore been used for calculations of the means (i.e., geometrical means) and for calculations of coefficients of correlation. Concentrations smaller than 0.01 fmole/μg

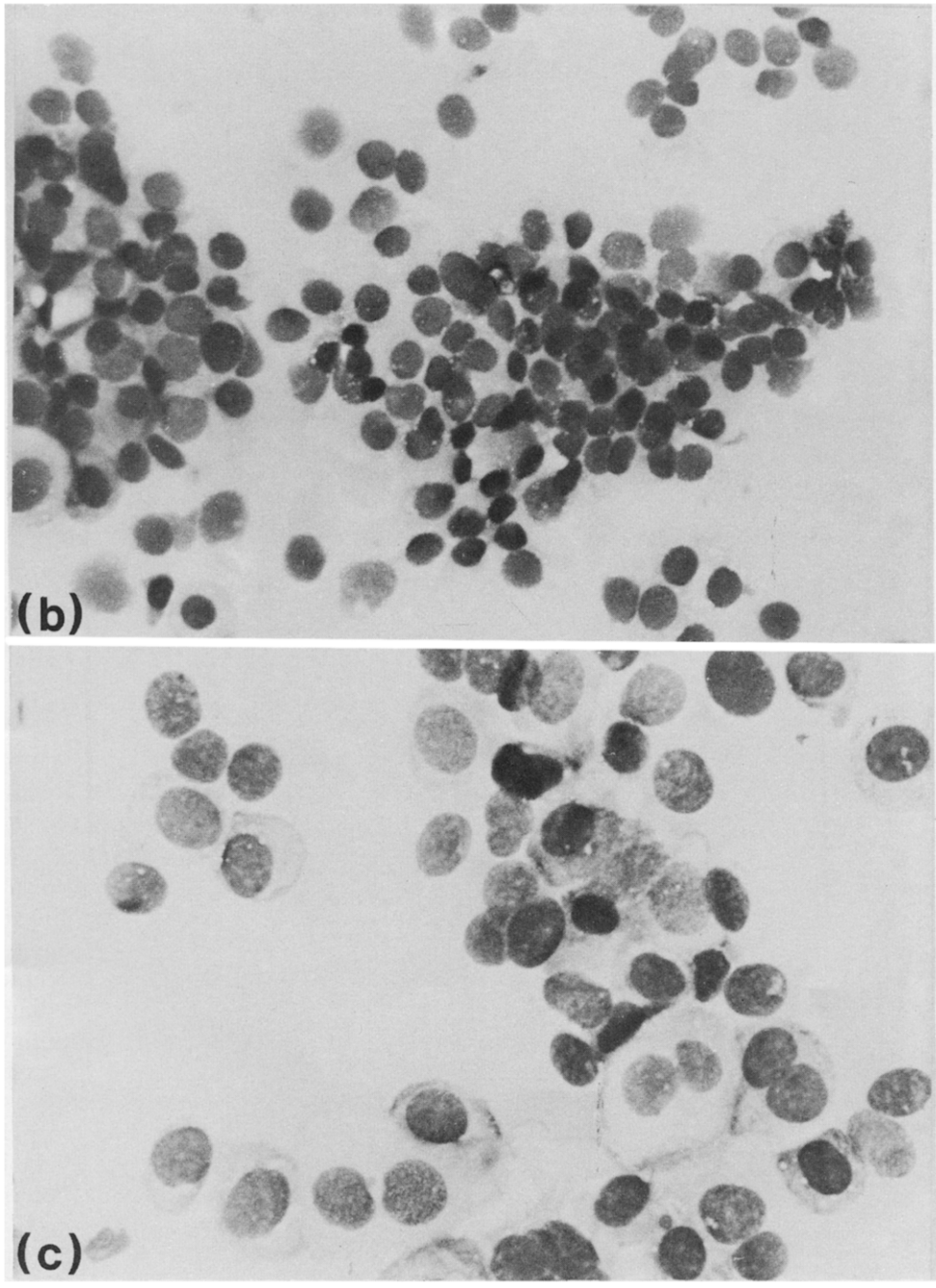

Fig. 1. (*b*) *and* (*c*). *The illustrated smears of the two aspirates contained abundant nuclei from cancer cells with very few contaminating normal cells. The total amount of DNA in the aspirates was* 40 μg *for patient A* (1*b*) *and* 55 μg *for patient B*(1*c*).

DNA could not be analysed and were considered as 0.01 fmole/μg DNA. The linear correlation coefficient (r) was calculated between the logarithms of the ER-concentrations in material obtained through fine needle aspiration and in surgical tissue specimens. The significance of the correlation coefficient was calculated. The significance of the differences between the logarithmic ER-concentrations in aspirates and surgical specimens in the same cases was tested by the paired t-test. For differences in the amount of DNA Student's t-test was used.

RESULTS

Cancer cells and the amount of DNA in aspirates

Carcinoma cell nuclei were easily identified in the smears of the diluted aspirates from 106 of 115 (92%) cases of primary or recurrent breast cancer. There was an extensive cytolysis caused by the hypotonic incubation solution. The microscopic examination failed to show any tumor cells in the control smears from four of 96 aspirates from primary tumors and from five of 19 aspirates from skin or lymph node recurrences (Table 1). The mean amount of DNA in the nine cases without identified tumor cells was 4.6 μg per aspirate (range 0.9–12.0 μg), and among 106 cases in which carcinoma cells were found it was 30.7 μg (range 2.4–310 μg). Increasing numbers of cells in the smears according to the subjective estimate correlated well with an increased amount of DNA in the aspirate (Table 2).

Correlation between ER-determination in aspirates and in surgical tissue specimens

ER-determinations were only performed in cases with microscopically demonstrable tumor cells in the control smears of the cytosols. In 67 cases ER-determinations were made in aspirates as well as in surgical tissue specimens. The mean amount of DNA of these aspirates was 21.9 μg (range 2.1–166 μg) and of the corresponding tissue specimens 287 μg (range 85–2280 μg). The ER-values in aspirates are plotted against the corresponding ER-values in tissue specimens in Fig. 2, which also shows that the ER-values were ≦0.01 fmole/μg DNA in eight cases in which the ER-assay in tissue specimens from the same cases showed values ≧0.1 fmole/μg DNA. Seven of these eight 'false negative' cases occurred in aspirates containing <10 μg DNA. The eighth case occurred in an aspirate containing 57 μg DNA, and the corresponding ER-value in the surgical specimen was 0.15 fmole/μg DNA. The correlation between the two ER-determinations was statistically significant ($r=0.68$, $P<0.0001$). The correlation between the double ER-determinations for 25 cases with aspirates containing <10 μg

Table 1. Number of aspirates for ER-assays from primary and recurrent breast cancer according to presence or absence of cancer cells in the corresponding smear and to the concomitant ER-assay in surgical tissue specimens

	Primary cancers	Recurrent cancers	Total
No. of cancer cells in aspirate	4	5	9
Cancer cells in aspirate	92	14	106
Surgical tissue available	60	7	67
No tissue ER-assay	32	7	39
Total	96	19	115

Table 2. Estimates of cellularity on smears and amounts of DNA in the aspirates

Cellularity	No. of cases	Mean amount of DNA±S.E. (μg)
Sparse	47	12.1±1.1
Moderate	24	29.8±4.4
Numerous	35	56.2±10.6
Total	106	30.7±4.1

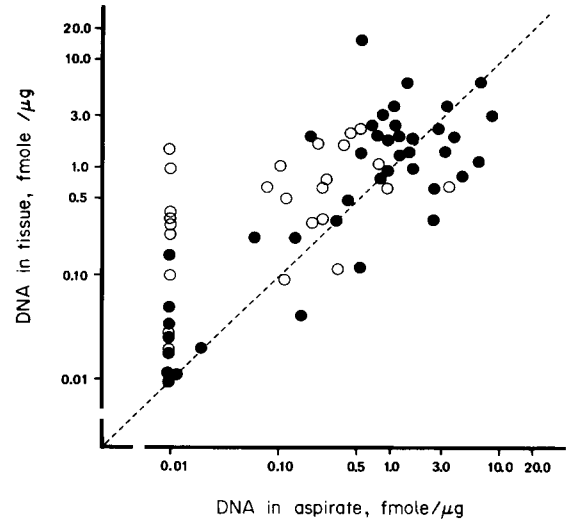

Fig. 2. Correlation between ER-levels in aspirates and in histologic tissue specimens from 67 cases of primary or recurrent breast cancer. Regression line for r=1.0 is included for comparison.
(--○--) <10 μg DNA, (--●--) >10 μg DNA in aspirate.

DNA was of low statistical significance ($r = 0.46$, $P < 0.05$), whereas 42 cases with $\geq 10\,\mu g$ DNA in the aspirates showed a strong correlation between the two ER-determinations ($r = 0.87$, $P < 0.0001$).

The ER-concentrations in aspirates were significantly lower than the corresponding values measured in surgical tissue specimens if the amount of DNA was less than $20\,\mu g$ in the aspirates (Table 3). Even if the 'false negative' cases were excluded, ER-concentrations in aspirates with less than $20\,\mu g$ DNA were significantly lower than the determinations in tissue specimens ($P < 0.001$). Aspirates containing $> 20\,\mu g$ DNA gave similar ER-values to those obtained from tissue specimens (Table 3).

Table 3. ER-concentrations (geometrical means) in 67 cases of breast cancer determined in aspirates as well as in surgical tissue specimens listed according to the amount of DNA in the aspirates

DNA in aspirates (μg)	No. of cases	ER-concentration (fmole/μg DNA)		Probability of difference (P)
		Aspirates	Tissue spec.	
< 5.0	10	0.09	0.50	< 0.01
5.0–9.9	15	0.13	0.51	< 0.05
10.0–19.9	22	0.21	0.36	< 0.05
≥ 20.0	20	0.79	0.60	> 0.40 (N.S.)
Total	67	0.25	0.48	< 0.01

ER-assays in aspirates from primary or recurrent breast cancers without surgical biopsy

In 32 cases of primary and in seven cases of recurrent breast cancer with aspirates containing tumor cells, no surgical specimens were obtainable for ER-assays, mainly because the lesions were considered inoperable (Table 1). The mean content of DNA was $45.8\,\mu g$ (range 2.4–$310\,\mu g$), which was higher than of the previous group of operable primary or recurrent carcinomas ($P < 0.01$). All but three aspirates contained more than $10\,\mu g$ DNA, and 27 (69%) contained more than $20\,\mu g$ DNA.

DISCUSSION

This study demonstrates that ER-analysis may be routinely performed on needle aspirates from human mammary carcinomas. It also demonstrates that aspirates that are rich in cells yield the most reliable receptor data. Of our eight 'false negative' aspirates, seven contained less than $10\,\mu g$ DNA ($<$approx. 10^6 cells). Thus data which indicate low levels of estradiol binding in aspirates with few cells must be interpreted with caution. A high level of estradiol binding (> 0.5 fmole/μg DNA) can, however, be trusted even if the aspirate contains only little DNA (Fig. 1). All but two of 22 cases with inoperable breast cancer contained more than $10\,\mu g$ DNA. This is promising since this patient category is best served without surgical intervention.

Five aspirations of 19, from patients with skin or lymph node recurrences, failed to yield adequate tumor material. Four of these five patients had local skin recurrences. This is not surprising since skin metastases from breast cancer are often small and quite acellular with a sclerotic stroma. For the remaining 14 cases reliable values were, however, obtained.

There are several potential advantages with estrogen assays using needle aspirates as compared to surgical biopsies. Firstly the method can be used in patients with inoperable or recurrent breast cancer, in whom surgical biopsy may be difficult and sometimes impossible. In cases selected for preoperative irradiation [2] the fine needle aspiration technique for ER-determination is the only method available. Aspirated material may be immediately chilled in ice-cold solution which reduces receptor deterioration.

The aspiration procedure selects for tumor cells and therefore diminishes benign cell contamination. Finally, a drop of the incubation solution may be reserved for microscopic characterization of the material used for estradiol binding (Fig. 1).

The disadvantages with assays of needle aspirates are firstly that the amount of material obtained is too small for repeated assays and assays using other steroid ligands. This may however, be overcome by repeated fine needle aspirations.

Secondly the contamination with serum protein is relatively larger which sometimes results in a peak of sex hormone-binding globulin (SHBG) focusing in the pH 5 region during isoelectric focusing. As illustrated in Fig. 1 this latter problem is not serious since the technique of electrofocusing readily differentiates between the true receptor at approximately pH 6.5 and SHBG at pH 5.

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