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ORIGINAL ARTICLE

Oestrogen and progesterone receptor status of individual foci in multifocal invasive ductal breast cancer

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Abstract

Widespread acceptance of breast conserving surgery for early breast cancer has led to renewed interest in multifocality, which is seen in 13–63% of breast cancers. According to current guidelines, oestrogen/progesterone receptor status is assessed on the sample obtained at initial core biopsy or the main tumour focus in multifocal breast cancer (more than one distinct tumour focus in a quadrant). We assessed receptor status of individual foci in multifocal breast cancer. Mastectomy specimens for 18 cases of multifocal breast cancer were identified. Immunohistochemical staining for oestrogen and progesterone receptors was performed on all tumour foci. On histological examination 11 patients demonstrated two independent tumour foci, three demonstrated three foci and four demonstrated four foci. Minor differences in oestrogen receptor score were seen between foci (attributed to the subjective nature of the scoring system), which did not affect the overall positive/negative classification. Sixteen patients (88%) were oestrogen receptor-positive. Progesterone receptor staining showed more variability between foci in two patients but, since the tumours were oestrogen receptor-positive this would not have affected clinical decision-making. No major differences in oestrogen receptor status between multiple tumour foci in the same quadrant were found in this pilot study.

It has been known for over 100 years that some breast cancers are hormone sensitive and that reduction of growth of these tumours can be achieved by removal of circulating oestrogens. Oestrogen receptors (ER) are required for oestrogen dependent breast tumours and 50–80% of breast cancers are ER-positive. Development of monoclonal antibodies for ER led to the introduction of an immunohistochemical method for receptor status determination in 1984 [1]. Hormone receptor status is used to identify patients suitable for hormone therapy and has prognostic implications [2]. According to current guidelines, ER and PR status is assessed using the tumour sample obtained at initial core biopsy. In cases of multifocal breast cancer (MFBC), defined by the appearance of more than one distinct tumour focus in a quadrant [3], only the main focus is examined. A diagnosis of MFBC has implications for prognosis [3,4], however the variability of ER and PR status between individual foci in these cases has not been widely studied. Poulsen et al. [5] presented a case report in 1981 of a patient with two foci of invasive ductal breast cancer in the same breast. One tumour was ER-positive while the other was ER-negative. Panahy et al. [6] studied ER and PR distribution in normal and cancerous breast tissue. Multifocal tumours with a varying phenotype for ER and PR status were identified and, in addition, variability was seen in different regions of large tumours. We aimed to examine the variability of ER and PR receptor status between individual foci in cases demonstrating MFBC.

Materials and methods

Patients

Local Research Ethics Committee approval was granted for the study. Eighteen women with a diagnosis of MFBC who underwent mastectomy in the Hull and East Yorkshire NHS Trust were identified.

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ER and PR immunohistochemistry

Immuonhistochemical staining of ER and PR was performed on each tumour focus using an avidin-biotin complex method essentially as previously described [7]. Briefly, formalin-fixed, paraffin-embedded tissue sections (4 μm thick) were prepared on SuperFrost Plus microscope slides (Menzel-Glaser, Germany) and dried for 24 hours in a 37°C incubator. Following dewaxing and rehydration the endogenous peroxidase activity was quenched and antigenic sites were retrieved by boiling the slides in 1 500 ml distilled water with 15 ml Antigen Unmasking Solution (Vector Laboratories Ltd, Burlingame, CA) in a pressure cooker for 3 min at 15 psi. Non-specific protein was blocked with 1x casein (Vector Laboratories Ltd) and endogeneous avidin and biotin were blocked by 15 min incubation periods with biotin and avidin, respectively (Avidin Biotin Blocking Kit, Vector Laboratories Ltd). Sections were then incubated at room temperature for 2 h with primary antibody. A final dilution of 1:100 was used for ER alpha monoclonal antibody clone 6F11 (#NCL-ER-6F11, Vector Laboratories Ltd) and PR monoclonal antibody clone 16 (#NCL-PGR-312, Vector Laboratories Ltd). A negative control was also included in each batch of slides, in which the primary antibody was omitted. The slides were scored by a breast histopathologist (EDL) using the Allred scoring system [8] where a score of 0–8 is given by taking into account both the staining intensity and heterogeneity (Table I). A total score of 3 or more represented a positive result.

Results

Sample characteristics

A total of 18 patients with MFBC were identified. All of these patients had undergone mastectomy as pimary treatment or following histological diagnosis of MFBC after an initial wide local excision. A breast pathologist confirmed the presence of multifocal disease following histological review of the mastectomy specimens. Of the 18 patients, 11 had two independent foci of cancer, three patients had three foci and four patients had four foci on histological examination. One patient (#9) had a focus of cancer in the contralateral breast and had undergone bilateral mastectomy. Histological examination of the resected specimens showed all the cancers to be invasive ductal carcinomas. Three patients (#5, 7 and 12) had multifocal disease with differing tumour grade for each of two foci (Table II).

Table I. Allred immunohistochemical scoring system [8]. The score for heterogeneity (proportion of nuclei staining) is added to the score for staining intensity to give a value of 0–8. A value of 3 or more represents a positive result.

Score for proportion	Score for intensity
0 = No staining	0 = No staining
1 = <1% nuclei staining	1 = weak staining
2 = 1–10% nuclei staining	2 = moderate staining
3 = 11–33% nuclei staining	3 = strong staining
4 = 34–66% nuclei staining	
5 = 67–100% nuclei staining	

Immunohistochemical status of ER

The results for ER status of each focus are given in Table II. Overall, the ER status (i.e. positive or negative) was the same for each tumour focus from all MFBC patients. A total of 16/18 (88%) patients were ER-positive. There were some minor variations in the score (0–8) for ER between foci, but this could be attributed to the subjective nature of the scoring system.

Immunohistochemical status of PR

The results for PR status of each focus are given in Table II. For 16/18 patients, all individual foci produced the same overall result i.e. positive (n = 13) or negative (n = 3). For 2/18 patients (#3 and 4) there were mixed PR results, although all foci were ER-positive. In one case (#3) the index (largest) tumour focus was PR-negative but one focus was PR-positive. However, since all foci were ER-positive, this would not have affected clinical decision-making.

Tumour grade

Three patients (#5, 7 and 12) demonstrated tumour foci of varying grades of differentiation. The ER and PR status, however, was the same in each case.

Contralateral breast cancer

One patient (#9) had a focus of cancer in the contralateral breast and had undergone bilateral mastectomy. All foci were grade three. A sample of this tumour focus was also included for assessment of ER and PR status. Interestingly, each tumour focus from the breast affected by MFBC was ER-positive and PR-positive whereas the single tumour focus from the other breast was completely negative for both receptors.

Discussion

ER and PR-positivity is used routinely to identify patients who are most likely to respond to

Table II. Clinical details of tumour foci from 18 patients with MFBC. The immunohistochemical score for ER and PR was assigned according to Table I.

Patient #	Tumour Focus #	Size (mm)	Grade	ER status (score)	PR status (score)
1	A5	23	2	+ (7)	+ (6)
	A9	4	2	+ (7)	+ (7)
	B4	3	2	+ (7)	+ (6)
2	A2	18	1	+ (7)	+ (7)
	A14	<5	1	+ (7)	+ (7)
	A20	<5	1	+ (7)	+ (8)
	A23	<5	1	+ (7)	+ (7)
3	A5	12	2	+ (4)	Neg (0)
	A12	7	2	+ (8)	+ (6)
	A9	6	2	+ (8)	+ (6)
	A8	5	2	+ (7)	+ (7)
4	A10	12	2	+ (5)	+ (8)
	A14	4	2	+ (5)	+ (5)
	A16	3	2	+ (7)	Neg (2)
5	A4	12.5	3	+ (7)	+ (7)
	A6	2	1	+ (7)	+ (7)
6	A5	24	2	+ (5)	+ (5)
	A10	13	2	+ (4)	+ (5)
7	A5	8	1	+ (7)	+ (8)
	A	N/A	2	+ (8)	+ (8)
8	A13a	22	3	Neg (0)	Neg (0)
	A13b	10	3	Neg (0)	Neg (0)
	A9	6	3	Neg (0)	Neg (0)
	A8	6	3	Neg (0)	Neg (0)
9	B3	17	3	+ (7)	+ (7)
	B4	5	3	+ (5)	+ (7)
	B5	5	3	+ (7)	+ (7)
	B7	5	3	+ (7)	+ (7)
	A4*	14	3	Neg (0)	Neg (0)
10	A3	17	3	Neg (0)	Neg (0)
	A3	5	3	Neg (0)	Neg (0)
11	A12	12	2	+ (7)	+ (7)
	A24	10	2	+ (7)	+ (7)
12	A7	26.5	2	+ (5)	+ (5)
	B5	8	1	+ (7)	+ (7)
13	A3	16	1	+ (8)	+ (8)
	A3	12	1	+ (8)	+ (8)
14	A7	25	2	+ (7)	+ (7)
	A12	6	2	+ (7)	+ (8)
15	A6	24	2	+ (6)	+ (7)
	B3	3	2	+ (7)	+ (3)
16	A1	30	2	+ (7)	+ (5)
	A3	2	2	+ (7)	+ (6)
	A8	2	2	+ (7)	+ (3)
17	A11	24	2	+ (5)	Neg (2)
	A11	3	2	+ (5)	Neg (2)
18	A4	8	1	+ (4)	+ (8)
	A4	8	1	+ (4)	+ (8)

*Contralateral tumour.

hormone therapy. In addition, it also provides valuable information regarding patient prognosis. The present study, although a pilot study involving 18 patients, showed that ER status was the same in all foci of MFBC. This was also true in a subset of patients who had foci of differing grade within the same breast quadrant.

PR status showed variability in 2/18 (11%) of MFBC patients, but in our series the ER status was positive and therefore hormone therapy would have been offered irrespective of the PR findings. There may, however, be a small number of patients with ER-negative MFBC, for whom PR testing of all tumour foci would be beneficial.

In a single case of bilateral breast cancer ER and PR status was completely different between breasts, despite the tumours being of the same grade. Interestingly, this is in contrast to the findings of

Coradini et al. [9] who retrospectively reviewed ER and PR status of 74 women with synchronous breast cancer and concluded that the receptor status was equivalent between the two sides. Our study was not designed to identify patients with bilateral breast cancer, but fortuitously included one such case. Further research with a larger study group will be required to assess this group of patients.

The current practice of establishing ER status in the primary focus is adequate in relation to hormone therapy. However, MFBC behaves more aggressively than unifocal breast cancer of a similar size hence there is a necessity for further research into its biology. It has been previously demonstrated by allelotyping that multiple breast lesions may exhibit distinct clonal patterns at the molecular level [10,11]. A similar study to identify molecular heterogeneity between tumour foci in MFBC, as defined in this paper, is underway.

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References

[1] King WJ, Greene GL. Monoclonal antibodies localize oestrogen receptor in the nuclei of target cells. Nature 1984;307:745–7.

[2] Regan MM, Gelber RD. Predicting response to systemic treatments: Learning from the past to plan for the future. Breast 2005;14:582–93.

[3] Andea AA, Bouwman D, Wallis T, Visscher DW. Correlation of tumor volume and surface area with lymph node status in patients with multifocal/multicentric breast carcinoma. Cancer 2004;100:20–7.

[4] Coombs NJ, Boyages J. Multifocal and multicentric breast cancer: Does each focus matter? J Clin Oncol 2005;23: 7497–502.

[5] Poulsen HS, Jensen J, Hermansen C. Human breast cancer: Heterogeneity of estrogen binding sites. Cancer 1981;48: 1791–3.

[6] Panahy C, Puddefoot JR, Anderson E, Vinson GP, Berry CL, Turner MJ, et al. Oestrogen and progesterone receptor distribution in the cancerous breast. Br J Cancer 1987;55: 459–62.

[7] Cawkwell L, Gray S, Murgatroyd H, Sutherland F, Haine L, Longfellow M, et al. Choice of management strategy for colorectal cancer based on a diagnostic immunohistochemical test for defective mismatch repair. Gut 1999;45:409–15.

[8] Harvey JM, Clark GM, Osborne CK, Allred DC. Estrogen receptor status by immunohistochemistry is superior to the ligand-binding assay for predicting response to adjuvant endocrine therapy in breast cancer. J Clin Oncol 1999;17: 1474–81.

[9] Coradini D, Oriana S, Mariani L, Miceli R, Bresciani G, Marubini E, et al. Is steroid receptor profile in contralateral breast cancer a marker of independence of the corresponding primary tumour? Eur J Cancer 1998;34:825–30.

[10] Tse GM, Kung FY, Chan AB, Law BK, Chang AR, Lo KW. Clonal analysis of bilateral mammary carcinomas by clinical evaluation and partial allelotyping. Am J Clin Pathol 2003; 120:168–74.

[11] Smeds J, Warngerg F, Norberg T, Nordgren H, Holmberg L, Bergh J. Ductal carcinoma in situ of the breast with different histopathological grades and corresponding new breast tumour events: Analysis of loss of heterozygosity. Acta Oncol 2005;44:41–9.