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Oestrogen-Receptor Determinations on Fine-Needle Aspirations from Malignant Tumours of the Breast

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Abstract—*Samples from benign and malignant breast tumours were assayed for oestrogen receptors in tumour biopsies as well as corresponding fine-needle aspirations. Oestrogen receptors could be demonstrated in fine-needle aspirations. In cytosols containing* 1 mg/ml *protein or more no significant difference in the number of receptor-positive tumours was observed in fine-needle aspirations as compared to receptor determinations of tumour biopsies. The binding capacities* (fmole *bound oestrogen*/mg *protein*) *were comparable in fine-needle aspirations and corresponding biopsies, but the* K_d *values were significantly lower in fine-needle aspirations. Many surgically biopsied receptor-positive tumours were receptor-negative in the fine-needle aspirations because of* (1) *too few cells*, (2) *non-representative aspirations.*

INTRODUCTION

OF PATIENTS with advanced breast cancer, 20–30% obtain a temporary remission when treated by endocrine ablative surgery or by oestrogen or other hormonal therapy.

Recently, it has been shown that some breast cancers contain cytoplasmic oestrogen receptors. It has also been shown that patients with tumours containing oestrogen receptors stand a significantly better chance of responding to surgery and adjuvant hormonal therapy than those without binding sites. Of patients with oestrogen-receptor positive tumours, 50–60% respond to endocrine therapy [1].

More recently, several investigators have reported that the concentration of cytoplasmic oestrogen receptors in a breast cancer, not simply its presence or absence, is correlated with the likelihood of response to endocrine manipulation [2, 3].

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Normally, receptor analyses are carried out in surgically removed breast biopsy specimens, but in a substantial part of the patients this is not possible because all the tumour tissue is used for histological examination by the pathologist. In patients with multiple tumour lesions some of the lesions may respond to endocrine therapy whereas others may not.

No current method enables us to investigate different lesions for their oestrogen receptor content in an atraumatic way, therefore, it was found of interest to investigate the feasibility of determining oestrogen-binding sites in fine-needle aspirations from patients with breast cancer in order to elucidate better the likelihood of a response to endocrine manipulation. This would be very helpful in the management of patients with disseminated disease.

MATERIALS AND METHODS

Patients

Fine-needle aspiration biopsies were ob-

Table 1. Characteristics of tissue, cystosol, oestrogen receptors (ER) from fine-needle aspirations (2–4 aspirations/patient) of surgically removed breast biopsies

No.	Histological diagnosis (WHO grade)*	Cytological diagnosis	Fibrosis (biopsies)†	mg protein/ml cytosol in biopsies	mg protein/ml cytosol in fine-needle aspirations	ER conc./mg protein in biopsies, K_d fmole/mg/K_d($\times 10^{-10}$M)‡	ER conc./mg protein in fine-needle aspirations, K_d fmole/mg/K_d($\times 10^{-10}$M)
1	benign	benign	3	3.7	0.6	0	0
2	—	—	3	5.6	1.2	10/1.6	0
3	—	—	3	6.2	0.8	0	0
4	—	—	3	4.8	0.6	0	0
5	—	—	3	5.8	0.6	0	0
6	—	—	3	5.5	0.6	0	0
7	—	—	3	3.5	0.7	0	0
8	—	—	3	4.2	0.4	0	0
9	—	malignant	3	7.2	2.2	0	0
10	—	benign	3	8.0	0.7	0	0
11	malignant (II)	malignant	3	4.1	1.3	448/13	56/2.9
12	— (II)		3	4.0	0.5	0	0
13	— (II)	—	1	4.3	0.5	28/6.5	0
14	— (III)		3	7.1	1.0	0	13/1.2
15	— (II)	benign	3	2.6	0	23/6.5	0
16	— (II)	malignant	1	2.6	0.04	30/4	0
17	— (II)	—	3	2.8	0.06	17/3.4	0
18	— (II)	—	3	3.3	3.8	0	0
19	— (II)	—	3	3.6	0.02	26/3.8	0
20	— (III)	—	1	5.0	0.3	60/12	0
21	(not possible)	—	1	8.3	3.9	0	0
22	(III)	—	3	8.0	5.4	98/87	94/0.83
23	(II)	—	3	3.8	0.2	0	0
24	(II)	—	3	2.9	0.1	81/2.2	0
25	(III)	—	3	5.0	0.2	0	0
26	(III)	—	1	4.0	1.0	368/11	340/1.1

*Scarff and Torloni [17].
†Alderson *et al.* [18].
‡fmole = 10^{-15} mole.

Table 2. Characteristics of tissue, cytosol, oestrogen receptors (ER) from fine-needle aspirations (2 aspirations/patient) of preoperative biopsies

Patient	Location	Histological diagnosis (WHO grade)*	Cytological diagnosis	Fibrosis (biopsies)†	mg protein/ml cytosol in biopsies	mg protein/ml cytosol in fine-needle aspirations	ER conc./mg protein in biopsies, K_d fmole/mg/K_d($\times 10^{-10}$M)‡	ER conc./mg protein in fine-needle aspirations, K_d fmole/mg/K_d($\times 10^{-10}$M)
A	breast	malignant (II)	malignant	3	3.3	6.1	0	0
B	neck gland	—	—	—	4.0	3.0	0	0
C	breast	— (not possible)	—	3	4.5	0.5	0	0
D	—	— (II)	benign	1	3.9	0.5	229/2.0	0
E	—	— (II)	malignant	3	2.9	0.8	137/14	362/1.6
F	—	— (II)	—	3	4.6	0.5	57/5.0	0
G	—	— (II)	—	3	5.9	0.5	106/3.9	0
H	—	— (III)	—	3	5.4	0	17/4.3	0

*Scarff and Torloni [17].
†Alderson *et al.* [18].
‡fmole = 10^{-15} mole.

Table 3. Characteristics of tissue, cytosol and oestrogen receptors (ER) from fine-needle aspirations (2 aspirations/patient) of diagnostic fine-needle biopsies

Patient	Location	Cytological diagnosis	mg protein/ ml cytosol	ER conc./mg protein, K_d fmole/mg/K_d ($\times 10^{-10}$ M)
I	breast	benign	0.02	0
J	—	—	1.4	0
K	—	—	1.1	0
L_1	—	malignant	0	0
L_2	—	—	1.9	46/2.2
L_3	—	acellular	0	0
L_4	—	malignant	4.2	0
L_5	—	acellular	0	0
M_1	—	malignant	2.1	0
M_2	—	—	0.4	0
M_3	—	—	0.4	0
N	axillary gland	—	1.4	0
O	—	acellular	0	0
P	breast	malignant	0.72	0
Q	neck gland	—	0.40	0
R	breast	—	0.83	0
S	—	—	2.4	839/1.0
T	—	—	0.08	0
U	—	—	0.7	0
V	—	—	0.2	0
X	—	—	4.9	0
Y	axillary gland	—	0.8	378/2.7

tained from 60 consecutive females with breast tumour.

In patients Nos. 1–26 the aspiration biopsies were obtained by aspirating directly from the surgically removed tumour mass. All the tumour specimens were obtained from the breast (Table 1). In patients A–Y fine-needle biopsies were obtained from preoperative or diagnostic aspiration biopsies before the patients underwent treatment (Tables 2 and 3).

Whenever possible the surgically removed biopsy specimens were investigated for the content of oestrogen receptors.

The material comprises pre- as well as postmenopausal patients. None had had any hormonal treatment within 3 months before the investigation.

Fine-needle aspiration technique

The technique has been described in detail elsewhere [4]. Briefly, a 10 ml syringe was mounted on a 'sprutpistol' (Cancco AB, Södertälje, Sweden) and a needle of 0.7–0.8 mm was routinely used. The needle was introduced into the lesion, the pistol retracted, and the needle moved back and forth in different areas of the lesion. The pistol was released before the needle was withdrawn and 2–4 aspirations obtained from each puncture site.

From each biopsy, part of the material was used for cytological examination and the rest was transferred to tubes containing 1.1 ml TE buffer (Tris 10 mM, EDTA 1.5 mM, pH 7.4). The tube was immediately frozen in liquid nitrogen and stored in liquid nitrogen until the sample was prepared for determination of oestrogen receptor content. The determination was carried out within a week after the removal of the biopsy.

(A) *Preparation of cytosols from fine-needle biopsies.* (1) The sample was alternately frozen in liquid nitrogen and thawed to 18°C four times. By this procedure most of the cells were disintegrated. (2) The homogenate was centrifuged at 100,000 ***g*** and 4°C (Beckman, Spinco Ultracentrifuge L50) for 1 hr and the supernatant (the cytosol) assayed for the amount of high affinity oestrogen receptors (see below, C).

(B) *Preparation of cytosols from surgically removed tumour biopsy specimens.* The tissue was minced with a pair of scissors, cooled in liquid nitrogen and homogenized in a Schwingmühle (Retch, West Germany). The homogenate was weighed and suspended in a three-fold volume of TE buffer. The homogenate was treated as described above (A, 2).

(C) *The oestrogen receptor assay.* The method used was originally described by Mester *et al.*

[5], Feherty *et al.* [6] and later modified by Daehnfeldt [7].

The assay is a dextran-coated charcoal assay which allows the calculation both of the total binding capacity of high affinity receptors as well as the dissociation constant.

Briefly, 50 μl cyrosol was incubated at 2–4°C with ^{3}H-17-beta-oestradiol (90 Ci/mmole. The Radiochemical Centre, Amersham, U.K.) and at least five different concentrations of 17-beta-oestradiol (5 μl solution in 99% ethanol + 25 μl TKE buffer) ranging from 10^{-10}M to 10^{-8}M for 2 hr. All these investigations were performed in duplicate. The TKE buffer consisted of: Tris 10 mM; KCl 50 mM; EDTA 1.0 mM; pH = 7.4.

The incubation was terminated by addition of 250 μl dextran-coated charcoal suspension (5.0 mg Dextran T-70, 500 mg charcoal, 200 ml TKE buffer). After absorption for 30 min at 2–4°C the charcoal was spun down (800 ***g***, 10 min, 4°C) and the radioactivity in an aliquot of the supernatant was determined by liquid scintillation (Packard Tricarb 3003-spectrophotometer, efficiency for tritium approx. 25%). Quench correction was carried out by the channel ratio method. To correct for unspecific binding, controls were used containing ^{3}H-17-beta-oestradiol, TKE buffer and 100-fold excess DES (diethylstilboestrol) (a modification compared to [8, 9]) and the radioactivity was subtracted from the experimental values.

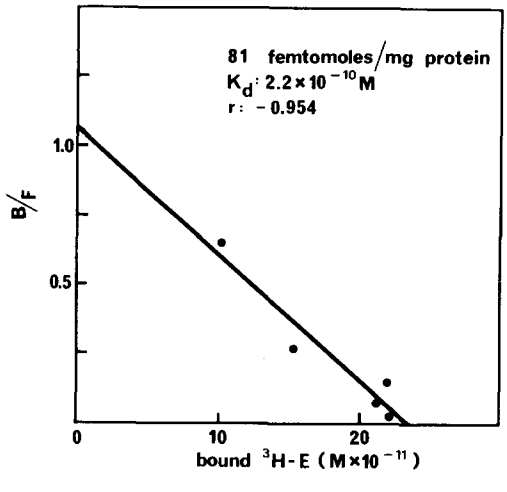

Fig. 1. Binding of oestradiol by carcinoma cytosol. The results are presented in a Scatchard plot with molar concentrations on the abscissa and ratios bound/free oestradiol on the ordinate. Regression line is calculated by the least square fit from the results in the steepest part of the curve. The interception of this line with the abscissa gives the binding capacity, whereas the slope of this line gives an approximate value of the association constant of the oestradiol receptor complex.

The binding capacities and K_d values were read from a Scatchard plot (Fig.1) [10, 11].

When the results were scattered along a sloping straight line the biopsies were defined as *receptor-positive* (Fig. 1). When the binding data were scattered along a straight line with no slope, when no straight line could be drawn with any confidence or when no significant binding of oestradiol was recorded the biopsies were defined as *receptor-negative.*

Protein measurements were carried out by the method described by Lowry *et al.* [12] and the binding capacities are presented as fmole oestrogen bound/mg cytosol protein.

Statistical evaluations were carried out using the Mann–Whitney U-test.

RESULTS

In order to evaluate the quality of information obtained from fine-needle aspirations and biopsy material from the same tumours the following parameters were compared: (1) biopsy histology vs cytology of aspirates; (2) presence/absence of oestrogen receptors, (3) K_d and binding capacities in receptor-positive tumours; (4) oestrogen content in relation to protein content.

Biopsy histology vs cytology of aspirates

From Tables 1, 2 and 3 it appears that one aspiration out of 10 from histologically verified benign breast tumours contained cytologically, apparently malignant cells (false positive) (patient No. 9). In two cases the aspiration of cells from histologically verified malignant tumours only revealed benign cells (false negative, patients No. 15 and D). In a third case multiple fine-needle biopsies were not comparable since 2 out of 5 samples were acellular (patient L_1).

Presence/absence of oestrogen receptors

From Tables 1 and 2 it appears that one out of 10 benign breast biopsies was receptor-positive (patient No. 2) whereas none of the corresponding fine-needle samples were receptor-positive. It also appears that 15 out of 24 malignant biopsies were receptor-positive, whereas only 5 out of 24 homologous fine-needle samples contained measurable oestrogen receptors, which is a significant difference ($P<0.05$). It patient No. 14 the fine-needle sample was receptor-positive, though no oestrogen receptors were detected in the corresponding biopsy.

K_d and binding capacities

The median K_d value in fine-needle biopsies

was (Tables 1 and 2) 1.35 (range 2.9–0.83 $\times 10^{-10}$M, $n=4$). The median K_d value in corresponding receptor-positive tumours was 13.5 (range 87–11 $\times 10^{-10}$M, $n=4$). This is a significant difference, $P<0.05$. The median binding capacities obtained in fine-needle aspirations (217.0, range 362–56, $n=4$) did not differ significantly from the results obtained from corresponding biopsies (252.5, range 448–98, $n=4$).

Furthermore, it appears from Tables 1 and 2 that the median binding capacities in biopsies in which the corresponding fine-needle aspirations also appeared to be positive were 252.5, range 448–98, $n=4$, whereas the median binding capacity in receptor-positive tumours without corresponding positivity in fine-needle aspirations was 30, range 229–17, $n=11$, which is a significant difference, $P<0.02$.

Oestrogen-receptor content in relation to protein content

Tables 1 and 2 show that protein content in cytosols from fine-needle aspirations was significantly smaller than in the corresponding biopsies. As seen from Fig. 2, a low protein

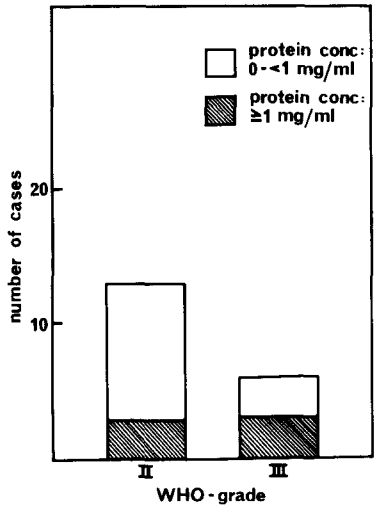

Fig. 2. Cytosol concentration (mg *protein*/ml *cytosol*) *in fine-needle samples from malignant aspirations in relation to the grade of malignancy of the tumours* (*WHO grade*).

content in fine-needle samples was not associated with a specific tumour type since there was no significant difference in the protein cytosol concentration in fine-needle samples between grades II and III ($P>0.05$). The protein concentration in the cytosols was not dependent on the relative fibrous content in these tumours (Fig. 3). No significant difference was found in tumours with dense fibrous

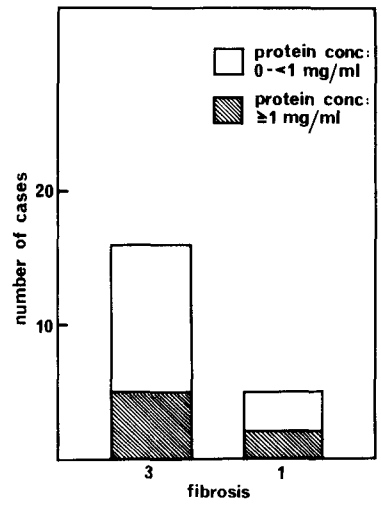

Fig. 3. Cytosol concentration (mg *protein*/ml *cytosol*) *in fine-needle samples from malignant aspirations in relation to relative fibrosis of target tissue.*

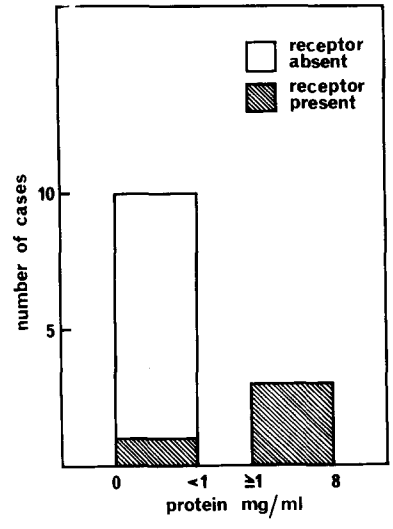

Fig. 4. Occurrence of oestrogen receptors in fine-needle samples from malignant aspirations as a function of protein in cytosol from receptor-positive biopsies.

tissue compared to tumours with loose fibrous tissue.

It is apparent (Fig. 4) that the number of receptor-positive fine-needle samples increased significantly ($P<0.05$) if the cytosol protein concentration was more than 1 mg protein/ml cytosol. In fine-needle samples from receptor-positive tumour biopsies with less protein, only 1 of 10 samples was receptor-positive (patient E, Table 2), whereas 3 of 3 samples with more than 1 mg protein/ml cytosol were receptor-positive in receptor-containing biopsies.

Altogether, 6 out of 14 malignant fine-needle samples with a protein content above 1 mg/ml cytosol were receptor-positive which does not differ significantly from the results

obtained from oestrogen determination of biopsies from malignant breast tissue (15 out of 24) (Fig.5).

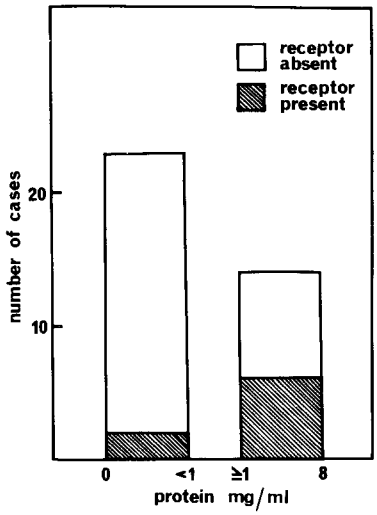

Fig. 5. The occurrence of oestrogen receptors in fine-needle aspirations from malignant samples as a function of protein concentration (mg *protein*/ml *cytosol*) *in the entire material.*

In one case (patient L) one fine-needle sample was receptor-positive (L_2) whereas another cytologically comparable sample (L_4) did not contain oestrogen receptors, although both samples contained more than 1 mg protein/ml cytosol (Table 3).

DISCUSSION

The present paper reports that oestrogen receptors can be demonstrated in fine-needle aspirations. To our knowledge this has not been described before. In cytosols containing 1 mg protein/ml or more no significant difference in the number of receptor-positive tumours was observed between fine-needle aspirations and tumour biopsies.

Others [13] have found that with an ordinary dextran charcoal (DCC) technique on tumour biopsies a protein concentration greater than 2 mg protein/ml cytosol is needed. They found that only 10% of biopsies were receptor-positive if the protein concentration in the cytosols was less than 2 mg/ml whereas 50–60% of tumour biopsies were receptor-positive if the protein concentration in the cytosol was greater than 2 mg/ml. In another paper [14] it is recommended that the protein concentration should not be less than 4 mg/ml. Our results are not directly comparable with these, because in the paper referred to above there is a great deal of contamination of serum proteins ranging from 10 to 30%, whereas the contamination in fine-needle aspirations is supposed to be negligible. Therefore, lower protein concentration is needed with the fine-needle aspiration technique.

From the results it seems that tumour biopsies with a high content of oestrogen receptors were also receptor-positive in fine-needle aspirations and the binding capacities were comparable. On the other hand, the K_d values were significantly lower in fine-needle aspirations compared to the biopsies. In another study we have found (H. S. Poulsen, to be published) that in uterine cytosols from rats there is a positive correlation between K_d values and protein concentration whereas the binding capacities are comparable. This observation fits very well with the results of this study.

In one case (patient L) one fine-needle aspiration was receptor-positive, whereas in another comparable aspiration no receptors were found. This lends support to the results published by others [15] that the receptor activity varies within the same tumour. This also suggests that it is not at all certain that a single fine-needle aspiration is representative of the tumour even if the protein content is more than 1 mg/ml cytosol—although it appears that there is a good agreement between results obtained by aspiration technique and biopsy technique.

However, it should be borne in mind that this agreement was obtained in fine-needle aspirations from surgically removed breast biopsies. This situation differs considerably from the *in vivo* situation, since it is much easier to make sure that the needle has been retracted in all areas of the tumour resulting in a presumbably more representative cell population than is obtained from direct *in vivo* aspirations.

The fine-needle method appears in this study to be inferior to receptor determinations based on tumour biopsy specimens, primarily because of lack of available material but also because some aspirations are not cytologically representative of the total tumour. But it is possible that other methods for measuring oestrogen receptors could be used, e.g., the hydroxylapatite assay which seems to be more sensitive than the DCC method, especially in tumour samples with cytosol protein concentrations below 1 mg/ml [16].

On the other hand, the results presented also suggest that in the ordinary biopsy technique the homogenization is quite insufficient since it was observed that in some fine-needle aspirations the protein concentration was only

one third of the protein concentration from the corresponding tumour biopsy.

In conclusion, the present results do indicate that this method is reliable provided the cytosol protein concentration is greater than 1 mg/ml and the aspirations are cytologically representative of the tumour.

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