30. Rugo HR, Roche H, Thomas E et al. Ixabepilone–capecitabine vs capecitabine in patients with triple negative tumors: a pooled analysis of patients from two large phase III clinical studies. In San Antonio Breast Cancer Symposium, San Antonio, TX, 2008; Abstract 3057.
31. Sledge G, Miller K, Moisa C et al. Safety and efficacy of capecitabine (C) plus bevacizumab (B) as first-line in metastatic breast cancer. J Clin Oncol 2007; 25: 1013.
32. Kotsori AA, Dolly S, Sheri A et al. Is capecitabine efficacious in triple negative metastatic breast cancer? Oncology 2010; 79: 331–336.

Annals of Oncology 24: 1225–1231, 2013
doi:10.1093/annonc/mds604
Published online 9 December 2012

Elevated levels of preoperative CA 15-3 and CEA serum levels have independently poor prognostic significance in breast cancer

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Received 10 July 2012; revised 8 October 2012; accepted 16 October 2012

Background: To evaluate the prognostic value of preoperative tumor markers, cancer antigen 15-3 (CA 15-3) and carcinoembryonic antigen (CEA), in breast cancers.
Patients and methods: Preoperative CA 15-3 and CEA levels of 1681 patients were measured. The association of both tumor markers levels with clinicopathological parameters and outcomes was investigated by univariate and multivariate analyses.
Results: Among 1681 patients, elevated preoperative CA15-3 and CEA levels were identified in 176 and 131 patients, respectively. Higher preoperative CA 15-3 and CEA levels were significantly associated with a larger tumor size, axillary node metastases, and advanced stage. Patients with elevated CA 15-3 and CEA levels showed worse survival, even in stage-matched analysis. Patients with normal levels of both CA15-3 and CEA showed better survival than those with one or both markers levels elevated. In multivariate analysis, elevated preoperative CA 15-3 and CEA levels were independent prognostic factors. The statistical significance of elevated preoperative tumor markers levels on survival was solidified with longer follow-up and larger study population.
Conclusions: Elevated preoperative CA 15-3 and CEA levels are associated with tumor burden and showed independent prognostic significance. Therefore, new treatment strategies are necessary for patients with elevated preoperative CA 15-3 and CEA levels in clinical practice.
Key words: breast cancer, CA 15-3, CEA, prognostic factor, tumor marker

introduction

Breast cancer is the most frequently occurring cancer in women from western countries and continues to be the most common fatal cancer together with lung, bronchus and colorectum cancers [1]. It is also the second most common malignancy in Korean women [2]. Despite the rising incidence of breast cancer, the survival rates have improved in recent years due to earlier detection and an increasing use of more effective systemic treatments based on prognostic factors [3]. Therefore, identifying prognostic and predictive factors is important to assist in decision making about treatment and to improve survival.

Along with the traditional prognostic factors such as tumor size, tumor grade, and lymph node status [4], the prognostic value of serum tumor markers has been investigated in breast cancer [5–8]. Some studies suggested that elevated carcinoembryonic antigen (CEA) and cancer antigen 15-3 (CA 15-3) levels provided the significant prognostic information; however, others reported no independent value of serum tumor markers [5–7]. Recently, Maric et al. [8] reviewed the role of serum tumor markers in breast cancer and they pointed out conflicting results of its prognostic value and rather emphasized the necessity of more extensive investigations for improved and a more cost-effective management of breast

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cancer. The American Society of Clinical Oncology (ASCO) guidelines do not currently recommend the use of serum CA 15-3 and CEA for breast cancer screening, as a routine surveillance tool or for therapeutic response monitoring due to inconsistent findings of their sensitivity and specificity [9]. However, serum tumor markers such as CA 15-3 and CEA are the most widely used serum tumor markers for surveillance purposes and treatment response in clinical practice [10–13].

Previously, we reported that patients with elevated preoperative levels of CA 15-3 and CEA had worse outcomes than those with normal levels by stage-matched and multivariate analyses [14]. However, our previous study had a relatively short follow-up duration and a small sample size. Therefore, we aimed to reconfirm the prognostic value of preoperative CA 15-3 and CEA levels while overcoming the previous limitations. We reinvestigated the data of our previous study cohort of 740 patients with extended follow-up and then added consecutively diagnosed breast cancer patients with a larger sample size.

patients and methods

patients selection

From April 1999 to December 2006, we investigated serum CA 15-3 and CEA concentration levels from a total of 1681 patients who were treated for stage I–III invasive breast cancer at Yonsei University Severance Hospital; 740 patients (group I) who had breast surgery between April 1999 and December 2003 [14] and another 941 patients (group II) who had breast surgery between January 2004 and December 2006. We excluded patients with stage IV disease at diagnosis, carcinoma *in situ*, unknown TNM stage and receiving neoadjuvant chemotherapy. All data including serum CA 15-3 and CEA levels at the time of diagnosis were obtained from the Severance Hospital Breast Cancer Registry, which is a prospectively maintained database that includes clinical and pathological information, treatment modality, and details of outcomes including disease recurrence and death. Retrospectively to confirm the significance of preoperative serum CA 15-3 and CEA levels in this longer follow-up study, we first reinvestigated the survival outcomes of group I and then evaluated the relationship between the level of tumor markers, clinicopathological characteristics and survival outcomes in a larger study population including groups I and II.

Management of all patients was based on international guidelines and adjuvant treatment with radiotherapy, chemotherapy and hormone therapy was not altered according to the marker levels. TNM staging was based on the sixth American Joint Committee on Cancer criteria. To detect local or distant relapse, clinical follow-up was carried out every 6 to 12 months, which included recording patient's history, physical examination, laboratory tests of CEA, CA 15-3, complete blood counts, and liver function test, chest radiography, mammography, breast and abdominopelvic ultrasonography, and bone scans. In addition, a computed tomography (CT) scan or a fluorine-18 fluorodeoxyglucose positron emission tomography (FDG PET)/CT scan was carried out if necessary.

tumor marker analysis

We measured the concentration of serum tumor markers by using automated immunoanalyzer systems using a chemiluminescent

Table 1. General characteristics of study population

Characteristics	Group I ($n = 740$)		Group II ($n = 941$)		P	Total population ($N = 1681$)	
	n	%	n	%		n	%
Age (years)							
≤35	75	10.1	54	5.7	0.001	129	7.7
>35	665	89.9	887	94.3		1552	92.3
Tumor size							
T1	410	55.4	589	62.6	<0.001	999	59.4
T2	308	41.6	343	36.5		651	38.7
≥T3	22	3.0	9	1.0		31	1.8
Nodal status							
N0	429	58.0	633	67.3	<0.001	1062	63.2
N1	183	24.7	221	23.5		404	24.0
N2	86	11.6	56	6.0		142	8.4
N3	42	5.7	31	3.3		73	4.3
TNM stage							
I	283	38.2	452	48.0	<0.001	735	43.7
II	329	44.5	400	42.5		729	43.4
III	128	17.3	89	9.5		217	12.9
HG ($n = 1420$)							
I	123	19.6	187	23.6	0.018	310	21.8
II	316	50.3	416	52.5		732	51.5
III	189	30.1	189	23.9		378	26.6
ER ($n = 1648$)							
Negative	267	37.6	295	31.4	0.009	562	34.1
Positive	443	62.4	643	68.6		1086	65.9
PR ($n = 1649$)							
Negative	387	54.4	349	37.2	<0.001	736	44.6
Positive	324	45.6	589	62.8		913	55.4
HER2 ($n = 1637$)							
Negative	434	62.1	801	85.4	<0.001	1235	75.4
Positive	265	37.9	137	14.6		402	24.6
CA 15-3							
Normal (≤20.11)	648	87.6	857	91.1	0.020	1505	89.5
Elevated (>20.11)	92	12.4	84	8.9		176	10.5
CEA							
Normal (≤3.88)	661	89.3	889	94.5	<0.001	1550	92.2
Elevated (>3.88)	79	10.7	52	5.5		131	7.8
Surgery							
BCS	218	29.5	352	37.4	0.001	570	33.9
TM	522	70.5	589	62.6		1111	66.1
Chemotherapy							
None	149	20.1	259	27.5	<0.001	408	24.3
Done	591	79.9	682	72.5		1273	75.7
Endocrine therapy							
None	247	33.4	270	28.7	0.037	517	30.8
Done	492	66.6	671	71.3		1163	69.2
Radiation therapy							
None	403	54.5	516	54.8	0.878	919	54.7
Done	337	45.5	425	45.2		762	45.3

TNM, tumor-node-metastasis; HG, histologic grade; ER, estrogen receptor; PR, progesterone receptor; HER2, human epidermal growth factor receptor 2; CA 15-3, cancer antigen 15-3; CEA, carcinoembryonic angigen; BCS, breast conserving surgery; TM, total mastectomy.

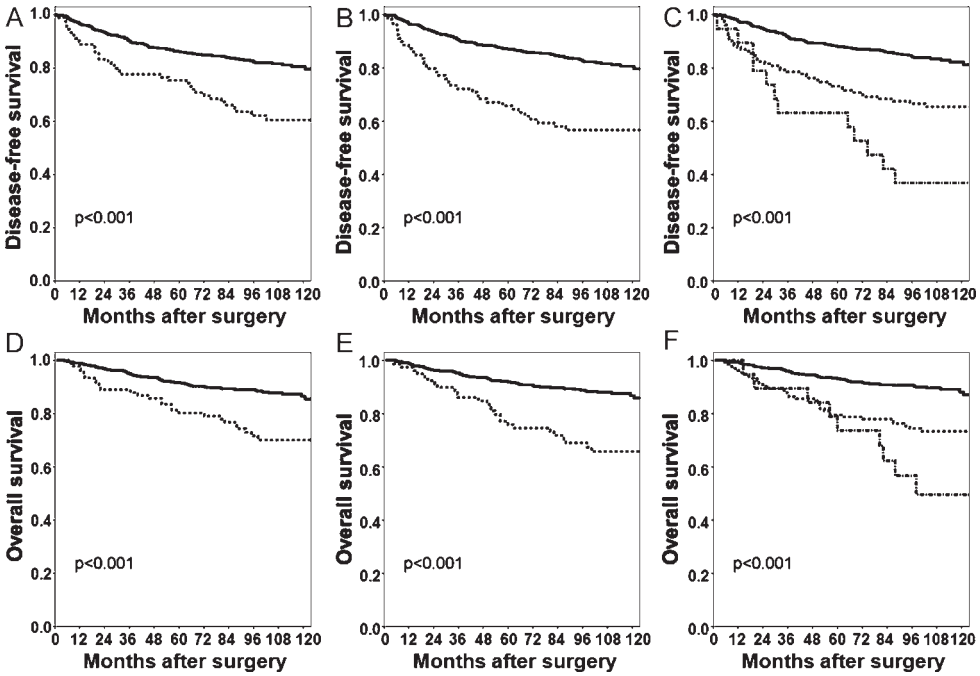

Figure 1. Survival curves of group I with an extended follow-up duration. Disease-free survival (DFS) according to preoperative cancer antigen 15-3 (CA 15-3) (A), carcinoembryonic antigen (CEA) (B), and combination of both markers levels (C) and overall survival (OS) according to preoperative CA 15-3 (D), CEA (E) and combination of both the markers (F). The bold line represents patients with normal levels and dotted line represents patients with elevated levels (A, B, D and E). The bold line represents patients with normal levels of both the markers, the dotted line represents patients with elevated either marker, and the chain line represents patients with elevated both markers (C and F).

immunoassay for CEA (ADVIA Centaur, Bayer HealthCare LLC Diagnostic Division, NY) and CA 15-3 (VITROS ECi Immunodiagnostic System, Ortho-Clinical Diagnostics, Inc., NY). We defined the cut-off values of tumor marker as the 95th percentile of healthy individuals, which was already used in our previous study (CA 15-3: 20.11 U/L, CEA: 3.88 ng/ml) [14]. Tumors with ≥10% nuclear-stained cells were considered positive for the estrogen receptor (ER) and progesterone receptor (PR). Human epidermal growth factor receptor 2 (HER2) immunohistochemistry (IHC) was carried out using the HercepTest™ (DAKO, Glostrup, Denmark) and interpreted as 0, 1+, 2+, or 3+. HER2 was considered positive in cases with an IHC 3+ score.

statistical analysis

The difference between proportions was evaluated by the chi-square test. Disease-free survival (DFS) was defined to be from the time of surgery to the locoregional recurrence, distant metastasis, and death before recurrence. Distant relapse-free survival (DRFS) was defined as the time from surgery to the distant recurrence. Overall survival (OS) was defined to be from the time of surgery to death from any cause. DFS and OS were estimated using the Kaplan–Meier method and the group differences in survival time were tested using the log-rank test. Multivariate Cox's proportional hazard analysis was carried out to compare and identify independent prognostic factors for DFS, DRFS and OS and to calculate hazard ratios. All significant parameters in the univariate analysis were entered into a multivariate model. All reported *P* values are two-sided, and *P* values <0.05 were considered significant. SPSS for Windows (version 15.0) was used for all statistical analyses.

results

The median age of the study population was 48 years (range 20–88 years) and the median follow-up time was 72 months (range 0.8–143.6 months). The median follow-up duration was extended to 98.8 months for the previously reported group I patients and was 63.0 months for the newly added 941 patients (group II). The general characteristics of the study population are summarized in Table 1. Compared with the group I patients, the group II patients were older, detected earlier, had favorable characteristics, and a few number of elevated preoperative tumor marker levels. During follow-up, among the total study population, recurrence occurred in 208 patients (first relapse: local recurrence alone $n = 32$, systemic recurrence alone $n = 124$, both local and systemic recurrences $n = 52$) and death occurred in 150 patients.

Survival curves of group I with an extended follow-up duration are shown in Figure 1. Elevated CA 15-3 or CEA levels were clearly associated with poor DFS and OS, respectively (Figure 1A, B, D, and E). Patients with normal levels of both CA 15-3 and CEA showed better DFS and OS than those with elevated either one or both markers levels ($P < 0.001$). Elevation of either one marker level was associated with significantly better DFS ($P = 0.022$) and an improved trend of OS ($P = 0.08$) than elevation of both markers (Figure 1C and F). Using the longer follow-up in this study, the statistical significance was reaffirmed from the previous study [14].

Elevated CA 15-3 and CEA levels were identified in 176 (10.5%) and 131(7.8%) patients, respectively, among whole group I and II patients (Table 1). The correlation between serum CA 15-3 and CEA levels and clinicopathological characteristics are shown in Table 2. Similar to the previous study [14], both CA 15-3 and CEA levels were correlated with

Table 2. Correlation between serum CA 15-3 and CEA level and clinicopathological factors in a total of 1681 patients

	CA 15-3			CEA		
	Mean	SD	*P*	Mean	SD	*P*
Age (years)						
≤35	12.6275	5.92382	0.290	1.5843	2.55522	0.148
>35	12.0246	6.24367		2.0067	3.23030	
Tumor size						
T1	11.1831	4.99665	<0.001	1.5955	1.35554	<0.001
T2	12.3598	6.76206		2.2388	4.28705	
≥T3	14.1071	7.40522		2.3687	3.13484	
Nodal status						
N0	11.5717	6.03416	<0.001	1.8288	2.54956	0.054
N1	12.2733	5.75976		2.1459	4.44273	
N2	13.9239	7.14502		2.2219	3.22985	
N3	14.6074	7.95814		2.6600	2.98600	
TNM stage						
I	11.1831	4.99665	<0.001	1.5955	1.35554	<0.001
II	12.3598	6.76206		2.2388	4.28705	
III	14.1071	7.40522		2.3687	3.13484	
HG						
I	11.2683	5.38122	0.023	1.8213	3.60024	0.629
II	12.2128	6.08949		1.9909	3.22634	
III	12.5184	6.99338		2.0506	2.86661	
ER						
Negative	12.3451	7.19094	0.194	2.0110	3.57443	0.534
Positive	11.9224	5.71791		1.9110	2.81601	
PR						
Negative	12.3183	6.72778	0.137	2.0408	3.28881	0.259
Positive	11.8575	5.85121		1.8677	2.92695	
HER2						
Negative	11.9643	6.31912	0.250	1.8405	3.28933	0.014
Positive	12.3789	6.11875		2.2762	2.42616	

CA 15-3, cancer antigen 15-3; CEA, carcinoembryonic angigen; SD, standard deviation; TNM, tumor-node-metastasis; HG, histologic grade; ER, estrogen receptor; PR, progesterone receptor; HER2, human epidermal growth factor receptor 2.

larger tumor size and advanced nodal stage, but not with ER nor PR expressions. Higher concentration of CA 15-3 was related to histologic grade III tumors and higher concentration of CEA was correlated with HER2 positivity in the current study. Since adjuvant treatment was not determined by tumor marker levels, there was no difference in adjuvant treatment according to CA 15-3 and CEA levels, but more patients with elevated CA15-3 levels received chemotherapy and this was not statistically significant (supplementary Table S1, available at *Annals of Oncology* online).

In our study population of 1681 patients, elevated CA 15-3 and CEA levels were significantly associated with worse DFS and OS (Figure 2). In this longer follow-up and larger study population, the statistical significance was more intensified and the survival curves were distinctively different according to tumor markers. In tumor stage-matched analysis, patients with elevated CA 15-3 levels showed significantly worse DFS and OS in stages I ($P = 0.042$ and 0.002, respectively) and II ($P < 0.001$ and $P = 0.034$, respectively), but not in stage III (supplementary Figure S1, available at *Annals of Oncology* online). By analysis of CEA levels, an elevated CEA group showed a significantly worse DFS and OS in all stages I–III (supplementary Figure S2, available at *Annals of Oncology* online).

Multivariate analysis adjusting for the most important conventional prognostic factors revealed that elevated preoperative CA 15-3 and CEA levels were independent prognostic factors in DFS, DRFS and OS. Traditional clinicopathological factors such as age, tumor size, node metastasis, and ER status also had independent prognostic power in the Cox's models (Table 3). Both CA 15-3 and CEA levels were elevated in 28 patients, either CA 15-3 or CEA was elevated in 251 patients, and the remaining 1402 patients had normal ranges of both the markers. Patients with normal levels of both the markers showed the best DFS and OS. Subsequently, those with either one elevated marker level demonstrated better survival, and finally, those with both elevated markers presented the worst survival (both normal versus either elevated, $P < 0.001$ for DFS, and $P < 0.001$ for OS; and both normal versus both elevated, $P < 0.001$ for DFS, and $P < 0.001$ for OS) (Figure 3).

discussion

The prognostic value of preoperative serum tumor marker CA 15-3 and CEA was demonstrated in our previous study, which included 740 patients with a median follow-up of 37.2 months [14]. Our previous findings were reconfirmed and even solidified in the present study with an extended follow-up of 98.8 months (Figure 1). Nine hundred and forty-one patients were added to the previous study population of 740, and the current analysis was carried out in a larger study population of 1681 with a median follow-up of 72 months.

With the activation of a national screening program, the proportion of screening-detection remarkably increased from 5.0% to 32.6% and the incidence of stage I breast cancer also increased from 19.6% to 34.8% between 1996 and 2008 in Korea [15]. As expected, the incidence of patients with elevated preoperative levels of serum CA 15-3 and CEA decreased along with the increase in early breast cancer patients in the current study (Table 1). This finding supports the proposed association of tumor burden and elevated levels of serum tumor markers. The preoperative serum CEA and CA 15-3 levels are associated with the tumor size and lymph node metastasis which represents tumor burden [14, 16, 17] and significantly higher levels of CEA and CA 15-3 were seen in patients with advanced disease than in those with locoregional breast cancer [9, 13, 18]. The current study also demonstrated the association of higher levels of CA 15-3 and CEA with tumor burden such as larger tumor size, node metastases, and advanced stage in a large study population with longer follow-up. Although the association of tumor markers and tumor biological factors is not well established [19], the serum CA 15-3 level was related to the poor histological grade in agreement with the recent study by Molina et al. [5, 20]. The relationship between higher levels of CEA and HER2 expression needs to be further investigated.

Since elevated levels of CA 15-3 and CEA are related to the tumor burden and higher levels may indicate vascularization of the tumor with an increased likelihood of occult systemic

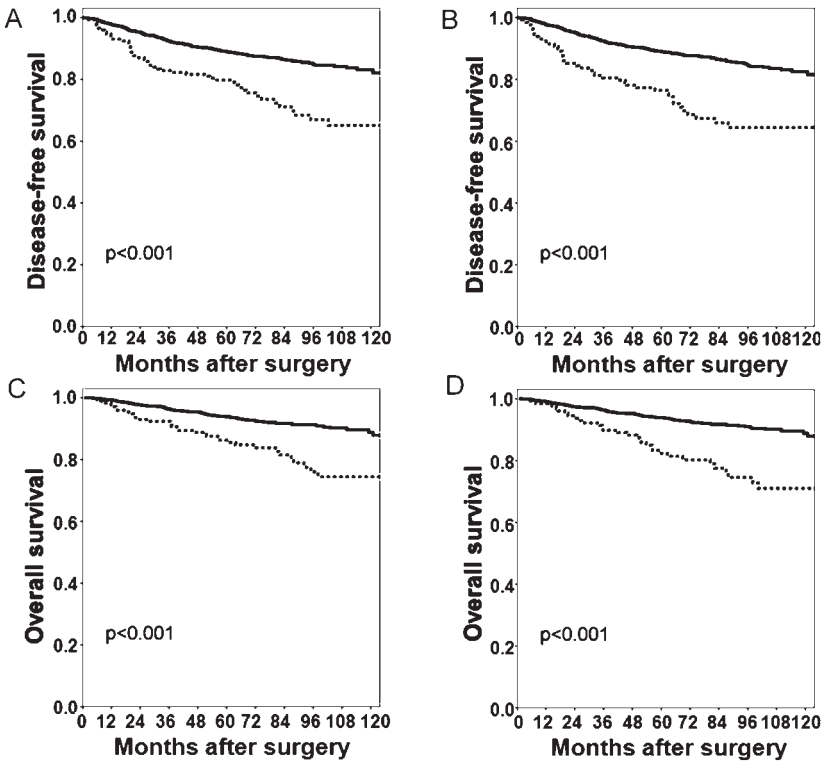

Figure 2. Disease-free survival (DFS) according to preoperative CA 15-3 (A) and CEA levels (B) and overall survival (OS) according to preoperative CA 15-3 (C) and CEA levels (D) in group I and II patients. The bold line represents patients with normal levels and the dotted line represents patients with elevated levels.

Table 3. Cox's regression analysis according to age, stage, estrogen receptor status, and serum markers in a total of 1681 patients

	DFS			DRFS			OS		
	HR	CI	*P*	HR	CI	*P*	HR	CI	*P*
Age (years)									
>35									
≤35	0.594	0.396–0.890	0.012	0.557	0.365–0.850	0.007	0.531	0.329–0.856	0.009
Tumor size									
≤2 cm									
>2 cm	1.431	1.094–1.871	0.009	1.499	1.125–1.998	0.006	1.540	1.090–2.174	0.014
Node									
Negative									
Positive	2.481	1.888–3.260	<0.001	2.632	1.962–3.531	<0.001	2.374	1.678–3.357	<0.001
ER									
Negative									
Positive	0.727	0.557–0.948	0.019	0.722	0.544–0.959	0.024	0.516	0.370–0.720	<0.001
CA15-3									
Normal									
Elevated	1.863	1.340–2.589	<0.001	1.972	1.398–2.781	<0.001	2.020	1.359–3.003	0.001
CEA									
Normal									
Elevated	2.134	1.493–3.051	<0.001	2.062	1.411–3.014	<0.001	2.601	1.709–3.958	<0.001

DFS, disease-free survival; DRFS, distant recurrence-free survival; OS, overall survival; HR, hazard ratio; CI, confidence interval; ER, estrogen receptor; CA 15-3, cancer antigen 15-3; CEA, carcinoembryonic angigen.

metastases [21, 22], elevated CA 15-3 and CEA concentration at initial presentation could be predictive of poor breast cancer outcome [23]. In agreement with other studies demonstrating significant association between the preoperative tumor marker levels and the survival outcome [14, 21, 24, 25], the elevated preoperative CA 15-3 or CEA levels were significantly

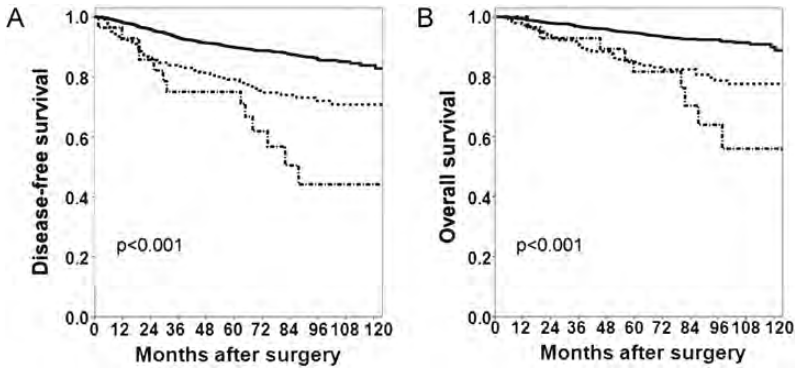

Figure 3. Disease-free survival (DFS) (A) and overall survival (OS) (B) according to the combination of both marker levels in group I and II patients. The bold line represents patients with normal levels of both markers, the dotted line represents patients with elevated either marker, and the chain line represents patients with both markers elevated.

associated with poor DFS ($P < 0.001$ and $P < 0.001$, respectively) and OS ($P < 0.001$ and $P < 0.001$, respectively) (Figure 2), even in a stage-matched analysis (supplementary Figures S1 and S2, available at *Annals of Oncology* online). With a longer follow-up and a larger population of the present study, the prognostic significance of tumor marker elevation became more confident and extended to almost all stages. The prognostic significance was also maintained in the multivariate analysis with the addition of other traditional prognostic factors such as age, tumor size, lymph node metastasis, and ER status (Table 3).

Although the ASCO panel does not recommend therapeutic decisions be based on the serum tumor marker status [9], several studies showed that the preoperative concentration of tumor markers could be useful in combination with other factors in deciding whether adjuvant chemotherapy should be administered [5, 13, 23, 26]. Furthermore, higher levels may reflect an increased likelihood of occult systemic metastases [21] and the study evaluating early treatment based on increasing tumor marker concentrations showed improved outcomes compared with controls [27]. Compared with the results of previous study [14], the prognostic value of the combination of both marker levels was further intensified with a longer follow-up and with a larger study population. Patients with either one or both markers elevated showed significantly worse survival outcomes than those with both normal ranges of markers in the current study. Therefore, elevated preoperative serum tumor markers could be useful in discriminating high-risk groups and in deciding adjuvant systemic treatment, for which the hypothesis should be verified.

In conclusion, our previous findings of independent prognostic significance of elevated preoperative serum CA 15-3 and CEA levels [14] are reconfirmed with the extended follow-up and larger study population in the present analyses. Preoperative serum CA 15-3 and CEA levels can provide additional prognostic information and may be useful in treatment implementation. Therefore, both the markers could be considered for the risk evaluation and determination of adjuvant treatment strategies in clinical practice, although this hypothesis should be further validated. Further clinical trials based on the tumor marker levels are necessary.

disclosure

The authors have declared no conflicts of interest.

references

1. Jemal A, Siegel R, Ward E et al. Cancer statistics, 2009. CA Cancer J Clin 2009; 59: 225–249.
2. Jung KW, Park S, Kong HJ et al. Cancer statistics in Korea: incidence, mortality, survival, and prevalence in 2009. Cancer Res Treat 2012; 44: 11–24.
3. Lonning PE. Breast cancer prognostication and prediction: are we making progress? Ann Oncol 2007; 18: viii3–viii7.
4. Elston CW, Ellis IO, Pinder SE. Pathological prognostic factors in breast cancer. Crit Rev Oncol Hematol 1999; 31: 209–223.
5. Molina R, Auge JM, Farrus B et al. Prospective evaluation of carcinoembryonic antigen (CEA) and carbohydrate antigen 15.3 (CA 15.3) in patients with primary locoregional breast cancer. Clin Chem 2010; 56: 1148–1157.
6. Sandri MT, Salvatici M, Botteri E et al. Prognostic role of CA15.3 in 7942 patients with operable breast cancer. Breast Cancer Res Treat 2012; 132: 317–326.
7. Uehara M, Kinoshita T, Hojo T et al. Long-term prognostic study of carcinoembryonic antigen (CEA) and carbohydrate antigen 15-3 (CA 15-3) in breast cancer. Int J Clin Oncol 2008; 13: 447–451.
8. Maric P, Ozretic P, Levanat S et al. Tumor markers in breast cancer—evaluation of their clinical usefulness. Coll Antropol 2011; 35: 241–247.
9. Harris L, Fritsche H, Mennel R et al. American Society of Clinical Oncology 2007 update of recommendations for the use of tumor markers in breast cancer. J Clin Oncol 2007; 25: 5287–5312.
10. Duffy MJ. CA 15-3 and related mucins as circulating markers in breast cancer. Ann Clin Biochem 1999; 36: 579–586.
11. Cheung KL, Graves CR, Robertson JF. Tumour marker measurements in the diagnosis and monitoring of breast cancer. Cancer Treat Rev 2000; 26: 91–102.
12. Nicolini A, Carpi A. Postoperative follow-up of breast cancer patients: overview and progress in the use of tumor markers. Tumour Biol 2000; 21: 235–248.
13. Duffy MJ. Serum tumor markers in breast cancer: are they of clinical value? Clin Chem 2006; 52: 345–351.
14. Park BW, Oh JW, Kim JH et al. Preoperative CA 15-3 and CEA serum levels as predictor for breast cancer outcomes. Ann Oncol 2008; 19: 675–681.
15. Jung YS, Na KY, Kim KS et al. Nation-wide Korean breast cancer data from 2008 using the breast cancer registration program. J Breast Cancer 2011; 14: 229–236.
16. Gion M, Boracchi P, Dittadi R et al. Prognostic role of serum CA15.3 in 362 node-negative breast cancers. An old player for a new game. Eur J Cancer 2002; 38: 1181–1188.
17. Tampellini M, Berruti A, Gerbino A et al. Relationship between CA 15-3 serum levels and disease extent in predicting overall survival of breast cancer patients with newly diagnosed metastatic disease. Br J Cancer 1997; 75: 698–702.

18. Molina R, Barak V, van Dalen A et al. Tumor markers in breast cancer- European Group on Tumor Markers recommendations. Tumour Biol 2005; 26: 281–293.
19. Yerushalmi R, Tyldesley S, Kennecke H et al. Tumor markers in metastatic breast cancer subtypes: frequency of elevation and correlation with outcome. Ann Oncol 2012; 23: 338–345.
20. Molina R, Auge JM, Escudero JM et al. Evaluation of tumor markers (HER-2/neu oncoprotein, CEA, and CA 15.3) in patients with locoregional breast cancer: prognostic value. Tumour Biol 2010; 31: 171–180.
21. Canizares F, Sola J, Perez M et al. Preoperative values of CA 15-3 and CEA as prognostic factors in breast cancer: a multivariate analysis. Tumour Biol 2001; 22: 273–281.
22. Gasparini G, Toi M, Gion M et al. Prognostic significance of vascular endothelial growth factor protein in node-negative breast carcinoma. J Natl Cancer Inst 1997; 89: 139–147.
23. Shering SG, Sherry F, McDermott EW et al. Preoperative CA 15-3 concentrations predict outcome of patients with breast carcinoma. Cancer 1998; 83: 2521–2527.
24. Ebeling FG, Stieber P, Untch M et al. Serum CEA and CA 15-3 as prognostic factors in primary breast cancer. Br J Cancer 2002; 86: 1217–1222.
25. Molina R, Filella X, Alicarte J et al. Prospective evaluation of CEA and CA 15.3 in patients with locoregional breast cancer. Anticancer Res 2003; 23: 1035–1041.
26. Duffy MJ, Duggan C, Keane R et al. High preoperative CA 15-3 concentrations predict adverse outcome in node-negative and node-positive breast cancer: study of 600 patients with histologically confirmed breast cancer. Clin Chem 2004; 50: 559–563.
27. Nicolini A, Carpi A, Michelassi C et al. 'Tumour marker guided' salvage treatment prolongs survival of breast cancer patients: final report of a 7-year study. Biomed Pharmacother 2003; 57: 452–459.

Annals of Oncology 24: 1231–1238, 2013
doi:10.1093/annonc/mds625
Published online 27 December 2012

Unbiased quantitative assessment of Her-2 expression of circulating tumor cells in patients with metastatic and non-metastatic breast cancer

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Received 11 July 2012; revised 29 October 2012; accepted 30 October 2012

Background: Circulating tumor cells (CTCs) can provide the basis for a liquid biopsy and may guide the use of targeted therapies. We report on unbiased quantification of Her-2 protein expression of CTCs.
Patients and methods: Her-2 assessment of CTCs was carried out using the CellSearch® system in 103 metastatic (M1) and 88 non-metastatic (M0) breast-cancer patients. Expression of Her-2 on CTCs was determined by a manual review and an automated algorithm using Her-2- fluorescein isothiocyanate (FITC) fluorescence of leukocytes to determine the Her-2-expression threshold in each sample.
Results: Her-2 expression of CTCs varied greatly within and among patients compared with Her-2 expression of leukocytes. In M1 patients, a threshold of 75% of Her-2 positive CTCs in patients with ≥5 CTCs was set. Applying this threshold, 9% of M1 patients with Her-2-negative primary tumors had Her-2-positive CTC status and 29% of M1 patients with Her-2-positive primary tumors had Her-2-negative CTC status. No Her-2 discrepancy was observed between CTCs and primary tumors in M0 patients.
Conclusions: Our findings demonstrate that Her-2 expression is heterogeneous among CTCs within each patient. We show the feasibility of unbiased quantitative and reproducible assessment of treatment targets on CTCs, opening a path towards personalized treatment.
Key words: automated analysis, breast cancer, circulating tumor cells, Her-2, quantitative assessment

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