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Neuron specific enolase-positive breast carcinomas

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Summary. Ninety-eight patients treated for breast carcinomas were followed from 54 to 75 months after primary diagnosis. All had undergone a modified radical mastectomy with removal of axillary lymph nodes.

36 breast carcinomas were NSE-positive and 62 were negative. NSE-positive tumours were significantly more frequently estrogen receptor-positive than the NSE-negative tumours, and the estrogen receptor values were higher in the NSE-positive groups.

Patients with NSE-positive tumours and patients with NSE-negative tumours did not differ with regard to presence of lymph node metastases at the time of primary surgery. However, the study showed that patients with NSE-positive tumours had a tendency towards more lymph node metastases after primary surgical intervention, but a better outcome than patients with NSE-negative tumours and metastases.

This study, with a 5-year follow up, failed to demonstrate any major prognostic significance of immunostaining for NSE.

Key words: Breast carcinoma - Immunocytochemistry - Neuron specific enolase

Introduction

Recent studies have shown that immunostaining with an anti-neuron specific enolase antibody is a good screening method for detecting breast carcinomas with neuroendocrine differentiation (Nesland et al., 1985, 1986 a,b).

Neuron specific enolase (NSE) immunoreactivity is not uncommon in ductal carcinomas, lobular carcinomas and in several other subgroups of breast carcinoma (Nesland et al., 1986c; Nesland et al., in press). In the majority of NSE-positive tumours, immunoreactivity for several hormones has been detected in tumour cells. Lack of reaction may be due to aberrant forms of hormones or to low hormone concentration. Hormones not tested for may also of course be present in the tumours.

In this study we have immunostained 98 cases of breast carcinomas for NSE to see if NSE-positivity is of any clinical importance.

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Materials and methods

Ninety-eight consecutive patients at the Norwegian Radium Hospital treated for breast carcinoma in 1980-81 were studied. All had undergone a modified radical mastectomy with removal of axillary lymph nodes. Survival evaluation was performed according to the life table method.

All histological sections from the tumours were examined by a pathologist not aware of the NSE staining results or of the clinical history, who classified and graded the tumours according to WHO recommendations.

Sections from formalin-fixed, paraffin-embedded material were used for immunocytochemical studies with the avidine-biotin-peroxidase complex (ABC) method (Hsu et al., 1981), using an antiserum raised against NSE (Dakopatts Corp.).

After deparaffinization, the sections were treated for 30 minutes with 0.3% hydrogen peroxide in methanol to block endogenous peroxidase before incubation for 20 minutes with normal goat serum diluted 1:75 in 0.01 M saline (PBS), pH 7.4, and containing 25% bovine serum albumin (BSA) to eliminate non-specific staining. The sections were then incubated at 4°C with anti-NSE (1:1000) for 18 to 22 hours, followed by 30-minute incubation with a 1:200 dilution of biotin-labelled secondary antibody and a 60-minute incubation with ABC (10 g/ml avidin and 2.5 g/ml biotin-labelled peroxidase) (Vector, Burlingame, CA). The sections were stained for 5 minutes with 0.05% 3'3 diaminobenzidine tetrahydrochloride, freshly prepared

in 0.05 Tris buffer, pH 7,6, containing 0.01% H_2O_2 and then counterstained with haematoxylin before dehydration and mounting. All dilutions were made with PBS containing 12.5% BSA as the diluent.

Sections from a medullary thyroid carcinoma were used as positive control. Negative controls included the use of nonimmune serum as first layer and absorption of the NSE antibody with NSE prior to primary incubation.

Results

NSE immunostaining

36 breast carcinomas were NSE-positive and 62 were negative. A diffuse cytoplasmic staining was seen in most tumour cells in the NSE-positive cases (Fig. 1). Nuclear staining was sometimes encountered in scattered cells (Fig. 2). Non-neoplastic myoepithelial cells were frequently stained in both NSE-positive and NSE-negative tumours.

The sections remained unstained when the primary antibody was absorbed with NSE prior to use or when a non-reactive serum was used instead of the anti-NSE antibody.

Sections from the medullary thyroid carcinoma were always positively stained with the anti-NSE antibody.

Light microscopy

The light microscopical classification of the tumours is seen in Table 1. Of the 98 tumours included in this study, 11 were lobular and 80 were ductal carcinomas. In 2 cases it was impossible to decide whether the tumours should be classified as ductal or lobular. 5 tumours were of rarely encountered types (2 mucinous, 2 tubular and 1 apocrine breast carcinoma).

Eight of the 18 ductal carcinomas of WHO grade 3, were NSE-positive, whereas only 1 NSE-positive tumour was seen among the 11 ductal carcinomas of WHO grade 1 (Table 2). Six of the 11 lobular carcinomas and both mucinous carcinomas were NSE-positive.

Clinical data

About 72% of the patients were above the age of 50. The age distribution was the same in NSE-positive and NSE-negative cases. 5.6% of the patients with NSE-positive tumours and 8.1% of the patients with NSE-negative tumours were less than 40 years old.

TNM classification

There were no major differences between the NSE-positive and -negative cases (Table 3). None of the patients had distant metastases at the time of surgery.

Estrogen receptor values (Table 4)

Six of the 11 patients with lobular carcinomas had estrogen receptor values higher than 100 pmol/g cytosol protein. Only 15 of the 80 patients with ductal carcinomas were had similar high values. Grade III ductal carcinomas were more often estrogen receptor-negative than Grade I ductal carcinomas, but 2 of the 18 patients with grade III ductal carcinomas had estrogen receptor values above 100 pmol/g cytosol protein.

Correlation between estrogen receptor values and NSE immunoreactivity

Six (16.7%) of the NSE-positive tumours and 20 (32.3%) of the NSE-negative tumours lacked estrogen receptors ($p < 0.006$) (Table 5). Whereas 15 (41.7%) of the patients with NSE-positive tumours had estrogen receptor values higher than 100 pmol/g cytosol protein, only 9 (14,5%) of the patients with NSE-negative tumours had the same values.

Correlation between progesteron receptor values and NSE immunoreactivity

Eleven of the patients with NSE-positive tumours (30,6%) lacked progesteron receptors (Table 6) whereas 14 (38.9%) of the patients had values higher than 100 pmol/g cytosol protein. Twenty six (41.9%) patients with NSE-negative tumours lacked progesteron receptors while 18 (29%) had values higher than 100 pmol/g cytosol protein.

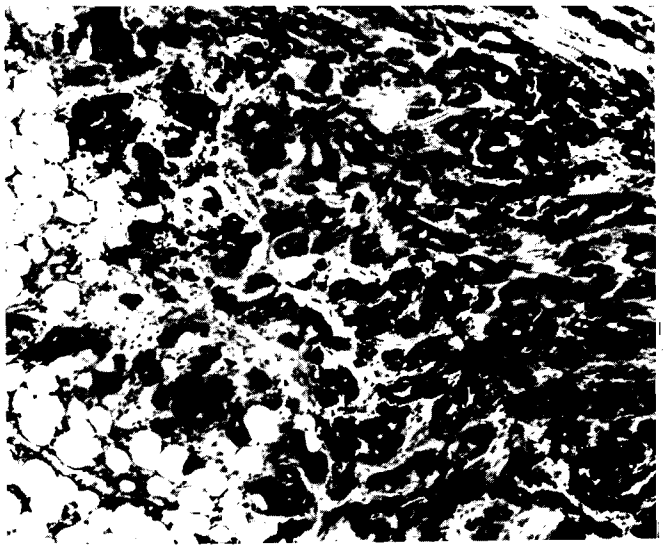
Follow up and outcome (See Tables 7 and 8)

Patients were followed up from 54 to 75 months after primary diagnosis and the crude survival was evaluated (Fig. 3), as well as survival for individual groups of patients with ductal carcinoma, grades I, II and III and lobular carcinoma (Fig. 4). Three (8.3%) patients with NSE-positive primary tumours developed regional lymph node metastases and 12 (33.3%) developed distant metastases. Among the group of patients with NSE-negative primary tumours, 3 (4.8%) developed regional lymph node metastases and 14 (22.6%) distant metastases. However, even though there is a tendency to more frequent metastases in the group with NSE-positive primary tumours, only 5 (13.9%) of these died because of breast cancer during the observation period, whereas 13 (21%) died in the other group.

Twenty (55.6%) patients with NSE-positive tumours and 40 (64.5%) patients with NSE-negative tumours were alive and without sign of malignancy at the end of the observation period.

Fig. 1. Groups of tumour cells with intense immunostaining for NSE (anti-NSE × 62)

Fig. 2. Infiltrating ductal carcinoma with NSE immunoreactivity. A diffuse staining is present in cytoplasm as well as in the majority of nuclei in this region (anti-NSE ×330)



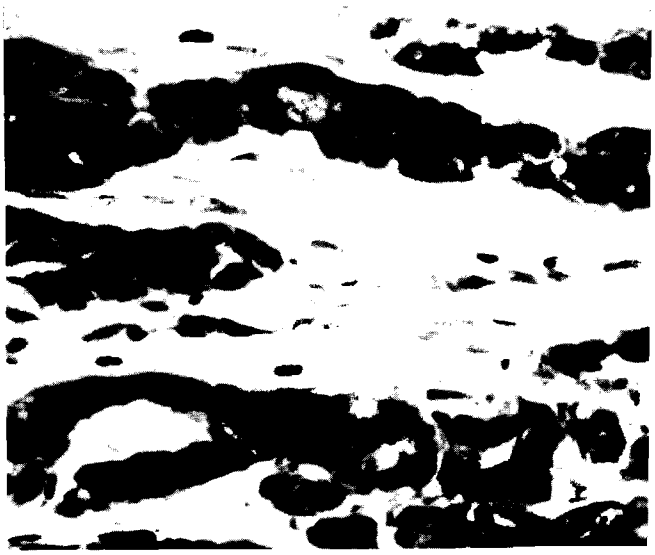


Table 1. Light microscopical classification

	No. of cases
Ductal carcinoma:	
WHO grade 1	11
WHO grade 2	51
WHO grade 3	18
Lobular carcinoma	11
Ductal?/lobular? carcinoma:	2
Mucinous	2
Tubular	2
Apocrine	1
	98

Table 2. Immunocytochemical results

	No. of NSE-positive cases	No. of NSE-negative cases
Ductal carcinoma:		
WHO grade 1	1	10
WHO grade 2	17	34
WHO grade 3	8	10
Lobular carcinoma	6	5
Ductal?/lobular? carcinoma:	2	0
Mucinous	2	0
Tubular	0	2
Apocrine	0	1
	36	62

Table 3. TNM classification

	NSE-positive cases	NSE-negative cases
T_1	23 (63.9%)	38 (61.3%)
T_2	12 (33.3%)	20 (32.3%)
T_3	1 (2.8%)	4 (6,5%)
N_0	22 (61.1%)	45 (72.6%)
N_1	14 (38.9%)	14 (22.6%)
N_3	0 (0.0%)	3 (4.8%)

Table 4. Correlations between histological type, grade and estrogen receptor values

	< 9 pmol/g	< 100 pmol/g	> 100 pmol/g
Ductal carcinoma grade I	2	7	2
II	15	25	11
III	9	7	2
Lobular carcinoma	1	4	6
Others	2	1	2
Lobular?/Ductal? carcinoma	0	0	2

Table 5. Correlation between estrogen receptor values and NSE immunoreacitivy

Estrogen receptor values	No. of NSE positive tumours	No. of NSE negative tumours
Negative < 9 pmol/g	6 (16.7%)	20 (32.3%)
< 10 - < 100 pmol/g	12 (33.3%)	30 (48.3%)
> 100 pmol/g	15 (41.7%)	9 (14.5%)
Unknown	3 (8.3%)	3 (4.8%)
	36	62

Table 6. Correlation between progesterone receptor values and NSE immunoreactivity

Progesterone receptor values	No. of NSE positive tumours	No. of NSE negative tumours
Negative < 9 pmol/g	11 (30.6%)	26 (41.9%)
< 10 - < 100 pmol/g	8 (22.8%)	15 (24.2%)
> 100 pmol/g	14 (38.9%)	18 (29%)
Unknown	3 (8.3%)	3 (4.8%)
	36	62

Table 7. Follow-up information

	Patients with NSE-positive tumours	Patients with NSE-negative tumours
Local recurrences	3 (8.3%)	6 (9.7%)
Regional lymph node metastases	3 (8.3%)	3 (4.8%)
Distant metastases	12 (33.3%)	14 (22.6%)

Table 8. Outcome

	Patients with NSE-positive tumours	Patients with NSE-negative tumours
Alive without carcinoma	20 (55.6%)	40 (64.5%)
Alive with carcinoma	7 (19.4%)	7 (11.3%)
Alive, carcinoma?	1 (1,8%)	0
Dead from breast carcinoma	5 (13.9%)	13 (21%)

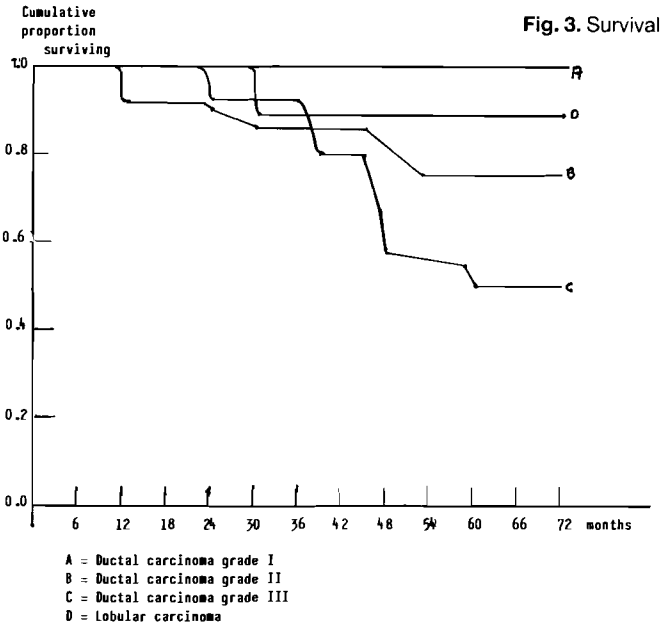


Fig. 3. Survival

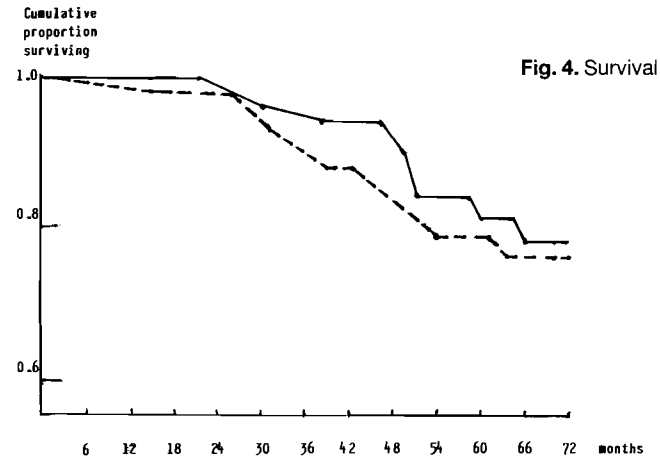


Fig. 4. Survival

Discussion

The outcome for breast cancer patients is difficult to predict. Tumour size (Fisher et al. 1969; Rosen et al., 1981), morphological type (Foote and Stewart, 1946; McDivitt et al., 1968; WHO, 1981), histopathological grade (Bloom, 1950; Bloom and Richardson, 1957; Eker et al., 1958; Schiødt, 1966; Nissen Meyer, 1969), nuclear grade (Black et al., 1950; Cutler et al., 1969; Hartveit, 1971), mean nuclear area (Mæhle and Skjærven, 1983), estrogen and progesteron receptor values (Ozzello et al., 1986; Pinotti et al., 1986), age (Mueller et al., 1978; Ribeiro and Swindell 1981; Palmer et al., 1982; Høst and Lund, 1986), number of lymph node metastases (Warren and Tompkins, 1943; Huvos et al., 1971; Fisher et al., 1978; Nemoto et al., 1980) and family history of breast cancer (Lynch et al. 1986) are all of prognostic significance.

We have previously shown that about 30% of breast carcinomas are immunoreactive for NSE (Nesland et al., 1985, 1986 a,b). Tumour cells with immunoreactivity for hormones are present in the majority of these NSE-

positive tumours (Nesland et al., 1986a,b). NSE immunoreactivity may be found in all histological types, although it is more frequent in lobular than in ductal carcinomas (Nesland et al., 1985, 1986 a). This is also confirmed in the present study where 6 of 11 lobular carcinomas were NSE-positive but only 26 of 80 ductal carcinomas. Within the group of ductal carcinomas, 8 of 18 grade III tumours were NSE-positive, but only 1 of 11 grade I tumours.

We have also previously shown that NSE-positive breast carcinomas are more often estrogen receptor-positive (Nesland et al., 1985) and have higher values than NSE-negative tumours. In this study, NSE-positive carcinomas are significantly more often estrogen receptor-positive ($p > 0{,}006$) than NSE-negative tumours.

Immunoreactivity for estrogen receptors is seen in the nucleus and only rarely in the cytoplasm. This indicates that estrogen receptor stimulation immediately leads to transport of the receptor-hormone complex to the nucleus where it is bound to a nuclear acceptor site. This causes an increased peptide-protein synthesis in cytoplasm and some of these peptides-hormones probably act as growth modulators. In vitro studies show that many hormones have a local growth effect. Some of them stimulate and some inhibit growth and thus have autocrine and paracrine properties (Rozengurt, 1983; Cuttitta et al., 1985).

Immunoreactivity for ACTH (Cohle et al., 1979; Woodward et al., 1981; Juntti-Berggren et al, 1983; Nesland et al., 1985, 1986a,b; Vinores et al., 1984), bombesin (Memoli et al., 1984; Nesland et al., 1985), serotonin (Raju and Fine, 1983; Memoli et al., 1984), prolactin (Raju and Fine, 1983), gastrin (Nesland et al., 1985), leu-enkephalin (Nesland et al., 1986a,b), pancreatic polypeptide (Nesland et al., 1986a,b), vasoactive intestinal peptide (Nesland et al., 1985), ß-endorphin (Nesland et al., 1986a,b) and substance P (Nesland et al., 1986) have all been demonstrated in breast carcinomas. In addition, biochemical analyses have shown the presence of ACTH (Liddle et al., 1963), PTH (Mavligit et al., 1971; Melick et al., 1972), and calcitonin (Hillyard et al. 1976) in selected cases. The links between the presence and stimulation of estrogen receptors, and the production of hormones have not yet been finally established. The importance of cytoplasmic p29 and its cellular function is also unknown, but p29 may be a product of estrogen action or a component of the estrogen receptor system (Coffer et al., 1985; King, 1986).

Some patients with estrogen receptor-positive tumours do not respond to tamoxyphen medication whereas some patients with estrogen receptor negative tumours do respond to this medication. Immunostaining with monoclonal antibodies raised against the receptor molecule have shown great variation in the staining results. The staining intensity varies from tumour to tumour and from area to area within one tumour. Some tumours consist almost exclusively of immunoreactive cells, while some are completely negative (Ozzello et al., 1986). The number of tamoxyphen-treated patients included in this study was too small to permit any conclusions to be made.

Patients with NSE-positive tumours and patients with NSE-negative tumours do not differ with regard to the presence of lymph node metastases at the time of primary surgery. In an earlier study (Nesland et al., 1985) we found patients with NSE-positive tumours to have less lymph node metastases than breast carcinoma patients in general. However, the present study shows that patients with NSE-positive tumours have a tendency to more lymph node metastases after primary surgical intervention, but a better outcome than patients with NSE-negative tumours and metastases. This could be explained by the higher frequency of estrogen receptor positivity in the group with NSE positive breast carcinomas.

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