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► To cite this version:

Giuseppe Viale, Nicole Rotmensz, Patrick Maisonneuve, Luca Bottiglieri, Emilia Montagna, et al..
Invasive ductal carcinoma of the breast with the “triple-negative” phenotype: prognostic implications of EGFR immunoreactivity. *Breast Cancer Research and Treatment*, 2008, 116 (2), pp.317-328.
10.1007/s10549-008-0206-z . hal-00478271

HAL Id: hal-00478271

<https://hal.science/hal-00478271>

Submitted on 30 Apr 2010

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Invasive ductal carcinoma of the breast with the “triple-negative” phenotype: prognostic implications of EGFR immunoreactivity

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Received: 4 April 2008 / Accepted: 19 September 2008 / Published online: 7 October 2008
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Abstract Invasive ductal carcinomas (IDC) of the breast with the triple negative phenotype (steroid hormone receptor absent, negative HER2 status) are characterized by poor clinical outcome. Additional tumor markers might allow identification of patients at higher risk. We evaluated clinical and biological features of 284 consecutive patients with pT1-3, pN1-3 M0 triple-negative IDC. Median follow-up was 70 months (interquartile range 59–94 months). Statistically significant worse disease-free and overall survival were observed in multivariate analysis, for patients with EGFR immunoreactivity in $\geq 50\%$ invasive tumor

cells (HR 2.39, 95% CI, 1.32–4.34, $P = 0.004$ for DFS; HR 2.34, 95% CI, 1.20–4.59 $P = 0.01$ for OS). Age ≥ 70 years and PVI were additional independent predictors of reduced overall survival. EGFR immunoreactivity significantly correlates with worse prognosis in patients with triple-negative IDC, supporting further studies on the correlation between the degree of EGFR expression and outcome of triple negative breast cancer.

Keywords Breast cancer · Triple negative · EGFR · Adjuvant treatment

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Introduction

Recent studies of gene expression profiling have led to the identification of breast cancer subtypes based on common molecular features [1, 2]. The basal like group is composed almost entirely of the so-called “triple negative” cancers, characterized by the lack of any estrogen (ER) and progesterone receptor (PgR) immunoreactivity and of HER2/neu over-expression.

Positive markers of this group of tumors are basal-cell cytokeratins including cytokeratins (CK) 5/6, 14 and 17 [3] which are normally found in the basal layer of stratified epithelia [2, 3]. Whether identified by the expression of basal immunohistochemical markers [4, 5] or by a basal-like RNA expression profile [6], these tumors represent about 15% of all breast cancers [7] and are characterized by an adverse clinical course, with an increased likelihood of disease recurrence and death [1, 8]. There is currently no specific targeted treatment for patients with triple-negative breast cancers, due to the lack of data on which to base treatment selection.

An easily obtainable immunohistochemical profile is the most suitable approach for the proper identification of

triple-negative breast cancers, but immunohistochemical markers are of little prognostic value for these tumors, largely because they have been assessed in old retrospective series with small sample sizes and collected over several years. Furthermore, it should be emphasized that triple-negative breast cancers currently include a heterogeneous group of tumors, and that the identification of tumor subtypes amenable to targeted treatments still represents a research priority. Immunohistochemical studies documented a high rate of epithelial growth factor receptor (EGFR; also known as HER1) expression in triple-negative breast cancers [8, 9]. In a recently published study, 57% of basal-like cancers overexpressed EGFR, compared with 8% ($P < 0.001$) of an independent series of cancers that were defined as non-basal-like by lack of CK 5/6 staining [9].

The aim of this retrospective study was to evaluate the possible prognostic role of a selected number of morphological and immunohistochemical features, according to REMARK recommendations [10]. We focused on the prognostic role of EGFR immunoreactivity in a large series of triple-negative breast cancer patients, who had a homogeneous diagnostic and therapeutic environment.

Materials and methods

Patients

For the present study, those patients with invasive ductal NOS breast cancer without any expression of ER and PgR and with no overexpression of HER2/neu were considered as eligible. Patients with invasive adenoid cystic, apocrine and typical medullary tumors were excluded from the analysis considering their peculiar clinicopathological features and favorable outcome [11–13]. Patients who presented with recurrent tumor, metastatic disease at presentation, non-invasive breast cancers, other previous tumor, bilateral tumors, or who had previously received neo-adjuvant treatment were also excluded.

All patients received adequate local treatment (breast conserving surgery or total mastectomy) with sentinel node (SLN) biopsy or complete axillary dissection. Patients with primary breast cancer were assigned to SLN biopsy in case of cytologically or histologically verified breast carcinoma 3 cm or less in size (measured clinically and/or by imaging techniques) and clinically uninvolved axillary lymph nodes. SLN biopsy was followed by axillary dissection only if the SLN contained metastasis or where there was minimal node involvement. The SLN was identified and isolated using a gamma probe as a guide, as previously published [14]. Postoperative breast irradiation (RT) was proposed to all patients who received breast-conserving surgery, excluding only those few elderly patients for

whom radiation was considered inappropriate [15]. Systemic adjuvant therapy was recommended according to recent St. Gallen Consensus Conferences Guidelines [16, 17]. Six months of adjuvant chemotherapy were considered for patients with triple-negative IDC. In particular, the selection of adjuvant chemotherapy was based upon indicators of risk. For patients with node-negative disease, classical CMF (oral cyclophosphamide, methotrexate and fluorouracil) for a duration of 6 cycles was considered [18]. For patients at higher risk (e.g., with tumors exhibiting PVI, pN1a disease) anthracycline containing chemotherapy was considered as the first option (e.g., AC, adriamycin and cyclophosphamide), for 4 cycles followed by classical CMF for three courses [19] or intensive CEF [20] according to the degree of patient risk.

Specimen characteristics and assay methods

Pathological assessment included evaluation of the primary tumor size, grade and histological type, and of lymph node status following axillary lymph node dissection or a SLN biopsy [21]. Tumor grade was evaluated according to Elston and Ellis [22] and peritumoral vascular invasion (PVI) was assessed according to Rosen et al. [23]. Central necrosis and fibrosis were assessed according to Tsuda et al. [24]. Estrogen (ER) and progesterone receptor (PgR) status, Ki-67 labeling index determined with the MIB1 monoclonal antibody, and HER2/neu overexpression were evaluated immunocytochemically as previously reported [25]. For evaluation of ER and PgR status and Ki-67 labeling index, the percentage of cells exhibiting definite nuclear staining over at least 2,000 neoplastic cells examined at 400× magnification was recorded. Only nuclear immunoreactivity was evaluated for ER, PgR, and MIB1. The threshold for the definition of triple-negative breast cancer was lack of any ER and PgR immunoreactivity and a 0–2+ scoring for HER2/neu as previously published [25].

Immunostaining for p63, CK 5/6, CK 14 and EGFR was performed using the following monoclonal antibodies: D5/16 B4 for CK5/6 (used at 1:100 dilution; Dako, Glostrup, Denmark); LL002 for CK 14 (at 1:80 dilution, NovoCastra, Newcastle Upon Tyne, UK); 4A4 for p63 (at 1:100 dilution, Dako) 31G7 for EGFR (at 1:20 dilution, Zymed Laboratories). Only cytoplasmic immunoreactivity was evaluated for CK 5/6 and 14, membrane and cytoplasmic for EGFR, and only nuclear immunoreactivity for p63. Immunohistochemical results for EGFR were recorded as the percentage of invasive tumor cells showing definite immunoreactivity for the corresponding antigen over at least 2,000 neoplastic cells examined at 400× magnification.

In accordance with Banerjee et al. [26], no threshold for cytokeratin positivity was used. Any definite

immunoreactivity in neoplastic cells was classified as positive. Similarly, any nuclear staining for p63 was considered positive in accordance with Ribeiro-Silva et al. [27].

Data were entered by surgeons into a ‘user-friendly’ Microsoft Access® database once weekly for a mean number of 25 patients per week, and checked by a data manager. The database was then used for an interdisciplinary discussion (among surgeons, medical and radiation oncologists and pathologists) resulting in the proposal for an adjuvant treatment program. Typically, a medical oncologist (and a radiation oncologist, where applicable) discussed the proposed treatment with the patient and verified the accuracy of the items entered into the database (internal quality control).

Study design

Information was collected on all consecutive breast cancer patients operated on at the European Institute of Oncology in Milan between April 1997 and December 2001. Data on the patient’s medical history, concurrent diseases, type of surgery, pathological assessment of morphological and biological features, and results of staging procedures (blood chemistry, hematological values, bone scan, chest film and upper abdominal ultrasound examination) were combined.

The primary endpoints were disease-free survival (DFS) and overall survival (OS). DFS was defined as the length of time from the date of surgery to any relapse (including ipsilateral breast recurrence), the appearance of a second primary cancer (including contralateral breast cancer), or death, whichever occurred first. OS was determined as the time from surgery until the date of death (from any cause) or the date of last follow-up. Secondary end points were distant metastasis free survival (DDFS), breast-related event-free survival (BREFS). BREFS was defined as the time from the date of surgery to any breast-related event.

Statistical analysis methods

The Mantel–Haenszel Chi-Square test for trend was used to assess the association between ordinal variables. Multivariate Cox proportional hazard regression analysis was used to assess the independent prognostic significance of various clinical and histopathological characteristics of the tumor on survival. Factors included in multiple regression analyses included age, pathological stage, degree of nodal involvement, PVI and EGFR expression set either as a categorical variable with three levels of expression (negative, 1–49, ≥ 50) or as a continuous variable with the hazard ratios given for a 10% increase of the expression.

All analyses were performed with the SAS software (Cary, NC). All tests were two-sided.

Results

Overall, 6,242 consecutive patients were referred for interdisciplinary evaluation between April 1997 and December 2001 and 4,993 had invasive, monolateral, non-metastatic, and untreated breast cancer.

For 2,177 of these patients HER2/neu was not determined, a further 2,481 patients had either endocrine responsive tumors and/or tumors overexpressing HER2/neu; 51 of the remaining patients had non ductal NOS tumors. Therefore, 284 patients with triple-negative IDC were eligible for the present analysis and their characteristics are shown in Table 1.

A total of 245 patients (86%) had tumors with a “basal-like” phenotype, based on immunoreactivity for p63, basal CKs and/or for EGFR. In particular, p63 immunoreactivity was documented in the tumors of 136 patients, CK14 in 153 cases, CK5/6 in 90 and EGFR in 163 cases, with 36 of the latter tumors exhibiting immunoreactivity in $\geq 50\%$ neoplastic cells. Tumors with the triple-negative phenotype were commonly of high grade (85% of patients) and had large size (> 2 cm, 54% of patients); 166 patients were classified pN0 whereas 115 patients had one to three metastatic axillary lymph nodes.

Treatments performed are shown in Table 2. Forty-eight patients had a total mastectomy as the primary treatment, 236 patients underwent breast conserving surgery and 86 had SLN biopsy. Radiotherapy was performed in 238 patients. The majority of the patients (263, 93%) were submitted to adjuvant chemotherapy. About half of them (138, 52%) were submitted to classical CMF (oral cyclophosphamide, methotrexate and fluorouracil) for a duration of six courses. Elderly patients compared with younger patients, were less likely to receive adjuvant chemotherapy as well as patients without PVI ($P < 0.0001$). No significant difference in the prescription of chemotherapy was observed according to EGFR expression (Table 2).

Analysis and presentation

Median follow-up was 70 months (interquartile range: 59–94 months). Types of events according to patient characteristics, and the event rates (defined as the incidence rate per 1,000 women/year) are shown in Table 3a.

We selected a cutoff of 50% membranous/cytoplasmic expression which corresponded to the 90th percentile of the distribution of EGFR in our series. The prognostic value of EGFR using other cutoff values (10 and 30%; Fig. 1) was

Table 1 Characteristics of 284 patients with triple-negative breast cancer and correlation with EGFR expression

| Characteristics ^a | All patients | EGFR expression | | | P-value |
|-------------------------------------|--------------|-----------------|-------------|------------|--------------|
| | | Negative | <50% | ≥50% | |
| All patients | 284 (100.0%) | 121 (42.6%) | 127 (44.7%) | 36 (12.7%) | |
| Age group | | | | | |
| <35 | 21 (7.4%) | 9 (42.9%) | 10 (47.6%) | 2 (9.5%) | |
| 35–49 | 126 (44.4%) | 49 (38.9%) | 63 (50.0%) | 14 (11.1%) | |
| 50–59 | 60 (21.1%) | 27 (45.0%) | 25 (41.7%) | 8 (13.3%) | |
| 60–69 | 54 (19.0%) | 22 (40.7%) | 22 (40.7%) | 10 (18.5%) | |
| 70+ | 23 (8.1%) | 14 (60.9%) | 7 (30.4%) | 2 (8.7%) | 0.50 |
| Tumor size (cm) | | | | | |
| <0.5 | 5 (1.8%) | 3 (60.0%) | 1 (20.0%) | 1 (20.0%) | |
| 0.5–1 | 25 (8.8%) | 8 (32.0%) | 13 (52.0%) | 4 (16.0%) | |
| 1–2 | 98 (34.5%) | 43 (43.9%) | 49 (50.0%) | 6 (6.1%) | |
| 2–5 | 139 (48.9%) | 56 (40.3%) | 59 (42.4%) | 24 (17.3%) | |
| >5 | 14 (4.9%) | 8 (57.1%) | 5 (35.7%) | 1 (7.1%) | 0.75 |
| PT | | | | | |
| pT1 | 130 (45.8%) | 55 (42.3%) | 63 (48.5%) | 12 (9.2%) | |
| pT2 | 138 (48.6%) | 56 (40.6%) | 59 (42.8%) | 23 (16.7%) | |
| pT3/4 | 13 (4.6%) | 7 (53.8%) | 5 (38.5%) | 1 (7.7%) | 0.24 |
| Number of positive nodes | | | | | |
| None | 166 (58.5%) | 71 (42.8%) | 79 (47.6%) | 16 (9.6%) | |
| 1 | 40 (14.1%) | 18 (45.0%) | 16 (40.0%) | 6 (15.0%) | |
| 2 | 20 (7.0%) | 10 (50.0%) | 8 (40.0%) | 2 (10.0%) | |
| 3+ | 55 (19.4%) | 22 (40.0%) | 22 (40.0%) | 11 (20.0%) | 0.065 |
| Tumor grade | | | | | |
| G1 | 2 (0.7%) | 1 (50.0%) | 0 (0.0%) | 1 (50.0%) | |
| G2 | 25 (8.8%) | 12 (48.0%) | 9 (36.0%) | 4 (16.0%) | |
| G3 | 241 (84.9%) | 98 (40.7%) | 113 (46.9%) | 30 (12.4%) | 0.27 |
| Proliferative fraction (Ki67) | | | | | |
| <50% | 131 (46.1%) | 58 (44.3%) | 51 (38.9%) | 22 (16.8%) | |
| ≥50% | 152 (53.5%) | 62 (40.8%) | 76 (50.0%) | 14 (9.2%) | 0.07 |
| PVI | | | | | |
| Absent | 212 (74.6%) | 88 (41.5%) | 100 (47.2%) | 24 (11.3%) | |
| Focal | 37 (13.0%) | 18 (48.6%) | 12 (32.4%) | 7 (18.9%) | |
| Diffuse | 32 (11.3%) | 14 (43.8%) | 14 (43.8%) | 4 (12.5%) | 0.56 |
| CA15.3 | | | | | |
| <16% | 71 (25.0%) | 30 (42.3%) | 31 (43.7%) | 10 (14.1%) | |
| ≥16% | 74 (26.1%) | 31 (41.9%) | 28 (37.8%) | 15 (20.3%) | 0.34 |
| “Basal like” phenotype ^b | | | | | |
| No | 36 (12.7%) | – | – | – | |
| Yes | 245 (86.3%) | – | – | – | |
| P63 | | | | | |
| Negative | 145 (51.1%) | 74 (51.0%) | 59 (40.7%) | 12 (8.3%) | |
| Positive | 136 (47.9%) | 44 (32.4%) | 68 (50.0%) | 24 (17.6%) | 0.012 |
| CK14 | | | | | |
| Negative | 128 (45.1%) | 66 (51.6%) | 45 (35.2%) | 17 (13.3%) | |
| Positive | 153 (53.9%) | 52 (34.0%) | 82 (53.6%) | 19 (12.4%) | 0.99 |
| CK5/6 | | | | | |
| Negative | 191 (67.3%) | 80 (41.9%) | 86 (45.0%) | 25 (13.1%) | |

Table 1 continued

| Characteristics ^a | All patients | EGFR expression | | | P-value |
|------------------------------|--------------|-----------------|-------------|------------|---------|
| | | Negative | <50% | ≥50% | |
| Positive | 90 (31.7%) | 38 (42.2%) | 41 (45.6%) | 11 (12.2%) | 0.84 |
| Type of surgery | | | | | |
| Breast conserving | 236 (83.1%) | 101 (42.8%) | 107 (45.3%) | 28 (11.9%) | 0.37 |
| Mastectomy | 48 (16.9%) | 20 (41.7%) | 20 (41.7%) | 8 (16.7%) | |

^a Information on tumor size, pT, pN, PVI, p63, CK14, CK5/6 and “basal like phenotype” are unknown for 3 patients, on tumor grade for 16 patients, on ki-67 expression for 1 patient and on CA15.3 for 139 patients

^b The “Basal like” phenotype corresponds to tumor expressing either CK14, CK5/6, EGFR or p63

Bold is statistically significant

evaluated and the prognostic value of EGFR as a continuous variable was also assessed (Table 4).

Four-year DFS for patients whose tumors showed EGFR immunoreactivity in <50 or ≥50% of the neoplastic cells was 79 versus 52% (log-rank $P < 0.0001$), and 4 years OS was 87 versus 81%, respectively, (log-rank $P = 0.0004$). Also, 4 year BREFS and DDFS were 81 versus 58%, (log-rank $P = 0.0002$) and 90 versus 68% (log-rank $P < 0.0001$) respectively.

Among the other clinico-pathological features investigated, tumor size, lymph node status, PVI, immunoreactivity for CK5/6 were correlated with DFS and/or OS (Table 3a).

Table 3b reports the results of the univariate analysis of event rates according to the type of treatments. Only the extent of surgery was significantly correlated with the risk

of BREFS, DFS and OS, while no correlation with the chemotherapy regimen was observed.

Multivariate analysis

We investigated the independent association between biological features and risk of relapse using the Cox proportional hazards regression analysis (Table 4). A statistically significant worse outcome was observed in multivariate analysis for patients with tumors exhibiting EGFR expression in ≥50% of the neoplastic cells versus the patients with tumors lacking any EGFR expression (HR 2.39, 95% CI, 1.32–4.34, $P = 0.004$ for DFS; HR 2.34, 95% CI, 1.20–4.59 $P = 0.01$ for OS; HR 3.39, 95% CI, 1.52–7.60, $P = 0.003$ for DDFS and HR 2.27, 95% CI,

Table 2 Treatment performed in 284 patients according to age, EGFR expression and perivascular invasion (PVI)

| | Surgery | | P-value | Radiotherapy | | P-value | Chemotherapy | | | | P-value |
|--------------|------------------|-------------------|---------|--------------|-------------|---------|--------------|-------------|-------------------|------------|---------|
| | Total mastectomy | Breast conserving | | No | Yes | | None | CMF × 6 | AC × 4 CMF × 3 | Other | |
| All patients | 48 (16.9%) | 236 (83.1%) | | 46 (16.2%) | 238 (83.8%) | | 21 (7.4%) | 138 (48.6%) | 38 (13.4%) | 87 (30.6%) | |
| Age group | | | | | | | | | | | |
| <35 | 3 (14.3%) | 18 (85.7%) | 0.08 | 2 (9.5%) | 19 (90.5%) | 0.17 | 0 (0.0%) | 12 (57.1%) | 4 (19.0%) | 5 (23.8%) | <0.0001 |
| 35–49 | 16 (12.7%) | 110 (87.3%) | | 17 (13.5%) | 109 (86.5%) | | 5 (4.0%) | 65 (51.6%) | 21 (16.7%) | 35 (27.8%) | |
| 50–59 | 14 (23.3%) | 46 (76.7%) | | 13 (21.7%) | 47 (78.3%) | | 5 (8.3%) | 33 (55.0%) | 6 (10.0%) | 16 (26.7%) | |
| 60–69 | 8 (14.8%) | 46 (85.2%) | | 9 (16.7%) | 45 (83.3%) | | 2 (3.7%) | 27 (50.0%) | 7 (13.0%) | 18 (33.3%) | |
| 70+ | 7 (30.4%) | 16 (69.6%) | | 5 (21.7%) | 18 (78.3%) | | 9 (39.1%) | 1 (4.3%) | 0 (0.0%) | 13 (56.5%) | |
| EGFR | | | | | | | | | | | |
| Negative | 20 (16.5%) | 101 (83.5%) | .37 | 19 (15.7%) | 102 (84.3%) | .71 | 10 (8.3%) | 60 (49.6%) | 15 (12.4%) | 36 (29.8%) | .47 |
| <50% | 20 (15.7%) | 107 (84.3%) | | 22 (17.3%) | 105 (82.7%) | | 6 (4.7%) | 64 (50.4%) | 16 (12.6%) | 41 (32.3%) | |
| ≥50% | 8 (22.2%) | 28 (77.8%) | | 5 (13.9%) | 31 (86.1%) | | 5 (13.9%) | 14 (38.9%) | 7 (19.4%) | 10 (27.8%) | |
| PVI | | | | | | | | | | | |
| Absent | 29 (13.7%) | 183 (86.3%) | 0.006 | 34 (16.0%) | 178 (84.0%) | .41 | 17 (8.0%) | 123 (58.0%) | 25 (11.8%) | 47 (22.2%) | <0.0001 |
| Focal | 9 (24.3%) | 28 (75.7%) | | 4 (10.8%) | 33 (89.2%) | | 4 (10.8%) | 11 (29.7%) | 6 (16.2%) | 16 (43.2%) | |
| Diffuse | 10 (31.3%) | 22 (68.8%) | | 8 (25.0%) | 24 (75.0%) | | 0 (0.0%) | 3 (9.4%) | 5 (15.6%) | 24 (75.0%) | |

BCS breast conserving surgery; CMF cyclophosphamide, methotrexate, 5-fluorouracil; AC adriamycin, cyclophosphamide

Table 3 Univariate analysis for distant disease-free survival, breast related event-free survival, disease-free survival and overall survival

| Characteristics ^a | All patients | Distant disease-free survival | | | Breast related event free survival | | | Disease free survival | | | Overall survival | | |
|--|--------------|-------------------------------|----------------------------|--------------|------------------------------------|----------------------------|-------------|-----------------------|----------------------------|-------------|------------------|----------------------------|-------------|
| | | Events | Event rate/100 women-years | Log-rank | Events | Event rate/100 women-years | Log-rank | Events | Event rate/100 women-years | Log-rank | Deaths | Death rate/100 women-years | Log-rank |
| <i>(a) According to clinical and biological features</i> | | | | | | | | | | | | | |
| All patients | 284 | 38 | 3.04 | | 71 | 5.68 | | 81 | 6.47 | | 57 | 3.32 | |
| Age group | | | | | | | | | | | | | |
| <35 | 21 | 3 | 4.05 | | 6 | 8.11 | | 6 | 8.11 | | 5 | 4.10 | |
| 35–49 | 126 | 14 | 2.43 | | 23 | 3.99 | | 28 | 4.86 | | 18 | 2.28 | |
| 50–59 | 60 | 5 | 1.84 | | 17 | 6.25 | | 18 | 6.62 | | 11 | 3.04 | |
| 60–69 | 54 | 10 | 4.29 | | 15 | 6.44 | | 18 | 7.73 | | 15 | 4.79 | |
| 70+ | 23 | 6 | 6.32 | 0.16 | 10 | 10.53 | 0.09 | 11 | 11.58 | 0.12 | 8 | 6.25 | 0.08 |
| Tumor size | | | | | | | | | | | | | |
| <0.5 cm | 5 | – | – | | 1 | 5.26 | | 1 | 5.26 | | 1 | 3.85 | |
| 0.5–1 cm | 25 | – | – | | 4 | 3.57 | | 6 | 5.36 | | 2 | 1.18 | |
| 1–2 cm | 98 | 10 | 2.25 | | 16 | 3.60 | | 17 | 3.83 | | 14 | 2.24 | |
| 2–5 cm | 139 | 24 | 4.04 | | 44 | 7.41 | | 50 | 8.42 | | 34 | 4.24 | |
| >5 cm | 14 | 3 | 4.17 | 0.13 | 4 | 5.56 | 0.14 | 5 | 6.94 | 0.08 | 4 | 4.71 | 0.13 |
| pT | | | | | | | | | | | | | |
| pT1 | 130 | 11 | 1.91 | | 23 | 3.99 | | 26 | 4.51 | | 19 | 2.31 | |
| pT2 | 138 | 23 | 3.88 | | 43 | 7.25 | | 49 | 8.26 | | 33 | 4.13 | |
| pT3/4 | 13 | 3 | 4.69 | 0.13 | 4 | 6.25 | 0.08 | 5 | 7.81 | 0.04 | 4 | 5.26 | 0.09 |
| Number of positive nodes | | | | | | | | | | | | | |
| None | 166 | 13 | 1.68 | | 31 | 4.01 | | 38 | 4.91 | | 25 | 2.39 | |
| 1 | 40 | 8 | 4.94 | | 12 | 7.41 | | 13 | 8.02 | | 10 | 4.39 | |
| 2 | 20 | 5 | 5.88 | | 6 | 7.06 | | 6 | 7.06 | | 4 | 3.54 | |
| 3+ | 55 | 12 | 5.50 | 0.005 | 21 | 9.63 | 0.01 | 23 | 10.55 | 0.03 | 17 | 5.57 | 0.05 |
| Tumor grade | | | | | | | | | | | | | |
| G1 | 2 | – | – | | – | – | | 0 | – | | 0 | – | |
| G2 | 25 | 4 | 3.51 | | 10 | 8.77 | | 10 | 8.77 | | 3 | 1.89 | |
| G3 | 241 | 33 | 3.13 | 0.86 | 58 | 5.51 | 0.36 | 68 | 6.46 | 0.51 | 51 | 3.53 | 0.41 |
| Proliferative fraction (Ki67) | | | | | | | | | | | | | |
| <50% | 131 | 19 | 3.28 | | 41 | 7.07 | | 44 | 7.59 | | 31 | 3.95 | |
| ≥50% | 152 | 19 | 2.86 | 0.72 | 30 | 4.52 | 0.06 | 37 | 5.57 | 0.17 | 26 | 2.81 | 0.23 |
| PVI | | | | | | | | | | | | | |
| Absent | 212 | 22 | 2.32 | | 46 | 4.85 | | 53 | 5.59 | | 35 | 2.68 | |
| Focal | 37 | 9 | 6.34 | | 12 | 8.45 | | 15 | 10.56 | | 12 | 5.83 | |
| Diffuse | 32 | 7 | 4.90 | 0.01 | 13 | 9.09 | 0.04 | 13 | 9.09 | 0.04 | 10 | 5.29 | 0.02 |
| CA15.3 | | | | | | | | | | | | | |
| <16% | 71 | 8 | 3.16 | | 12 | 4.74 | | 13 | 5.14 | | 11 | 2.96 | |
| ≥16% | 74 | 13 | 4.71 | 0.42 | 24 | 8.70 | 0.10 | 26 | 9.42 | 0.09 | 18 | 4.96 | 0.18 |
| “Basal like” phenotype ^b | | | | | | | | | | | | | |
| No | 36 | 4 | 2.47 | | 11 | 6.79 | | 11 | 6.79 | | 9 | 4.17 | |
| Yes | 245 | 33 | 3.06 | 0.70 | 58 | 5.37 | 0.43 | 68 | 6.30 | 0.77 | 46 | 3.09 | 0.38 |
| P63 | | | | | | | | | | | | | |
| Negative | 145 | 20 | 2.96 | | 39 | 5.78 | | 43 | 6.37 | | 30 | 3.39 | |
| Positive | 136 | 17 | 3.00 | 0.99 | 30 | 5.29 | 0.68 | 36 | 6.35 | 0.97 | 25 | 3.04 | 0.69 |

Table 3 continued

| Characteristics ^a | All patients | Distant disease-free survival | | | Breast related event free survival | | | Disease free survival | | | Overall survival | | |
|------------------------------------|--------------|-------------------------------|----------------------------|---------------|------------------------------------|----------------------------|---------------|-----------------------|----------------------------|---------------|------------------|----------------------------|---------------|
| | | Events | Event rate/100 women-years | Log-rank | Events | Event rate/100 women-years | Log-rank | Events | Event rate/100 women-years | Log-rank | Deaths | Death rate/100 women-years | Log-rank |
| CK14 | | | | | | | | | | | | | |
| Negative | 128 | 16 | 2.79 | | 31 | 5.40 | | 34 | 5.92 | | 25 | 3.21 | |
| Positive | 153 | 21 | 3.14 | 0.64 | 38 | 5.69 | 0.77 | 45 | 6.74 | 0.53 | 30 | 3.24 | 0.96 |
| CK5/6 | | | | | | | | | | | | | |
| Negative | 191 | 21 | 2.47 | | 37 | 4.35 | | 45 | 5.29 | | 32 | 2.70 | |
| Positive | 90 | 16 | 4.10 | 0.17 | 32 | 8.21 | 0.01 | 34 | 8.72 | 0.04 | 23 | 4.41 | 0.10 |
| EGFR | | | | | | | | | | | | | |
| Negative | 121 | 16 | 3.01 | | 32 | 6.02 | | 35 | 6.58 | | 25 | 3.53 | |
| 1–49% | 127 | 10 | 1.68 | | 22 | 3.69 | | 27 | 4.52 | | 17 | 2.08 | 0.0007 |
| ≥50% | 36 | 12 | 9.84 | 0.0001 | 17 | 13.93 | 0.0003 | 19 | 15.57 | 0.0001 | 15 | 7.89 | |
| Necrosis | | | | | | | | | | | | | |
| Absent | 104 | 16 | 3.54 | | 28 | 6.19 | | 30 | 6.64 | | 23 | 3.70 | |
| Present | 180 | 22 | 2.76 | 0.44 | 43 | 5.39 | 0.55 | 51 | 6.39 | 0.86 | 34 | 3.11 | 0.51 |
| <i>(b) According to treatments</i> | | | | | | | | | | | | | |
| All patients | 284 | 38 | 3.04 | | 71 | 5.68 | | 81 | 6.47 | | 57 | 3.32 | |
| Type of surgery | | | | | | | | | | | | | |
| Breast conserving | 236 | 28 | 2.68 | | 52 | 4.97 | | 58 | 5.54 | | 40 | 2.76 | |
| Mastectomy | 48 | 10 | 4.88 | 0.08 | 19 | 9.27 | 0.01 | 23 | 11.22 | 0.002 | 17 | 6.42 | 0.002 |
| Radiotherapy | | | | | | | | | | | | | |
| No | 45 | 8 | 3.56 | | 17 | 7.56 | | 21 | 9.33 | | 13 | 4.55 | |
| Yes | 238 | 30 | 2.92 | 0.67 | 54 | 5.26 | 0.21 | 60 | 5.85 | 0.08 | 44 | 3.08 | 0.30 |
| Chemotherapy | | | | | | | | | | | | | |
| None | 21 | 4 | 4.49 | | 5 | 5.62 | | 6 | 6.74 | | 4 | 3.39 | |
| CMF × 6 | 138 | 14 | 2.27 | | 31 | 5.03 | | 36 | 5.84 | | 26 | 3.08 | |
| AC × 4/ CMF × 3 | 38 | 6 | 3.87 | | 9 | 5.81 | | 9 | 5.81 | | 4 | 1.88 | |
| Other | 87 | 14 | 3.59 | 0.45 | 26 | 6.67 | 0.65 | 30 | 7.69 | 0.58 | 23 | 4.25 | 0.28 |

^a Information on tumor size, pT, pN, PVI, p63, CK14, CK5/6 and “basal like phenotype” are unknown for 3 patients, on tumor grade for 16 patients, on ki-67 expression for 1 patient and on CA15.3 for 139 patients. Breast related events include locoregional relapses, distant metastases, contralateral breast cancers and death

^b As documented by immunoreactivity for basal cytokeratins, p63 and/or EGFR

Bold is statistically significant

1.21–4.26, $P = 0.01$ for BREFS). EGFR immunoreactivity evaluated as a continuous variable (HRs for 10% increase in the number of positive cells) was also associated with worse DFS (HR 1.10, 95% CI 1.02–1.19, $P = 0.01$), DDFS (HR 1.15, 95% CI 1.05–1.27, $P = 0.004$), BREFS (HR 1.09, 95% CI 1.01–1.18, $P = 0.03$) and with OS (HR 1.10, 95% CI 1.01–1.20, $P = 0.03$). Survival curves for DFS, DDFS and OS according to different cut-off levels of EGFR expression (10, 30 and 50%) are shown in Fig. 1. A statistically significant worse outcome was also observed at the multivariate analysis for older patients (aged 70 or

over), for those with larger tumors (pT2 disease) and for those with tumors showing PVI (Table 4). None of the remaining clinico-pathological parameters retained statistical significance at multivariate analysis. Since in univariate analysis systemic treatment was not shown to affect recurrence and survival it was not included in multivariate analysis model. Since a different proportion of patients with low and high EGFR immunoreactivity did not receive adjuvant chemotherapy, we performed a subset analysis excluding patients untreated with chemotherapy. Also after the exclusion of untreated patients (21 pts),

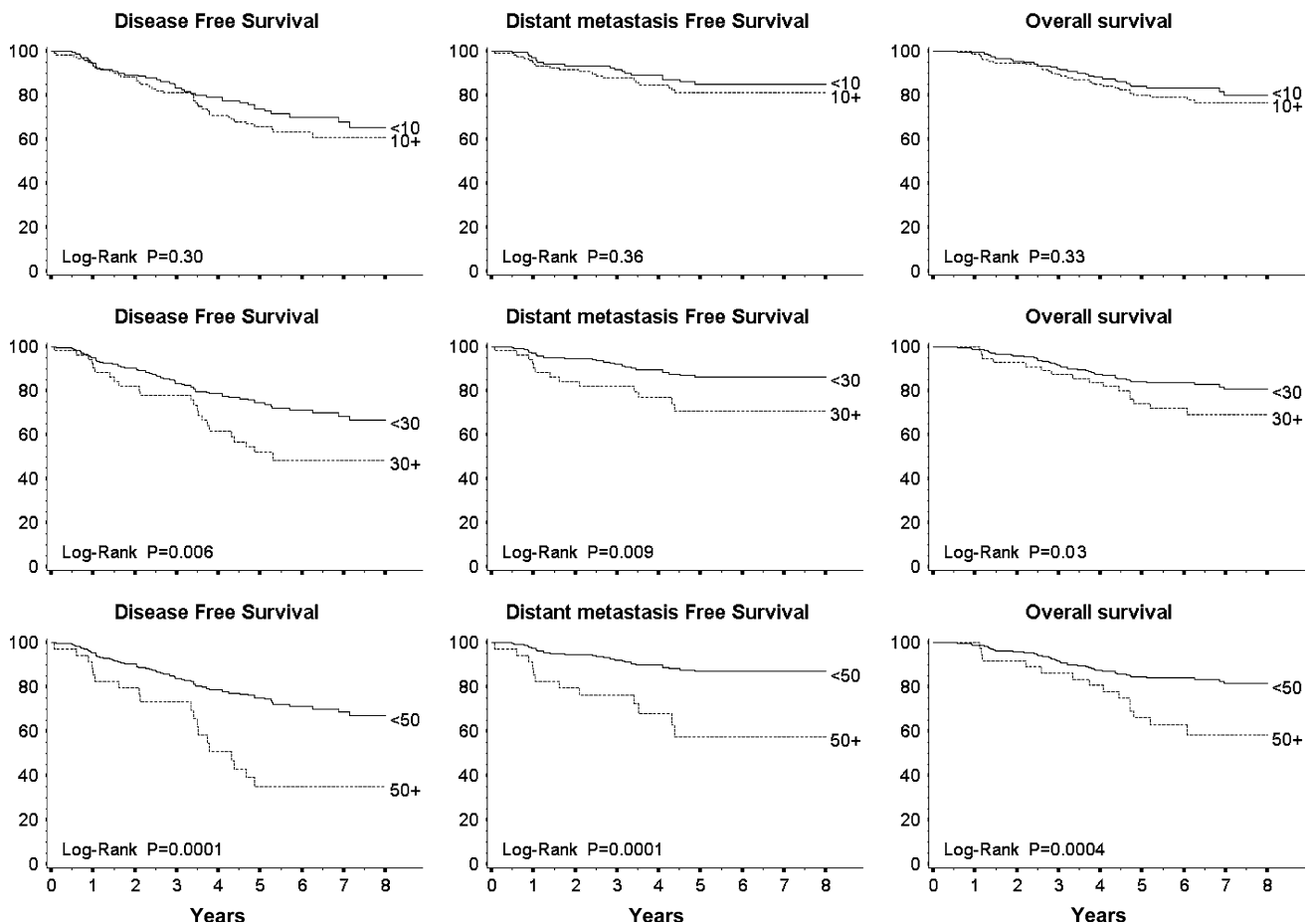


Fig. 1 Kaplan Meier curves of disease free survival (DFS), distant metastasis free survival (DDFS) and overall survival (OS) according to different cut off levels of EGFR expression

EGFR immunoreactivity significantly correlated with disease-free and overall survival (HR 2.22, 95% CI, 1.18–4.15, $P = 0.01$ for DFS and HR = 2.19 95% CI 1.07–4.48, $P = 0.03$ for OS for EGFR $\geq 50\%$ vs. EGFR 0–49%, respectively).

Discussion

Emerging data on the clinical implications of IDC with the triple-negative phenotype indicates an aggressive course of the disease. Despite the widespread acknowledgment of the poor clinical outcome, the prognostic value of specific morphological and biological features of these tumors continues to raise a substantial degree of uncertainty and controversy [5, 8, 28]. Data from past series include information on several characteristics of the disease collected in the earlier period, when neither systemic treatments nor various prognostic and predictive factors were available as they are today. Adjuvant systemic therapies and precise assessment of the biological features of primary breast cancers are probably the most relevant

innovations in the current treatment of breast carcinoma patients. Recent therapeutic strategies emphasize the paramount importance of targeted therapies wherever possible, though acknowledging that supplementation with less target-specific chemotherapy may be required [29].

This study provides useful insights into the treatment and prognosis of breast cancer since it is based on a large number of patients, collected in a relatively short time, thus allowing the adoption of modern procedures. The pathologists, surgeons and medical oncologists used consistent approaches during the years of reference. Adjuvant treatment proposed was largely based on the degree of nodal involvement as well as on known prognostic features according to recent St. Gallen Consensus Conferences Guidelines [16, 17].

From the current study, the extent of EGFR immunoreactivity in triple-negative IDC of the breast emerges as a clinically relevant prognostic parameter. Recently reported studies already indicated that EGFR expression is associated with significant worse DFS in early breast cancer [30]. A comparison between triple negative tumors (defined as ER, PgR and HER2 negative) and the so-called “five marker” tumors, defined also by the presence of EGFR and

Table 4 Multivariate analysis

| Characteristics | Disease free survival (DFS) | | Distant metastasis free survival (DDFS) | | Breast related event free survival (BREFS) | | Overall survival (OS) | |
|-------------------|-----------------------------|----------|---|----------|--|----------|-----------------------|----------|
| | HR (95% CI) | <i>P</i> | HR (95% CI) | <i>P</i> | HR (95% CI) | <i>P</i> | HR (95% CI) | <i>P</i> |
| Age group | | | | | | | | |
| <35 | 1.92 (0.78–4.72) | 0.16 | 2.25 (0.62–8.14) | 0.22 | 2.30 (0.92–5.77) | 0.07 | 2.04 (0.75–5.60) | 0.16 |
| 35–49 | 1.00 | | 1.00 | | 1.00 | | 1.00 | |
| 50–59 | 1.27 (0.70–2.32) | 0.43 | 0.72 (0.26–2.01) | 0.53 | 1.46 (0.78–2.75) | 0.24 | 1.31 (0.62–2.80) | 0.48 |
| 60–69 | 1.35 (0.73–2.52) | 0.34 | 1.44 (0.60–3.44) | 0.41 | 1.35 (0.69–2.68) | 0.38 | 1.78 (0.86–3.66) | 0.12 |
| 70+ | 3.10 (1.41–6.83) | 0.005 | 3.40 (1.17–9.89) | 0.02 | 3.11 (1.34–7.20) | 0.008 | 2.81 (1.10–7.15) | 0.03 |
| pT | | | | | | | | |
| pT1 | 1.00 | | 1.00 | | 1.00 | | 1.00 | |
| pT2 | 1.79 (1.08–2.98) | 0.02 | 1.72 (0.79–3.73) | 0.16 | 1.70 (0.99–2.92) | 0.05 | 1.61 (0.89–2.93) | 0.12 |
| pT3/4 | 1.55 (0.55–4.32) | 0.41 | 2.04 (0.51–8.14) | 0.31 | 1.38 (0.45–4.30) | 0.58 | 2.12 (0.66–6.83) | 0.20 |
| Positive nodes | | | | | | | | |
| None | 1.00 | | 1.00 | | 1.00 | | 1.00 | |
| 1–2 | 0.99 (0.58–1.69) | 0.96 | 1.71 (0.82–3.56) | 0.15 | 1.13 (0.64–1.98) | 0.67 | 1.02 (0.55–1.93) | 0.93 |
| 3+ | 0.65 (0.28–1.51) | 0.32 | 1.19 (0.41–3.48) | 0.75 | 0.81 (0.34–1.90) | 0.62 | 0.44 (0.15–1.32) | 0.14 |
| PVI | | | | | | | | |
| Absent | 1.00 | | 1.00 | | 1.00 | | 1.00 | |
| Present | 1.64 (0.97–2.76) | 0.07 | 1.83 (0.87–3.87) | 0.11 | 1.67 (0.95–2.91) | 0.07 | 1.98 (1.09–3.61) | 0.03 |
| EGFR | | | | | | | | |
| Negative | 1.00 | | 1.00 | | 1.00 | | 1.00 | |
| 1–49% | 0.74 (0.44–1.24) | | 0.68 (0.30–1.52) | 0.34 | 0.68 (0.39–1.18) | 0.16 | 0.70 (0.37–1.32) | 0.27 |
| ≥50% | 2.39 (1.32–4.34) | 0.004 | 3.39 (1.52–7.60) | 0.003 | 2.27 (1.21–4.26) | 0.01 | 2.34 (1.20–4.59) | 0.01 |
| EGFR ^a | | | | | | | | |
| Continuous | 1.10 (1.02–1.19) | 0.01 | 1.15 (1.05–1.27) | 0.004 | 1.09 (1.01–1.18) | 0.03 | 1.10 (1.01–1.20) | 0.03 |

Hazards Ratios (HR) and 95% confidence intervals (CI) obtained from a multivariate Cox proportional Hazard regression model

^a Obtained from a separate multivariate Cox model in which EGFR is set as a continuous variable and in which the Hazards ratios are given for an increase of 10% of the level of expression

CK 5 and 6, showed a poorer prognosis for the latter group, underscoring the relevance of EGFR expression among basal-like tumors [31]. However, the actual value of the quantitative evaluation of EGFR immunoreactivity is still uncertain.

The present study confirms previous published results indicating a different outcome for patients with triple negative IDC, according to the extent of EGFR immunoreactivity (defined as percentage of positive cells). Indeed, patients whose tumors lack EGFR immunoreactivity or show low levels of expression have similar outcomes as opposed to patients with tumors characterized by more extensive EGFR immunoreactivity (i.e., ≥50% of the neoplastic cells) who exhibit poorer DFS (HR 2.39; *P* = 0.004) and OS (HR 2.34; *P* = 0.01). A theoretical implication of these results may be the exploration of selective HER1 inhibitors in the adjuvant treatment of IDC of the breast with the “triple-negative” phenotype and extensive immunoreactivity for EGFR. Limited activity was recently shown in a small group of patients, unselected

for EGFR expression, and treated with an EGFR tyrosine kinase inhibitor [32]. The results of the present study support further investigations to exploit the role of these agents in patients whose tumors express EGFR.

Setting thresholds in a biological continuum as the extent of EGFR immunoreactivity may well be considered arbitrary, but despite this it may prove clinically useful to identify patients at higher risk who may benefit from targeted treatments. The percentage of neoplastic cells exhibiting peculiar biological features is already considered relevant in the assessment of prognostic and predictive factors in early breast cancer. A similar arbitrary cut-off of >30% immunoreactive tumor cells has recently been proposed for the assessment of HER2/neu-positive breast cancers [33]. Also the percentage of neoplastic cells immunoreactive for hormone receptors is currently considered of paramount value in assessing tumor endocrine responsiveness and in planning targeted treatments [29, 34].

In the present study we evaluated the prognostic value of EGFR either as a continuous variable and through different

cut-off values (10, 30 and 50%). We selected a cut-off of 50% (which corresponded to the 90th percentile in our series) bearing in mind that this cut-off has been used in other series of solid tumors [35].

It has been shown that the EGFR gene copy number was associated with a worse prognosis in non small cell lung cancer (NSCLC) [36]. Moreover, a correlation between EGFR immunoreactivity and EGFR gene copy number has been demonstrated in NSCLC [37]. Considering that EGFR immunohistochemistry is a widely applicable and inexpensive test, in contrast to the evaluation of EGFR copy number and mutations, the assessment of the percentage of immunohistochemically positive cells might be a useful approach to defining the degree of EGFR expression.

Similarly, HER-2 gene copy number has been closely associated with HER-2 protein expression, both predicting worse prognosis [29]. In a previous study we showed that the extent of HER2 protein expression was significantly correlated with HER2 gene amplification. In fact, only 21.4% of the tumors with positive immunostaining in $\leq 50\%$ of cells showed gene amplification which compares with 85.7% of the tumors presenting a positive staining in $>50\%$ of cells [38]. These results support the hypothesis that an higher expression of positivity through IHC (e.g., $>50\%$ of the cells) might identify subgroups of tumors with gene amplification.

The results of the present study show that age ≥ 70 years is an important prognostic factor, associated with a three-fold increased risk of relapse, metastases and breast related events when compared with younger patients. However, these results were observed in a population subjected to an adjuvant therapy program which might have interfered with the outcome. As shown in Table 2, elderly patients received significantly less chemotherapy and in particular anthracycline-containing chemotherapy than younger patients. In fact, according to the limited data available in the literature on the value of adjuvant chemotherapy in elderly patients [20, 39], women aged ≥ 70 yrs were typically not submitted to an adjuvant chemotherapy program. The poor prognosis observed in the present analysis for this subgroup of patients, supports appropriate discussions with patients on the costs and benefits of adjuvant chemotherapy.

In the multivariate analysis other known prognostic factors, such as pathological stage, were found to be significantly associated with the outcome of the patients. As shown in Table 4, the occurrence of PVI was an independent predictor of overall mortality (HR 1.98) and was associated with worse DFS, DDFS and BREFS although these last associations were of borderline statistical significance. Although these data are in line with recently published data and support a prognostic role for PVI in early breast cancer [40], limited information is available on the role of PVI in the cohort of patients with triple-negative

IDC. All these factors should be properly taken into account in the treatment decision-making procedure.

It has been previously demonstrated that expression of basal cytokeratins (CK 5/6 and/or CK 14/17) predicts poor outcome [8, 9] in breast carcinoma patients, and in particular in the node-positive group of patients [41]. The prognostic role of basal cytokeratins might be related to the identification of the subgroup of patients with basal-like phenotype. Limited and controversial data, however, are available on the prognostic value of basal cytokeratins in the cohort of patients with triple-negative IDC. Jumppanen et al. found that within the subgroup of ER-negative tumors, the clinical outcome of tumors characterized by the expression of basal e cytokeratins (CK5/14/17) was similar to that of ER-negative tumors lacking any immunoreactivity for these markers [42].

Large central necrosis or fibrosis have also been reported to correlate with poor prognosis in early breast cancer [43], and to be strongly associated with basal-like [44] and ER- negative breast cancer [45]. Information on their prognostic value within the subgroup of triple-negative IDC is, however, lacking. In the current study immunoreactivity for basal cytokeratins and occurrence of central necrosis were not independent predictors of survival. A significant proportion of the patients with triple-negative IDC might have tumors concomitantly showing intratumoral necrosis [28], a EGFR and basal cytokeratins immunoreactivity [46]. This may explain why these closely associated factors are not all independently predicting DFS or OS when taken together in the full regression models.

The efficacy of adjuvant systemic therapy for early breast cancer depends on several variables, which include features of the tumor, the patient and the treatment itself. In the present study, conducted in a single institution, we demonstrated that the extent of EGFR immunoreactivity significantly correlates with prognosis in triple-negative IDC. Despite the statistically significant detrimental prognostic effect associated with EGFR expression, the potential for bias still exists due to the retrospective nature of the evaluation and the arbitrary cut-off selection. Further studies using database analyses or prospective trials are required to confirm the prognostic value of the extent of EGFR expression. If the results of the present study are confirmed, EGFR expression should be included in the routine immunohistochemical assessment of triple negative breast cancer.

References

1. Perou CM, Sorlie T, Eisen MB et al (2000) Molecular portraits of human breast tumours. *Nature* 406:747–752. doi:10.1038/35021093

2. Sorlie T, Perou CM, Tibshirani R et al (2001) Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. *Proc Natl Acad Sci USA* 98:10869–10874. doi:10.1073/pnas.191367098
3. Yehiely F, Moyano JV, Evans JR et al (2006) Deconstructing the molecular portrait of basal-like breast cancer. *Trends Mol Med* 12:537–544. doi:10.1016/j.molmed.2006.09.004
4. Abd El-Rehim DM, Pinder SE, Paish CE et al (2004) Expression of luminal and basal cytokeratins in human breast carcinoma. *J Pathol* 203:661–671. doi:10.1002/path.1559
5. Livasy CA, Karaca G, Nanda R et al (2006) Phenotypic evaluation of the basal-like subtype of invasive breast carcinoma. *Mod Pathol* 19:264–271. doi:10.1038/modpathol.3800528
6. Abd El-Rehim DM, Ball G, Pinder SE et al (2005) High-throughput protein expression analysis using tissue microarray technology of a large well-characterised series identifies biologically distinct classes of breast cancer confirming recent cDNA expression analyses. *Int J Cancer* 116:340–350. doi:10.1002/ijc.21004
7. Rakha EA, El-Sayed ME, Green AR et al (2007) Prognostic markers in triple-negative breast cancer. *Cancer* 109:25–32. doi:10.1002/ncr.22381
8. Dent R, Trudeau M, Pritchard KI et al (2007) Triple-negative breast cancer: clinical features and patterns of recurrence. *Clin Cancer Res* 13:4429–4434. doi:10.1158/1078-0432.CCR-06-3045
9. Nielsen TO, Hsu FD, Jensen K et al (2004) Immunohistochemical and clinical characterization of the basal-like subtype of invasive breast carcinoma. *Clin Cancer Res* 10:5367–5374. doi:10.1158/1078-0432.CCR-04-0220
10. McShane LM, Altman DG, Sauerbrei W et al (2006) REporting recommendations for tumor MARKer prognostic studies (REMARK). *Breast Cancer Res Treat* 100:229–235. doi:10.1007/s10549-006-9242-8
11. Japaze H, Emina J, Diaz C, Schwam RJ et al (2005) ‘Pure’ invasive apocrine carcinoma of the breast: a new clinicopathological entity? *Breast* 14:3–10. doi:10.1016/j.breast.2004.06.003
12. Azoulay S, Laé M, Fréneau P, Merle S et al (2005) KIT is highly expressed in adenoid cystic carcinoma of the breast, a basal-like carcinoma associated with a favorable outcome. *Mod Pathol* 8:1623–1631
13. Orlando L, Renne G, Rocca A et al (2005) Are all high-grade breast cancers with no steroid receptor hormone expression alike? The special case of the medullary phenotype. *Ann Oncol* 16:1094–1099. doi:10.1093/annonc/mdi213
14. Veronesi U, Paganelli G, Viale G et al (2003) A randomized comparison of sentinel-node biopsy with routine axillary dissection in breast cancer. *N Engl J Med* 349:546–553. doi:10.1056/NEJMoa012782
15. Gennari R, Curigliano G, Rotmensz N et al (2004) Breast carcinoma in elderly women: features of disease presentation, choice of local and systemic treatments compared with younger postmenopausal patients. *Cancer* 101:1302–1310. doi:10.1002/ncr.20535
16. Goldhirsch A, Wood WC, Senn HJ et al (1995) Meeting highlights: international consensus panel on the treatment of primary breast cancer. *J Natl Cancer Inst* 87:1441–1445. doi:10.1093/jnci/87.19.1441
17. Goldhirsch A, Glick JH, Gelber RD et al (1998) Meeting highlights: international consensus panel on the treatment of primary breast cancer. *J Natl Cancer Inst* 90:1601–1608. doi:10.1093/jnci/90.21.1601
18. Colleoni M, Liman HJ, Castiglione-Gertsch M et al (2002) Duration of adjuvant chemotherapy for breast cancer: a joint analysis of two randomised trials investigating three versus six courses of CMF. *Br J Cancer* 86:1705–1714. doi:10.1038/sj.bjc.6600334
19. Fisher B, Brown AM, Dimitrov NV et al (1990) Two months of doxorubicin-cyclophosphamide with and without interval reinduction therapy compared with 6 months of cyclophosphamide, methotrexate, and fluorouracil in positive-node breast cancer patients with tamoxifen-nonresponsive tumors: results from the National Surgical Adjuvant Breast and Bowel Project B 15. *J Clin Oncol* 8:1483–1496
20. Levine MN, Bramwell VH, Pritchard KL et al (1998) Randomized trial of intensive cyclophosphamide, epirubicin, and fluorouracil chemotherapy compared with cyclophosphamide, methotrexate, and fluorouracil in premenopausal women with node-positive breast cancer. National Cancer Institute of Canada Clinical Trial Group. *J Clin Oncol* 16:2651–2658
21. Veronesi U, Paganelli G, Galimberti V et al (1997) Sentinel-node biopsy to avoid axillary dissection in breast cancer with clinically negative lymph nodes. *Lancet* 349:1864–1867. doi:10.1016/S0140-6736(97)01004-0
22. Elston CW, Ellis IO (2002) Pathological prognostic factors in breast cancer I. The value of histological grade in breast cancer: experience from a large study with long-term follow-up. *C Histopathology* 41(3A):154–161. doi:10.1046/j.1365-2559.2002.14691.x
23. Rosen PP, Oberman H (1993) Tumors of the mammary gland. Armed Forces Institute of Pathology, Washington, DC
24. Tsuda H, Takarabe T, Hasegawa F, Fukutomi T, Hirohashi S (2000) Large, central acellular zones indicating myoepithelial tumor differentiation in high-grade invasive ductal carcinomas as markers of predisposition to lung and brain metastases. *Am J Surg Pathol* 24:197–202. doi:10.1097/00000478-200002000-00005
25. Colleoni M, Orvieto E, Nolè F et al (1999) Prediction of response to primary chemotherapy for operable breast cancer. *Eur J Cancer* 35:574–579. doi:10.1016/S0959-8049(99)00005-2
26. Banerjee S, Reis-Filho JS, Ashley S et al (2006) Basal-like breast carcinomas: clinical outcome and response to chemotherapy. *J Clin Pathol* 59:729–735. doi:10.1136/jcp.2005.033043
27. Ribeiro-Silva A, Ramalho LN, Garcia SB et al (2005) p63 correlates with both BRCA1 and cytokeratin 5 in invasive breast carcinomas: further evidence for the pathogenesis of the basal phenotype of breast cancer. *Histopathology* 47:458–466. doi:10.1111/j.1365-2559.2005.02249.x
28. Cleator S, Heller W, Coombes RC et al (2007) Triple-negative breast cancer: therapeutic options. *Lancet Oncol* 8:235–244. doi:10.1016/S1470-2045(07)70074-8
29. Goldhirsch A, Wood WC, Gelber RD (2007) Progress and promise: highlights of the international expert consensus on the primary therapy of early breast cancer 2007. *Ann Oncol* 18:1133–1144. doi:10.1093/annonc/mdm271
30. Nieto Y, Nawaz F, Jones RB et al (2007) Prognostic significance of overexpression and phosphorylation of epidermal growth factor receptor (EGFR) and the presence of truncated EGFRvIII in locoregionally advanced breast cancer. *J Clin Oncol* 25:4405–4413. doi:10.1200/JCO.2006.09.8822
31. Cheang MCU, Voduc D, Bajdik C et al (2008) Basal-like breast cancer defined by five biomarkers has superior prognostic value than triple-negative phenotype. *Clin Cancer Res* 14:1368–1376. doi:10.1158/1078-0432.CCR-07-1658
32. Guix M, s Granja N, Meszoely I et al (2008) Short preoperative treatment with Erlotinib inhibits tumor cell proliferation in hormone receptor-positive breast cancer. *J Clin Oncol* 26:897–906. doi:10.1200/JCO.2007.13.5939
33. Wolff AC, Hammond ME, Schwartz JN et al (2007) American Society of Clinical Oncology/College of American Pathologists guideline recommendations for human epidermal growth factor receptor 2 testing in breast cancer. *Arch Pathol Lab Med* 131:18

34. Stendahl M, Ryden L, Nordenskjold B (2006) High progesterone receptor expression correlates to the effect of adjuvant tamoxifen in premenopausal breast cancer patients. *Clin Cancer Res* 12:4614–4618. doi:[10.1158/1078-0432.CCR-06-0248](https://doi.org/10.1158/1078-0432.CCR-06-0248)
35. Khalifa MA, Rowsell CH, Gladdy RA, Ko YJ, Hanna S, Smith A et al (2006) Expression of epidermal growth factor receptor in primary colorectal adenocarcinoma predicts expression in recurrent disease. *Am J Clin Pathol* 125:229–233
36. Hirsch FR, Dziadziuszko R, Thatcher N et al (2008) Epidermal growth factor receptor immunohistochemistry. *Cancer* 112:1114–1121. doi:[10.1002/cncr.23282](https://doi.org/10.1002/cncr.23282)
37. Hirsch FR, Varella-Garcia M, Bunn PA Jr et al (2003) Epidermal growth factor receptor in non-small cell lung cancer carcinomas: correlation between gene copy number and protein expression and impact on prognosis. *J Clin Oncol* 1521:3798–3807
38. Torrisi R, Rotmensz N, Bagnardi V et al (2007) HER2 status in early breast cancer: relevance of cell staining patterns, gene amplification and polysomy 17. *Eur J Cancer* 43:2339–2344. doi:[10.1016/j.ejca.2007.07.033](https://doi.org/10.1016/j.ejca.2007.07.033)
39. Dellapasqua S, Colleoni M, Castiglione M et al (2007) New criteria for selecting elderly patients for breast cancer adjuvant treatment studies. *Oncologist* 12:952–959. doi:[10.1634/theoncologist.12-8-952](https://doi.org/10.1634/theoncologist.12-8-952)
40. Colleoni M, Rotmensz N, Maisonneuve P et al (2007) Prognostic role of the extent of peritumoral vascular invasion in operable breast cancer. *Ann Oncol* 18:1632–1640. doi:[10.1093/annonc/mdm268](https://doi.org/10.1093/annonc/mdm268)
41. Van de Rijn M, Perou CM, Tibshirani R et al (2002) Expression of cytokeratins 17 and 5 identifies a group of breast carcinomas with poor clinical outcome. *Am J Pathol* 161:1991–1996
42. Jumppanen M, Gruvberger-Saal S, Kauraniemi P et al (2007) Basal like phenotype is not associated with patient survival in estrogen-receptor-negative breast cancers. *Breast Cancer Res* 9:R16. doi:[10.1186/bcr1649](https://doi.org/10.1186/bcr1649)
43. Colpaert C, Vermeulen P, Jeuris W et al (2001) Early distant relapse in “node-negative” breast cancer patients is not predicted by occult axillary lymph node metastases, but by the features of the primary tumours. *J Pathol* 193:442–449. doi:[10.1002/path.829](https://doi.org/10.1002/path.829)
44. Fulford LG, Easton DF, Reis-Filho JS et al (2006) Specific morphological features predictive for the basal phenotype in grade 3 invasive ductal carcinoma of breast. *Histopathology* 49:22–34. doi:[10.1111/j.1365-2559.2006.02453.x](https://doi.org/10.1111/j.1365-2559.2006.02453.x)
45. Putti TC, El-Rehim DM, Rakha EA et al (2005) Estrogen receptor-negative breast carcinomas: a review of morphology and immunophenotypical analysis. *Mod Pathol* 18:26–35. doi:[10.1038/modpathol.3800255](https://doi.org/10.1038/modpathol.3800255)
46. Siziopikou KP, Cobleigh M (2007) The basal subtype of breast carcinomas may represent the group of breast tumors that could benefit from EGFR-targeted therapies. *Breast* 16:104–107. doi:[10.1016/j.breast.2006.09.003](https://doi.org/10.1016/j.breast.2006.09.003)