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Carcinoembryonic antigen expression and peanut agglutinin binding in primary breast cancer and lymph node metastases; lack of correlation with clinical, histopathological, biochemical and morphometric features

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Carcinoembryonic antigen expression and peanut agglutinin binding in primary breast cancer and lymph node metastases; lack of correlation with clinical, histopathological, biochemical and morphometric features

In a group of 335 patients with primary breast carcinoma the presence of immunoreactive carcinoembryonic antigen (CEA) and the binding of the lectin peanut agglutinin (PNA) in the primary carcinoma and in axillary lymph node metastases were investigated. The correlation between these results and a variety of established clinical, histopathologic, morphometric and biochemical prognosticators was studied. These features included lymph node status, tumour diameter, tumour type, nuclear grade, histologic grade, oestrogen receptor status, mitotic activity index and a number of nuclear measurements. The results indicate that CEA immunoreactivity of and PNA binding to tumour cells in primary breast carcinomas or lymph node metastases do not correlate with established prognostic factors in breast cancer.

Keywords: breast cancer, carcinoembryonic antigen, peanut agglutinin lectin, prognostic factors

Introduction

Since Gold & Freedman in 1965 described carcinoembryonic antigen (CEA) as a specific marker for adenocarcinoma of the large bowel it has been shown that CEA was a marker for many other 'epithelial' cancers (Goldenberg 1978, Gold, Shuster &

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Freedman 1978, Neville 1981). In several publications CEA immunoreactivity in breast cancer was found to be correlated with a worse prognosis (Shousha & Lyssiotis 1978, Wharen *et al.* 1978, Kuhajda, Offut & Mendelsohn 1982, Mansour *et al.* 1983). Other results, however, were also published (Persijn & Korsten 1977, Walker 1980).

Lotan *et al.* (1975) described the purification, composition and specificity of the lectin peanut agglutinin (PNA) which is thought to be specific for beta-D-galactosyl(1→3)-*N*-acetyl-D-galactosamine. The presence of PNA as a marker for mammary epithelial cell differentiation was introduced by Newman, Klein & Rudland (1979).

The percentage of breast carcinomas that showed CEA immunoreactivity varied considerably, from 1.6 to 88%, in different studies (Heyderman & Neville 1977, Goldenberg, Sharkey & Primus 1978, Shousha & Lyssiotis 1978, Walker 1980). Variations in the methods for demonstrating CEA immunoreactivity, the specificity of the anti-serum used, the small numbers of cases investigated and differences in the way CEA positive tumour cells were scored explain some of these variations (Delellis *et al.* 1979, Walker 1980, Neville 1981, Kuhajda *et al.* 1982, Nap, ten Hoor & Fleuren 1983, Nap *et al.* 1985). In this study we have tried to overcome these problems by using a well absorbed CEA anti-serum, a large number of patients, and a well defined scoring method.

The purpose of the present study was twofold. First, to investigate the presence of CEA immunoreactivity and PNA binding to neoplastic cells in breast carcinoma, using strictly defined criteria for the scoring of CEA immunoreactivity and PNA binding to neoplastic cells. The second aim was to study the relationship between established single features with prognostic significance (patient's age, tumour size, lymph node status, histological tumour type, nuclear and histologic grade, oestrogen receptor content, mitotic activity index, cellularity index and nuclear features) and CEA immunoreactivity or PNA binding to neoplastic cells (Bloom & Richardson 1957, Black & Speer 1957, Fisher, Gregorio & Fisher 1975, Parl & Wagner 1980, Baak *et al.* 1982, Fisher, Sass & Fisher 1984).

Materials and methods

PATIENTS AND CLINICAL DATA

All primary invasive breast carcinomas (n=335) derived from female patients without other overt diseases in the period of January 1, 1981 until December 31, 1982 from six different hospitals were included in this study; average age 60.6 year (range 25–91, SD 14.21); mean tumour diameter 2.7 cm (range 0.4–15, SD 1.9); 39% (n=130) lymph node negative; 39% lymph node positive.

HISTOLOGICAL METHODS

Primary tumours and lymph node metastases were fixed in 4–5% buffered formalin and subsequently embedded in paraplast. The 4 μm thick sections were stained with

H & E. Lymph node status and tumour size were assessed according to accurate routine pathological examination. The following histologic indices were established:

Histological typing. All 335 cases of breast carcinoma were classified (Azzopardi *et al.* 1982) as ductal (n=273), lobular (n=20), medullary (n=4) and others (like adenoidcystic, papillary and combinations, 29); nine were unclassifiable.

Nuclear grade and histologic grade. Nuclear grading of the sections was performed according to Black, Barclay & Hankley (1975). Histologic grading was performed according to Bloom and Richardson (1957). The grading was only assessed in ductal carcinomas.

MORPHOMETRY

For the application of morphometry to breast cancer reference should be made to the studies of Baak and his colleagues (1982, 1983, 1984) and to that by Weibel & Elias (1967). Briefly, morphometry was performed on standard 4 μm thick, H & E sections. The following features were assessed in the areas where the tumour cells were most densely packed: the mitotic activity index; the cellularity index; nuclear features including perimeter (p), area (a), shortest axis, longest axis, axes ratio and shape factor calculated as 4π times the area divided by the perimeter squared i.e. $4\pi a/p^2$.

OESTROGEN RECEPTOR CONTENT

The oestrogen receptor content was determined with a biochemical assay as described by Persijn & Korsten (1977), and the EORTC Breast Cancer Cooperative Group (1980). According to this procedure a value of more than 11 000 fmol bound oestradiol per g protein was classified as positive. Values were regarded as negative if less than 9000 fmol/g protein were present.

IMMUNO-HISTOCHEMICAL METHODS

For the detection of CEA in tissue sections, an indirect immunoperoxidase method was used (Tejada, Pascal & Fenoglio 1977). To eliminate reactivity of non-specific cross-reacting antigen, a protocol was used for immunoabsorption (Nap *et al.* 1983, 1985). This included absorption of the anti-CEA serum by white blood cells, normal lung tissue, liver powder and normal stomach mucosa powder. Normal lung and spleen served as negative controls. Anti-CEA was visualized by Swine-anti-Rabbit-PO (Dakopatts A/S, Denmark). After incubation no reaction was seen with gastric mucosa and, at most, a very weak reaction with granulocytes. Positive controls included sections of primary colon carcinoma and squamous cell carcinoma of the lung both known to contain CEA. As a negative control normal rabbit serum was substituted for the rabbit anti-CEA serum. The anti-CEA serum (rabbit-anti-CEA) was obtained from Dakopatts A/S, Denmark.

In order to investigate the quality of the CEA assessment in our laboratory, reactivity of anti-CEA kindly purified for us by Nap, and anti-CEA purified in our own laboratory according to Nap's protocol (Nap *et al.* 1983) were compared in a test

set. The same tumour cells showed CEA immunoreactivity, thus proving the quality of the purification procedure.

Visualization of PNA binding was done with the direct immunoperoxidase method using peanut agglutinin conjugated to horse-radish peroxidase (E-Y lab. Inc., Santa Mateo CA) (Howard, Ferguson & Batsakis 1981, Moller 1982, Lehman, Cooper & Mullholland 1984). No PNA binding was found in our sections after incubation with D-galactose (Howard *et al.* 1981).

IMMUNOREACTIVITY SCORING

Immunoreactivity of CEA and binding of PNA were evaluated semi-quantitatively, with the following scoring table:

I no positive tumour cells detectable
II less than 20% of the tumour cells showed reactivity
III 20%–50% of the tumour cells showed reactivity
IV 51%–70% of the tumour cells showed reactivity
V more than 70% of the tumour cells showed reactivity

Each slide was graded independently by two of the authors. Fifteen cases, in which differences in grading were obtained, were re-evaluated by both authors and consensus was reached.

A number of analyses, using different combinations (I versus the others; I+II versus the others) were performed. It turned out that CEA and PNA grade I gave exactly the same results as I+II taken together. Therefore, CEA and PNA scores I+II were regarded as 'negative or weakly positive', III as 'moderately positive' and IV+V as 'strongly positive'.

STATISTICAL ANALYSIS

Statistical analysis of data was performed using chi-square contingency tables and linear regression. Values with $P<0.05$ were regarded as significant.

The following numbers of patients were available for statistical evaluation: lymph node status in 260, tumour type in 326, histological grade and nuclear grade in 273, morphometry in 286, oestrogen receptor assay in 219, CEA immunoreactivity in 312 of the primary tumours and 89 of the lymph node metastases and PNA binding to the primary tumour cells in 257 and to 77 of the lymph node metastases.

Results

CARCINOEMBRYONIC ANTIGEN

The distribution of the CEA scores established in the 312 primary breast carcinomas is presented in Table 1. There was a weakly significant positive correlation between CEA positivity and age ($r=0.13$, $P<0.05$).

Table 1. Frequency tables of CEA immunoreactivity in and PNA binding to primary breast carcinoma and their lymph node metastases

Score	CEA		PNA	
	Primary	Lymph node	Primary	Lymph node
I	63 (20.2%)	31 (34.8%)	60 (23.4%)	21 (27.3%)
II	75 (24.0%)	13 (14.6%)	73 (28.4%)	17 (22.1%)
III	47 (15.1%)	7 (7.9%)	44 (17.1%)	7 (9.1%)
IV	80 (25.6%)	23 (25.8%)	53 (20.6%)	18 (23.3%)
V	47 (15.1%)	15 (16.9%)	27 (10.5%)	14 (18.2%)
Total	312 (100%)	89 (100%)	257 (100%)	77 (100%)

The presence of CEA immunoreactivity in the neoplastic cells of the primary breast carcinoma showed no correlation with the presence of lymph node metastases (n=243). Forty-six per cent (n=57) of the CEA negative or weakly positive primary tumours had metastases and 46% (n=54) had no metastases. Of the primary tumours that were strongly positive for CEA 42% (n=52) had metastases and 40% (n=47) had none.

There was no significant correlation between CEA immunoreactivity in the primary breast carcinoma and tumour size, histological tumour type, nuclear grade, histological grade, oestrogen receptor status or any of the morphometric features.

A correlation was found between the scores of CEA immunoreactivity in the primary breast carcinoma and PNA binding to the neoplastic cells in the same tumour (r=0.22, P<0.05 n=257). This correlation was not found between CEA immunoreactivity in the primary tumour and PNA binding in lymph node metastases. Similarly no correlation was found between CEA immunoreactivity in the axillary lymph node metastases and the PNA binding to the neoplastic cells in the primary tumour.

Table 1 shows the distribution of the CEA immunoreactivity scores established in the tumour cells in lymph nodes. There was a correlation (r=0.65, P<0.005, n=89) between the CEA immunoreactivity in the neoplastic cells of the primary breast carcinoma and that in the lymph node metastases. Moreover, the CEA scores in the lymph node metastases and the primary tumours were equally distributed over the groups. In both groups about 40% of the patients were strongly positive for CEA (42.7% and 40.7% respectively) and 45% negative or weakly positive (49.4% and 44.2% respectively); the others were doubtful. CEA immunoreactivity in the lymph node metastases showed no correlation with any of the other investigated features.

BINDING OF PNA

There was a correlation between PNA binding to neoplastic cells in the primary breast carcinoma and the PNA binding to axillary lymph node metastases (r=0.60, P<0.005, n=77). Table 1 shows the distribution of PNA binding in the primary tumour and in the metastases. In the two groups 52% and 49% of the cases respectively were negative or weakly positive for PNA.

Table 2. Significant correlations found in present study

CEA in primary tumour–CEA in lymph node metastases	(r=0.65, P<0.005)
CEA in primary tumour–PNA in primary tumour	(r=0.22, P<0.05)
PNA in primary tumour–PNA in lymph node metastases	(r=0.60, P<0.005)
PNA in primary tumour–mitotic activity index	(r=−0.14, P<0.05)
PNA in primary tumour–shortest nuclear axis	(r=−0.14, P<0.05)

There was a tendency towards a negative correlation between PNA binding and the mitotic activity index (r=−0.14, P=0.04). There was also a negative correlation between PNA binding in the primary breast carcinoma and the mean of the short axis of the nuclei measured in the primary tumour (r=−0.14, P<0.05), indicating that less PNA binding is associated with larger nuclei.

No correlations were found between PNA binding to tumour cells in the primary breast carcinoma and lymph node status, nuclear grade, histological grade or any of the other features investigated. Similarly PNA binding of tumour cells in axillary lymph nodes showed no significant correlations with these indices.

The significant correlations are summarized in Table 2.

Discussion

In this study it was found that CEA immunoreactivity and PNA binding to neoplastic cells of primary breast cancer and their lymph node metastases were not correlated with the established prognosticators in breast cancer. In particular no correlations were found with tumour spread to the axillary lymph nodes. The results do not support the prognostic significance of CEA immunoreactivity and PNA binding as suggested in several studies (Shousha & Lyssiotis 1978, Newman *et al.* 1979, Walker 1980, Howard *et al.* 1981, Mansour *et al.* 1983).

CARCINOEMBRYONIC ANTIGEN

The CEA immunoreactivity was established in 312 primary breast tumours. Of these 41% were strongly positive, 15% moderately positive and 44% negative or weakly positive (Table 1). This is in agreement with others, using the same protocol, who found 42% of primary breast cancers positive for CEA (Nap *et al.* 1983, 1985).

Shousha & Lyssiotis (1978) found a correlation between the presence of lymph node metastases and CEA positivity in the primary tumour, 39% of CEA positive and 8% of CEA negative tumours having metastases. However, in the present study no correlation was found between the lymph node status and CEA immunoreactivity. They also found that in their 21 patients with lymph node metastases all the tumour tissue in the metastatic deposits was positive for CEA. On the contrary, we found that 31 of the 89 cases (35%) of the lymph node metastatic deposits had no positive cells (Table 1). One of the possible explanations for this difference is the small number of patients investigated in the study of Shousha & Lyssiotis (1978). Other explanations may be differences in the anti-sera used, the methods of purification and the scoring of positivity.

Walker (1980) also failed to find a correlation between CEA positivity and the presence of axillary lymph node metastases. Indeed in her 90 cases the presence of CEA correlated with good histological differentiation. This was not confirmed in our study.

Kuhajda *et al.* (1982) in a study of 93 ductal carcinomas found that tumours smaller than 3 cm were strongly positive for CEA, and that this was associated with axillary lymph node metastases. However, we could not confirm these results in the present study.

Persijn *et al.* (1977), using a CEA assay on extracts of breast tumours, found no correlation between CEA positivity and oestrogen receptor presence, an observation which was confirmed by Mansour *et al.* (1983). Shousha & Lyssiotis (1978) and Walker (1980) did not find correlations between CEA immunoreactivity and tumour type. These results concur with the present study.

No correlations were found for CEA immunoreactivity with any of the parameters of morphometry. Thus, in this large material, with strictly defined criteria, CEA positivity or negativity seems to be a phenomenon not associated with any other tumour characteristic. The CEA immunoreactivity in the primary tumour is correlated with PNA positivity, and with CEA immunoreactivity in the lymph node metastases. The biological implications of these correlations are not clear.

BINDING OF PNA

Peanut lectin binding was investigated by us because in two independent studies correlations were found between PNA positivity and the grade of differentiation (Newman *et al.* 1979, Howard *et al.* 1981). In the present study histological grade and nuclear grade did not correlate with PNA binding. A strong correlation was found between PNA binding to primary carcinoma cells and PNA binding to their lymph node metastases. A weak negative correlation was found between PNA binding to neoplastic cells in the primary breast carcinoma and both the mitotic activity index and the mean of the short axis of the tumour cell nuclei; this correlation was not found for PNA binding to the lymph node metastatic deposits. No correlation was found between PNA binding and spread of the tumour to the axillary lymph nodes.

In conclusion therefore we could not demonstrate that CEA immunoreactivity of and PNA binding by the tumour cells could be used as predictors of prognosis in patients with breast carcinoma. In a small number of patients (n=28) CEA immunoreactivity and/or PNA binding were not concordant as between the primary tumour and the lymph node metastases. This is further evidence that CEA and PNA reactivity appear not to be related to tumour spread. In a separate study, however, we are now investigating the relationship between these markers and long-term follow-up.

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