Prognosis in hormon receptor negative breast cancer patients according to ERBB2 status

L. DOGAN, C. ATALAY*, K. B. YILMAZ, C. OZASLAN

Department of General Surgery, Ankara Oncology Hospital, Ankara, Turkey, e/mail: atalay_can@hotmail.com

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Breast carcinomas represent a heterogenous group of tumors and recent studies have demonstrated several subtypes of breast cancer by gene expression profiles. This study aimed to compare hormon receptor negative (ER-/PR-/ERBB2+) and triple negative (ER-/PR-/ERBB2-) patients in terms of prognosis and to show that molecularly defined subtypes can be distinguished by conventional laboratory methods. Patients treated between 2001-2007 for hormon receptor negative breast cancer were retrospectively studied. In addition to the conventional prognostic factors, effect of ERBB2 status of the patients on disease-free and overall survival was evaluated. Hormon receptor and ERBB2 status were determined by immunuhistochemistry and fluorescence in-situ hybridization. 141 patients were eligible for the study. Number of patients with ERBB2 positive and triple negative tumors was 70 and 71, respectively, and two groups were comparable in terms of study parameters. Tumor size, grade, axillary status, patient groups, and adjuvant chemotherapy and radiotherapy showed significant impact on disease-free survival and overall survival was significantly dependent on axillary status, type of surgery, and patient groups in univariate analysis. In multivariate analysis, patient groups, tumor grade, and axillary status were independent prognostic factors for disease-free survival whereas patient groups, extent of surgery, and axillary status were independent prognostic factors for overall survival. This study has indicated that ERBB2 negative patients had worse survival among hormon receptor negative breast cancer patients and showed that molecularly defined subtypes of breast cancer can be differentiated by immunuhistochemistry in terms of prognosis.

Key words: breast cancer; hormon receptor negative; triple negative; survival

Breast carcinomas represent a heterogenous group of tumors that are diverse in behavior, outcome, and response to treatment. Breast cancer patients with the same clinical prognostic profiles can have markedly different clinical outcomes. Current classifications of breast cancer group molecularly distinct diseases into clinical classes based mainly on histopathological properties. However, there is a desire to examine and characterize tumors of poor prognosis to ensure adequate therapy and to improve patient outcome. Several previous studies have addressed these issues and tried to define various subtypes of breast cancer at molecular level [1–3]. In their landmark study, Perou et al. grouped breast cancer samples according to their similarities in gene expression profiles and clearly showed the molecular heterogeneity of breast cancer [1]. Estrogen receptor (ER) status was the primary factor that distinguished between the subtypes of breast cancer. ER positive group had at least two subgroups, luminal A and B, that are differing in ER and related genes expressions and prognosis. On the other hand, ER negative group composed of three subgroups as ERBB2 overexpressing, normal-like, and basal-like or ERBB2 negative.

Emerging data demonstrated that stratification of tumors by gene expression profiles divides breast cancer into several subgroups. Relating gene expression patterns to clinical phenotypes and outcomes is a key issue in understanding the biological diversity of breast cancer. Thus, the clinical importance of these molecularly defined subgroups is yet to be determined. Previous studies compared the patients with triple negative breast cancer to the remaining patients with breast cancer and reported significant differences in prognosis [4–6]. However, this study aimed to compare the two breast cancer subtypes with poor clinical outcome and to show that molecularly defined subtypes can be distinguished by conventional methods such as immunohistochemistry (IHC). For this purpose, both ER and progesterone receptor (PR) negative patients were grouped ac-

* Corresponding author

cording to their ERBB2 status to depict a possible difference in prognosis between hormon receptor negative (ER-/PR-/ERBB2+) and triple negative (ER-/PR-/ERBB2-) patients.

Patients and methods

Breast cancer patients diagnosed and treated between January 2001 and July 2007 were retrospectively evaluated and both ER and PR negative patients without systemic metastases at the time of diagnosis were included in the study. Patients' demographic and clinical characteristics such as age, gender, menopausal status, and family history, histopathological data such as tumor size, grade, presence and number of axillary lymph node metastases, and treatment methods were collected from the hospital files. ER and PR are considered negative if staining of tumor cell nuclei is less than 5% with IHC. ERBB2 status was assessed with IHC and fluorescence in-situ hybridization (FISH). IHC results were scored from 0 to 3+ based on staining intensity, with 0 and 1+ classified as negative, 2+ as borderline, and 3+ as positive. FISH was scored quantitatively with less than two copies of the ERBB2 gene classified as negative. Patients were stratified into two groups according to ERBB2 status. Those patients who had 3+ result on IHC examination or gene amplification on FISH were considered as ERBB2 positive (Group 1) whereas remaining patients were assigned to ERBB2 negative group (Group 2). Patients were also stratified according to age as <50 and >50 years, to menopausal status as premenopausal and postmenopausal, and to tumor grade as grade II and III since none of the patients had grade I tumor. Family history was accepted as positive if at least one of the first degree relatives of the patients had breast cancer.

Patients were staged according to the American Joint Committee on Cancer (AJCC) Cancer Staging Manual, 6th ed [7]. Patients were grouped according to tumor size as T1 (<2 cm), T2 (2-5 cm), T3 (>5 cm), and T4 (tumor fixed to chest wall and/or skin) and to lymph node status as N0 (node negative), N1 (1-3 positive), N2 (4-9 positive), and N3 (>9 positive). Surgery was primarily performed for the patients in stages I to IIIA as modified radical mastectomy (MRM) or breast conserving surgery (BCS) and axillary dissection. Axillary status was determined with axillary dissection. Adjuvant chemotherapy (CT) and/or radiotherapy (RT) were administered according to the accepted practice guidelines. Neoadjuvant CT was applied to clinically stage IIIB patients. Combination CT protocols, namely CAF (cyclophosphamide, doxorubicin, 5-fluorouracil), CEF (cyclophosphamide, epirubicin, 5-fluorouracil), CA (cyclophosphamide, doxorubicin), CE (cyclophosphamide, epirubicin), and their combinations with taxanes (paclitaxel or docetaxel) were utilized. ERBB2 positive patients did not receive trastuzumab in the adjuvant setting.

Patients were followed every three months in the first two years, every six months between two to five years, and yearly afterwards. In addition to a thorough physical examination, liver function tests, chest radiography, abdominal ultrasonography, and bone scintigraphy were performed according to the patient's complaints. Mammography and/or breast ultrasonography were taken yearly. Time to local recurrence, distant metastasis, and death were calculated from the day of initial surgery to the last follow-up or the occurence of the relevant event in all groups. For the survival analyses, patients' age, menopausal status, family history, tumor size and grade, axillary lymph node status, and treatment methods such as surgery, CT, and RT were evaluated in addition to the groups formed according to ERBB2 status of the patients.

Statistical analysis. Survival values were determined using Kaplan-Meier method. Univariate analysis for the prognostic factors affecting disease-free survival (DFS) and overall survival (OS) was performed with log-rank test. Cox stepwise regression analysis was performed to determine the independent prognostic factors affecting DFS and OS. The frequencies of different variables in two patient groups were compared with Pearson's chi-square or Fisher's exact tests as appropriate. Statistical analyses were performed with SPSS 10.0 statistical software package (SPSS Inc., Chicago, IL). All of the tests applied were two-way and level of significance (p) was accepted as significant when it is <0.05.

Results

One hundred and forty-one patients with a median age of 49 (range, 27 to 90) were included in the study. All except one patient were female. Eighty-seven patients (62.1%) were postmenopausal and family history was positive in 17 patients (12.1%). Histopathological diagnosis was invasive ductal carcinoma in all patients. Clinicopathological characteristics of the patients are shown in Table 1. MRM was performed in 120 patients (86.3%). Median tumor size was 3 cm (range, 0.5-11 cm). More than half of the patients (57.4%) had T2 tumors and 55 patients (39.6%) did not have any axillary lymph node metastasis. Median number of dissected and metastatic lymph nodes was 22 (range, 8-51) and one (range, 0-33), respectively. Number of patients with 1-3, 4-9, and >9 histologically involved lymph nodes was 38 (27.3%), 24 (17.3%), and 22 (15.8%), respectively.

Nineteen patients with clinical stage IIIB breast cancer received neoadjuvant CT and two patients showed progression during CT with the development of distant metastases and surgery was not performed in these patients. Number of patients receiving adjuvant CT and RT was 115 (81.6%) and 96 (68.1%), respectively. In CT group, number of patients receiving CAF and CEF was 41 (29.1%) and 29 (20.6%), respectively. Taxanes were sequentially added to anthracycline based CT protocols in 22 (15.6%) patients.

Characteristics of the two study groups are compared in Table 2. The number of patients with ERBB2 positive and triple negative tumors was 70 and 71, respectively. Groups formed according to ERBB2 status did not have a significant

Table 1. The clinicopathologic properties of the patients

	N	%
Age	Median 49	Range 27-90
<50	78	55.3
≥50	63	44.7
Menopausal status		
Premenopausal	53	37.9
Postmenopausal	87	62.1
Family history		
Negative	124	87.9
Positive	17	12.1
Surgery		
MRM	120	86.3
BCS	19	13.7
Axillary status		
N0	55	39.6
N1	38	27.3
N2	24	17.3
N3	22	15.8
Tumor		
T1	25	17.7
T2	81	57.4
T3	16	11.3
T4	19	13.5
Tumor grade		
II	48	34
III	93	66

MRM: modified radical mastectomy; BCS: breast conserving surgery

Table 2. The clinicopathologic properties of the patients in groups formed according to ERBB2 status

	Group 1	Group 2	P*
Age			
<50 y	40	38	
≥50 y	30	33	0.74
Family history			
Negative	67	57	0.008
Positive	3	14	
Menopausal status			
Premenopausal	28	25	
Postmenopausal	42	45	0.73
Surgery			
MRM	64	56	
BCS	6	13	0.09
Tumor			
T1	11	14	
T2	37	44	
T3	8	8	
T4	14	5	0.16
Tumor grade			
II	28	20	
III	42	51	0.16
Axillary status			
N0	24	31	
N1	22	16	
N2	13	11	
N3	11	11	0.57
Adjuvant CT			
Positive	67	67	
Negative	3	4	0.71
Adjuvant RT			
Positive	46	50	
Negative	24	21	0.59

*with chi-square test; MRM: modified radical mastectomy; BCS: breast conserving surgery; CT: chemotherapy; RT: radiotherapy . Group 1: ER-/PR-/ERBB2+/, Group 2: ER-/PR-/ERBB2- .

difference in any of the study parameters except family history. More patients in ERBB2 negative group had positive family history (19.7% vs 4.3%).

The median follow-up time was 36 months (range, 3-75). Sixty-four patients (45.3%) had disease recurrence and 30 patients (21.3%) died during follow-up. Sixty patients had distant metastases whereas only in eight patients the disease recurred locally, four of which had even concomittant distant metastases. Median OS and DFS durations were 26 (range, 6-75) and 15 (range, 3-65) months, respectively. Five-year DFS and OS were 28% and 66%, respectively. Median OS and DFS were 66 and 24 months for triple negative patient group whereas median DFS was 49 months and median OS was not reached yet for ERRB2 positive patient group.

Prognostic factors affecting DFS and OS are depicted in Tables 3 and 4. In univariate analysis, tumor size and grade, axillary status, patient groups, and treating patients with adjuvant CT and RT were found to be significantly affecting DFS. Patients without axillary lymph node metastasis and treated with adjuvant CT and RT showed better DFS whereas those with ERBB2 negative and larger tumors with higher grade recurred more frequently. On the other hand, OS was significantly dependent on axillary status, type of surgery, and patient groups. Patients treated with MRM, with negative axilla, and a tumor with ERBB2 positivity showed longer OS. In multivariate analysis, patient groups, tumor grade, and axillary status were found as independent prognostic factors for DFS whereas patient groups, extent of surgery, and axillary status were independent prognostic factors for OS.

Discussion

In previous studies, ER status of the tumor was shown to be the most important discriminator of breast cancer subtypes and studies evaluating the prognostic differences only between hormon receptor negative breast cancer subtypes are rare [1–3]. The results of this study have indicated the presence of a group of patients with poorer survival among the hormon receptor negative breast cancer patients. Triple negative breast cancer patients showed decreased DFS and OS compared to those hormon receptor negative patients with ERBB2 positivity. Regardless of conventional prognostic parameters such as tumor size, grade, and axillary lymph node metastases, having a triple negative tumor re-

Table 3. Prognostic factors affecting disease-free survival

Variable	Univariate* (p)	Multivariate** (p)
Age (<50 vs. ≥50)	0.92	—
Family history (neg. vs. pos.)	0.7	—
Menopausal status (premenopausal vs. postmenopausal)	0.15	—
Type of surgery (MRM vs. BCS)	0.79	—
Tumor size (≤3 vs. >3 cm)	0.02	0.72
Tumor grade (II vs. III)	0.0037	0.027
Axillary status (N0 vs. N+)	0.0003	0.003
Adjuvant chemotherapy (yes vs. no)	0.035	0.076
Adjuvant radiotherapy (yes vs. no)	0.0034	0.25
Patient groups (triple neg. vs. ERBB2+)	0.0029	0.015

* log-rank ** Cox-regression MRM: modified radical mastectomy; BCS: breast conserving surgery

Table 4. Prognostic factors affecting overall survival

Variable	Univariate* (p)	Multivariate** (p)
Age (<50 vs. ≥50)	0.87	–
Family history (neg. vs. pos.)	0.07	–
Menopausal status (premenopausal vs. postmenopausal)	0.3	–
Type of surgery (MRM vs. BCS)	0.0035	<0.0001
Tumor size (≤3 vs. >3 cm)	0.25	–
Tumor grade (II vs. III)	0.5	–
Axillary status (N0 vs. N+)	0.0034	<0.0001
Adjuvant chemotherapy (yes vs. no)	0.12	–
Adjuvant radiotherapy (yes vs. no)	0.13	–
Patient groups (triple neg. vs. ERBB2+)	0.0014	0.02

* log-rank ** Cox-regression MRM: modified radical mastectomy; BCS: breast conserving surgery

mained an independent prognostic factor for this group of patients with breast cancer.

There could be various reasons for poorer outcome in triple negative patients. Hormon receptor status of invasive breast cancer is a useful prognostic and predictive factor. Hormon receptor positivity predicts the response rate to endocrine therapy or ovarian ablation. Hormon receptor negative breast cancers are known to be more aggressive tumors with poor prognosis and endocrine therapy is not an effective treatment option for these tumors. ER and PR positive tumors respond to endocrine manipulation in 85% of cases whereas the likelihood of responding decreases to less than 10% in double receptor negative patients [6]. Similar to hormon receptor negative counterparts, triple negative breast cancers are not amenable to conventional treatment strategies such as endocrine therapy and targeted therapies with monoclonal antibodies like trastuzumab. Although ERBB2 negative breast cancer is known to have a favourable prognosis compared to ERBB2 positive disease, the opposite is true in case of triple negative breast cancers. Aggressive clinical behaviour of these tumors due to higher proliferative capacity and lack of effective therapies may contribute to the poor clinical outcome. In addition, sporadic cancers with triple negative phenotype carry similar molecular characteristics to BRCA-1 associated cancers [3]. This may be due to suppression of BRCA-1 gene via several different signalling pathways resulting in the silencing of this gene [3, 8]. In accordance with this, patients with triple negative breast cancer had more frequent positive family history in the current study supporting the possibility of BRCA-1 associated dysfunction although these patients were not evaluated for BRCA-1 mutations.

Patients with triple negative breast cancers were previously reported to have poorer prognosis compared to all other breast cancer patients and patients with ERBB2 positive cancers [3, 5, 6, 9]. This was supported in studies defining basal-like breast cancers based solely on IHC which may be the reflection of molecularly defined subtypes of breast cancer in histopathological examination [10]. Bauer et al. reported 5-year survival in triple negative and non-triple negative breast cancer patients as 77% and 93%, respectively [5]. Similarly, Rakha et al. found that patients with triple negative tumors showed higher recurrence (25% vs 17.7%) and death (17% vs 8.6%) rates compared to other breast cancer patients [6]. In contrast, Carey et al. reported a better survival for basal-like breast cancers compared to hormon receptor negative and ERBB2 positive tumors (75% vs 52%) [4]. However, this study compared all breast cancer subtypes and did not aim to show particular survival differences between the two previously mentioned subtypes.

Triple negative breast cancers resemble basal-like tumors described in molecular studies [1, 2, 11]. Basal-like tumors account for 15% of breast cancer cases and characterized by ER, PR, and ERBB2 negativity in addition to positive staining for basal cytokeratins 5/6 and 17. However, previous cDNA microarray and IHC studies have reported that up to 80-90% of triple negative tumors are basal-like [5, 6]. In this retrospective study, additional IHC staining was not performed to differentiate basal-like tumors, since breast cancer subtypes originally identified by gene expression analysis were matched to IHC profiles by performing both microarray analyses and IHC [4]. Variations in the expression of ER gene correlated with clinical measurement of ER protein levels in tumors [3]. IHC-based assays were reported to be effective in discriminating between intrinsic subtypes and, thus, knowing ER, PR, and ERRB2 status of patients would be enough to define triple-negative and hormon receptor negative breast cancers. Triple-negative phenotype can be used as a surrogate marker for basal-like breast cancer in clinical practice.

Hormon receptor negative and triple negative breast cancers are known as high grade tumors with less differentiation [4–6]. Supporting this finding, only grade II and III tumors were encountered in this study and tumor grade was still found to be an independent prognostic factor affecting DFS in the current study. Efficacy of chemotherapy increases in triple negative breast cancers due to their higher proliferation rate. Triple negative patients had poorer survival when chemo-

therapy was not administered whereas significant decrease in survival abolishes in the chemotherapy group [6].

Presence of axillary lymph node metastases in breast cancer is an important predictive and prognostic factor and it was an independent prognostic factor affecting both DFS and OS in this study. Triple negative breast cancers are not commonly associated with involvement of axillary lymph nodes. Early and frequent distant metastases encountered in this group of tumors may reflect a predominantly hematogenous pattern of dissemination [4]. In addition, incidence of visceral metastases is higher than bone metastases [12]. In accordance with these findings, although triple negative breast cancers had decreased DFS compared to hormon receptor negative breast cancers, axillary lymph node metastases were encountered less (55% vs 66%) in triple negative tumors.

Currently, there is no specific systemic therapy that is recommended for the treatment of triple negative breast cancers. However, previous neoadjuvant chemotherapy studies have reported increased response rates when anthracyclines and taxanes were used for the treatment of triple negative breast cancers [13, 14]. In the current study, all patients were treated with anthracyclines and/or taxanes as the best treatment option available. In addition, several potential systemic treatments based on pathological and molecular characteristics of triple negative breast cancers are being tested in clinical trials. Triple-negative breast cancers were shown to be sensitive to alkylating agents, platinum drugs, mitomycin C, etoposide, and bleomycin and resistant to taxanes and vinca alkaloids in in-vitro studies. These tumors are thought to respond better to platinum-based drugs and targeted therapies involving their molecular structure. Drugs developed against molecular targets such as EGFR, c-KIT, and PARP-1 are being tested in phase I and II studies [12].

In conclusion, molecularly defined subtypes of breast cancer can be differentiated by IHC-based assays in terms of prognosis. IHC is a widely used and less expensive method which is useful in patient populations where fresh tissues are not available and it seems to be the only alternative to use in defining breast cancer subtypes until molecular methods become easily attainable. Since triple negative breast cancers show poor prognosis and carry molecularly defined targets, tumor type specific treatments should be urgently developed and, only when this goal is achieved, better survival rates are to be expected in this group of patients. Until the results of clinical trials on new targeted therapies are available, triple negative breast cancer patients should be informed about their poorer prognosis and closely followed in the clinics.

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