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ORIGINAL ARTICLE

Correlation between c-erbB2 expression, lymphovascular invasion and other biological and clinical prognostic factors and preoperative tumor markers in patients with early-stage and locally advanced breast cancer

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Summary

Purpose: *To evaluate the correlation between c-erbB2 expression, lymphovascular invasion and other biological and clinical prognostic variables and preoperative CA 15-3 and CEA levels in patients with early-stage and locally advanced breast cancer.*

Methods: *Preoperative serum concentrations of CA 15-3 and CEA were measured in 123 patients undergoing surgical treatment for stage I-III breast cancer and the association between these markers and clinical and biological variables were evaluated.*

Results: *With cut-off values of 45 U/ml (CA 15-3) and 2.5 ng/ml (CEA), the sensitivity for CA 15-3 and CEA was 10% and 24% and their mean values were 23 U/ml and 2.32 ng/ml, respectively. A significant correlation between preoperative levels of CA 15-3 and CEA was noticed (p=0.023). Preoperative CA 15-3 levels were significantly higher in patients with tumors > 5 cm (p<0.0001), with positive axillary lymph nodes (p=0.04), with increasing nodal burden (p= 0.025) and in patients with stage III disease (p=0.003). Tumor size >5 cm (p=0.002), increasing axillary nodal burden (p=0.02) and stage III disease (p<0.0001) were also significantly correlated with CEA values above the cut-off level. There were no correlations between CA 15-3 and CEA levels and other variables including c-erbB2 expression, age, grade, hormone receptor status, and lymphovascular invasion.*

Conclusion: *Preoperative CA 15-3 and CEA levels are significantly correlated with tumor size, axillary nodal status and stage in patients with non-metastatic breast carcinoma. No correlation between preoperative values of CA15-3/CEA and c-erbB2 status, lymphovascular invasion and other prognostic factors was detected.*

Key words: breast cancer, c-erbB2, CA 15-3, CEA, prognostic factors, tumor markers

Introduction

Breast cancer is the foremost cause of cancer death in women along with lung cancer, and 1 of every 10 women in the United States is anticipated to develop breast carcinoma. However, there is increasing evidence that early diagnosis with the use of screening methods is associated with reduced cancer mortality for women 50 years of age and older and today more than 90% of patients have only localized disease at the time of initial diagnosis [1,2].

The optimum management of patients with breast cancer requires a multidisciplinary approach and several factors are determined for assessing prognosis or predicting response to therapy. In 1999 The College of American Pathologists presented a Consensus Statement summarizing the prognostic factors in breast cancer by dividing them into 3 categories. Factors in category I that were proven to be of prognostic importance included tumor size, lymph node status, micrometastasis, histologic grade, mitotic count and hormonal receptor status. The expression of c-erbB2, p53 mutations, lymphovascular invasion and DNA ploidy were included in category II and tumor angiogenesis, epidermal growth factor receptor (EGFR), transforming growth factor-alpha, Bcl-2 and overexpression of cathepsin-D were included in category III [3].

CEA and CA 15-3 are the best investigated tumor

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markers in patients with breast cancer but their sensitivity and specificity are low and therefore the ASCO panel in 1996 concluded that "routine use of CA 15-3 and CEA to monitor the course of therapy cannot be recommended." [4]. However, in the same panel it was also stated that "in exceptional circumstances such as the presence of osseous metastasis, which are difficult to evaluate clinically, the marker level may be able to support the clinical estimate of disease status." Furthermore, although their clinical benefits are still controversial, many clinicians, especially in Europe, still use widely CEA and CA 15-3 for the follow-up of women with diagnosed breast cancer.

Despite this statement, there are also some studies stating that high levels of preoperative CEA and CA 15-3 in patients with breast cancer are related to poor prognosis, preoperative CEA and CA 15-3 are correlated exclusively with the size of the tumor and preoperative CA 15-3 level is positively correlated with the number of level I and II positive lymph nodes [5-13]. Evaluating 368 patients with breast cancer, Schering et al. showed that preoperative CA 15-3 levels were significantly correlated with tumor size and nodal burden and the concentrations were also significantly related to both disease-free survival ($p < 0.001$) and overall survival ($p < 0.001$) [9]. Duffy et al. also analyzed the correlation between preoperative CA 15-3 level and prognosis and other biological and clinical variables and they also reported that CA 15-3 concentrations were significantly higher in patients with larger tumors ($p=0.002$), with increasing nodal burden ($p=0.004$) and in those younger than 50 years. The authors also reported that patients with high preoperative concentrations of CA 15-3 (>30 U/ml) had a significantly shorter overall survival than those with low concentrations and CA 15-3 predicted outcome in different subgroups of patients, and was also predictive of outcome irrespective of the type of adjuvant therapy administered [13]. In these two large studies, preoperative CEA levels were not evaluated; however, there are also some smaller studies stating that there is also a correlation between the CEA levels at diagnosis and the stage of cancer, and a positive relationship between serum CEA levels and biologic characteristics of breast cancer were also reported [14,15].

However, the relationship between c-erbB2 expression and CA 15-3 and CEA are evaluated in only a few series in the literature and the results are variable [20-22]. Saghatchian et al. [16] stated that pretreatment levels of c-erbB2 correlated positively with CA 15-3 while Ali et al. showed that serum CA 15-3 and c-erbB2 were weakly correlated ($r = 0.39$; $p < 0.0001$) [17]. Despite these findings, in their study, Molina et al. reported that serum c-erbB2 level was not correlated with either CEA or CA 15-3 levels [18].

Regarding these results, the aim of our study was to evaluate the correlation between c-erbB2 expression, lymphovascular invasion and other biological and clinical prognostic variables and preoperative CA 15-3 and CEA levels in patients with early-stage and locally advanced breast cancer and discuss the recent findings in the literature.

Methods

In the present study, 123 patients with stage I, II and III breast cancer referred to Kocaeli University, Faculty of Medicine between July 2002 and September 2005 were reviewed. Tumors with direct extension to the chest wall or skin (pT4) were not included. The other exclusion criteria were the presence of another cancer or previous cancer history, receiving any neoadjuvant treatment and bilateral or multifocal tumors.

Patient median age was 50 years (range 28-86). Of 123 patients, 94 (76%) had stage I and II disease while the remaining 29 patients (24%) had stage III disease. All patients underwent either modified radical mastectomy or breast-conserving surgery. The presence of distant metastases was excluded by chest X-ray or computed tomography (CT), liver ultrasound, bone scanning and laboratory tests prior to surgical treatment. Preoperative CEA and CA 15-3 serum levels were measured one or two weeks before the operation in all patients. CA 15-3 levels estimation was carried out by two-step sequential chemiluminescent enzyme-linked immunometric assay (Immulite 2000 BR-MA, DPC, Diagnostic Products Corporation, Los Angeles, USA). CEA levels estimation was carried out using solid-phase, two-site sequential chemiluminescent enzyme-linked immunometric assay (Immulite 2000 CEA, DPC, Diagnostic Products Corporation, Los Angeles, USA). Cut-off limits were taken as 2.5 ng/ml for CEA and 45 U/ml for CA 15-3, as recommended by the manufacturer.

Thirty-one (25%) patients had pT1 disease, 74 (60%) pT2 and 18 (15%) had pT3 disease. The median number of dissected axillary lymph nodes was 11 (range 5-29) and nodes were negative (pN0) in 55 (44%) patients, 1-3 positive in 35 (28%), 4-9 positive in 18 (15%) and ≥10 positive in 15 (13%) patients. Estrogen (ER) and progesterone (PR) receptors were both positive in 40 (33%) patients and positive c-erbB2 expression was present in 47 (38%) patients. These variables and other patient characteristics are listed in Table 1.

Statistical analysis

Statistical analysis was conducted with SPSS

Table 1. Patient characteristics

Characteristics	*n*	*%*
Histopathology		
Invasive ductal carcinoma (IDC)	98	80
Invasive lobular carcinoma (ILC)	6	5
IDC+ILC	11	9
Others	8	6
Age (years)		
Range 28-86		
< 50	59	47
≥ 50	64	53
Tumor size (cm)		
0- < 2 (T1)	31	25
2-5 (T2)	74	60
>5 (T3)	18	15
Axillary nodal status		
Negative nodes	55	44
1-3 positive (N1)	35	28
4-9 positive (N2)	18	15
≥10 positive (N3)	15	13
Stage		
I-II	94	76
III	29	24
Histological grade		
1	20	16
2	81	66
3	22	18
Estrogen receptor		
Negative	40	33
Positive	83	67
Progesterone receptor		
Negative	40	33
Positive	83	67
c-erbB2 expression		
Negative	76	62
Positive	47	38
Intraductal component		
Negative	70	57
Positive	53	43
Lymphovascular invasion		
No	75	61
Yes	48	39

package program, version 12.0. The variables that were analyzed for their relationship between CA 15-3 and CEA levels and prognostic significance were age, lymphovascular invasion, stage, histological grade, tumor size, nodal status, ER and PR status and the expression of c-erbB2. The Mann-Whitney *U*-test was used for relating CA 15-3 and CEA concentrations to other variables in the case of non-normal distribution (i.e., T stage, histological grade) and the chi-square test was used for comparison of qualitative variables, and to evaluate the linear relationship between pairs of quantitative variables. A value of $p < 0.05$ was considered to be statistically significant.

Results

According to the cut-off level of 45 U/ml the sensitivity of CA 15-3 for the whole group was 10% (12/123 patients) and the mean CA 15-3 value was 23 U/ml (range 8.8-445). For CEA the sensitivity for the cut-off level of 2.5 ng/ml was 24% (29/123 patients) and the mean value was 2.32 ng/ml (range 0.45-23.1) (Table 2). A significant correlation between preoperative levels of CA 15-3 and CEA was present (p=0.023). There was also a strong correlation between ER and PR rates ($p < 0.0001$) and ER and c-erbB2 expression (p=0.024). A strong correlation between tumor size and axillary nodal status (positive or negative) was also determined (p=0.003) (Table 3).

Relationship between CA 15-3 and prognostic factors

Preoperative CA 15-3 levels were significantly higher in patients with tumors >5 cm ($p < 0.0001$), with positive axillary lymph nodes (p=0.04), with increasing nodal burden (p=0.025) and in patients with stage III disease (p=0.003) (Table 3).

Among 105 patients with tumor size < 5 cm, only 5 (4%) patients had CA 15-3 levels above the cut-off level.

Table 2. Mean CA 15-3 and CEA levels and their correlation

	Sensitivity (%)	*(Patients no./total)*	*Mean value*	*Range*	*p-value*
CA 15-3	10	(12/123)	23 U/ml	8.8-445	0.023
CEA	24	(29/123)	2.32 ng/ml	0.45-23.1	

Table 3. Association between preoperative serum CA 15-3 concentrations and tumor size, axillary nodal status, number of positive lymph nodes and stage

Characteristics	*Percent of patients with increased serum CA 15-3 (no. of patients/total)*		*p-value*
Tumor size (cm)			<0.0001
<2	6	(2/31)	
2-5	4	(3/74)	
>5	38	(7/18)	
Lymph node status			0.04
Negative	3	(2/55)	
Positive	15	(10/68)	
Number of positive nodes			0.025
1-3	11	(4/35)	
4-9	17	(3/18)	
> 10	20	(3/15)	
Stage			0.003
I-II	5	(5/94)	
III	24	(7/29)	

Analyzing serum CA 15-3 concentrations above the cut-off level, a significant difference was found between patients with positive axillary lymph nodes compared with those without nodal involvement (p=0.04).

Increasing nodal burden was also significantly related with preoperative CA 15-3 values above the cut-off, since 6 of 84 patients (7%) with ≤ 3 positive lymph nodes had increased CA 15-3 levels while this rate raised to 22% (6 of 27 patients) in patients with ≤ 4 positive nodes (p=0.025).

CA 15-3 concentrations were also significantly higher in patients with stage III disease (5 of 94 patients with stage I-II disease vs. 7 of 29 patients with stage III disease, p=0.003).

Table 3 summarizes the relationship between CA 15-3 concentrations and tumor size, nodal status and stage.

There were no correlations between CA 15-3 level and other variables including age (p=0.6), grade (p=0.64), ER status (p=0.95), PR status (p=0.2), lymphovascular invasion (p=0.5) and c-erbB2 expression (p=0.2) (Table 4).

Relationship between CEA and prognostic factors

Tumor size >5 cm (p=0.002), increasing axillary nodal burden (p=0.02) and stage III disease (p=0.001) were also significantly correlated with CEA values above the cut-off level. Unlike CA 15-3, axillary lymph node positivity did not reach statistical significance, however there was also a trend in node positive patients for having higher CEA levels (p=0.09). Like CA 15-3, the other variables also had no significant relationship with preoperative CEA concentrations. The results for CEA are summarized in Table 5.

Table 4. Association between CA 15-3 concentrations and histological grade, c-erbB2 expression, hormone receptor status and lymphovascular invasion

Characteristics	*Percent of patients with increased serum CA 15-3 (no. of patients/total)*		*p-value*
Age (years)			0.6
<50	8	(5/59)	
≥50	11	(7/64)	
Histological grade			0.6
1	5	(1/20)	
2	10	(8/81)	
3	13	(3/22)	
c-erbB2 expression			0.2
Negative	6	(5/76)	
Positive	15	(7/47)	
Estrogen receptor status			0.95
Negative	10	(4/40)	
Positive	9	(8/83)	
Progesterone receptor status			0.2
Negative	15	(6/40)	
Positive	7	(6/83)	
Lymphovascular invasion			0.5
Negative	8	(6/75)	
Positive	12	(6/48)	

Discussion

In patients with breast cancer, prognostic factors are useful in identifying those whose outcome is favorable and whose prognosis is poor with conventional approaches, hence warranting consideration for more aggressive therapies. Tumor size, histological grade and nodal status are the major prognostic factors. Among these factors the most significant prognostic indicator for patients with early-stage breast cancer is the presence or absence of axillary lymph node involvement. However, with the advent of mammography, approximately two thirds of newly diagnosed breast cancer patients have node-negative disease and in some patients without axillary nodal involvement the appropriate treatment, especially the use of adjuvant chemotherapy, is under debate regarding the prognostic factors that are already used. Therefore, the need for new variables for determining prognosis has become clear. Also, the incorporation of tumor markers such as alpha-fetoprotein (AFP), human chorionic gonadotropin (HCG), and lactate dehydrogenase (LDH) into the Union Internationale Contre le Cancer (UICC) staging system for testicular germ cell tumors and the recommendation to add

Table 5. Association between preoperative serum CEA concentrations and tumor size, nodal status, number of positive lymph nodes and stage

Characteristics	*Percent of patients with increased serum CEA (no. of patients/total)*		*p-value*
Tumor size (cm)			0.002
<2	19	(6/31)	
2-5	17	(3/74)	
>5	55	(10/18)	
Lymph node status			0.09
Negative	16	(9/55)	
Positive	15	(20/68)	
Number of positive nodes			0.02
1-3	11	(6/35)	
4-9	17	(7/18)	
≥10	20	(7/15)	
Stage			0.001
I-II	16	(15/94)	
III	48	(14/29)	

preoperative CEA concentrations to the staging system for colorectal cancer by the American Joint Committee on Cancer (AJCC) have led the investigators to test the role of preoperative CA 15-3 and CEA concentrations in determining prognosis in patients with breast cancer.

CA 15-3 is a breast-associated antigen encoded by the MUC-1 gene and is the most widely used tumor marker in breast cancer. However, there is a general acceptance that the current role of CA 15-3 in clinical practice includes the diagnosis of symptomatic metastases and the monitoring of response to treatment in patients with metastatic breast carcinoma since its sensitivity and specificity are lower. CEA is a glycoprotein that is normally found in embryonic endodermal epithelium but is also produced by malignant tissues. It is elevated in 30-50% of patients with symptomatic metastatic breast cancer but its specificity is also lower since increased levels have been also detected in patients with colorectal cancer and gastrointestinal, lung, ovarian, prostate, liver and pancreatic cancers.

Despite their low sensitivity and specificity, the results of the studies testing the relationship between these markers and prognosis showed that high preoperative CA 15-3 and/or CEA concentrations predict adverse outcome in patients with breast cancer [10-16] and they are also independent prognostic factors [16-22]. The relationship between these tumor markers and traditional prognostic factors in breast cancer is also tested in these series.

In the Schering et al. study, that evaluated 368 patients with early breast cancer, a significant positive association was found between CA 15-3 concentrations and both tumor stage and the number of involved axillary lymph nodes. Patients with high concentrations of CA 15-3 had a significantly worse prognosis compared with those with low concentrations. The probability of disease-free survival at 5 years was 44% in patients with high CA 15-3 levels compared with 65% in patients with low CA 15-3 levels ($p=0.002$). Overall survival was 67 and 83%, respectively ($p=0.001$) [9].

Gion et al. evaluated 362 patients with breast carcinoma and also reported that there was a significant positive correlation between tumor size and CA 15-3 level. According to their findings, the risk of relapse increased progressively starting from a value of approximately 10 U/ml of CA 15-3 [19].

Another study by Duffy et al. also confirmed the findings of these studies by reporting that CA 15-3, as a prognostic factor, was independent of tumor size, axillary node status, and age and patients with high preoperative CA 15-3 levels (>30 units/L) had a significantly shorter overall survival than those with low concentrations. CA 15-3 was also predictive of outcome irrespective of the type of adjuvant therapy administered (hormone therapy/ chemotherapy or radiotherapy) [13].

Lumachi et al. reported that CEA and CA 15-3 correlated exclusively with the size of the tumor [6] while Seker et al. stated that the only variable that CA 15-3 was positively correlated was the number of level I and II positive lymph nodes [7].

In the Kumpulainen et al. study the disease-specific survival (DSS) at 5 years was 86% and 45% with normal and abnormal CA 15-3 values, respectively ($p=0.0006$). Five-year DSS in patients with stage I and III was 95% and 81%, while it was 70% and 33% in stage III and IV patients, respectively ($p=0.00023$) [20].

There are also some studies stating that CEA level is also correlated with prognosis and some other biologic and clinical variables in patients with breast cancer. Schwartz et al. analyzed the relationships between CEA and ER, PR, age, menstrual status, histological grade, race and pathological stage and found that preoperative CEA level was related only to pathologic stage [15].

Lokich et al., in a different study, showed that rising CEA levels were correlated with subsequent progression of disease in all patients with elevated baseline levels at a minimum of 8 weeks before the progression was clinically evident [14].

However, the relationship between these markers and c-erbB2 expression is tested only in limited studies [16-18]. In the Saghatchian et al. trial, pretreatment levels of c-erbB2 correlated positively with CA 15.3 ($p=0.0169$), pathological tumor size ($p=0.0082$), number of involved lymph nodes ($p=0.0160$) and histological grading ($p=0.0086$) [16]. Evaluating 566 patients with non-metastatic breast cancer, Ali et al. also stated that serum CA 15-3 and c-erbB2 were weakly correlated ($r=0.39$; $p < 0.0001$) [17]. Despite these findings Molina et al. prospectively evaluated 3 tumor markers (c-erbB2, CEA and CA 15-3) and stated that all these markers were independent prognostic factors for disease-free survival and reported that serum c-erbB2 level was not correlated with either CEA or CA 15-3 levels [18].

Like the Molina et al. results, our data also confirm that there is no correlation between c-erbB2 status, lymphovascular invasion and other prognostic factors between preoperative CA 15-3 and CEA levels. However, these markers are significantly correlated with tumor size, axillary nodal status and stage in patients with non-metastatic breast carcinoma.

In a recent study, Lialiaris et al. stated that ER and E-cadherin were expressed more commonly in tumors of low histological grade and small number (≤ 3) of metastatic lymph nodes, whereas c-erbB2 and the p53 gene were usually expressed in breast tumors of high

histological grade and increased number (>3) of metastatic lymph nodes [23].

Kesisis et al. and Baskic et al. also showed that biological markers exhibit prognostic and predictive significance in breast cancer and could be used to guide personalized treatment by estimating patient prognosis and risk of relapse and tailor accordingly therapeutic approaches [24,25].

Regarding our findings we believe that-correlated with tumor size, histological grade and hormonal receptor status-these markers can provide independent prognostic information, especially in node-negative breast cancer, and can be used as complementary tools when deciding to administer adjuvant chemotherapy or not. High preoperative CA 15-3 and CEA concentrations may also guide physicians to consider more aggressive treatments in patients with axillary nodal involvement. Considering the development in molecular and genetic analyses in breast cancer further clinical studies may be warranted to optimize the use of these markers in advanced disease as well as to utilize their use in screening and diagnosis of early breast tumors.

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