Is apocrine differentiation in breast carcinoma of prognostic significance?

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Summary. Apocrine differentiation in human breast cancers has been assessed using immunohistochemistry to detect zinc α2 glycoprotein and the findings related to standard prognostic factors, disease free interval (DFI) and survival in 145 women with early breast cancer. Breast tumour samples from women with a minimum follow-up of 5 years were assessed. Routinely fixed and processed tissue was used throughout. Sixty-six (45%) tumours did not stain with the antibody. Fifty-two (36%) exhibited positive apocrine staining while for 27 (19%) only a few cells were reactive. The presence of apocrine differentiation was unrelated to lymph node status, menstrual status, tumour grade or size, oestrogen receptor (ER) or progesterone receptor (PR). However, patients whose tumours exhibited apocrine staining had a shorter disease-free interval (DFI) \( (P = 0.03) \) and survival \( (P = 0.03) \). A Cox's multiple regression analysis of the data found that the presence of staining added significantly \( (P = 0.047) \) to the predictive value of node status \( (P = 0.001) \), menstrual status \( (P = 0.0001) \), tumour size \( (P = 0.0026) \) and \( \text{ER} \text{R} \) status \( (P = 0.0014) \) for patient survival. The other seven prognostic factors tested did not reach significance and were rejected from the model. Apocrine differentiation in breast cancer appears to be an independent predictor of poor prognosis tumours.

The importance of apocrine differentiation in breast carcinomas is unknown because the incidence of apocrine change is difficult to establish. No general agreement exists on the criteria and extent of change required for categorisation. The percentage of primary cancers displaying evidence of apocrine characteristics in different reported series has varied between 0.3 and 57% (Azzopardi, 1979; Bonser et al., 1961; Fisher et al., 1975; Haagensen, 1986). Haagensen has defined apocrine carcinomas as tumours composed of large cells with acidophilic cytoplasm, with evidence of cytoplasmic 'snouts' in areas of tubular differentiation.

However, using immunohistochemical techniques employing antibodies to proteins found in apocrine secretions (breast cyst proteins), more recent studies (Mazoujian et al., 1983; Bundred et al., 1987a) have suggested that 40–50% of unselected carcinomas exhibited apocrine differentiation, and have demonstrated a good correlation between staining and histological criteria.

Carcinomas showing apocrine differentiation have high 5α reductase activity, are capable of metabolising androgens and possess androgen receptors, implying that they have a different endocrine drive (Miller et al., 1988).

In the present study we have used an antibody to zinc α2 glycoprotein, a known marker of apocrine cell differentiation (Bundred et al., 1987a), to examine the relationship between expression of apocrine antigens and various prognostic factors and to determine whether the presence of apocrine differentiation in tumours conferred any additional prognostic information over the more standard prognostic factors.

Materials and methods

Patients

One hundred and forty-five women who presented to the Breast Clinic of the University Department of Clinical Surgery, Edinburgh, with primary operable breast cancer were studied. Patients were treated by mastectomy, with either axillary node sampling \( (n = 38) \), node sampling and radiotherapy \( (n = 69) \) or axillary clearance \( (n = 38) \). All patients were followed up clinically every 3 months for 18 months then every 6 months up to 5 years and once a year thereafter. A minimum follow-up of 5 years or to a patient's death was available for all the women. Forty-five women received adjuvant therapy (Table I). The number of women who received adjuvant therapy was equally matched between groups.

Prognostic factors

The following known prognostic factors were studied. Lymph node status was obtained from the main histopathology report and all patients were designated node positive or node negative. The methods used to assess tumour grade, cellularity, elastosis, oestrogen receptor and progesterone receptor have been described in a previous publication (Hawkins et al., 1987). Tumour histology was classified as either 'special type' or 'no special type'. Menstrual status was classified by designating the patients as premenopausal (less than 12 months since last menstrual period) or post-menopausal (more than 12 months since last menstrual period, hysterectomised-ovariectomy or hysterectomised and 50 or more years of age) (Table I).

Immunohistochemistry

Zinc α2 glycoprotein antibody, a polyclonal rabbit antiserum, was purchased from Behring Diagnostics UK. All tissues were formalin fixed, paraaffin embedded. The immunoperoxidase technique has been described elsewhere (Bundred et al., 1987a) and was a three stage peroxidase-antiperoxidase complex method.

Zinc α2 glycoprotein antibody was applied diluted 1 in 1000 in phosphate buffered saline pH 7.4. The intermediate step reagents were used at the following dilutions: Swine anti rabbit immunoglobulin serum 1 in 30, rabbit peroxidase antiperoxidase complex 1 in 50. These reagents were obtained from DAKO Ltd. The diaminobenzidine hydrogen peroxide reaction was employed to detect the peroxidase, with Mayer's Haemalum as a nuclear counterstain. Controls were the use of non-immune rabbit antiserum in place of the primary antiserum. Previous absorption studies have been undertaken (Bundred et al., 1987a).

The extent of staining was assessed in a semi-quantitative method by two observers (R.A.W., N.J.B.) without prior knowledge of the outcome of the patient. The number of tumour cells staining was assessed and scored as: negative, no staining, equivocal, 0–5% of total tumour cells stained; or positive, 5–100% of total tumour cells stained (Figure 1).

The relationship of apocrine staining to other prognostic factors was analysed by the test for trend, Spearman rank correlation or \( \chi^2 \) test as appropriate. Disease-free interval and survival curves were calculated by the Kaplan–Meier method. Cox's proportional hazards model was used to assess the importance of each prognostic factor, both univar-
Cox's multivariate regression analysis of the data from these 118 women found that the presence of zinc $\alpha^2$ glycoprotein added significantly ($P = 0.006$) to the predictive value of node status ($P = 0.0002$), menstrual status ($P = 0.0006$), tumour size ($P = 0.0008$) and ER status ($P = 0.0004$) for patient survival. The other seven factors tested did not reach significance and were rejected from the model. Likewise in a similar analysis the presence of apocrine staining added significantly ($P = 0.016$) to the predictive value of node status ($P = 0.004$), menstrual status ($P = 0.004$), tumour size ($P = 0.036$) and oestrogen receptor status ($P = 0.002$) for patient relapse-free interval.

To take account of the equivocally staining tumours a second analysis was then carried out comparing 79 women whose tumours showed any apocrine staining (i.e. positive and equivocally staining tumours grouped together) compared with the 66 women whose tumours did not stain. On this analysis, the test for linear trend found that tumour staining was associated with better grade tumours ($P = 0.09$). No significant correlation between any of the other prognostic factors was however found.

The second univariate analysis revealed that seven of the 12 prognostic factors (node status $P = 0.0001$; menstrual status $P = 0.0001$; tumour grade $P = 0.026$; tumour size $P = 0.016$; ER levels $P = 0.005$; PgR status $P = 0.02$ and apocrine stain result $P = 0.03$) (see Table III) were significantly predictive of survival. A Cox's multivariate analysis was then performed and factors added to the model until no significant improvement in predicting the DFI and survival could be made. Significant levels for exclusion from or inclusion into the model were calculated from the likelihood ratio test (Tables IV and V). Apocrine stain result again added significantly to the prognostic value of the model. Other factors included in the model for survival were node status, menstrual status, tumour size and ER level, for both relapse free interval and survival. None of the other seven factors was found to be significant in this form of analysis for patients survival.
Glycoprotein antibody showing (a) and (c) negative staining. Sections stained (b) equivocal and (c) negative staining.

Table II Breast carcinomas stained for zinc α-2 glycoprotein

| Type of carcinoma | Number of cases | Stain |
|-------------------|-----------------|-------|
| Infiltrating ductal | 133 | + | ± | - |
| Infiltrating lobular | 7 | 1 | 1 | 5 |
| Medullary | 2 | 0 | 0 | 2 |
| Mucoid | 2 | 0 | 2 | 0 |
| Papillary | 1 | 0 | 0 | 1 |
| Total | 145 | 52 | 27 | 66 |

Table III Relationship of prognostic factors to disease-free interval and survival by univariate analysis

| Significance level | DF1 | Survival |
|--------------------|-----|----------|
| Node status | 0.0001 | 0.0001 |
| Menstrual status | 0.0035 | 0.0001 |
| Tumour grade | 0.042 | 0.026 |
| Tumour size | 0.013 | 0.016 |
| ER level | 0.0029 | 0.005 |
| PgR status | 0.11 | 0.021 |
| Elastosis | 0.3 (NS) | 0.3 (NS) |
| Cellularity | 0.6 (NS) | 0.4 (NS) |
| Histology | 0.5 (NS) | 0.7 (NS) |
| Initial treatment | 0.2 (NS) | 0.2 (NS) |
| Adjuvant therapy | 0.3 (NS) | 0.4 (NS) |
| Apocrine stain | 0.03 | 0.03 |

NS, not significant. Tumour size was as a continuous variable. Stain was as present or absent.

Discussion

Krompecher first described apocrine breast carcinoma in 1916 but difficulties in categorising apocrine change have made attempts at determining the clinical significance of such differentiation impossible.

The diagnosis of apocrine carcinoma has rested on the presence of large eosinophilic cells with basophilic nuclei and bulbous apical snouts seen on haematoxylin and eosin stained sections of breast cancer tissue (Azzopardi, 1979). The subjectiveness of this diagnosis is illustrated by the variety of claims of the incidence of apocrine change in breast cancer, ranging from 0.3 to 57%.

Haagensen (1986) has suggested that this sub-group of breast carcinomas may arise from areas of gross cystic disease with apocrine metaplasia. Zinc α2 glycoprotein forms 36% of the total protein content of apocrine sweat (Jirka, 1968) and is one of the major protein components of another apocrine secretion, breast cyst fluid (Haagensen et al., 1979). A previous study (Bundred et al., 1987a) has shown that staining of tumour cells with an antiserum to zinc α2 glycoprotein is a reliable immunohistochemical marker of apocrine differentiation in tumours. Workers using an antibody to another cyst protein, GCDFP 15, have confirmed that tumour apocrine differentiation can be objectively diagnosed using immunohistochemistry and found an incidence of 50–80% of tumours exhibiting some degree of apocrine change (Mazoujian et al., 1983; Miller et al., 1988; Le Dousal et al., 1985). The incidence of 55% of tumours exhibiting staining in this study is in broad agreement with these figures.

Mossler et al. (1980), have claimed that apocrine carcinomas are deficient in oestrogen and progesterone receptors but we have been unable to find any relationship with either oestrogen or progesterone receptor status.

Two previous studies of tumour apocrine change using antisera to GCDFP 15 have shown an association between better differentiated tumours and immunohistochemically determined GCDFP 15 expression (Le Dousal et al., 1985) and high cytosol GCDFP 15 levels (Silva et al., 1982). We were able to substantiate these findings, the better differentiated carcinomas being less likely to be negative in comparison to the poorly differentiated tumours although the association was a weak one.
Table IV  Relationship of prognostic factors to disease-free interval by multivariate analysis (145 cases)

| Factor          | Groups (codes) | Coeff. (β) | s.e. (β) | P-value* |
|-----------------|---------------|------------|----------|---------|
| Node status     | – ve (0)      | 1.433      | 0.283    | <0.0001 |
|                 | + ve (1)      |            |          |         |
| Adjuvant therapy| none (A1 = 0, A2 = 0) (A1) | -0.860 | 0.326 | 0.0001 |
|                 | tam (A1 = 1, A2 = 0) (A2) | -1.630 | 0.457 |         |
|                 | other (A1 = 0, A2 = 1) |            |          |         |
| Tumour size     | Not applicable| 0.202      | 0.075    | 0.011   |
| ER level        | <5 (0)        | -0.672     | 0.264    | 0.014   |
|                 | >5 (0)        |            |          |         |
| Apocrine stain  | 0% (0)        | 0.247      | 0.121    | 0.038   |
|                 | >0% (2)       |            |          |         |
| Elastosis       | 0 (E1 = 1, E2 = 0) (E1) | 0.758 | 0.350 | 0.035   |
|                 | 1 (E1 = 1, E2 = 1) (E2) | 0.031 | 0.297 |         |
|                 | 2 (E1 = 0, E2 = 1) |            |          |         |

0 = none, 1 = minimal, 2 = marked. *Obtained from likelihood ratio test.

Table V  Relationship of prognostic factors to survival by multivariate analysis (145 cases)

| Factor          | Groups (codes) | Coeff. (β) | s.e. (β) | P-value* |
|-----------------|---------------|------------|----------|---------|
| Node status     | – ve (0)      | 1.058      | 0.289    | 0.0001  |
|                 | + ve (1)      | -1.322     | 0.386    | 0.0001  |
| Menstrual status| pre (1)       |            |          |         |
|                 | post (0)      |            |          |         |
| Tumour size     | Not applicable| 0.265      | 0.081    | 0.0026  |
| ER level        | <5 (0)        | -0.926     | 0.274    | 0.0014  |
|                 | >5 (1)        |            |          |         |
| Apocrine stain  | 0% (0)        | 0.263      | 0.135    | 0.047   |
|                 | >0% (2)       |            |          |         |

*Obtained from likelihood ratio test.

Figure 2  Log rank curve of relapse-free interval (DFI) stratified by apocrine stain status (P<0.05).

Figure 3  Log rank curve of survival in 145 breast cancers stratified by the presence or absence of apocrine staining: positively staining tumours have a poorer survival (P<0.03).

In many centres the only breast cancer tissue that is available will be formalin fixed and paraffin embedded. Various histological parameters can be assessed from haematode Yin and eosin stained sections of such material and some of these, e.g. histological grade, can be of prognostic significance (Elston et al., 1982). However, a major criticism of these assessments is that they are subjective (Stenkvist et al., 1979). The widespread use of immunohistochemistry has allowed the determination of more objective assessments of tumour behaviour. Immunohistochemical staining with the monoclonal antibody NCRC II, which recognises components of the milk fat globule membrane, can give prognostic information (Ellis et al., 1985). However, the extent of staining relates to histological grade and oestrogen receptor status. Other prognostic markers which can be defined by immunohistochemistry such as epidermal growth factor receptor (Sainsbury et al., 1987) require fresh tumour tissue for analysis. In this respect the immunohistochemical identification of apocrine change provides an independent prognostic parameter which does not rely on a complex staining scale and can be applied to routinely fixed and processed tissue.

In many centres the trend towards conservative surgery for breast cancer (without determining node status) and the absence of oestrogen receptor assay facilities has meant that two of the most important prognostic factors are unavailable. Apocrine staining may prove a useful objective prognostic parameter in these patients and could be used to select patients who require adjuvant therapy. Furthermore, the recognition of apocrine differentiation in breast carcinomas as a clinicopathological entity with a distinct natural history may help therapeutically as such carcinomas are more likely to express androgen receptors (Miller, 1988) and less likely to respond to hormonal manipulation (Bundred et al., 1987b).

Why apocrine differentiation should be associated with such a poor prognosis is not clear and must await further investigation. Despite the excess of women undergoing mastectomy and clearance whose tumours did not stain, the initial treatment made no difference to the prognosis of the carcinomas. This study shows that the identification of apocrine differentiation is of prognostic value and adds significantly to the predictive ability of the other standard prognostic factors.

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