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Original Paper

Large Core Biopsy for Diagnostic and Prognostic Evaluation of Invasive Breast Carcinomas

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Large core biopsy is a recently introduced method for pre-operative evaluation of breast lumps. The aim of this study was to evaluate the usefulness of this technique in providing pre-operative diagnostic and prognostic information that can lead to a correct line of treatment. We compared 41 cases of breast carcinomas diagnosed both by core biopsies and surgically removed samples. A high (93%) diagnostic agreement was obtained. Moreover, we found a significant correlation for mitotic count ($r = 0.76$), oestrogen receptor ($r = 0.78$), progesterone receptor ($r = 0.80$), p53 ($r = 0.86$) and c-erbB-2 ($r = 0.90$) analysis between core biopsy and definitive surgical pathology. An agreement for histological grading evaluation between the two techniques was obtained in 32 out of 40 cases ($k = 0.65$) whereas in the other cases, a lower grade was assigned by evaluating core biopsies. These findings suggest that percutaneous core breast biopsy is a valid tool for pre-operative management of breast lesions, but this should be confirmed in larger, prospective studies.

Key words: breast cancer, core biopsy, surgical pathology, prognosis, grading, steroid receptors, p53, c-erbB-2, immunohistochemistry

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INTRODUCTION

PERCUTANEOUS IMAGE-GUIDED large core biopsy (PLCB) is a recently introduced method for the diagnosis of breast lesions [1, 2]. It provides histological-quality tissue overcoming some of the drawbacks of fine-needle aspiration biopsy (FNAB), and its diagnostic accuracy seems to approach that of open surgical biopsy [2–5].

To determine the most appropriate surgical treatment for a patient with a breast lump, it is necessary to perform a correct histological diagnosis and to obtain as much information as possible on prognosis. To date, most of the prognostic studies have been performed on relatively large surgical specimens, whereas there are only few studies on characterisation of breast cancer on FNAB material [6, 7].

The aim of the current study was to evaluate if PLCB can be used as a routine procedure in pre-operative prognostic evaluation of patients with breast cancer. For this purpose, we compared histological diagnosis based on the evaluation of the large core biopsy specimens with the corresponding results at surgery. In addition, we performed a comparative study of immunohistochemically evaluated oestrogen and progesterone receptor status and other biological parameters from core biopsies and surgically resected samples.

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MATERIALS AND METHODS

Between June 1993 and June 1994, 65 consecutive large core biopsies were performed in mammographically evident breast lesions detected in women receiving a mammography as a regular control in postmenopausal age or because of a clinic suspicion of breast cancer. Of the patients, 41 (63%) had histologically confirmed carcinoma and were included in the study. The mean age was 59.3 years (range 33–89). The arbitrary cut-off point, greater than 50 years of age, was chosen for classification of menopausal status. Informed consent was obtained prior to biopsy. The needle core biopsies were used as a proper pre-operative diagnostic technique and, when a diagnosis of malignancy was performed, the surgical treatment was planned according to tumour-related (namely histopathological type, ratio of tumour size to breast size, location) and patient-related (namely attitude, age, contraindications to

radiotherapy) factors. All core biopsies were carried out with a biopsy gauge with 18-gauge, 15-cm long needles in conjunction with dedicated ultrasound guidance. For each case, 1–3 (mean 1.8) large core biopsies were taken. The patients' compliance to the procedure was good and no complications requiring treatment occurred. A corresponding surgically resected sample from the total or partial mastectomy were obtained.

All specimens were fixed in buffered neutral formalin for 2 h, washed in tap water and embedded in paraffin wax. A specimen from the resected sample was immediately frozen in isopenthane–liquid nitrogen. Haematoxylin and eosin sections of the core biopsies (Figure 1) and resected samples were evaluated for histological diagnosis and for mitotic count. Histological typing was determined according to the guidelines of the World Health Organisation (WHO). pT category and pN category were determined on resected samples according to the TNM classification [8].

In 40 of the 41 cases it was possible to perform microscopic grading (grades 1–3) according to the criteria suggested by Elston and Ellis [9]. On core biopsies, mitotic figures were counted in the ten most cellular high-power fields where the number of mitoses was supposed to be higher. In fact, because of the relatively small size of the biopsy sample, ten high power fields at the periphery of the tumour were not available in any of the biopsies.

In addition, we also expressed the mitotic index as the number of mitoses per 1000 tumour cells. Both in biopsy and in resected samples, the count was started from the maximally cellular area, avoiding regions showing necrosis or inflammation. Only bona fide mitotic figures were counted. Cells in prophase were excluded.

Immunohistochemical analysis for oestrogen receptor (ER) (Figure 2a) and progesterone receptor (PR) (Figure 2b) status was carried out in 33 cases. Evaluation of p53 (Figure 2c) and c-erB-2 (Figure 2d) oncoproteins by immunohistochemistry was respectively performed on 37 and 36 specimens. Using the paraffin blocks of the core biopsies, five micron sections were cut, rehydrated and the endogenous peroxidase was blocked. The slides were heated in a 750 W microwave oven set at maximum power for 2 min and at 100 W for 8 min, then were cooled and washed twice in phosphate buffered saline (PBS). Monoclonal undiluted rat anti-ER and anti-PR antibodies (Abbott, Chicago, Illinois, U.S.A.) were incubated overnight at 4°C followed by incubation with the label reagent streptavidin: biotinylated peroxidase (Stravigen supersensitive multilink; Biogenex, San Ramon, California, U.S.A.). Sections were immunostained for p53 and c-erbB-2 oncoprotein by avidin–biotin–peroxidase complex (Vectastain ABC Elite Kit, Vector, Burlingame, California, U.S.A.) using monoclonal antibody DO7 (Dako-p53, DO7) and rabbit anti-c-erbB-2 oncoprotein (Dako A/S, Glostrup, Denmark). The slides were incubated overnight in primary mouse DO7 (1/300 diluted in PBS) and in rabbit anti-c-erbB-2 (1/200 in PBS).

Each step was followed by washing slides in phosphate buffered saline (PBS), and the peroxidase reaction was developed in 3,3′-diaminobenzidine tetrahydrochloride (Sigma, St. Louis, Missouri, U.S.A.) and H_2O_2. The slides were counterstained in Mayer's haematoxylin, dehydrated and coverslipped with Permount. Appropriate negative controls were carried out. The percentage of ER, PR, p53 and c-erbB-2 positive cells was evaluated by counting all neoplastic cells and the number of stained cells present in the sections from core biopsies and those present in ten high power fields randomly selected on the sections from the resected samples.

Frozen sections from the surgically resected tumours were cut for detection of ER and PR using the Abbot ER-ICA and Abbot-PR-ICA Monoclonal Kit.

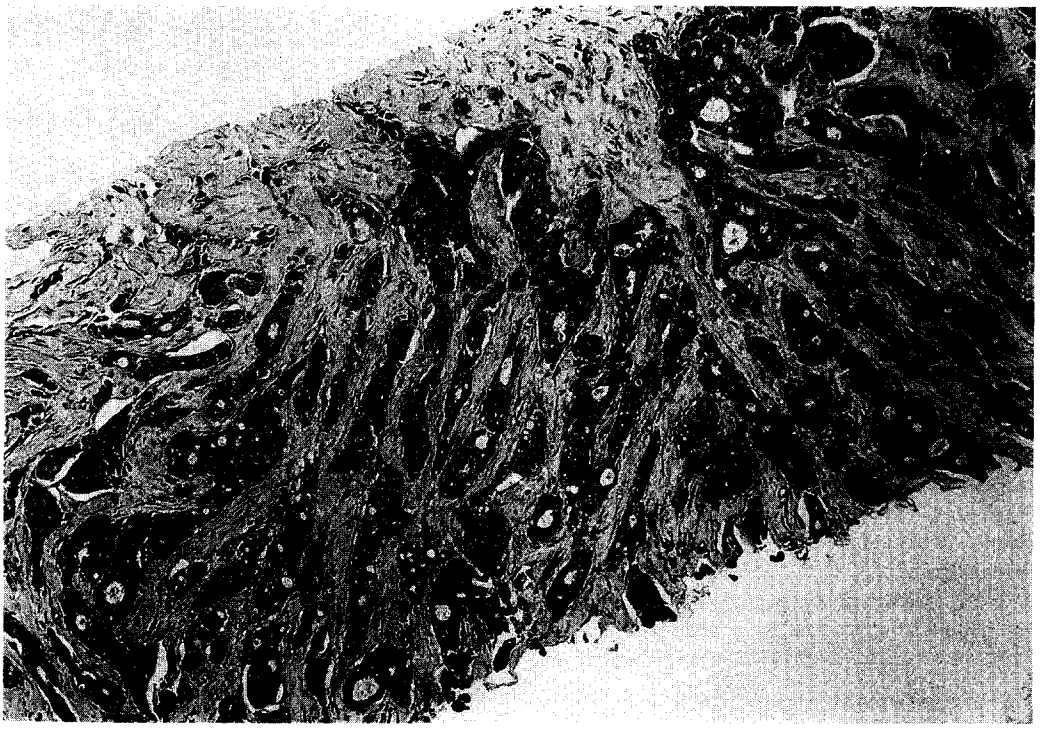

Figure 1. Invasive breast carcinoma. HE-stained section from a large core breast biopsy (×100).

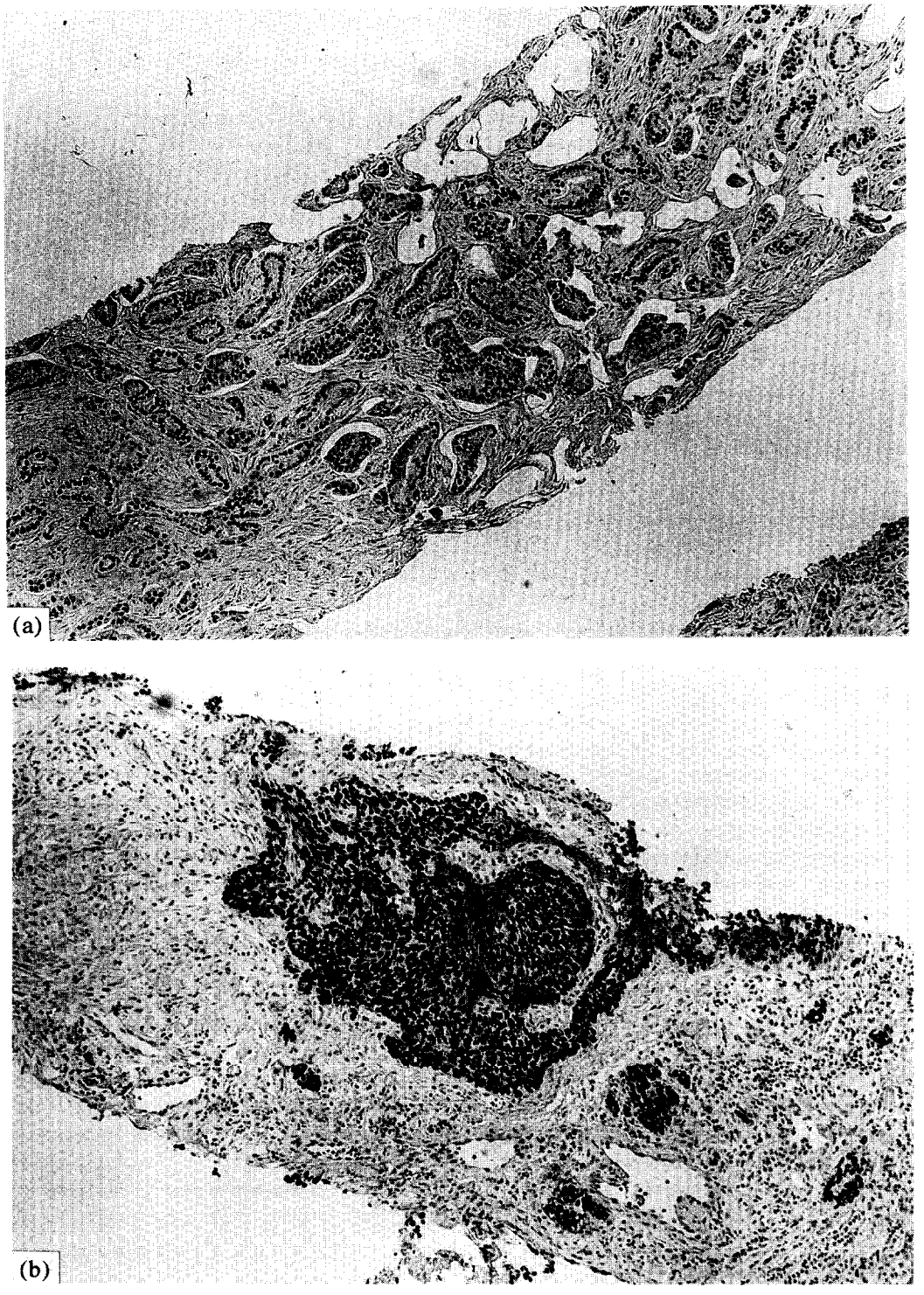

Figure 2. Positive immunostaining of the tumour cells for (a) oestrogen receptor; (b) progesterone receptor; (c) p53 oncoprotein; and (d) c-erB-2 oncoprotein is evident on large core biopsy (× 50).

Statistical analysis

The correlation between prognostic indicators in the core biopsy and definitive surgical pathology was tested with the Spearman's rank correlation coefficient. The level of agreement between the two diagnostic procedures for the determination of tumour grade and steroid receptors status was assessed by the *k* statistic [10]. Test sensitivity was calculated as the percentage of ER and PR positive formalin-fixed biopsies that were positive in frozen sections from the surgically removed sample (percentage true positive). Test specificity was calculated as percentage of ER and PR negative formalin-fixed biopsies that were negative in frozen sections from the surgically removed sample (percentage true negative). The association between menopausal status and steroid receptor immunoreactivity on core biopsy was tested by using a Fisher's exact test, as was the relationship between ER status and PR status.

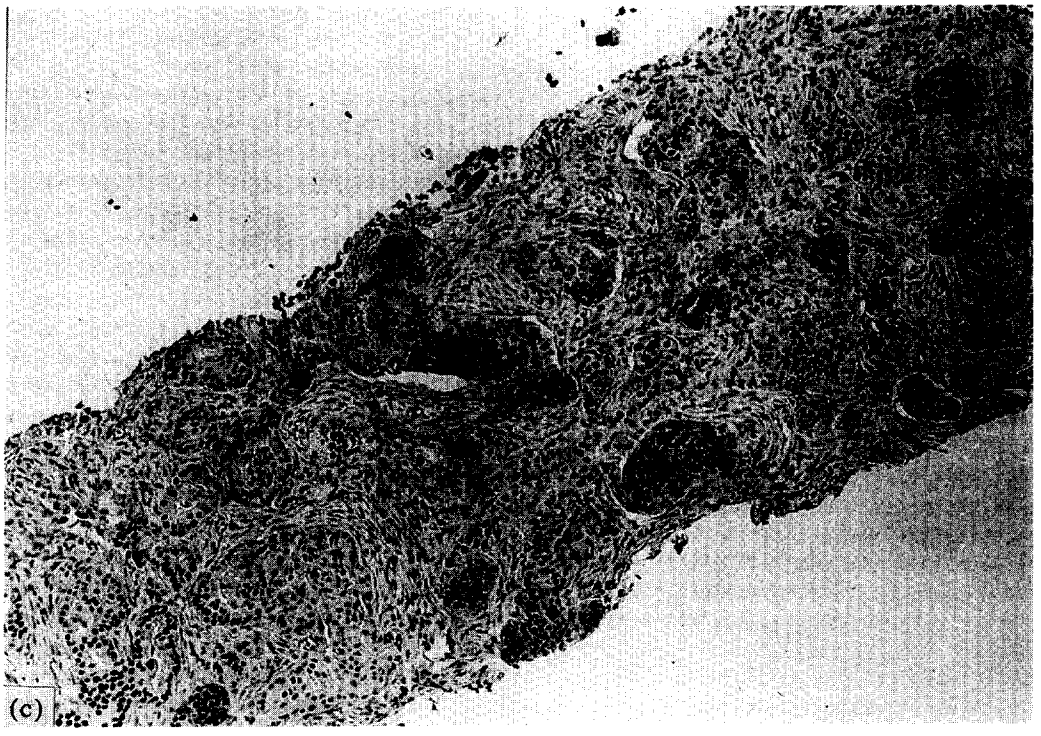

Figure 2. Continued.

RESULTS

In 38 out of 41 cases (93%), a perfect agreement between the needle core biopsy and definitive surgical pathology was found: all cases were diagnosed as ductal invasive breast carcinoma of no special type on the needle core samples and the diagnosis was confirmed at surgery. In the other 3 cases, the diagnosis by examining the large core biopsy was of invasive breast carcinoma, but it was not possible to specify with certainty the histological type (ductal versus lobular). The subsequent evaluation of the three lesions on the resected sample allowed a definitive diagnosis of lobular invasive carcinomas. No invasive ductal carcinomas of special type were

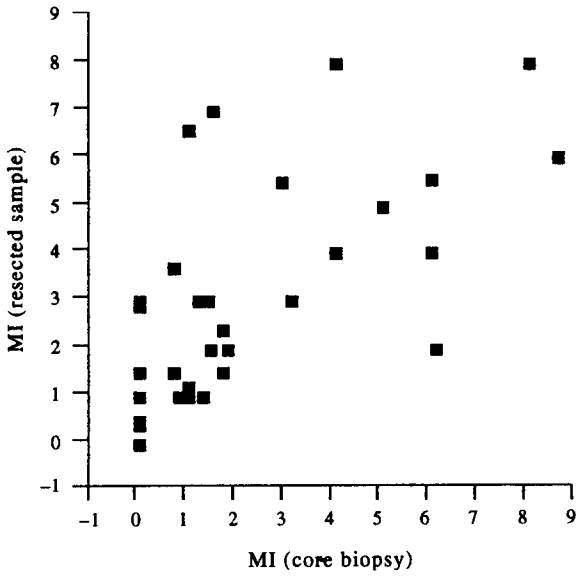

Figure 3. Correlation between mitotic index (MI) in the core biopsy and surgically resected tumours (Spearman's correlation coefficient=0.76, $P < 0.001$).

observed in our series. Sixteen (39%) tumours were in category pT1, 20 (49%) in category pT2, 1 (2%) in category pT3, and 4 (10%) in category pT4. The smallest tumour was 0.5 cm in diameter. Fifteen (36.5%) were categorised as pN0, 24 (58.5%) as pN1 and 2 (5%) as pN2.

The correlation between mitotic indices of large core biopsies and surgically removed samples is presented in Figure 3. There was a significant correlation ($r = 0.76$, $P < 0.001$) between percutaneous biopsy and traditional surgical excision. However, the former tends to underestimate mitotic activity in most cases, with a few cases overestimated. The mean mitotic index of core tissue was 1.69 (95% confidence interval (CI) 0.98–2.40), whereas on resected samples it was 2.43 (95% CI 1.73–3.13). In 10 of the 41 cases, the mitotic index was the same in both specimens, but in the majority of cases (24/41) mitotic activity was lower in needle core biopsies.

A comparison of the histological grading between the core biopsy and resected samples is shown in Table 1. A perfect agreement was obtained in 32 out of 40 cases (agreement=80%, $k = 0.65$, $z = 5.44$) whereas in the other cases (20%) a lower grade was assigned by evaluating biopsy samples.

Figure 4 shows the correlation between steroid receptor status of the two diagnostic methods. A significant correlation was found both for ER ($r = 0.78$, $P < 0.001$) or for PR ($r = 0.80$, $P < 0.001$) analysis. By interpreting results as positive when unequivocal staining was detected in more than 20% of tumour nuclei, there was a concordance of negative or positive results in 91% of cases ($k = 0.79$, $z = 4.5$) for ER and in 81.2% of cases ($k = 0.61$, $z = 3.5$) for PR evaluation. By considering steroid receptor analysis on frozen tissue as a reference, we calculated a sensitivity of 95% and 69% and a specificity of 82% and 90%, respectively for ER and PR status evaluated on core biopsy samples (Table 2). In addition, we found that steroid receptor immunostaining was associated with menopausal status ($P < 0.05$ and $P < 0.02$, respectively for ER and PR analysis on core biopsy) (Table 3). A significant association was also observed between ER and PR immunoreactivity in the core biopsy ($P < 0.02$) (Table 4). Finally, good correlation of the c-erbB-2 ($r = 0.90$, $P < 0.001$) and p53 ($r = 0.86$, $P < 0.001$) protein expression (Figure 5) was seen between the core biopsy and surgical specimens.

Table 1. Histological grading of large core and surgical biopsies (agreement = 80%, k = 0.65, z = 5.44)

Resected sample	Core biopsy Grade 1	Grade 2	Grade 3	Total
Grade 1	**20**	0	0	20
Grade 2	1	**12**	0	13
Grade 3	0	7	**0**	7
Total	21	19	0	**40**

DISCUSSION

In recent years, the role of percutaneous large core breast biopsy in establishing the diagnosis of breast disease has increased. Recent studies have reported the accuracy of this technique in pre-operative diagnostic evaluation of breast lesions [2–5]. Large core biopsy provides a histological instead of a cytological sample. This in turn provides definitive diagnosis in most cases by allowing a distinction between invasive and non-invasive tumours. No false-positive results have been reported by using this technique. As a consequence, by obtaining definitive diagnosis with a core sample, it should be possible, in many cases, to avoid the excisional surgical biopsy with frozen section. To date, available data show almost perfect agreement between needle-core and surgical diagnoses [11–13]. Although controversy exists about the appropriate clinical role for the image-guided large core biopsy, the majority of authors maintain that this technique is particularly indicated for evaluating mammographically indeterminate impalpable lesions [5–11]. Since a possible drawback of the core biopsy may be missing the critical foci of malignant lesions that are associated with diffuse areas of epithelial proliferative lesions, an excisional biopsy should be performed when a mammographically indeterminate lesion is diagnosed as ductal atypia by means of core biopsy. The role of the core biopsy for the mammographically suspected lesions is directed towards the attainment of a definitive diagnosis, together with the main data on the biology of the tumour. Therefore, large core breast biopsy provides a larger amount of material in which the main prognostic factors can be evaluated.

An early choice of the most appropriate treatment and the possibility of stratifying patients that can benefit from neoadjuvant regimes of chemotherapy are the main purposes of obtaining pre-operative prognostic data.

In addition, the ability to consult the patient on therapy options is another interesting aspect. From this point of view, sceptical physicians could object that it is very difficult to plan a correct line of treatment without evaluating some of the main parameters provided by examination of the surgically removed sample (i.e. tumour size, multicentricity, proportion of invasive and *in situ* components).

Currently, tumour size, lymph node status and histological grading are considered the most important prognostic indicators in breast cancer. However, within the past few years, a large number of biological indicators have assumed a significant role in predicting prognosis and in providing the basis for management of patients with breast cancer [14]. Therefore,

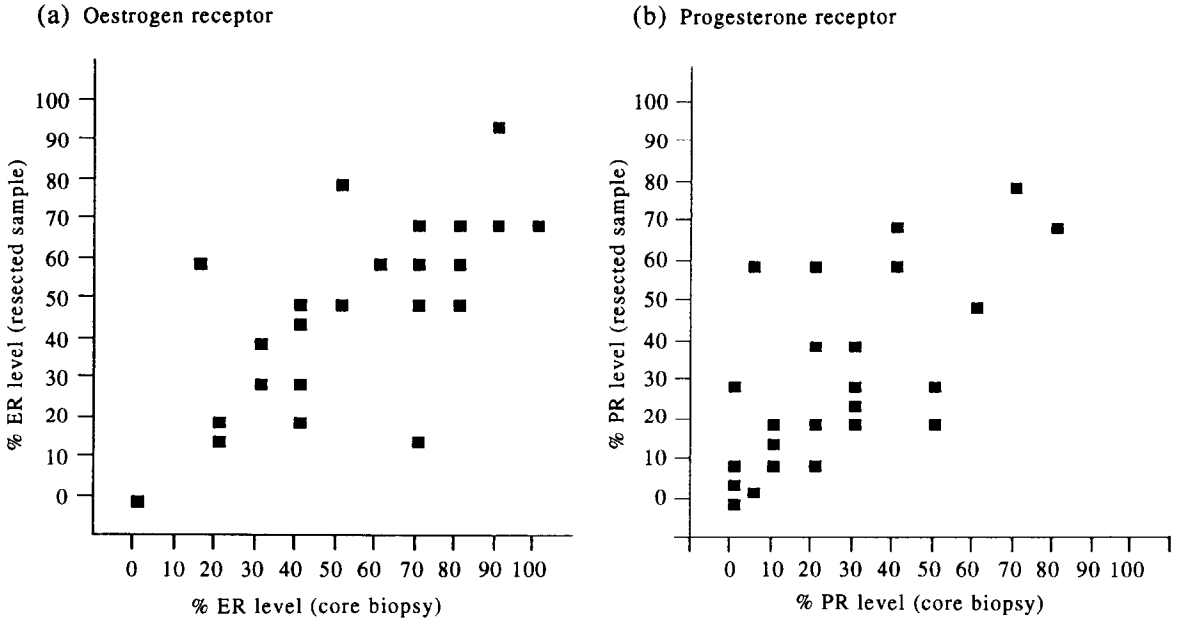

Figure 4. Correlation between (a) oestrogen receptor level (%) (Spearman's correlation coefficient=0.78, $P < 0.001$), and (b) progesterone receptor level (%) (Spearman's correlation coefficient=0.80, $P < 0.001$) in the core biopsy and surgically resected tumours.

Table 2. Relationship between ER (a) and PR (b) receptor status on core biopsy and surgically removed samples

(a) Oestrogen receptor-resected sample	Oestrogen receptor-Core biopsy Negative	Positive	Total
Negative	9(tn)	2(fp)	11
Positive	1(fn)	21(tp)	22
Total	10	23	33

(b) Progesterone receptor-resected sample	Progesterone receptor-Core biopsy Negative	Positive	Total
Negative	18(tn)	2(fp)	20
Positive	4(fn)	9(tp)	13
Total	22	11	33

tp, true positive; tn, true negative; fp, false positive; fn, false negative.

Table 3. Association between steroid receptor immunostaining in the core biopsy and menopausal status (Fisher's exact, P <0.05 for ER and P <0.02 for PR)

Menopausal status	ER+	ER−	PR+	PR−
≤50	4	6	0	10
>50	19	4	11	12
Total	23	10	11	22

Table 4. Association between ER and PR status in the core biopsy (Fisher's exact, P <0.02)

	ER−	ER+	Total
PR−	10	12	22
PR+	0	11	11
Total	10	23	33

we carried out the present study to test the feasibility of using large core breast biopsies as a pre-operative prognostic tool. By analysing some of the main biological indicators on core biopsy and definitive surgical pathology, we found a significant correlation for the evaluation of mitotic activity. However, as the core biopsy contains less tissue than the surgically removed sample, mitotic count was often lower in the former. This is in turn associated with an underestimation of the tumour's histological grade in some core biopsies versus surgical samples. Nevertheless, in most cases, there was concordance in histological grade performed on core biopsy and resected tumours.

ER and PR status are currently considered useful means of predicting the response to endocrine therapy and prognosis of carcinoma of the breast [15–18]. The statistical results of this study revealed the reliability of ER and PR evaluation by immunohistochemical analysis on core biopsy material. In addition, our data also indicated a reasonable correlation for steroid receptor immunohistochemical analysis in frozen and paraffin sections. However, the low sensitivity for PR evaluation in core biopsy sections suggests caution in interpretation of negative results. This high rate (31%) of false-negative results has also been observed in other studies [19–20] and several possibile explanations were provided. A loss of immunoreactivity may result from different variables, such as delays in fixation and time and temperature of fixation. In addition,

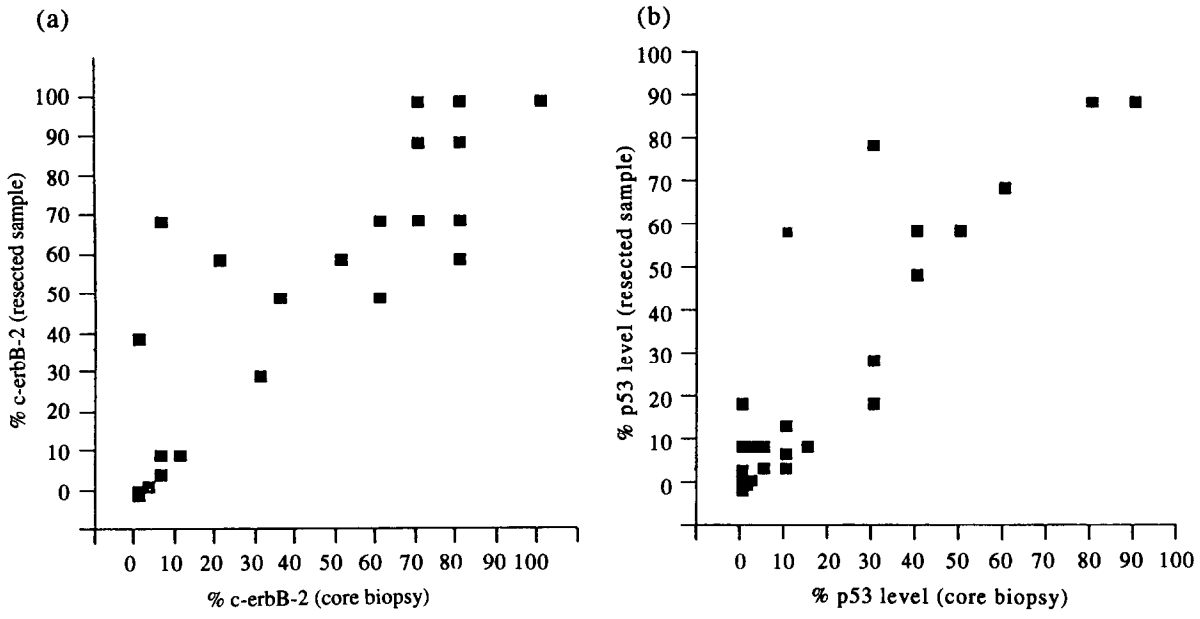

Figure 5. Correlation between (a) c-erbB-2 protein expression (Spearman's correlation coefficient=0.90, P <0.001) and (b) p53 protein expression (Spearman's correlation coefficient=0.86, P <0.001) in the core biopsy and surgically resected tumours.

heterogeneity of staining has been reported as another possible cause of false-negative results. This seems to be a plausible explanation in our series, because the core biopsy could come from a negative zone of an otherwise positive tumour.

Overexpression of the *TP53* and *HER-2/neu* genes are the most common genetic abnormalities associated with breast cancer. Different studies have reported a good correlation between immunohistochemically detected p53 protein accumulation and the presence of gene mutation in breast cancer [21–23]. Furthermore, there are several reports concerning the prognostic role of p53 [23–27]. Evaluation of the prognosis of patients with p53 positive breast cancer was not the purpose of this study. However, we looked for the ability of the core biopsy to provide enough tumour tissue for the evaluation of this oncoprotein. A significant correlation between the core biopsies and definitive resected tumours was obtained. The same results were obtained by determining c-erbB-2 protein, a proto-oncogene product that seems to be correlated with a poorer prognosis, especially shorter recurrence-free interval [27–30].

In conclusion, the current study indicates the potential value of image-guided large core breast biopsy as a diagnostic tool, but in particular provides new information on the use of the technique in pre-operative evaluation of invasive carcinoma. However, the sample size of the study is small and the results should be confirmed in a larger, prospective study.

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