RESEARCH ARTICLE

Correlation of Preoperative Ki67 and Serum CA15.3 Levels with Outcome in Early Breast Cancers - a Multi Institutional Study

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Abstract

Background: To investigate the association between preoperative pathological Ki-67 labeling index and serum tumor marker cancer antigen 15-3 (CA 15-3) with clinic-pathological parameters and treatment outcomes in early breast cancer. Materials and Methods: A retrospective study at 4 cancer centers in Saudi Arabia and Egypt was performed. Data were collected for female patients diagnosed with unilateral early breast cancer between March 2010 and October 2013. Cases treated with neoadjuvant chemotherapy (NACT) followed by surgery and radiotherapy were included. NACT included 6-8 cycles of anthracycline and taxane based regimens. Trastuzumab and hormonal treatments were added according to HER2 and hormone receptor status. Baseline serum CA15.3 and pathological Ki67 levels were evaluated and correlated with disease free survival (DFS) and overall survival (OS). Results: A total of 280 pts was included. The median age was 49 years (38-66 y) and median overall survival was 35 (20-38) months (mo). Estrogen receptors (ER), progesterone receptors (PR) and HER 2 receptors were positive in 233 (83.2%), 198 (70%) and 65 cases (23.2%), respectively. High preoperative Ki67 and CA15.3 were noted in 177 (63.2%) and 131 (46.8%). A total of 45 (16%) patients had distal or local recurrence and 24 (8.6%) died of their disease. Most of the relapsed cases had high preoperative Ki-67 (n=41, 91%) and CA15.3 (n=28, 62%) values. All of the patients who died had a high Ki-67 but CA15.3 was high in 9 (37%) only. Mean DFS/OS in patients with high preoperative Ki-67 was 32 months /32 months as compared to 37 months/35 months in those with normal Ki-67 (p<0.001). Correlation of preoperative CA15.3 and survival was statistically not significant. Conclusions:Preoperative Ki-67 can be a predictive and prognostic marker. Higher levels are associated with poor DFS and OS in patients with early BC.

Keywords: Early breast cancer - Ki67 - CA15.3 - chemotherapy - DFS and OS

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Introduction

By definition, the early-stage breast cancer (EBC) is the cancer that has not spread beyond the breast or axillary lymph nodes, including ductal carcinoma in situ, stages I, IIA, IIB, and IIIA (Fotinos-Ioannis D, et al, 2015).

Early-stage breast cancer is potentially curable. In developed countries more than 80% of patients(pts) with EBC have long-term survival after surgery with or without adjuvant treatment for some patients (Coleman M, et al, 2008).

Prognosis of recurrent breast cancer (BC) after the initial treatment is poor. Tumor size, nodal status, hormone receptor status and human epidermal growth factor receptor 2 (HER2) expressions are the prognostic factors used in clinical practice, but identification of more prognostic and predictive markers are needed to guide clinicians in treating those patients more effectively. The aim of our study was to evaluate the Correlation of Ki67 and CA15.3 with survival.

Materials and Methods

Patients

In this retrospective study, performed across 4 oncology centers in Saudi Arabia and Egypt, we evaluated 280 women who were diagnosed with unilateral early breast cancer from between March 2010 and October 2013. Patients received standard neoadjuvant chemotherapy (6-8 cycles of an anthracycline and Ttaxane-containing

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regimen) with or without trastuzumab according to the human epidermal growth factor receptor 2 (HER2) status. After excluding patients who underwent breast conservative surgery because of a possible high incidence of recurrence, the included patients underwent modified radical mastectomy with axillary clearance followed by local radiotherapy with/without hormonal treatment for Candide patients, according to the hormonal receptor status and menopausal statuses.

Methods

In this retrospective study, we evaluated the baseline serum CA 15-3 levels and tissue Ki-67 labeling index in women with unilateral early breast cancer. Tumor staging was based on the sixth American Joint Committee on Cancer Criteria, and the choice of staging work- up, treatment protocols, and follow- up were based on the NCCN guidelines.

Baseline evaluation of serum levels of CA 15-3 was performed for patients with confirmed breast cancer. Approximately 5 ml of venous blood was collected for measuring CA 15-3 levels by using an automatic electrochemistry luminescence immunoassay system. The cutoff values was 25 U/ml.

We classified the Ki-67 labeling index into two groups - as low or high based on the percentage of cells stained among the cells in the field selected in the tissue section. (Among the 4 centers in this study, 3 of them used 14% as the cutoff value, while 1 center used 25% as the cutoff value.

The scoring method for HER2 expression by immunohistochemistry (IHC) was based on the cell membrane staining pattern. The results were classified as follows: 3+: positive HER2 expression (uniform intense membrane staining of more than 30% of invasive tumor cells); 2+: equivocal HER2 protein expression (complete membrane staining that is either nonuniform or weak in intensity, but has a circumferential distribution in at least 10% of cells); and 0 or 1+, negative for HER2 protein expression.

Fluorescence in situ hybridization (FISH) was used to establish the HER2 status when IHC yielded equivocal results. The interpretation of HER2 FISH results (HER2-to-CEP 17 ratio and gene copy number) was as follows: positive HER2 amplification, FISH ratio > 2.2 or HER2 gene > 6.0; equivocal HER2 amplification, FISH ratio of 1.8-2.2 or HER2 gene copy of 4.0-6.0; and negative HER2 amplification, FISH ratio <1.8 or HER2 gene copy < 4.0.

Statistical Analysis

SPSS version 17.0 was used for data analysis. Quantitative data are presented as the mean and standard deviation (SD). Parametric and non-parametric t-tests were used for comparison of two independent groups. The Kaplan-Meier method was used to estimate overall survival. Overall survival was measured from the first day of treatment until death, while disease- free survival was defined as the period from the date of diagnosis until the date of first recurrence, either loco-regional or systemic.

The log rank test was used to compare survival between the groups. The χ^2 and Fisher exact tested were used to determine the proportion of independents. The log-rank test was used to calculate P- values for the differences between groups. For all the above- mentioned statistical tests, the threshold of significance was fixed at the 5% level. The P value of >0.05: indicates non-significant results.

Table 1. Patient Characteristics

Variable	Number	%
Age		
Range	38-66 y	
Mean	50.52	
Median	49	
<45	65	23.20%
≥45	215	76.70%
Menopausal state		
Pre-menopausal	139	49.60%
Post- menopausal	141	50.40%
Tumor size		
T1	19	6.80%
T2	131	46.80%
≥T3	130	46.40%
Nodal status		
N0	46	16.40%
N1	119	42.50%
N2	86	30.70%
N3	29	10.40%
TNM stage		
I	83	29.65%
II	84	30.00%
III	113	40.35%
Pathological type		
DCIS	261	93.20%
LCIS	19	6.80%
Grade		
G I	19	6.80%
G II	168	60.00%
G III	93	33.20%
LVI		
Yes	112	40.00%
No	168	60.00%
ER status		
Negative	47	16.80%
Positive	233	83.20%
PR status		
Negative	84	30.00%
Positive	196	70.00%
HER2 Status		
Negative	215	76.80%
Positive	65	23.20%
CA15.3		
Normal	149	53.20%
High	131	46.80%
Ki67		
Low	103	36.80%
High	177	63.20%
Recurrence		
No	235	83.90%
Yes	45	16.10%
Mortality		
Alive	256	91.40%
Died	24	8.60%

Table 2. Correlation between CA15.3 and Ki 67 with Different Patients and Disease Variables

Variable	CA 15-3			Ki67		
	Normal 149 (53.2%)	High 131 (46.8%)	P value	Normal 103 (36.8%)	High 177 (63.2%)	P value
Age						
<45y 65(23.2%)	9(13.8%)	56(86.2%)	<0.000	0 (0.0%)	65 (100.0%)	<0.000
≥45y 215(76.7)	140(65.1%)	75(34.9%)		103 (47.9%)	112 (52.1%)	
Menopausal state						
Pre-menopausal 139(49.6%)	64 (46.0%)	75 (54.0%)	0.017	36 (25.9%)	103 (74.1%)	0
Post- menopausal 141(50.4%)	85 (60.3%)	56 (39.7%)		67 (47.5%)	74 (52.5%)	
Tumor size						
T1 19(6.8%)	19(100%)	0(0.00%)	<0.00	2(10.5%)	17(89.5%)	0.001
T2 131(46.8%)	66(50.4%)	65(49.6%)		57(43.5%)	74(56.5%)	
≥T3 130(46.4%)	64(49.2%)	66(50.8%)		46(35.4%)	84(64.6%)	
Nodal status						
N0 46(16.4%)	9(19.6%)	37(80.4%)		19 41.3%)	27 (58.7%)	0
N1 119(42.5%)	83(69.7%)	36(30.3%)	<0.00	27 22.7%)	92 (77.3%)	
N2 86(30.7%)	47(54.7%)	39(45.3%)		38 44.2%)	48 (55.8%)	
N3 29(10.4%)	10(34.5%)	19(65.5%)		19 65.5%)	10 (34.5%)	
TNM stage						
I 83 (%)	61(73.7%)	22(26.5 %)		4(4.9%)	79(95.1%)	
II 84 (%)	33(39.3%)	51(60.7 %)	0 .05	53(63.1%)	31(36.9%)	
III 113(%)	64(56.7 %)	49(43.3%)		46(40.7%)	67(59.3%)	
Pathological type						
DCIS 261(93.2%)	140 (53.6%)	121 (46.4%)	0.38	84 (32.2%)	177 (67.8%)	<0.000
LCIS 19(6.8%)	9 (47.4%)	10 (52.6%)		19 100.0%)	0(0.0%)	
Grade						
G I 19(6.8%)	10 (52.6%)	9(47.4%)	0.002	9(47.4%)	10 (52.6%)	<0.000
G II 168(60.0%)	103 (61.3%)	65(38.7%)		84 (50.0%)	84 (50.0%)	
G III 93(33.2%)	36 (38.7%)	57(61.3%)		10(10.8%)	83(89.2%)	
LVI						
Yes 112 (40%)	28 (25%)	84 (75%)	0	37(33%)	75(67%)	0.313
No 168 (60%)	121(72%)	47 (28%)		66 (39.3%)	102(60.7%)	
ER						
Negative 47(16.8%)	19(40.4%)	28(59.6%)	0.057	0(0.0%)	47(100.0%)	<0.000
Positive233(83.2%)	130(55.8%)	103(44.2%)		103(44.2%)	130(55.8%)	
PR						
Negative 84(30.0%)	47(56.0%)	37(44.0%)	0.6	10(11.9%)	74(88.1%)	<0.000
Positive 196(70.0%)	102(52.0%)	94(48.0%)		93(47.4%)	103(52.6%)	
HER2 Status						
Negative 215(76.8%)	112(52.1%)	103(47.9%)	0.571	93(43.3%)	122(56.7%)	0
Positive 65(23.2%)	37(56.9%)	28(43.1%)		10(15.4%)	55(84.6%)	

Results

The median age of these patients was 49 years (range, 38-66 years); only 23.3% of the patients were aged <45 years. Only 50% in this study were pre-menopausal. The TNM stage was I, II, and III in 29.6%, 30%, and 40.3 of the patients, respectively. Most of the patients (93.2%) had ductal carcinoma in situ.

Regarding the hormone receptor status, estrogen receptor (ER) was confirmed as positive in 83.2% of the patients and progesterone receptor (PR) was positive in 70%. Confirmed HER 2 positivity was observed in 23.2% of the patients.

Pre-treatment evaluation showed that the Ki-67 labeling index was high in 63.2% of the patients, while serum CA 15-3 level was high in 46.8% of the patients. Recurrence was observed in 45 of 280 patients (16.1%); this recurrence was observed as local recurrence and distant metastasis in 11 and 34 patients, respectively (Table 1).

Of 24 patients who died of disease in our study, 15 were aged > 45 years while the remaining 9 patients were aged ≤ 45 years (62.5% *vs*. 37.5%, respectively; p=0.083).

For statistical analysis, we divided the patients into two groups by age (<45 years and ≥45 years); as shown in Table 2, all the patients with a high Ki-67 labeling index were aged <45 years (p=0.00) and 86.2% of the patients in the same age group had higher CA15-3 levels (p=0.00).

Both the CA 15-3 levels and the Ki-67 labeling index were correlated with the tumor size, lymph node involvement, pathological grade, and pathological stage. However, this correlation was not statistically significant for the pathological type (ductal carcinoma in situ or lobular carcinoma in situ) considering CA 15-3 levels (p=0.38), but they remained significant considering the ki-67 labeling index (p=0.00).

Regarding the hormone receptor status, although there was a significant correlation between the Ki-67 labeling index with both ER and PR overexpression, there was only a borderline significant correlation between CA 15-3 levels and ER expression. In addition, even though there was no significant correlation between CA 15-.3 levels and PR over-expression (p=0.6), there was a significant correlation between the Ki-67 labeling index with PR

Table 3. Correlation between CA15.3 and Ki 67 with Survival

Variable	Normal 149(53.2%)	High 131 (46.8%)	P value	Normal 103 (36.8%)	High 177 (63.2%)	P value
Recurrence						
No 235(83.9%)	132(56.2%)	103(43.8%)	0.033	99(42.1%)	136(57.9%)	0
Yes 45(16.1%)	17(37.8%)	28(62.2%)		4(8.9%)	41(91.1%)	
Mortality						
Alive 256(91.4%)	134(52.3%)	122(47.7%)	0.396	103(40.2%)	153(59.8%)	0
Died 24(8.6%)	15 (62.5%)	9(37.5%)		0(0.00%)	24(100.0%)	
Survival(Months)						
Mean			33.29 ±0.304			
Median			35.00±5.082			
Range			20-38			
DFS						
(Mean) months	35.114(±0.663)	32.748(±0.882)	0.025	37.029(0. 476)	32.242(±0. 792)	0
Overall	34.005 (±0.548)			34.005(±0. .548)		
OS						
(Mean) months	36.270(±0.424)	37.000(±0.322)	0.327	34.82 (±0.30)	32.41 (±0..43)	0
Overall	36.612 (±0.272)			33.29 (±0. 304)		

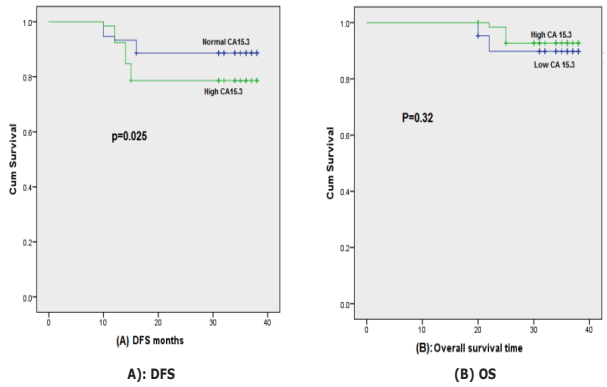

Figure 1. Survival Curves for Baseline CA15.3 Level

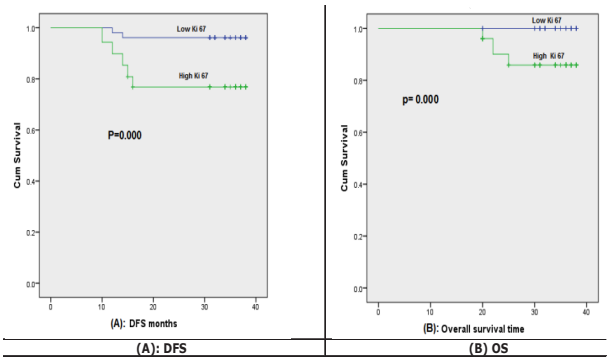

Figure 2. Survival Curves for Baseline Ki 67 Level

overexpression (p=0.00).

HER2 overexpression showed a significant correlation with the Ki-67 labeling index (p=0.00) and a borderline significant correlation with the CA 15-3 level (p=0.57).

The median follow- up in our study was 33.18 months (range, 20-38 months). Disease- related mortality was observed in 24 patients; all of them had a high Ki- 67 labeling index (p=0.00), but only 37.5% of the patients who died had high baseline CA 15-3 levels (p=0.39). On the other hand, there was a significant correlation between both the CA 15-3 level and the Ki- 67 labeling index with the recurrence rate (p=0.033 and p=0.00 respectively).

The baseline CA 15-3 level was significantly correlated with the disease-free survival (p=0.025) but not with the overall survival (36.2 ±0.424 months for patients with a normal baseline level *vs*. 37.0±0.322 months for patients with high levels; P=0.327) (Table 3).

The baseline Ki-67 labeling index was statistically significantly correlated with both the disease-free survival and the overall survival (p=0.00 for both) (Figure1, 2).

Discussion

The first known attempt to determine markers for malignancy was performed 2000 years ago; it has been described in an Egyptian papyrus scroll, where breast cancer was distinguished from mastitis (Waxman, 1995).

The three most important characteristics of an ideal tumor marker are (a) high specificity for the given tumor type, (b) lead- time after clinical diagnosis, and (c) highly sensitive to avoid false- positive results. In addition, the levels of the marker should be correlated reliably with the tumor burden, accurately reflecting any tumor progression or regression, along with a short half-life, thereby allowing frequent serial measurements.

Clinical applications of tumor markers can be broadly classified into 4 four groups: screening and early detection, diagnostic confirmation, prognosis and prediction of therapeutic response, and monitoring of disease and recurrence (Sokoll L and Chan D, 2004).

A prognostic factor is a measurement taken at the time of diagnosis or surgery that is associated with the outcome (e.g: overall survival, disease- free survival and local control). Predictive factors predict the response or the lack of it to specific treatments (DeVita et al, 2014).

In the field of oncology, the prognostic and predictive factors are divided as either serum markers or pathological tissue markers, with distinct advantages and disadvantages.

The CA 15-3 level is directly associated with the tumor burden in the host, and the presence of serum tumor-associated antigens in serum at diagnosis indicates vascularisation of the tumor with possible micrometastases (Gasparini G, et al, 1997); preoperative levels of serum tumor markers could be related to poor outcomes.

Despite some controversies, the levels of both CA 15-3 and carcinoembryonic antigen could provide independent prognostic information that could be used

along with conventional markers measured in tumour tissues (Gasparini G, 1998).

In patients with operable breast cancer, presurgical CA15.3 value is an independent prognostic factor for metastases and deaths. CA15.3 provides additional information to the common prognostic factors and should be considered in the adjuvant therapeutic algorithm (Sandri M, 2012).

Shao et al., 2015 showed that the univariate and multivariate Cox's regression analysis revealed that elevated preoperative CEA and CA 15-3 levels were independent prognostic factors for DFS and OS and the preoperative serum levels of CEA and CA 15-3 are independent prognostic parameters for breast cancer.

Significant association was found among changing patterns of serum CA15-3 levels and breast cancer recurrence rate (Chu W, and Ryu D, 2016).

In the current study, the serum CA 15-3 level was associated with a larger tumor size, presence of positive lymph nodes, and a high tumor grade. This significant finding is in concordance with that shown previously with Tampellini M, et al, 1997, Molina R, et al ,1998, Canizares F, et al ,2001, Molina R, et al ,2003, and Duffy MJ, et al ,2004, Hashim Z, 2014.

A meta-analysis involving 12,155 patients demonstrated that the Ki-67 positivity confers a higher risk of recurrence and a worse survival rate in patients with early breast cancer. Even though this meta-analysis could not scrutinize if Ki-67 had independent prognostic value beyond the standard clinic-pathological variables, it confirmed that high levels of Ki-67 are associated with worse prognoses (De Azambuja E, et al, 2007).

Recently, the "International Ki-67 in Breast Cancer Working Group" published their recommendations based on current evidence concerning the evaluation and the interpretation of Ki-67, pursuing to increase the interlaboratory comparability and analytical validity of this marker in clinical practice, which is one of the most robust biomarkers measured by immunohistochemistry (IHC). They emphasized the potential of Ki-67 involving prognosis, prediction of relative response or deficiency to chemotherapy, and as a dynamic biomarker of the treatment effectiveness (Gnant M, et al, 2011).

While the prognostic values of the other molecular markers were not significant, combined Ki67 and p53 status was an independent prognostic factor by multivariate analysis (Kobayashi T, et al, 2013).

High Ki67 labeling index (LI), and high nuclear grade were significantly worse prognostic factors for distant metastasis (Kurebayashi J, et al, 2014).

In this study, we also evaluated the Ki-67 labeling index also as a prognostic marker for disease-free survival and overall survival in pre-treatment core biopsies. Many studies have investigated the use of the Ki-67 labeling index as a prognostic marker for breast cancer (Colozza M, et al ,2005, Stuart-Harris R, et al ,2008, Jones RL, et al ,2009, Yerushalmi R, et al ,2010, and Tanei T, et al , 2011). In most of those studies, a higher Ki-67 labeling index was associated with a poor prognosis.

In conclusion, the CA 15-3 level and the Ki-67 labeling index are reliable, simple, easily applicable, and effective prognostic and predictive factors for patients with early breast cancer who received proper staging work- up, underwent surgery by a qualified expert surgical oncologist, and received neoadjuvant and/or adjuvant treatment as per the standard guidelines.

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