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PROGNOSTIC SIGNIFICANCE OF Ep-CAM AND Her-2/neu OVEREXPRESSION IN INVASIVE BREAST CANCER

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To assess the frequency and prognostic impact of Ep-CAM and Her-2/neu overexpression in patients with breast cancer and to determine its relationship with other prognostic markers, 205 breast cancer patients with a median follow-up of 10.8 years were enrolled in this retrospective study. Overexpression of Ep-CAM and Her-2/neu in tumor tissue samples was assessed by immunohistochemistry. Tumors presenting a Her-2/neu 2+ staining were additionally analyzed by FISH to exclude false positive results. Ep-CAM and Her-2/neu overexpression was found in 35.6% and 19.5% of the tumor samples, respectively. Both Ep-CAM and Her-2/neu overexpression were predictive for poor disease-free (DFS) and disease-related overall survival (DROS). Concurrent Ep-CAM and Her-2/neu overexpression was present in 13.2% of tumor specimens and had an additive negative impact on DFS and DROS. This minority of patients had a median time to relapse of only 34 months, whereas the median time to relapse was not reached in the patient population without Her-2/neu and Ep-CAM overexpression. By multivariate analysis Ep-CAM overexpression proved to be an indicator of poor prognosis, independent of tumor size, histologic grade, hormone receptor expression and Her-2/neu overexpression. In conclusion, overexpression of Ep-CAM and Her-2/neu complement each other as predictors for poor prognosis in patients with invasive breast cancer. Determination of these tumor markers should help in assigning breast cancer patients to 1 of 3 distinct risk categories.

Key words: *breast; cancer; Ep-CAM; Her-2/neu*

The search for new prognostic factors in breast cancer reflects the need for a more adequate risk assessment to select the most appropriate treatment modalities for individual patients. Ep-CAM (also called 17-1A, ESA, EGP40, 323/A3) is a 40-kDa transmembrane glycoprotein expressed on most human epithelial cells.[1] The encoding gene (GA733-2) has been sequenced and localized on chromosome 4.[2] Reportedly, Ep-CAM glycoprotein functions as a homotypic intercellular adhesion molecule.[3] In normal human mammary glands, Ep-CAM is mainly expressed in luminal epithelium.[4] Thus, tumors originating from luminal epithelium are likely to present Ep-CAM overexpression. We have recently reported that Ep-CAM overexpression, assessed by immunohistochemistry, in breast cancer predicts poor disease-free and overall survival,[5] corroborating findings from a previous study by Tandon *et al.*[6] using a western blotting technique.

Among a variety of prognostic factors, Her-2/neu (also known as c-erbB-2) has recently attracted major scientific interest. Her-2/neu oncogene amplification appears to be an important step in breast cancer tumorigenesis. The Her-2/neu gene encodes a transmembrane 185-kD protein that shows homology to the epidermal growth factor family.[7,8] Her-2/neu gene amplification correlates with overexpression of the Her-2/neu protein.[9] Gene amplification and overexpression of Her-2/neu protein has been reported in 20% to 30% of breast carcinomas.[10] Her-2/neu overexpression was found to be associated with poor grade of differentiation,[11,12] hormone receptor negativity,[13] and poor survival.[14–20] Patients with strong Her-2/neu expression seem to have a poorer response to hormonal agents such as tamoxifen[21] and a poorer response to non-anthracycline based chemotherapy.[22] Thus, Her-2/neu may become an important predictive marker in guiding therapeutic decisions. Recently, treatment with trastuzumab (Herceptin™), a humanized monoclonal antibody specific for Her-2/neu, either alone or in combination with chemotherapy has been shown to be efficacious in patients with Her-2/neu-overexpressing breast carcinomas.[23–25] To screen for the Her-2/neu status it is recommended to use immunohistochemistry. Tumors with 2+ staining should additionally be assessed by FISH for evaluating gene amplification. In tumors with 3+ staining no additional diagnostic benefit can be expected by using FISH.[26]

Ep-CAM can reportedly play a dual role in tumorigenesis.[27] Because Ep-CAM functions as an intercellular adhesion molecule, it has been claimed that overexpression could prevent shedding of cancer cells due to increased intercellular adhesion of tumor cells in primary lesions. This suggests that Ep-CAM overexpression might inhibit metastases. Only few studies support this hypothesis.[28,29] Ep-CAM overexpression was found to suppress cadherin-mediated cell adhesion.[30] It is known that E-cadherin functions as an invasion suppressor molecule.[31] Thus, Ep-CAM overexpression might promote tumor cell invasion and metastasis. Our results on the negative prognostic impact of Ep-CAM overexpression in breast cancer favor the latter hypothesis.[5] Studies in other epithelial cancers such as cervical cancer and hepatocellular carcinoma point to the same direction.[33,34] Furthermore, also Her-2/neu can disrupt the cadherin-catenin-mediated cell adhesion system,[35] suggesting a possible common molecular pathway of Ep-CAM and Her-2/neu.

Because Her-2/neu is commonly used in the hospital setting and because specific antibody therapies are available for these 2 particular markers we investigated in our study the relationship between Her-2/neu and Ep-CAM overexpression in breast cancer and the prognostic impact of both tumor markers.

PATIENTS AND METHODS

A total number of 205 patients were included in this retrospective study. This patient sample represents one-third of all cases with localized invasive breast cancer who were operated at the Department of Surgery, Innsbruck University Hospital, from 1980–91. All cases for which paraffin-embedded tissue samples were still retrievable from the local pathology repository and for

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which clinical follow-up data were available, were included. Only patients with lymph node status and without evidence of distant metastases at the time of primary surgery were eligible for this analysis. Otherwise, no case selection was evident. The median age of the patients was 54.2 years (range 29–85 years). Patients younger than 50 years were considered premenopausal. Of the women 108 (52.7%) were node-positive, 97 (47.3%) node-negative. After primary surgery the clinical status was documented by re-evaluating each patient at least once annually at the Department of Surgery. The evaluation procedure included physical examination, mammography, abdominal ultrasound and chest radiography. The median follow-up time was 10.8 years (range 36–240 months). During this observation period 96 patients relapsed. As first event, 20 patients had locoregional recurrence and 76 patients presented with distant metastases. Median time to relapse of patients with locoregional recurrence was 31 months (range 7–127 months). Median time to relapse of patients with distant metastatic disease was 32 months (range 5–200 months). From a total of 96 deaths, 85 were tumor related, while 11 patients died without documented disease recurrence. The latter cases were excluded from overall survival analysis. In the group of patients who died from breast cancer, 15 presented with locoregional recurrence and 70 with distant metastases as first event. Median survival time after disease recurrence in patients with locoregional recurrence was 21 months (range 3–99 months), of patients with distant metastases 18 months (range 0–106 months). Eighty-five (41.5%) patients received documented systemic adjuvant therapy (hormonal therapy or chemotherapy).

Histopathology

All tumor samples were formalin-fixed, embedded in paraffin wax and stored at the local pathology repository for varying periods of time. The storage time for paraffin-embedded tumor specimens did not differ between tumor samples with (13.1 years) and without Her-2/neu overexpression (13.6 years) or between tumor samples with (13.1 years) and without (13.7 years) Ep-CAM overexpression. Hematoxylin- and eosin-stained slides were prepared from each tumor specimen using routine methods and then examined by light microscopy. Histologic type and tumor grade were assessed by one co-author (P.O.) in a blinded fashion using standard pathology criteria.

Immunohistochemistry

Ep-CAM and Her-2/neu overexpression were determined by immunohistochemistry using the murine monoclonal antibodies ESA (NovoCastra, Medac GmbH, Hamburg, Germany) and c-erbB-2/Her-2/neu (NeoMarkers, Laborchemie, Vienna, Austria), respectively. Briefly, 5-μm sections were cut from paraffin-embedded tissue blocks, mounted on adhesive-coated glass slides, deparaffinized and rehydrated. Endogenous peroxidase was blocked with methanol containing hydrogen peroxide 30% over 20 min. Pretreatment consisted in a 20 min incubation period in a pronase solution for Ep-CAM and 15 min incubation period in a water bath at 80°C for Her-2/neu. After washing in Tris buffer, slides were incubated over 30 min at room temperature with the primary antibody (ESA and c-erbB-2/Her-2/neu, dilution 1:50). Afterwards a peroxidase-conjugated goat anti-mouse antibody ready for use (EnVision™, DAKO, Vienna, Austria) was added over 60 min for immunostaining. Slides were then placed into the chromogen that consisted of diaminobenzidine solution containing hydrogen peroxide 30%. Finally, slides were counterstained with Mayer's Hemalum solution. Positive and negative controls were included in each run. All cases that presented a 2+ staining for Her-2/neu where re-evaluated by FISH to exclude false-positive results. The Neomarkers Her-2/neu antibody was compared to the FDA-approved reagent from the DAKO HercepTest™ on control slides, consisting of three pelleted, formalin-fixed and paraffin-embedded human breast cancer cell lines with staining intensity scores of 0, 1+ and 3+ (supplied in the HercepTest™ kit).

FISH assay

Tumor specimens were cut, mounted on coated glass slides, deparaffinized, rehydrated and air dried. Pretreatment consisted in immersion of slides in 0.2 N HCl over 20 min. Further, manufacturer's instructions were followed using manufacturer's solutions (Vysis, Inc.) After pretreatment slides were dehydrated by soaking in 70%, 85% and 100% ethanol for 2 min each and air dried.

A total of 10 μl of PathVysion Her-2 DNA probe mixture (Vysis, Inc. Abbott, Vienna, Austria) containing a Her-2 DNA probe (190-kb Spectrum-Orange directly labeled DNA probe) and the CEP 17 DNA probe (5.4-kb SpectrumGreen directly labeled fluorescent DNA probe specific for the α satellite DNA sequence) was applied to the target area. After coverslipping, the slides were placed in the HYBrite (Vysis, Inc.) unit. Denaturation was performed at 85°C for 5 min and hybridization at 37°C for 20 hr. To remove non-specifically bound probe, after removing coverslips slides were placed in a solution containing SSC and NP40 at 73°C over 2 min, then air dried in the dark and 10 μl of DAPI counterstain was applied. Thereafter, sections were coverslipped and sealed.

Evaluation of slides

Ep-CAM and Her-2/neu overexpression was evaluated by 2 independent assessors (G.S., P.O.) using light microscopy. Reading of tissue slides was blinded and both assessors were unaware of clinical outcome. Antigen expression was defined as the presence of specific staining on the surface membranes of tumor cells. Ep-CAM overexpression was evaluated by calculating a total immunostaining score as the product of a proportion score and an intensity score. The proportion score described the estimated fraction of positive stained tumor cells (0, none; 1, <10%; 2, 10–50%; 3, 50–80%; 4, >80%). The intensity score represented the estimated staining intensity (0, no staining; 1, weak; 2, moderate; 3,

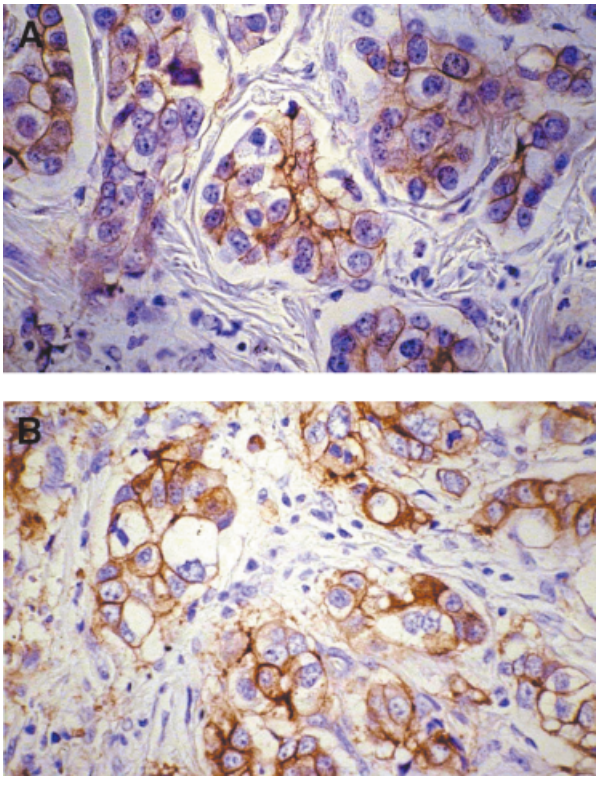

FIGURE 1 – Example of a tumor sample overexpressing both Ep-CAM (*a*, score 9) and Her-2/neu (*b*, 3+ staining). Invasive ductal carcinoma, pN1, pT2, Grade III, ER positive and PR negative.

TABLE I – RELATIONSHIP OF EP-CAM AND HER-2/NEU OVEREXPRESSION AND CONVENTIONAL CLINICAL AND TUMOR PARAMETERS

Characteristics	Patients	Her-2/neu overexpression			Ep-CAM overexpression		
		No	Yes	p^1	No	Yes	p^1
Age at diagnosis							
<50	91	74 (81%)	17 (19%)	0.789	59 (65%)	32 (35%)	0.905
≥50	114	91 (80%)	23 (20%)		73 (64%)	41 (36%)	
Histological type							
Ductal	144	110 (76%)	34 (24%)	0.061	84 (58%)	60 (42%)	0.020
Lobular	40	37 (93%)	3 (7%)		32 (80%)	8 (20%)	
Other types	21	18 (86%)	3 (14%)		16 (76%)	5 (24%)	
Histologic grade							
I	12	12 (100%)	0 (0%)	<0.001	9 (75%)	3 (25%)	0.001
II	124	108 (87%)	16 (13%)		90 (73%)	34 (27%)	
III	62	40 (65%)	22 (35%)		28 (45%)	34 (55%)	
NE[2]	7						
Nodal status							
pN0	97	81 (84%)	16 (16%)	0.302	68 (70%)	29 (30%)	0.105
pN1/2/3	108	84 (78%)	24 (22%)		64 (59%)	44 (41%)	
Tumor size							
<2cm	78	66 (85%)	12 (15%)	0.104	54 (69%)	24 (31%)	0.086
2–5cm	97	79 (81%)	18 (19%)		59 (61%)	38 (39%)	
>5cm	9	5 (56%)	4 (44%)		3 (33%)	6 (67%)	
Unknown[3]	21						
Estrogen receptor							
ER neg: 0–9 fmol	42	27 (64%)	15 (36%)	0.001	25 (60%)	17 (40%)	0.547
ER pos: >9 mol	133	115 (86%)	18 (14%)		86 (65%)	47 (35%)	
Unknown[3]	30						
Progesterone receptor							
PR neg: 0–9 fmol	60	41 (68%)	19 (32%)	0.002	36 (60%)	24 (40%)	0.496
PR pos: >9 fmol	115	101 (88%)	14 (12%)		75 (65%)	40 (35%)	
Unknown[3]	30						

[1]χ^2 test.–[2]Not evaluable.–[3]Unknown cases are excluded from *p*-value calculation.

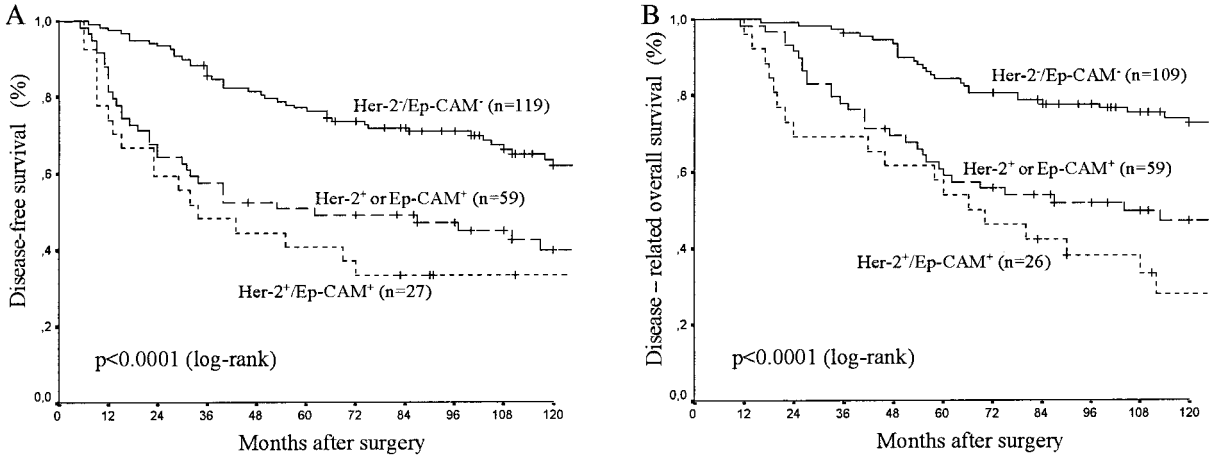

FIGURE 2 – Relationship between Ep-CAM and Her-2/neu overexpression and disease-free survival (DFS) (*a*) and disease related overall survival (DROS) (*b*). DROS was calculated on patients that died with documented disease recurrence ($n = 194$). Ep-CAM$^+$/Her-2$^+$: Tumors overexpressing both antigens. Ep-CAM$^+$ or Her-2$^+$: Tumors overexpressing only 1 of the 2 antigens. EP-CAM$^-$/Her-2$^-$: Tumors without overexpression of the antigens.

strong). The total score ranged from 0–12. Regarding the total score the tissue samples were bimodally distributed with the nadir at a total score of 3–4. Therefore, Ep-CAM 'overexpression' was arbitrarily defined as a total score >4. Her-2/neu overexpression was defined as tumors presenting at least weak to moderate staining of the entire membrane in more than 10% of the tumor cells. These criteria were in accordance with the scoring system of the DAKO HercepTest™. The cases that presented a 2+ staining were additionally assessed by FISH by one observer (C.E.) using a fluorescence microscope (Olympus BX50 with fluorescence unit.) Each slide was analyzed using DAPI and single (orange and green) bandpass filters. All cases with more than a mean number of 4 fluorescence signals per 2 signals of the centromere of chromosome 17 were considered amplified. Tumors were considered negative when less than 10% of tumor cells exhibited gene amplification.

Statistical methods

Statistical analysis was performed with the SPSS software program for Windows™. The primary end points in our study were disease-free survival (DFS) and disease-related overall survival (DROS). Thus, survival curves were calculated according to the method of Kaplan and Meier. *p*-Values were evaluated using the log-rank test for censored survival data. Follow-up time was censored if the patient was lost to follow-up. Patients who died without documented disease recurrence were considered censored

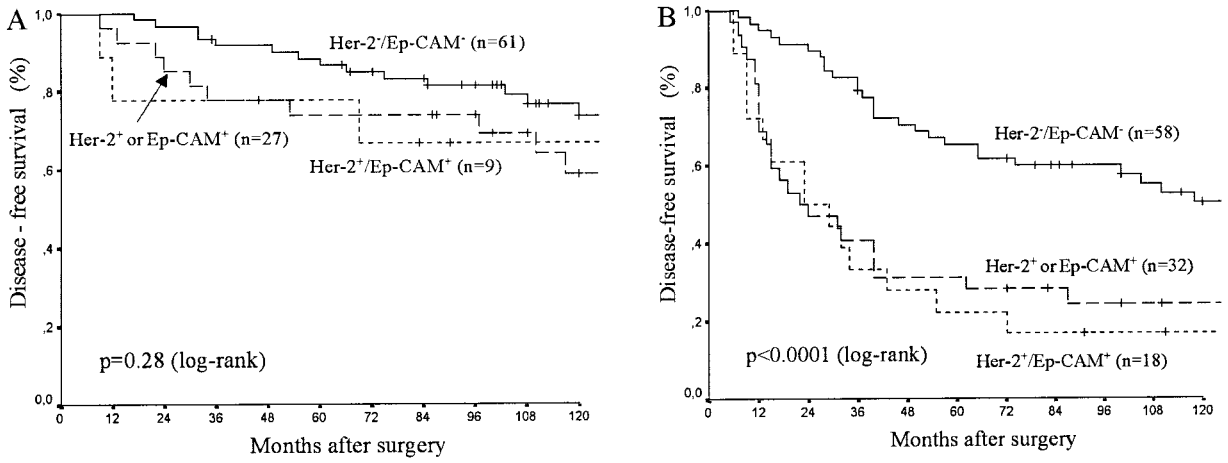

FIGURE 3 – Kaplan-Meyer analysis of disease free survival (DFS) in the subgroup of lymph node negative (*a*, $n = 97$) and lymph node positive (*b*, $n = 108$) patients. Ep-CAM$^+$/Her-2$^+$: Tumors overexpressing both antigens. Ep-CAM$^+$ or Her-2$^+$: Tumors overexpressing only one of the two antigens. EP-CAM$^-$/Her-2$^-$: Tumors without overexpression of the antigens.

TABLE II – SEPARATE PRESENTATION OF CLINICAL AND TUMOR CHARACTERISTICS OF PATIENTS WITH OVEREXPRESSION OF BOTH EP-CAM ANF HER-2/NEU

Characteristics	Patients	Her-2$^+$/Ep-CAM$^+$
Histological type		
ductal	144	23
lobular	40	2
other types	21	2
Histologic grade		
I	12	
II	124	9
III	62	16
NE[1]	7	2
Nodal status		
pN0	97	9
pN1/2/3	108	18
Tumor size		
<2cm	78	9
2–5cm	97	13
>5cm	9	4
unknown[2]	21	1
Estrogen receptor		
ER neg: 0–9 fmol	42	13
ER pos: >9 mol	133	11
unknown	30	3
Progesterone receptor		
PR neg: 0–9 fmol	60	15
PR pos: >9 fmol	115	9
unknown[2]	30	3

[1]Not evaluable-[2]Not reported on data files.

for DFS and were excluded from overall survival analysis. The relationship between antigen overexpression and other clinical or tumor parameters was calculated with the χ^2 test. To determine the relative importance of HER-2/neu and Ep-CAM overexpression and established prognostic markers, these variables were subjected to multivariate analysis (Cox regression). For multivariate analysis histological grade had to be re-categorized due to the small number of events in the group of Grade 1 tumors. Thus, Grade 1 and 2 tumors were taken together and compared to Grade 3 tumors.

RESULTS

Prognostic significance of Ep-CAM overexpression

Ep-CAM antigen was found to be overexpressed (Fig. 1*a*) in 73 (35.6%) of the 205 primary tumor samples. Ep-CAM overexpression correlated significantly with poor DFS ($p < 0.0001$) and DROS ($p = 0.0001$). In univariate analysis, Ep-CAM overexpression was associated with histologic grade ($p = 0.001$), histologic subtype ($p = 0.02$) and Her-2/neu overexpression ($p < 0.001$; χ^2 test) but not with other clinical or tumor parameters (Table I). When normal tissue was present on evaluated tissue slides, a weak to moderate staining of Ep-CAM could be seen predominantly in luminal epithelium of mammary glands. Only few cases presented a strong Ep-CAM expression in benign epithelium.

Prognostic significance of Her-2/neu overexpression

The Neomarkers antibody gave identical staining results in terms of intensity and staining pattern as compared to the DAKO antibody. On cells with strong Her-2/neu expression, both antibodies yielded a 3+ staining, whereas on cells with low Her-2/neu expression, both antibodies gave a 1+ staining. Further, cells with no Her-2/neu expression stained negatively with both antibodies. Her-2/neu overexpression (Fig. 1*b*) according to the criteria defined by the DAKO HercepTest™ was found in 49 of 205 tumor specimens. Tumors presenting a 2+ staining (15 cases) were re-evaluated by FISH. Of these tumor samples 6 cases showed a Her-2/neu gene amplification confirming Her-2/neu overexpression. Taken together, overexpression of Her-2/neu was found in 40 of 205 (19.5%) tumor specimens and correlated with poor DFS ($p = 0.001$; log-rank) and DROS ($p < 0.0003$; log-rank). By univariate analysis Her-2/neu overexpression was associated with poor histologic grade ($p < 0.001$), with lack of estrogen receptor ($p = 0.001$) and progesterone receptor expression ($p = 0.002$). No relationship was found with other clinical or tumor parameters such as age, nodal status, tumor size and histologic subtype (Table I).

Relationship between Her-2/neu and Ep-CAM overexpression

Despite the finding that in univariate analysis there was a significant relationship between Her-2/neu and Ep-CAM overexpression ($p < 0.001$; χ^2 test), both markers only partially overlapped. In fact, only 27 (13.2%) of the tumor samples showed overexpression of both markers, whereas 119 (58%) were negative for Her-2/neu and Ep-CAM overexpression. 46 (22.4%) of tumor samples showed Ep-CAM overexpression and Her-2/neu negativity. Thirteen (6.3%) tumor samples revealed Her-2/neu overexpression and Ep-CAM negativity. Regarding the prognostic impact of Her-2/neu and Ep-CAM, the patient sample was stratified into 3 distinct subgroups with different prognosis (Fig. 2). Concerning DFS (Fig 2*a*) and DROS (Fig 2*b*), patients with tumors overexpressing both antigens had the worst prognosis. Median time to relapse and median overall survival time in this patient population

TABLE III – MULTIVARIATE ANALYSIS OF VARIOUS PROGNOSTIC MARKERS INCLUDING EP-CAM AND HER-2/NEU[1]

	DFS			DROS		
	p	RR	95% CI	*p*	RR	95% CI
Nodal status	0.002	2.3	1.4–3.8	<0.001	3.1	1.7–5.6
Ep-CAM overexpression	0.004	2.1	1.3–3.6	0.02	2.0	1.1–3.5
Tumor size						
<2cm *vs.* 2–5cm	NS			NS		
<2cm *vs.* >5cm	0.001	4.2	1.8–10.0	0.002	4.0	1.6–9.9
Histological grade I+II *vs.* III	NS			0.05	1.8	1.0–3.1
Progesterone receptor	0.04	1.8	1.0–3.0	NS		
Estrogen receptor	NS			NS		
Her-2/neu overexpression	NS			NS		

[1]Histologic grade had to be re-categorized due to small number of events in grade I tumors. DFS, disease-free survival; DROS, disease-related overall survival; RR, relative risk; CI, confidence interval; NS, not signifigant.

were 34 months and 66 months, respectively. Patients overexpressing only 1 of the antigens had an intermediate prognosis with a median time to relapse of 62 months and a median survival time of 104 months. Tumors without Her-2/neu and Ep-CAM overexpression had the best prognosis and median time to relapse and median survival time were not reached. Notably, in node negative cases, Her-2/neu and Ep-CAM overexpression was not discriminating for survival (Fig. 3*a*). In the subgroup of lymph node positive patients, the median disease-free survival time was 23 months for tumors overexpressing both antigens, 22 months for tumors overexpressing only 1 antigen and 127 months for tumors without Her-2/neu and Ep-CAM overexpression (Fig. 3*b*). In the patient cohort receiving systemic adjuvant therapy (hormonal or chemotherapy), tumors with Ep-CAM and Her-2/neu overexpression, Ep-CAM or Her-2/neu overexpression and no Ep-CAM and Her-2/neu overexpression had a median disease-free survival of 23, 24 and 127 months, respectively. The clinico-pathological characteristics of tumors with overexpression of both antigens ($n = 27$) are presented in Table II.

Multivariate analysis

Multivariate analysis for DROS revealed that nodal status, Ep-CAM overexpression, tumor size and histological grade were significant prognostic factors. Hormone receptor expression and Her-2/neu overexpression were not significant predictors of DROS. For DFS, nodal status, Ep-CAM overexpression, tumor size and progesterone receptor expression were significant prognostic factors. Her-2/neu overexpression, histologic grade and estrogen receptor expression had no prognostic value for disease-free survival (Table III).

DISCUSSION

In our study, we show for the first time that simultaneous Her-2/neu and Ep-CAM overexpression occurs in about 13.2% of patients with invasive carcinoma of the breast. Immunohistochemical assessment of these 2 antigens helps in assigning breast cancer patients to distinct prognostic subgroups. In a recent study, we focused particularly on the frequency and prognostic value of Ep-CAM overexpression.[5] The Ep-CAM glycoprotein is known to mediate Ca^{2+}-independent homotypic cell-cell adhesions,[3,36] but the definite role of Ep-CAM in breast cancer tumorigenesis and progression remains elusive. The reason for Ep-CAM overexpression in tumor cells has not been reported so far. Studies are in progress at our institute to assess various mechanisms of Ep-CAM overexpression such as gene amplification, enhanced transcription, increased mRNA stability or a prolonged half-life of the Ep-CAM glycoprotein.

Slamon *et al.*[14] were the first to show the negative impact of Her-2/neu gene amplification on disease-free and overall survival in breast cancer. Numerous studies have confirmed their findings using different methods for the detection of Her-2/neu gene amplification and overexpression.[15–20,37,38] Due to its broad availability, immunohistochemistry using various Her-2/neu-specific antibodies was the preferred technique for determining the Her-2/neu status. In our study, we used the murine monoclonal antibody c-erbB-2/Her-2/neu (diluted 1:50), which gave identical staining results in terms of intensity and staining pattern as compared to the FDA-approved reagent from the DAKO HercepTest™. Cases with 2+ expression were additionally tested by FISH, as suggested by Lebeau and coworkers,[26] to exclude false positive results in this group. Overall, Her-2/neu overexpression was found in 19.5% of tumor samples. Moreover, our study confirms the prognostic significance of Her-2/neu overexpression for DFS and DROS. The relationship of Her-2/neu immunopositivity to poor histological grade and with absent or low progesterone and estrogen receptor expression was also reported previously.[11–13]

Because accepted guidelines for the definition of Ep-CAM are lacking, Ep-CAM overexpression was calculated as the product (total score) of an intensity score and a proportion score. The cut-off point of the total score for antigen overexpression (>4) was set at the nadir of a bimodal distribution pattern of total scores among the 205 tumor specimens.

The relationship between Ep-CAM overexpression and clinical outcome in breast cancer is in accordance with data reported by Tandon *et al.*[6] In our study, however, Ep-CAM overexpression was an independent prognostic marker by multivariate analysis. Furthermore, Tandon *et al.*[6] reported a relationship between Ep-CAM expression, tumor size and progesterone receptor expression, but unlike us not with histological grade, histological type or Her-2/neu overexpression. Future retrospective and prospective studies on large patient cohorts with standardized detection systems for Ep-CAM will be necessary to solve these discrepancies.

The main finding of our study is that simultaneous Her-2/neu and Ep-CAM overexpression in breast cancer has an additive negative impact on survival. Given that, as described previously, Ep-CAM overexpressing tumors originate from luminal epithelium[4] and tumors that originate from luminal epithelium are Her-2/neu negative,[39] a co-overexpression of these 2 markers in the same tumor type appears unlikely. Our study shows, however, that co-overexpression of both antigens occurs in 13.2% of tumor samples, defining a patient population with highly aggressive breast cancer. In conclusion, using Her-2/neu and Ep-CAM overexpression as prognostic markers, breast cancer patients can be stratified in a low, intermediate and high risk population.

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