CLINICAL RESEARCH

Immunocytochemical staining of breast carcinoma with the monoclonal antibody NCRC 11: a new prognostic indicator

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Abstract

The staining of breast cancer with a new monoclonal antibody, NCRC 11, was studied in a series of 126 women with primary breast carcinoma. Tumour samples embedded in paraffin were tested, and the minimum duration of follow up was five years or to death. Altogether 119 tumours stained positively. There was a strong relation between the intensity of staining, divided on a four point scale, and patient survival. Patients whose tumours exhibited intense staining had an improved survival compared with those with less intensely staining tumours ($p < 0.0001$). Staining related weakly to histological grade but not significantly to oestrogen receptor state or the pathological stage of lymph node disease. Mathematical analysis showed the relation to survival to be independent of the other known prognostic factors.

Inclusion of intensity of staining with other factors in a prognostic index might permit a more accurate estimation of prognosis in patients with breast cancer.

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Introduction

A prognostic index based on various factors, in particular tumour size, histological grade, and lymph node state, will indicate the likely prognosis of a patient presenting with primary breast carcinoma.[1] Expression of antigen by tumours is another factor that has been shown to be of prognostic value in certain instances. Loss of expression of blood group antigen by transitional cell carcinomas is associated with high grade and invasion[2]; and loss of expression of a human milk fat globule membrane antigen by breast carcinoma is associated with a poor prognosis and extracellular staining with a favourable prognosis.[3]

As part of the Nottingham Tenovus study a monoclonal antibody, NCRC 11, has been raised against human mammary carcinoma cells.[4] The antigen it recognises has a specific distribution in normal tissues similar to that of antibodies raised to human milk fat globule membrane[5,6] and is particularly expressed on the luminal surface of exocrine gland epithelium. Epithelial tumours, especially adenocarcinomas, also express the antigen. Initial studies showed that most breast carcinomas stained positively, but the degree of expression varied between individual tumours.

To investigate this variability of expression of antigen by breast carcinomas we studied a series of primary breast carcinomas using immunocytochemical staining of tissue sections by monoclonal antibody NCRC 11.

Patients and methods

We studied 126 women who presented consecutively with primary operable breast carcinoma. All were treated by simple mastectomy with node sampling from the low axilla, the apex of the axilla, and the internal mammary chain at the second intercostal space. All were followed up every three months to 18 months, then every six months to five years, and once a year thereafter. No prophylactic radiotherapy was given. A few women were given adjuvant chemotherapy, but this failed to influence survival.[1]

Detailed clinical and pathological information was available on all patients, including histological grade, lymph node state, and follow up history to five years or death. In most cases oestrogen receptor state was known. Histological grade was assessed by a modification of the

method of Bloom and Richardson[7] described by Elston.[8 9] Oestrogen receptor state was determined by a method using dextran coated charcoal.[10] Tumours were considered to be positive for oestrogen receptor if values greater than 5 fmol/mg cytosol protein were found.

MONOCLONAL ANTIBODY NCRC 11

NCRC 11 is an IgM antibody raised by immunising Balb/c mice with dissociated human mammary carcinoma cells. Although not specific for breast carcinoma, expression of the antigen recognised is virtually confined to epithelial sites. The distribution of staining is similar to that of some of the antibodies raised to human milk fat globule membrane. A detailed description of the production of NCRC 11 and its immunohistological characterisation has been published elsewhere.[4]

IMMUNOHISTOLOGY

All the tissues examined had been fixed in phosphate buffered formalin, processed routinely for histological examination, and embedded in paraffin wax. From each case one representative tumour block was selected and tissue sections 5 μm thick cut. These tissue sections were stained by the peroxidase antiperoxidase method[11] using diaminobenzidine as the chromogen. A haematoxylin counterstain was used.

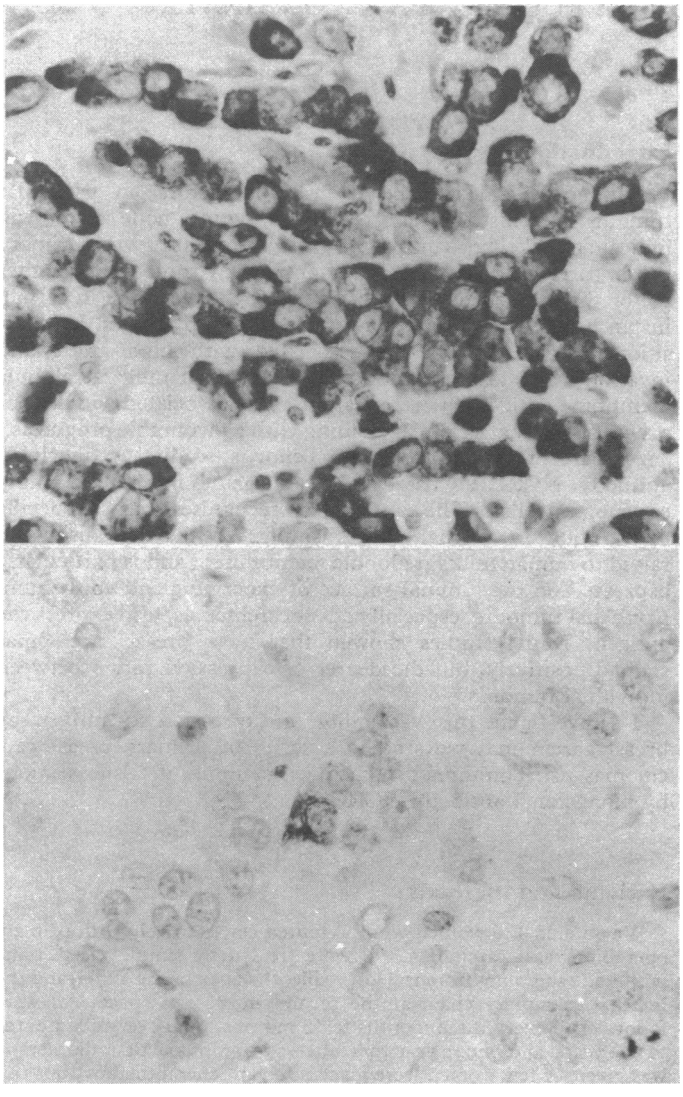

FIG 1—Immunohistological staining, using NCRC 11, of two breast carcinomas. One (top) shows positive staining of virtually all the tumour cells, giving a score of ++++; the other (bottom) shows positive staining of only one cell in the centre of the field, giving a score of +. Both figures ×426 (original magnification).

NCRC 11 was applied as cell culture supernatant fluid. The intermediate step reagents were used in the following dilutions: rabbit antimouse immunoglobulin, 1 in 1000; swine antirabbit immunoglobulin, 1 in 40; rabbit peroxidase antiperoxidase, 1 in 80. These reagents were supplied commercially by Dako (Mercia Brocades). A control serial section from each case was stained using a mouse monoclonal IgM antibody against sheep erythrocytes (Sera-Lab) as the primary antibody.

SCORING OF INTENSITY OF STAINING

The degree of staining of each tumour was assessed in a semiquantitative manner by two observers (IOE, JMacN) synchronously using a double headed light microscope, without prior knowledge of other clinical or pathological details. Each slide was scanned entirely at low power magnification (×63) and selected areas at high power magnification (×160 and ×400). The number of tumour cells staining positively was assessed and scored on a four point scale based on the proportion of the total number of tumour cells: 1-25% = +, 26-50% = ++, 51-75% = +++, 76-100% = ++++. A cell was regarded as being positively stained if stain product was present on all or part of its surface membrane or intracellularly. Figure 1 shows two stained tumours. In this study no account was taken of the difference in staining patterns (surface or cytoplasmic) of the tumour cells.

In the poorly staining (+) group several tumours had only a few positive cells: in some cases fewer than 10 positive cells were identified in the entire tissue section. In seven cases no positive tumour cells were seen despite extensive scanning at high power magnification.

The reproducibility of the scoring method was checked, and found to be satisfactory, by repeat scoring of 20 cases selected in a random, blind manner. Sixteen cases were scored identically (80%). The four cases scored differently were changed by only one grade—that is, +++ to ++, or +++ to ++++.

Results

Of the 126 tumours, 119 stained positively with NCRC-11 and seven did not. These seven formed a non-homogeneous group: histologically two were grade I, two grade II, and three grade III; lymph node state was negative in three cases and positive in four; and oestrogen receptor state was negative in three cases and positive in four. Three of the seven patients died (at six, 30, and 36 months); the remainder were still alive after 90, 102, 102, and 108 months.

The 119 tumours that expressed the antigen to some degree were studied to investigate the relation between the intensity of staining and other factors relating to differentiation and survival.

Histological grade—Table I shows the relation between the intensity of the staining and histological grade. There was a tendency for tumours exhibiting strong (+++ or ++++) staining to be better differentiated (grade I or II) and for those exhibiting weak (+ or ++) staining to be less well differentiated (grade III). This was significant ($\chi^2 = 4{\cdot}52$, df = 1, $p < 0{\cdot}05$).

Oestrogen receptor state—Table II shows the relation between the

TABLE I—*Relation between intensity of staining and histological grade*

Grade	Staining intensity				Total
	+	++	+++	++++	
I	5	5	8	3	21
II	9	8	17	5	38
III	18	20	17	4	60
Total	32	33	42	12	119

TABLE II—*Relation between intensity of staining and oestrogen receptor state in 109 patients in whom oestrogen receptor state was known*

Oestrogen receptor state	Staining intensity				Total
	+	++	+++	++++	
Positive	18	14	19	9	60
Negative	14	11	21	3	49
Total	32	25	40	12	109

intensity of staining and oestrogen receptor state. There was no significant association ($\chi^2 = 2{\cdot}88$, df = 3), although there was an apparent predominance of tumours containing oestrogen receptor in the intensely staining (++++) group (nine oestrogen receptor positive, three oestrogen receptor negative).

Lymph node state—Table III shows the relation between the intensity of staining and lymph node disease at mastectomy. No significant association was seen ($\chi^2 = 3{\cdot}57$, df = 3).

Survival—Figure 2 shows survival curves for patients according to the intensity of staining of their tumours. There was a highly significant relation between intensity of staining and survival. Patients whose tumours showed little staining with the antibody had a poor outlook compared with those whose tumours stained more intensely (Mantel's life table analysis[12] $\chi^2 = 63{\cdot}35$, $p < 0{\cdot}0001$).

Multivariate analysis—To test whether the relation with survival was independent of other factors and would therefore be of additional value in predicting the prognosis a Cox multivariate analysis was performed[13] with regard to intensity of staining, lymph node stage, histological grade, tumour size, and oestrogen receptor state. Table IV shows the results of this analysis. The Cox analysis generates a value (β) that relates the contribution of the factor to the hazard (in this case death). A positive value for β indicates that higher values of the factor are associated with higher risk. A negative value for β indicates that higher values of the factor are associated with lower risk. To test the significance of β the ratio of its absolute value to its standard error is calculated (Z). Values of Z greater than 1·96 are significant at the 5% level. In our analysis the values for the intensity of staining indicated that the relation between the intensity of staining and survival was independent of the other factors analysed.

TABLE III—*Relation between intensity of staining and lymph node state*

Lymph node disease	Staining intensity				Total
	+	++	+++	++++	
Present	14	19	16	7	56
Absent	18	14	26	5	63
Total	32	33	42	12	119

TABLE IV—*Values of β and Z obtained when each prognostic factor was included in Cox analysis*

	β	Z
Lymph node state	1·029	5·16**
Histological grade	0·781	3·57**
Tumour size	0·232	2·47*
Oestrogen receptor state	0·429	1·74
Intensity of staining	0·722	4·66**

*p < 0·05, **p < 0·001.

Discussion

The staining pattern of NCRC 11 in normal and malignant tissues is similar to that of antibodies raised to human milk fat globule membrane.[5 6] The antigen recognised has been called epithelial membrane antigen.[14] In a competition assay NCRC 11 showed partial cross reactivity with one of these monoclonal antibodies, LICR LON/M8.[4] A correlation between staining and prognosis using another of these antibodies, HMFG 1, has been described.[3] In that study complete absence of staining was associated with a poor prognosis and extracellular staining with a favourable prognosis, but both of the groups were small.

We showed a clear relation between the staining characteristics of primary breast cancers using NCRC 11 and the clinical course of the disease. Expression of the antigen may perhaps provide a marker of differentiation of the tumour cells. The relation with histological grade, although weak, tends to support this hypothesis, but it is surprising that no such relation existed between staining characteristics and the presence of oestrogen receptor as a relation exists between grade and oestrogen receptor.[15]

Intense staining (++++) of a tumour with NCRC 11 appears to indicate an extremely good prognosis. Of the 12 patients in this group, only one died. She presented a year after her mastectomy with rectal carcinoma (Dukes's stage C) and died subsequently from liver metastases, which may have been from the advanced rectal tumour. This group of patients were not uniform in their other tumour characteristics, and the good prognosis is surprising as most (seven cases) had lymph node metastases at the time of mastectomy and one third of the tumours were histological grade III.

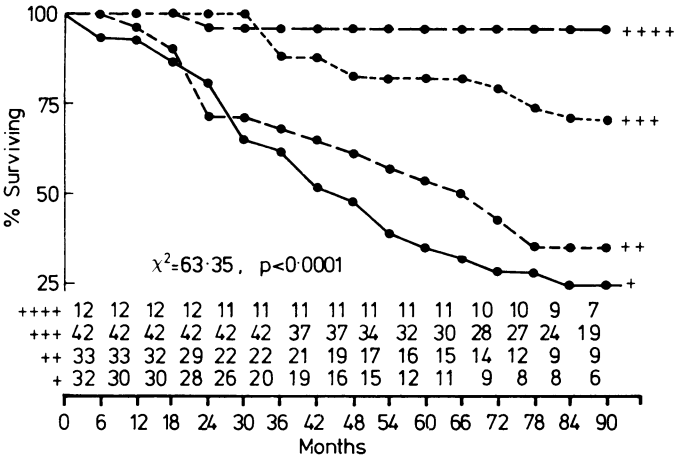

FIG 2—Survival curves for patients according to intensity of staining of their tumours (numbers at risk at each time interval in each group shown at base of graph).

Currently, the two most potent predictors of prognosis in patients with operable breast cancer are the presence or absence of lymph node metastases at the time of mastectomy and the histological grade of the primary tumour.[1] In this series of patients the intensity of staining provided information that was of a similar magnitude to these two factors but independent of them. We believe that using the intensity of staining in combination with other factors will enable the prognosis of a patient with breast cancer to be predicted more accurately. We are currently carrying out a study with a view to including intensity of staining in a prognostic index.

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References

1 Haybittle JL, Blamey RW, Elston CW, *et al.* A prognostic index in primary breast cancer. *Br J Cancer* 1982;**45**:361-6.
2 Coon JS, Pauli BU, Weinstein RS. Precancer of the urinary bladder. *Cancer Surveys* 1983;**2**:479-94.
3 Wilkinson MJS, Howell A, Harris M, Taylor-Papadimitriou J, Swindell R, Sellwood RA. The prognostic significance of two epithelial membrane antigens expressed by human mammary carcinomas. *Int J Cancer* 1984;**33**:299-304.
4 Ellis IO, Robins RA, Elston CW, Blamey RW, Ferry B, Baldwin RW. A monoclonal antibody, NCRC 11, raised to human breast carcinoma. 1. Production and immunohistological characterization. *Histopathology* 1984;**8**:501-16.
5 Foster CS, Edwards PAW, Dinsdale EA, Neville AM. Monoclonal antibodies to the human mammary gland. *Virchows Arch [Pathol Anat]* 1982;**394**:279-93.
6 Arklie J, Taylor-Papadimitriou J, Bodmer W. Differentiation antigens expressed by epithelial cells in the lactating breast are also detectable in breast cancers. *Int J Cancer* 1981;**28**:23-9.
7 Bloom HJG, Richardson WW. Histological grading and prognosis in breast cancer: a study of 1409 cases of which 359 have been followed for 15 years. *Br J Cancer* 1957;**11**:359-401.
8 Elston CW. Assessment of histological differentiation in breast cancer. *Aust NZ J Surg* 1984;**54**:11-6.
9 Elston CW, Gresham GA, Rao GS, *et al.* The Cancer Research Campaign trial (King's/Cambridge) for early breast cancer: clinicopathological aspects. *Br J Cancer* 1982;**45**:655-69.
10 Maynard PV, Blamey RW, Elston CW, Haybittle JL, Griffiths K. Oestrogen receptor assay in primary breast cancer and early recurrence of the disease. *Cancer Res* 1978;**38**:4292-5.
11 Sternberger LA. *Immunocytochemistry*. 2nd ed. New York: John Wiley and Sons, 1979.
12 Mantel N. Evaluation of survival data and two new rank order statistics arising in its consideration. *Cancer Chemotherapy Reports* 1966;**50**:163-70.
13 Cox DR. Regression models and life tables. *Journal of the Royal Statistical Society* 1972;**34**:187-220.
14 Heyderman E, Steele K, Ormerod MG. A new antigen on the epithelial membrane: its immunoperoxidase localization in normal and neoplastic tissue. *J Clin Pathol* 1979;**32**:35-9.
15 Elston CW, Blamey RW, Johnson J, Bishop HM, Haybittle JL, Griffiths K. The relationship of oestradiol receptors (ER) and histological tumour differentiation with prognosis in human breast carcinoma. In: Mouridson HT, Palshof T, eds. *Breast cancer: experimental and clinical aspects*. Oxford: Pergamon Press, 1980:59-62

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