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Relation of elastosis to biochemical and immunohistochemical steroid receptor findings, Ki-67 and epidermal growth factor receptor (EGFR) immunostaining in invasive ductal breast cancer

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Abstract. We studied 1073 cases of invasive ductal breast cancer, NOS for their elastic content (DEL, ductal + periductal elastosis; TEL, tumour elastosis) and compared the findings with the results of biochemical and immunohistochemical steroid hormone receptor examination. Tumours of patients up to 50 years of age and older were examined separately. In a number of tumours elastosis was also examined in relation to Ki-67 and epidermal growth factor receptor (EGFR) immunostaining. Sensitivity and specificity of DEL and TEL for predicting the receptor, Ki-67 and EGFR findings were estimated. Sensitivity of DEL and TEL for oestrogen and progesterone receptors is dependent on the degree of tumour differentiation and the degree of elastosis, increasing from DEL 1° and TEL 1° to DEL 3° and TEL 3°. It was more evident in grade 1 (G1) and G2 than in G3 carcinomas. Elastosis is a useful predictor of positive receptor findings particularly in G1 and G2 tumours with moderate and high-grade elastosis. It is a similarly useful predictor of negative receptor values in G3 carcinomas. The predictive value of DEL and TEL for the results of Ki-67 and EGFR immunostaining gradually decreases with increasing elastosis, consistent with the assumption that Ki-67 and EGFR identify the degree of tumour proliferation and invasion, while elastosis correlates with the degree of differentiation of breast cancer. Elastosis is a poor predictor of Ki-67 and EGFR findings in any individual breast cancer. Moderate and high-grade elastosis points to positive steroid hormone receptor assays in G1 and G2 carcinomas. In contrast, the lack of elastosis in G3 carcinomas may indicate a negative receptor assay. Both findings have a high degree of reliability.

Key words: Breast cancer – Elastosis – Steroid hormone receptors – Ki-67 – Epidermal growth factor receptor (EGFR)

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Introduction

Estimation of oestrogen and progesterone receptor activity by means of the dextran-coated charcoal assay (DCC) or by direct visualization of the receptor proteins by an immunocytochemical assay (ICA) are useful methods for planning therapy and for the prognostic evaluation of breast cancer patients. Elastosis in breast cancer tissue has importance as a clue for prognosis and some aspects of its relation to steroid hormone receptors are still a matter of debate. The present studies were performed to answer some of these questions, particularly with regard to the possible role of elastosis as a predictor of oestrogen receptor (ER) and progesterone receptor (PR) findings. A large number of invasive ductal breast carcinomas were examined by both biochemical and immunohistochemical receptor assays and the results compared to the two types of elastosis [ductal or periductal elastosis (DEL) and tumour elastosis (TEL)] in breast cancer tissue. Moreover, the results of Ki-67 and (EGFR) immunostaining in a number of cases have been related to elastosis.

Material and methods

A total of 1073 cases of invasive ductal breast cancer, NOS, observed at the Department of Pathology, Community Hospital Wiesbaden (Germany) from 1985 to 1992 were used in this study. The tumours were divided into three groups according to the histological classification of Bloom and Richardson (1957). The patients were divided into two groups (up to 50 years and older) in order to realize possible differences in pre- and postmenopausal women.

To estimate the extent of DEL and TEL, elastic fibres were stained by conventional elastica-van-Gieson staining of paraffin sections, 4 μm thick. In most cases, the sections contained both central and peripheral portions of the tumour. All sections were examined by the same author (W.R.) without knowledge of the receptor findings. DEL was graded as follows: 0, no elastosis; 1°, mild elastosis of at least three neoplastic ducts; 2°, moderate elastosis of at least three ducts; 3°, severe elastosis of at least three ducts, frequently involving the whole wall and its immediate neighbourhood. TEL was graded as follows: 0, no elastic fibres between

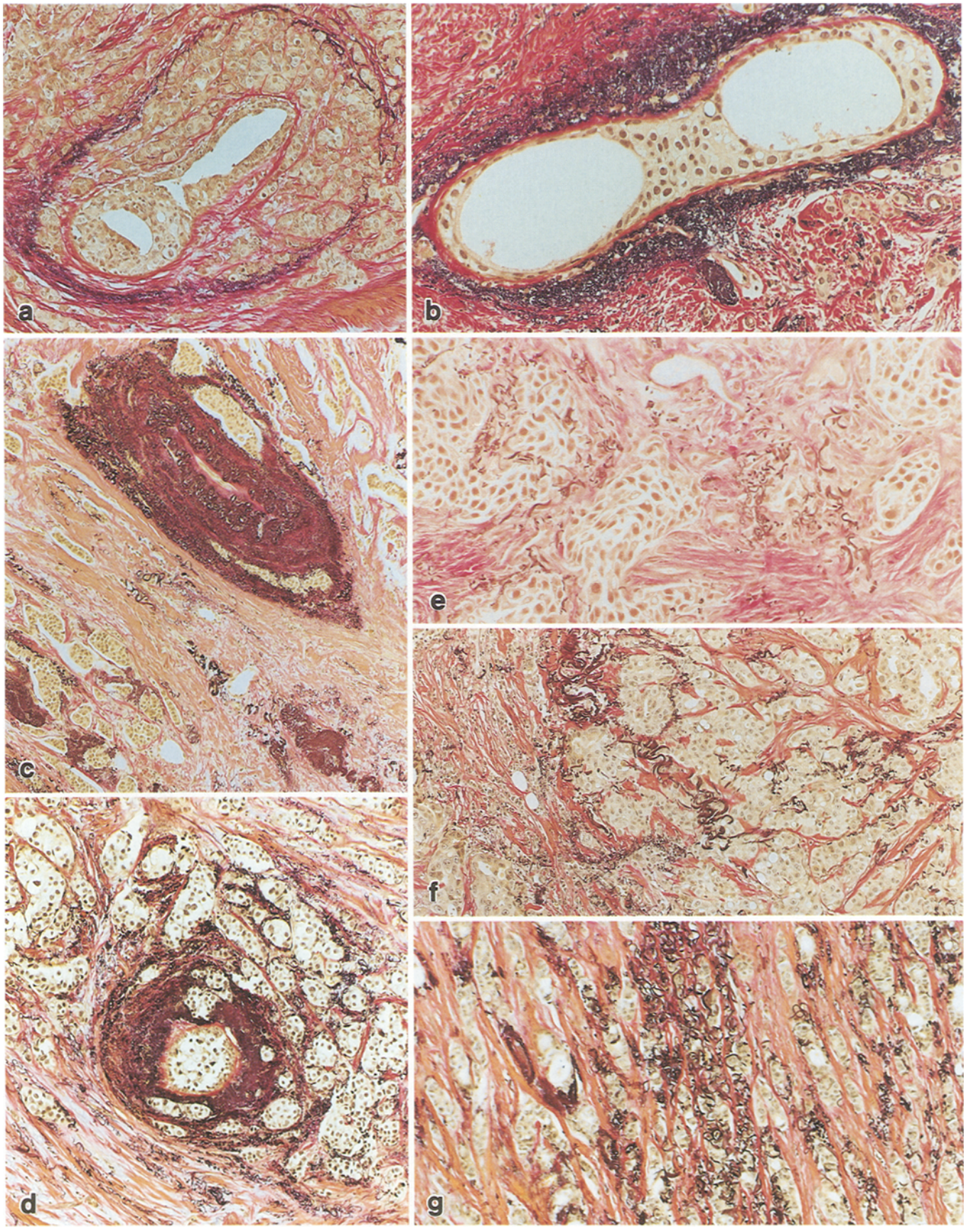

Fig. 1a–g. Different degrees of ductal/periductal elastosis (DEL) and tumour elastosis (TEL) in invasive ductal breast cancer. All stained with elastia-van Gieson. **a** DEL 1°. Mild elastosis of the periphery of a neoplastic duct. × 54. **b** DEL 2°. Moderate elastosis of the wall of a neoplastic duct. × 140. **c** DEL 3° and TEL 2°. Severe elastosis of a neoplastic duct (*top*) with compression of the lumen. Moderate elastosis between the invasive cancer cells (*bottom*). × 35. **d** DEL 3° and TEL 2°. Severe elastosis of a duct. Moderate elastosis between the tumour cells immediately around the duct. × 54. **e** TEL 1°. Mild elastosis between the invasive cancer cells. × 140. **f** TEL 2°. Moderate elastosis (increase of thin and coarse elastic fibres) between the cancer cells. × 54. **g** TEL 3°. Severe elastosis (mostly coarse elastic fibres) between the invasive cancer cells. × 54

Table 1. Sensitivity of elastosis: prediction of positive receptor findings from elastosis

Grade of tumour differentiation	Receptor assay	Ductal elastosis (DEL)			Tumour elastosis (TEL)		
		1°	2°	3°	1°	2°	3°
Patients ≤50 years							
G1	ER-DCC	64.7^{17}	66.7^{18}	85.7^{21}	70.8^{24}	70.0^{20}	88.9^{9}
	ER-ICA	88.9^{18}	84.2^{19}	90.9^{22}	92.0^{25}	82.6^{23}	100.0^{8}
	PR-DCC	70.6^{17}	70.6^{17}	95.2^{21}	70.8^{24}	85.0^{20}	100.0^{9}
	PR-ICA	76.5^{17}	94.4^{18}	91.5^{21}	87.0^{23}	87.0^{23}	100.0^{8}
G2	ER-DCC	65.8^{38}	81.4^{43}	78.6^{14}	75.0^{60}	78.3^{23}	100.0^{4}
	ER-ICA	65.9^{41}	69.6^{46}	75.0^{16}	69.7^{66}	66.7^{24}	80.0^{5}
	PR-DCC	57.9^{38}	67.4^{43}	92.9^{14}	63.3^{60}	78.3^{23}	75.0^{4}
	PR-ICA	66.7^{42}	76.1^{46}	93.8^{16}	72.3^{65}	79.2^{24}	80.0^{5}
G3	ER-DCC	38.1^{42}	70.0^{20}	88.9^{9}	52.3^{39}	83.3^{12}	100.0^{3}
	ER-ICA	35.6^{45}	52.2^{23}	100.0^{10}	47.6^{42}	92.3^{13}	100.0^{3}
	PR-DCC	42.8^{42}	55.0^{20}	100.0^{9}	53.9^{39}	83.3^{12}	100.0^{3}
	PR-ICA	33.3^{45}	43.5^{23}	90.0^{10}	43.9^{41}	76.9^{13}	100.0^{3}
Patients >50 years							
G1	ER-DCC	72.4^{29}	89.5^{76}	91.3^{92}	84.6^{65}	84.6^{65}	97.4^{39}
	ER-ICA	90.6^{32}	94.0^{83}	96.2^{106}	94.7^{75}	94.1^{85}	97.7^{44}
	PR-DCC	62.1^{29}	72.4^{76}	75.0^{92}	72.3^{65}	77.3^{75}	71.8^{39}
	PR-ICA	71.0^{31}	71.8^{78}	86.3^{102}	76.7^{73}	83.1^{83}	85.0^{40}
G2	ER-DCC	78.5^{65}	87.0^{108}	88.7^{124}	86.8^{106}	86.4^{110}	93.1^{58}
	ER-ICA	82.4^{74}	84.8^{118}	91.9^{135}	85.1^{114}	88.8^{125}	96.8^{62}
	PR-DCC	55.4^{65}	61.1^{108}	73.8^{124}	61.3^{106}	65.5^{110}	75.9^{58}
	PR-ICA	66.2^{74}	62.8^{113}	79.0^{133}	71.4^{112}	70.3^{121}	80.0^{60}
G3	ER-DCC	44.6^{56}	62.2^{45}	73.5^{34}	65.4^{52}	60.9^{46}	88.9^{9}
	ER-ICA	41.0^{61}	57.5^{47}	72.5^{40}	65.5^{58}	55.1^{59}	80.0^{10}
	PR-DCC	32.1^{56}	28.9^{45}	70.6^{34}	44.2^{52}	41.3^{46}	77.8^{9}
	PR-ICA	31.6^{57}	40.4^{47}	71.8^{39}	53.6^{56}	47.9^{48}	90.0^{10}

The first (large) number identifies the sensitivity. The second (small) number (top) identifies the total number of cases. ER, oestrogen receptor; PR, progesterone receptor; DCC, dextran-coated charcoal assay; ICA, immunocytochemical assay

the tumour cells; 1°, few (mostly thin, rarely coarse) elastic fibres between the tumour cells in up to 10% of the total slide; 2°, elastosis between the tumour cells in approximately up to 50% of the total slide; 3°, elastosis (frequently coarse elastic fibres) between the tumour cells in more than 50% of the total slide. Intra-observer variation was satisfactory; total agreement between the first and second examination was found in 77% for DEL and 81% for TEL. A difference of +/− one point was observed in 18% for DEL and 15% for TEL and a difference of +/− two points in 5% for DEL and 4% for TEL.

Biochemical receptor studies were performed by Bioscientia, Institut für Laboruntersuchungen, Ingelheim/Rh and by the Department of Experimental Endocrinology of the University Hospital for Gynaecology and Obstetrics, Mainz. Both laboratories take part in regular quality control studies. The preparation of tumour samples for DCC has been described earlier (Remmele et al. 1982). In the present study, tumours were classified as receptor-negative if the receptor values did not exceed 25 fmol/mg protein. Tumours with higher receptor values were classified as positive. No attempt was made towards further subclassification of positive tumours.

Immunohistochemical receptor studies were performed on cryostat sections from cancer tissue blocks cooled in ice and delivered to the Institute of Pathology within 1 h following removal from the patient. The antibodies were supplied by Abbott Laboratories [Chicago, Ill., (German branch: Wiesbaden-Delkenheim)]. The receptor content of the cryostat sections was graded from 0 to 12 as previously described (Remmele and Stegner 1987).

For Ki-67 immunohistochemistry the primary antibody (Ki-67 monoclonal mouse antibody for proliferating cells) was supplied by Dakopatts (Hamburg). Bridging antibody and ABC reagent came from Vectastain (Camon, Wiesbaden). Control slides consisted of normal mouse serum instead of primary antibody. Nuclei were evaluated as positive if they showed unequivocal light to dark brown staining. The percentage of positive cells was estimated as the average percentage of positive cells observed in the whole section (0, 5%; 10%; further 10% steps). Evaluation was always done by the same author (W.R.).

For EGFR immunohistochemistry the primary antibody was supplied by Amersham-Buchler (Braunschweig, FRG). Bridging antibody and ABC reagent were again from Vectastain. For control slides immunostaining for desmin was used (1:100) and the intrinsic control was the myoepithelial cells of the ducts present in the slides. Tumours were evaluated as negative or positive (grade 1–4) according to their overall staining intensity and the percentage of positive cells.

Sensitivity and specificity were calculated from the number of positive and negative cases according to the following definitions:

$$\text{Sensitivity} = \frac{\text{true positives}}{\text{true positives} + \text{false negatives}} \times 100$$

$$\text{Specificity} = \frac{\text{true negatives}}{\text{true negatives} + \text{false positives}} \times 100$$

Table 2. Specificity of elastosis: prediction of negative receptor findings from missing elastosis

Grade of tumor differentiation	Age (years)	Ductal elastosis (DEL)				Tumour elastosis (TEL)			
		ER-DCC	ER-ICA	PR-DCC	PR-ICA	ER-DCC	ER-ICA	PR-DCC	PR-ICA
G1	≤50	0^{3}	33.3^{3}	0^{3}	50.0^{2}	14.3^{7}	28.6^{7}	14.3^{7}	40.0^{5}
	>50	28.6^{7}	14.3^{7}	28.6^{7}	16.7^{6}	69.2^{26}	11.5^{26}	46.2^{26}	39.1^{23}
G2	≤50	27.3^{11}	38.5^{13}	63.6^{11}	38.5^{13}	43.5^{23}	42.3^{26}	52.2^{23}	40.7^{27}
	>50	33.3^{18}	36.9^{19}	44.4^{18}	55.6^{18}	39.6^{53}	36.8^{57}	52.8^{53}	55.6^{54}
G3	≤50	91.3^{23}	88.0^{25}	89.0^{23}	91.7^{24}	85.1^{47}	88.5^{52}	83.0^{47}	90.0^{50}
	>50	63.2^{19}	79.0^{19}	84.2^{19}	94.4^{18}	68.8^{48}	77.0^{52}	81.3^{48}	89.6^{48}

For layout and abbreviations see Table 1

Results

The sensitivity of elastosis (DEL and TEL) as a predictor of the biochemical and immunohistochemical receptor findings is summarized in Table 1. High-grade elastosis (DEL 3°, TEL 3°) predicts the receptor findings in G1 carcinomas with a sensitivity mostly exceeding 90, although it must be mentioned that some values, particularly in the younger age group, are based upon only few cases. In DEL 2° and TEL 2° sensitivity is also high and sometimes reaches comparable values to DEL 3° and TEL 3°. This is again true particularly for patients over 50 years old, but the lower number of cases in the younger patients does not permit final conclusions. Even mild elastosis (DEL 1°) may show a sensitivity of approximately 90 for ER-ICA in G1 carcinomas. As a general rule, sensitivity decreases with decreasing degree of tumour differentiation, some high values (up to 100) are probably due to the extremely low number of cases. In G2 carcinomas in older patients DEL 2°/3° and TEL 2°/3° predict the ER findings with a sensitivity of 85–97, and even DEL 1° and TEL 1° show a sensitivity between 79 and 87. In G3 carcinomas, no reliable prediction is possible. High sensitivity (up to 100) observed in patients up to 50 years old is based upon only a few cases.

In the younger age group, prediction of PR values is somewhat better than prediction of ER values, but again this may be due to the lower number of cases when compared with the older age group. In the patients over 50 years of age, sensitivity of elastosis for ER is usually better than for PR.

No significant differences were observed between the predictive value of DEL and TEL, although a tendency towards a higher sensitivity of TEL than for DEL may exist. The generally minor differences between the two age groups are probably due to the different number of cases.

Even if the sensitivity reaches values of 90 or more, the difference between this and 100 indicates the occurrence of tumours with 3° elastosis but without measurable receptor content. These cases will be further discussed below.

Calculation of specificity (Table 2) reveals low values for the prediction of receptor findings from DEL and TEL in G1 and G2 carcinomas ranging from 0 to 69 in G1 and from 27 to 64 in G2 tumours. Only in G3 carcinomas does sensitivity reach values from 83 to 92 in the younger patients and from 77 to 94 (exception: 63 for ER-DCC, DEL) in the older patients.

The prediction of DEL from TEL is possible in to 86–100% of cases irrespective of the grade of elastosis, the grade of tumour differentiation or the patients' age. With few exceptions, specificity increases from G1 to G3 carcinomas. Prediction of TEL from DEL is generally lower than prediction of TEL for DEL and progressively decreases from G1 to G3 carcinomas while specificity is higher than specificity of TEL for DEL. It also increases from G1 to G3 carcinomas.

Numerous studies have shown that the percentage of ER-positive tumours is higher in postmenopausal than in premenopausal women. The present findings confirm this observation (Table 3). The percentage of ER-positive tumours increases by 17.5% (ER-DCC) and 19.7% (ER-ICA). The postmenopausal increase of PR-positive cases is much lower (0.8% and 4.7%), again consistent with previous results in a smaller number of cases (Remmele, unpublished data, 1989). Other authors even report a postmenopausal decrease of the PR-DCC-positive cases (Thorpe and Rose 1986). DEL and TEL behave similarly to the ER values, showing an increase of 8.5% and 11.8%, respectively.

Cases with an entirely negative Ki-67 result were extremely rare and so the apparent sensitivity of DEL and TEL for Ki-67 ranges from 97.5 to 100 if "zero" cases are compared with positive cases. Specificity is also extremely low (0–1.8). No differences are observed between cases of different age, different tumour differentiation and varying degrees of elastosis. With regard to the biological importance of the Ki-67 antigen as an important marker of cellular proliferation, it appears highly improbable that a cut-off line separating approximately 98% of positive and 2% of negative cases would be consistent with the tumour biology. Further, the occasional occurrence of positively staining nuclei in the "zero" cases, particularly in other areas than those immunohistochemically examined, cannot be ruled out. Therefore, the cut-off value was placed at the 5% and 10% level of positively staining cells, respectively. At the 5% level, sensitivity is usually high, particularly in tumours without or with only mild elastosis (Table 4). Specificity (not mentioned in Table 4) is still very low,

Table 3. Percentage of positive elastosis and receptor cases in pre- and postmenopausal women

Elastosis type/ receptor assay	≤ 50 years		> 50 years		Difference >50/≤50 years (percent)
	Number of cases	Percent	Number of cases	Percent	
DEL	246	85.4	705	93.9	+ 8.5
TEL	212	70.4	631	82.2	+11.8
ER-DCC	166	60.4	537	77.9	+17.5
ER-ICA	180	60.2	607	79.9	+19.7
PR-DCC	162	58.9	412	59.7	+ 0.8
PR-ICA	177	60.6	477	65.3	+ 4.7

DEL, Ductal and periductal elastosis; TEL, tumour elastosis; others as Table 1

Table 4. Sensitivity of DEL and TEL for Ki-67 immunohistochemical findings

Ki-67 cut-off value	Age (years)	Ductal elastosis (DEL)			Tumour elastosis (TEL)		
		1°	2°	3°	1°	2°	3°
0	≤50	97.5^{79}	100.0^{68}	100.0^{39}	99.0^{97}	100.0^{45}	100.0^{12}
	>50	98.5^{135}	98.3^{181}	97.2^{212}	99.5^{195}	97.8^{182}	95.2^{82}
5%	≤50	87.3^{79}	80.9^{68}	71.8^{39}	85.6^{97}	80.0^{45}	58.3^{12}
	>50	84.4^{135}	72.4^{181}	61.8^{212}	75.4^{195}	65.4^{182}	61.0^{82}
10%	≤50	63.3^{79}	61.8^{68}	41.0^{39}	59.8^{97}	60.0^{45}	25.0^{12}
	>50	57.8^{135}	42.5^{181}	28.3^{212}	46.2^{195}	35.7^{182}	28.1^{82}

For explanation see Table 1

Table 5. Sensitivity of DEL and TEL for EGFR immunohistochemical findings

(EGFR) cut-off value	Age (years)	Ductal elastosis (DEL)			Tumour elastosis (TEL)		
		1°	2°	3°	1°	2°	3°
0*	≤50	51.5^{68}	42.6^{54}	29.6^{27}	46.9^{81}	27.0^{37}	14.3^{7}
	>50	56.5^{85}	42.1^{107}	48.1^{158}	44.3^{131}	51.3^{115}	44.1^{59}
1**	≤50	41.2^{68}	29.6^{54}	11.1^{27}	28.4^{81}	16.2^{37}	0^{7}
	>50	34.1^{85}	28.0^{107}	22.8^{158}	23.7^{131}	27.0^{115}	18.6^{59}
2***	≤50	25.0^{68}	13.0^{54}	0^{27}	16.1^{81}	0^{37}	0^{7}
	>50	18.8^{85}	9.4^{107}	8.2^{158}	9.2^{131}	9.6^{115}	5.1^{59}

*Negative = EGFR 0, positive = EGFR 1–4
**Negative = EGFR 0 and 1, positive = EGFR 2–4
***Negative = EGFR 0 to 2, positive = EGFR 3–4
For explanation, see Table 1

ranging from 9 to 13.3. At the 10% level, sensitivity decreases but specifity improves to values of 21.2 (DEL, younger age group) to 30 (TEL, patients of the older age group). Both cut-off values are associated with a continuous decrease of sensitivity from tumours with elastosis 1° to tumours with DEL 3° and TEL 3°. These findings confirm a negative correlation between the grade of elastosis and the Ki-67 findings: the higher the grade of elastosis, the lower the percentage of Ki-67-positive cells within the cancer tissue.

In examining elastosis and EGFR immunostaining it is evident that although the higher number of negative cases (29.4%) permits a cut-off value at the "zero" lvel, two other cut-off values were examined (1° and 2°; Table 5). Sensitivity is highest if the cut-off point is "zero", but specificity then ranges from 20.0 (DEL, younger patients) to 39.7 (TEL, older patients) only. If tumours with EGFR 1° are also considered to be negative, both sensitivity and specificity fall in the range of about 50 in elastosis-negative tumours. If the cut-off value is EGFR 2°, specificity increases to values ranging from 61.5 to 78.9, but sensitivity even in tumours with elastosis 1° is less than 20–30. Possibly these findings point to a "biological" cut-off value of EGFR "zero" to EGFR 1. The gradual decrease of EGFR with increasing elastosis is similar to the findings in Ki-67 immunostaining.

Discussion

Breast cancer tissue may contain large amounts of elastin within and around the neoplastic ducts and between the tumour cells. The chemistry and immunohistochemistry of the elastic material have been studied (Lundmark 1972; Davies and Mera 1987; Mera and Davies 1987). In spite of intense immunohistochemical and electron microscopic studies, however, the question remains open whether the material is produced by the tumour cells themselves or by fibroblasts and myofibroblasts of the tumour stroma stimulated by the cancer (Douglas and Shivas 1974; Tremblay 1974, 1976; Martinez-Hernandez et al. 1977; Reyes et al. 1982; Lima-de-Almeida et al. 1985; Bogomoletz 1986; Kao and Stern 1986; Mera and Davies 1987).

The possible relation of elastosis to steroid hormone receptor findings has been examined by numerous authors (Masters et al. 1976; Rolland et al. 1980; Fisher et al. 1981; Contesso et al. 1983; Underwood et al. 1983; Glaubitz et al. 1984; Mantouvalos et al. 1984; Montesco et al. 1984; Lima-de-Almeida et al. 1985; Rasmussen et al. 1985; Muresan et al. 1986; Reiner et al. 1988), but most papers are based upon low numbers of cases, and only few reports deal with PR and with immunohistochemical receptor findings. Moreover, a number of papers lack precise information concerning the type of elastosis (DEL or TEL). A statistical correlation between ER and elastosis is denied by Hanselmann and Genton (1983). Giri et al. (1987) describe a positive correlation of ER and elastosis only for the central portions of the tumour but deny a similar correlation of elastosis and the peripheral portions. Finally, Bell et al. (1986) did not find any correlation of ER and elastosis at all.

In view of these incomplete and sometimes controversial results, our studies are based upon an uncommonly high number of tumours, and the study was confined to the most frequent type of breast cancer subdivided according to the degree of tumour differentiation. DEL and TEL were examined separately, and both types of elastosis were compared with biochemical and immunohistochemical receptor findings. We found a high sensitivity of DEL and TEL for the biochemical and immunohistochemical receptor findings, particularly in highly differentiated carcinomas G1 with severe elastosis DEL 3° and/or TEL 3°. Moderate elastosis (DEL 2° and TEL 2°) still permits a good prediction of the receptor values in G1 carcinomas, at least in the patients older than 50 years of age, while the lower number of cases in patients beneath this age does not permit a conclusion. Elastosis 2° and 3° further predicts the ER findings in G2 carcinomas of the older age group with a sensitivity of 85–92 (DEL) and 86–97 (TEL). In G3 carcinomas, no reliable prediction appears possible since the high sensitivity of up to 100 is generally based upon only few cases, and the only subclass with a higher number of tumours (older patients, DEL) shows a sensitivity of only 71–74. In contrast, specificity is best in elastosis-free G3 tumours. Further, both DEL and TEL are useful variables in predicting receptor values although TEL apparently renders somewhat better results. Finally, in the older age group with the much higher number of cases, prediction of ER is generally better than prediction of PR.

Our results confirm previous reports on the correlation of elastosis and tumour differentiation (Lundmark 1972; Anastassiades et al. 1979; Rolland et al. 1980; Rasmussen et al. 1981, 1985; Howat et al. 1983; Baak and Persijn 1984; Glaubitz et al. 1984; Jacquemier et al. 1984; Urdiales-Viedma et al. 1986). However, our material contains some cases of G1 carcinomas with severe elastosis and negative receptor findings. Similar tumours have been described by Mantouvalos et al. (1984). Urdiales-Viedma et al. (1986) speculate that massive elastosis might result in destruction of epithelial cells due to either mechanical destruction or nutritional deficiency. Since elastosis may be triggered by ER- and PR-dependent cells (Rolland et al. 1980; Lima-de-Almeida et al. 1985; Rasmussen et al. 1985) another possible explanation is the existence of hormone-independent (myo)fibroblasts which may be influenced by other unknown factors towards enhanced elastin production or which might be able to produce elastin independently of any other stimuli.

According to our results in both age groups, DEL and TEL give a better prediction of the immunohistochemical (ER-ICA and PR-ICA) than the biochemical (ER-DCC and PR-DCC) receptor findings, at least in G1 carcinomas. Similar findings have been mentioned briefly by Reiner et al. (1988), who described a 97% agreement of ER-ICA and high-grade elastosis compared to an only 67% agreement of ER-DCC and high-grade elastosis. These observations may be explained by the fact that elastosis and immunohistochemical findings both represent morphological features of the tumour and hence may be better correlated than elastosis and biochemical assay because the DCC measures the functional ability of cancer cells to bind oestrogen.

The largely parallel increase of the percentage of elastosis and ER-positive cases in patients beyond 50 years of age may point to some biological correlation of elastosis and receptor content. It appears more probable, however, that both features are independent of each other. Usually, the postmenopausal increase of ER-positive cases is interpreted as a consequence of lower serum oestrogen levels leading to more available free receptor molecules. However, the increase of elastosis-positive cases may be due to less aggressive tumour growth in older patients favouring a more intense production of elastin by the tumour cells or the tumour stroma. The precise mechanism of enhanced elastin production in older patients remains poorly understood.

Our results are of practical value. Not all breast cancers are examined for receptor content, either by biochemistry or by immunohistochemistry. Moreover, carcinomas may be too small for biochemical receptor estimation, or they may be sent to the pathologist following fixation in formalin. Of course, such tumours may be

examined for their receptor content by means of immunohistochemistry on paraffin sections, but the method is not performed by many pathologists since it is expensive and time-consuming. In such cases, simple elastic staining of breast cancer specimens (at the centre and periphery of the tumour) may represent a simple and useful method, replacing the receptor assays with a high degree of reliability. Finally, the large number of cases examined in our study is apt to answer the relation of elastosis and receptor findings definitely, irrespective of its practical value.

To our knowledge, no other studies have been reported on the relationship of elastosis and Ki-67 and EGFR immunohistochemistry. Our findings are consistent with the observation that Ki-67 indicates the degree of tumour proliferation and EGFR the risk of tumour invasion. Tumours without elastosis show the highest sensitivity for both Ki-67 and EGFR while carcinomas with elastosis 3° show the lowest sensitivity. Our findings, if the cut-off point in the examination of Ki-67 is placed at the 5% or 10% level, are of particular interest in view of two recently published papers reporting a cut-off point of 14–19% Ki-67-positive cells in patients with good and poor prognosis (Wintzer et al. 1991) and a progressively worse prognosis in patients with less than 4%, 4–12% and 13% or more Ki-67-positive cells in breast cancer tissue (Sabin et al. 1991). Obviously, the biological cut-off point separating tumours with favourable and unfavourable prognosis may lie somewhere between 5% and 15% Ki-67-positive cells.

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