ORIGINAL ARTICLE

The reliability of assessment of oestrogen receptor expression on needle core biopsy specimens of invasive carcinomas of the breast

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Aim: To assess the reliability of assessment of oestrogen receptor expression on needle core biopsy specimens of invasive carcinomas of the breast. Previous studies have mostly been small, with a range of agreement from 62% to 100%.

Methods: Retrospective audit of 338 tumours surgically excised within 60 days of core biopsy, that had had oestrogen receptor assessed on both the core biopsy and tumour specimens. Surgical specimens were incised when fresh to ensure good fixation. External controls including a weakly positive tumour were included in each immunohistochemistry run.

Results: Oestrogen receptor expression was bimodal, with H score in both specimens of either 0 or >50 in 96% of tumours. Using H score cut-off of 10 for positivity, there was an agreement between core and excision in 334 of 338 tumours (98.8%). All discrepancies were between weakly positive and negative tumours. Intratumoral heterogeneity could explain the one tumour that was negative on core and positive on excision. H score tended to be slightly higher on core than excision (means 146 and 136). Better fixation on the core is the most likely explanation for this and for the three tumours that were positive on core and negative on excision. Repeat staining on tumours with discrepant results gave similar results in all except one case. An internal control was present in 97% of excisions and 55% of cores of oestrogen receptor-negative tumours; the internal control stained positively in all except two sections.

Conclusion: Oestrogen receptor can be assessed reliably on needle core biopsies of invasive carcinomas of the breast.

Oestrogen receptor status is a powerful predictive factor for response to adjuvant endocrine therapy. [1] In this situation, assessment of oestrogen receptor is usually made using immunohistochemistry on sections from the surgical specimen. Since the introduction of improved automated core devices, core needle biopsy has become the method of choice for diagnosing lesions of the breast in many units, including Nottingham. In addition, there is also an increasing interest in primary hormone therapy, particularly in women with invasive carcinoma of the breast who are not fit for surgery or who have locally advanced or disseminated disease. In these circumstances, the information yielded preoperatively regarding the oestrogen receptor status of the tumour affects patient management directly.[2–5] Previous studies have shown that needle core biopsy can be used to assess receptor status. However, most of these studies included only a small number of patients and did not investigate the reasons for discrepancies. To assess the reliability of oestrogen receptor status in core biopsy, we compared oestrogen receptor, assessed using immunohistochemistry on core biopsy and subsequent excision biopsy in 379 invasive carcinomas.

METHOD

A retrospective audit of consecutive patients who had oestrogen receptor status assessed on preoperative core biopsy and on subsequent excision specimen for invasive breast adenocarcinoma at Nottingham City Hospital between 1999 and 2004 was performed. This project was discussed with the chair of the Nottingham City Hospital Research Ethics Committee, who considered that it was an audit and therefore did not require formal ethical approval. Core biopsies were fixed in formalin for at least 8 h and processed overnight. Surgical resection specimens were received fresh, and the tumour incised and fixed in formalin for 48 h.

Immunohistochemistry for oestrogen receptor was performed on formalin-fixed paraffin-wax-embedded sections using a streptavidin–biotin complex method with diaminobenzidine as the chromagen, with methyl green counterstain. Before application of the primary antibody, the sections underwent antigen retrieval in 0.01 M citrate buffer in an 800W microwave[6] for a total of 20 min (10 on full power and 10 on simmer). The primary antibody used was 1D5 (Dako), diluted 1/100. An external control section with three tumours (strongly positive, weakly positive and negative for oestrogen receptor) was included with every run. The run was repeated if

Table 1 Comparison between H score on core biopsy and excision specimen of patients with <60 days between core biopsy and surgery

	Core biopsy					
Excision	**0**	**1–9**	**10–49**	**50–99**	**100–300**	**Total**
0	88	0	2	0	0	90
1–9	2	0	1	0	0	3
10–49	1	0	1	3	1	6
50–99	0	0	1	3	10	14
100–300	0	0	2	6	217	225
Total	91	0	7	12	228	338

Table 2 Details of the positive/negative discrepancies using the cutoff of H score of 10

H score		Internal control				
Core biopsy	Excision	Core biopsy	Excision	Time between core biopsy and surgery (days)	Systemic treatment before surgery	Tumour type and grade
0	30	Positive	Positive	40	None	NST, grade 3
0	55	Positive	Positive	124	None	NST, grade 2
10	0	Positive	Positive	31	None	NST, grade 3
20	0	Positive	Positive	31	None	NST, grade 3
40	2	Positive	Positive	43	None	NST, grade 3
10	0	None present	Positive	97	Chemotherapy	NST, grade 3
80	5	Positive	Positive	134	Chemotherapy	NST, grade 3

NST, no special type.

staining of the external control was suboptimal. It was also standard practice to repeat the staining in oestrogen receptor-negative tumours if the internal control was negative. Comments about the staining of internal controls in oestrogen receptor-negative tumours were retrieved from the original report.

Staining of tumour cell nuclei was assessed semiquantitatively according to McCarty's H-scoring system.[7] The percentage of weakly stained cells was multiplied by 1, the percentage of moderately stained cells by 2 and the percentage of strongly stained cells by 3; the total of these three was the final H score. In negative tumours, the presence and positivity of any internal control was also recorded. A tumour with an H score of $\geqslant$10 was considered oestrogen receptor positive. The oestrogen receptor scoring as recorded in the original report was used. Scoring is routinely performed by one consultant and sometimes by a trainee pathologist as well. For this study, immunohistochemical staining was repeated for patients with a discrepancy between core and excision biopsy oestrogen receptor results using the cut-off of H score of 10.

The series was divided into two groups for comparison of the H score on core biopsy and surgical specimens. The group with <60 days between core biopsy and surgery was designed to exclude patients who had received primary systemic treatment. Most of the patients with >60 days between core biopsy and surgery had primary systemic treatment.

RESULTS

In all, 379 tumours from 373 patients were studied (six patients had two tumours). The patient sample was biased towards those with locally advanced disease or >70 years in whom primary endocrine treatment was being considered. The median age of the patients was 71 years (range 28–90).

Table 3 Comparison between percentage staining on core biopsy and excision specimen of patients with <60 days between core biopsy and surgery

	Core biopsy							
Excision	0	<1	1–5	6–10	11–33	34–66	67–100	Total
0	88	0	0	1	1	0	0	90
<1	0	0	0	0	0	0	0	0
1–5	2	0	0	0	1	0	0	3
6–10	2	0	0	0	1	1	0	2
11–33	1	0	0	0	1	1	1	4
34–66	0	0	0	0	1	1	4	6
67–100	0	0	0	2	0	8	223	233
Total	91	0	0	3	5	11	228	338

The percentage categories correspond to those used in the Allred system with an additional cut-off at 5%; <1 = staining present in <1%.

Altogether, 338 tumours were excised within 60 days of core biopsy. In this group, the oestrogen receptor level in the core biopsy tended to be higher than in the excision specimen. Mean H score on core was 146 and mean H score on excision was 136 (Wilcoxon signed rank test, $p<0.001$). Table 1 summarises the results of oestrogen receptor H scores on core and excision specimens. Most tumours were either clearly negative or clearly positive on both core and excision specimens. In all, 88 tumours had an H score of 0 on both specimens and 236 had an H score of $\geqslant$50 or above on both specimens and only 14 tumours had intermediate results.

Using the cut-off of an H score of 10, the core and excision agreed in 334 of 338 tumours (98.8%; κ statistic = 0.97). Table 2 shows the details of the patients with a discrepancy. There was one false-negative core biopsy result (1%), which had patchy staining in the excision specimen. Three patients had a weakly positive core and negative excision specimen.

Table 3 shows a comparison of the percentage of positive tumours cells in the core biopsy and surgical specimen of patients with <60 days between core biopsy and surgery. Using the percentage cut-offs proposed by the Allred system,[8] there was complete agreement between the core and excision specimen in 313 (93%) of 338 tumours. If cases with a difference of one percentage category between the core and excision specimen are included, the agreement was 97%.

In all, 41 tumours were excised >60 days after the core biopsy. In this group, the oestrogen receptor on core biopsy was higher than in the excision specimen: mean H score on core 121, and mean H score on excision 97 (Wilcoxon signed rank test $p = 0.03$). Table 4 summarises the results of oestrogen receptor staining on core and excision specimens.

Using the cut-off of an H score of 10, the core and excision agreed in 38 (93%) of 41 tumours. Table 2 shows the details of the patients with a discrepancy. There was one false-negative core biopsy result, which had patchy staining in the excision specimen, in which the heterogeneity of oestrogen receptor expression corresponded to morphological heterogeneity. Two patients had a positive core and negative excision specimen.

For the seven patients with a discrepancy, immunohistochemistry for oestrogen receptor was repeated in the 13 specimens for which the blocks could be found. An internal control was present in 13 of the 14 specimens and the only tumour without an internal control was scored as positive. In all cases except one, the results were similar on the original and repeat staining. The exception had an H score of 20 on the original staining of the core biopsy (H score of 12 on review) and the two repeat stainings had H scores of 0 and 1. The surgical specimen of this tumour had an H score of 0 on both stainings. Table 5 shows the details of internal controls in oestrogen receptor-negative tumours.

Table 4 Comparison between H score on core biopsy and excision specimen of patients with >60 days between core biopsy and surgery

	Core biopsy					
Excision	**0**	**1–9**	**10–49**	**50–99**	**100–300**	**Total**
0	14	0	1	0	0	15
1–9	0	0	0	1	0	1
10–49	0	0	1	0	0	1
50–99	1	0	1	0	3	5
100–300	0	0	0	0	19	19
Total	15	0	3	1	22	41

Table 5 Details of internal controls in tumours that were oestrogen receptor negative (H score <10)

Internal control	Core	Excision
Positive	42 (55%)	68 (97%)
Negative*	2 (3%)	0
None present	33 (43%)	2 (3%)
Total with comment in report	77	70
No comment in report	28	39
Total	106	109

*Despite repeated staining.

DISCUSSION

Most tumours in this study were either clearly negative or clearly positive for oestrogen receptor: in the group with surgery within 60 days of core biopsy, 96% of tumours had either an H score of 0 on both specimens or had an H score of ⩾50 on both specimens. This bimodal distribution is in agreement with recent studies[9 10] and contrasts with the distribution observed by Harvey *et al*.[8] In this study, we used standardised optimal fixation methods and modern, highly sensitive immunohistochemistry methods. We believe that this reflects good current practice. Harvey *et al*[8] used frozen samples referred from other centres that were pulverised and later fixed, with less control over specimen quality. Our clear separation of most tumours into two groups means that oestrogen receptor classification on core biopsy should be reliable in most tumours. The major area of difficulty of reproducibility of oestrogen receptor staining is the small middle group with low expression, in which there is the risk of false-negative results.[11] Inadequate assay sensitivity has been shown to be the main cause of poor results in oestrogen receptor immunohistochemistry.[12]

Most previous studies have not addressed the reasons for discrepancies between oestrogen receptor in core and the surgical specimens. The explanation for the two tumours in this study that were oestrogen receptor negative on the core and oestrogen receptor positive on the excision specimen is probably intra-tumoral heterogeneity of oestrogen receptor expression, which correlated with morphologically distinct areas in one. This is reinforced by a recent case we have seen with two distinct areas. One area was cohesive with tubule formation and an H score of 10; this part was sampled in the core biopsy. A second area, only apparent in the surgical specimen, was invasive lobular carcinoma with an H score of 250. Such marked intra-tumoral heterogeneity for oestrogen receptor apparent at low power is rare; it was described in only 5 (0.5%) of 980 tumours by Douglas-Jones *et al*.[13] Sometimes stronger staining is seen at the edge of the tumour in surgical specimens; this is probably due to poor fixation centrally.[13] This is a problem we see rarely as surgical specimens are incised when fresh. An ''edge artefact'' is well recognised with some antibodies, such as antibodies to c-erbB-2/Her-2, which is particularly seen in core biopsies. We have not seen this artefact in core biopsies stained for oestrogen receptor.

In the group with tumours excised within 60 days of surgery, the most likely explanation for the three tumours that were weakly oestrogen receptor positive on the core and oestrogen receptor negative on the excision specimen is suboptimal fixation in the excision specimen. We routinely incise tumours on receipt in the laboratory, but occasionally tumours are not well incised. Theoretically, the positive staining in the core biopsies could be false positives, perhaps due to over-retrieval, but we consider that this is most unlikely. Adequate fixation is essential for reliable oestrogen receptor immunohistochemistry, with a minimum of 6–8 h of fixation required for consistent results.[14] The slightly higher H score on excision than core specimens in this and another study is consistent with superior fixation in the core specimen.[13]

It appears that the discrepancy between the core and the subsequent excision biopsy can only rarely be attributed to the immunohistochemical technique as identical results were obtained on repeat staining in all except one specimen. The weakly positive tumour external control section is particularly important as it highlights problems far better than the strongly positive control. An internal control was almost always present in the surgical sections and was always positive in this study;

Table 6 Previous studies of oestrogen receptor on core biopsy

Study	Tumours (n)	Core negative, excision positive	Core positive, excision negative	Overall discrepancy rate (%)	Cut-off value for oestrogen receptor-positive
Railo *et al*, 1996[16]	70	6	2	11	10%
Di Loretto *et al*, 1996[17]	33	1	2	9	20%
Zidan 1997[18]	26	0	2	8	H score 50
Gotzinger *et al*, 1998[19]	103	1	2	3	No details
Jacobs *et al*, 1998[20]	54	0	0	0	10%
Mayer *et al*, 1999[21]	35	No details	No details	3	>0%
Connor *et al*, 2002[22]	44	1	0	2	10%
Taucher *et al*, 2003[23]	180	7	10	9	10%
Taucher *et al*, 2003[23]*	191	14	13	14	10%
Harris *et al*, 2004[24]	95	No details	No details	5	No details
Badoual *et al*, 2005[25]	103	3	7	10	10%
Cavaliere *et al*, 2005[26]	68	No details	No details	38	10%
Mann *et al*, 2005[15]	100	1	13	14	10%
Present study	338	1	3	1	H score 10

n, Number of tumours.
*Post chemotherapy.

Take-home messages

- Oestrogen receptor expression in invasive carcinoma of the breast has a bimodal distribution with most tumours either completely negative or convincingly positive.
- Oestrogen receptor can be assessed reliably on needle core biopsies of invasive carcinomas of the breast.

an internal control was present in only half of core biopsies and was almost always positive. Repeat staining if any control is negative is essential to reduce the chance of false-negative results. One study by Mann *et al*[15] with a high rate of 13% of tumours that were positive on core and negative in the surgical specimen had several tumours with a negative internal control in the surgical specimen, suggesting that inadequate fixation of the surgical specimen was the explanation of the high discrepancy rate. Consistent with this hypothesis, Mann *et al* had several tumours that were strongly positive on core biopsy with no staining in the surgical specimen. By contrast, all the discrepancies in the present study were between negative and weakly positive results.

Previous studies have shown a range of rates of discrepancy between core and surgical specimens of 0–14%, apart from one study with a rate of 38% (table 6). The present study, with a rate of discrepancy of 1%, is at the lower end of this range. We believe that with attention to adequate fixation and repeat staining if internal or external controls are negative, it is possible to obtain a satisfactory rate of concordance. We now routinely assess oestrogen receptor in the core biopsy for many invasive carcinomas. We repeat the staining in the surgical specimen if the core biopsy shows weak staining (H score 1–50) or if the tumour shows morphological heterogeneity in the surgical resection and was oestrogen receptor negative on the core biopsy.

In conclusion, this study shows that the oestrogen receptor status of carcinoma of the breast can be assessed reliably on core biopsy.

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