·临床研究·

术前 CA 15-3 和 CEA 水平在接受托瑞米芬治疗的管腔型可手术乳腺癌患者中的预后价值

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摘 要: 【目的】为了探讨在使用托瑞米芬治疗的管腔型可手术早期乳腺癌患者中,术前 CA15-3 及 CEA 水平与预后的相关性。【方法】共纳入 368 名于 2000 年至 2009 年在本中心使用托瑞米芬进行辅助内分泌治疗的早期乳腺癌患者。使用卡方检验或 Fisher 检验进行分类变量的比较,使用 Kaplan-Meier 曲线描述无病生存期(DFS)和总生存期(OS),同时应用 log-rank 检验比较差异,并使用单因素及多因素 Cox 回归分析和分层分析法来寻找和 DFS 及 OS 相关的预后指标。【结果】在这 368 例患者中,分别有 29 和 47 例患者术前检查发现 CEA 和 CA15-3 的升高。CEA 和 CA15-3 升高与淋巴结状态,HER2 状态及是否进行辅助放疗相关。CEA 和 CA15-3 升高的患者较瘤标正常患者预后更差。多因素分析和分层分析提示,术前 CA15-3 水平是管腔 A 样和管腔 B 样(HER2 阴性)患者 DFS 的独立预后因素。【结论】在托瑞米芬进行辅助内分泌治疗患者中,术前 CA15-3 水平在管腔 A 样及管腔 B 样(HER2 阴性)亚型中为独立预后因素。在管腔 A 样及管腔 B 样(HER2 阴性)亚型患者中术前 CA15-3 及 CEA 升高提示预后更差。

关键词: 乳腺癌;托瑞米芬;预后;肿瘤标志物
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Predictive Value of Preoperative CA 15-3 and CEA Levels in Luminal Subtype Operable Breast Cancer Patients Treated with Toremifene

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Abstract: 【Objective】Whether preoperative cancer antigen 15-3 (CA 15-3) and carcinoembryonic antigen (CEA) levels can forecast prognosis of various luminal subtype operable breast cancer treated with toremifene remain unknown. In this study, we retrospectively investigated the relationship between CA 15-3, CEA, clinicopathological characteristics, and patient outcomes in these group patients. 【Methods】 A total of 368 early breast cancer patients who underwent surgery and treated with toremifene as adjuvant endocrine therapy between 2000 and 2009 at Sun Yat-sen University Cancer Center were analyzed. Chi-square test or Fisher exact test was used to compare categorical data when appropriate. Kaplan-Meier method was used to construct disease-free survival (DFS) and overall survival (OS) curves, and the log-rank test was used to evaluate the statistical significance of differences. Univariate and multivariate Cox analyses and stratified analysis were performed to identify prognostic factors for DFS and OS. 【Result】 Among 368 patients, elevated preoperative CEA and CA 15-3 levels were identified in 29 and 47 patients, respectively. Elevated preoperative CEA and CA 15-3 level were associated with axillary lymph node status, Her2 status, and adjuvant radiotherapy. The

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prognosis of patients with higher CEA and CA 15-3 concentrations was worse than that of those with serum tumor markers (STMs) normal. Multivariate analysis and stratified analysis indicated that elevated preoperative CA 15-3 levels were independent prognostic factors for DFS in luminal A-like and luminal B-like (HER2-negative) patients. 【Conclusion】 Preoperative CA 15-3 and CEA elevated were associated with worse clinical outcome in patients with luminal A-like and luminal B-like (HER2-negative) molecular subtype. CA 15-3 and CEA elevated were associated with worse clinical outcome in patients with luminal A or luminal B (HER2-negative) molecular subtype.

Key words: breast cancer; toremifene; prognosis; tumor marker

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Breast carcinoma is a clinically heterogeneous disease[1-2]. Molecular subtype of breast cancer has been proposed on the basis of gene expression profiling[1-2] or immunohistochemistry assay[3] of human tumors. In 2011, St. Gallen International Breast Cancer Conference Expert Panel adopted a new approach to classify breast cancer into luminal A, luminal B (HER2-negative), luminal B (HER2-positive), HER2-positive (non-luminal), and triple negative (ductal) subtypes by using immunohistochemical testing[3-4]. These subtypes are of great difference in epidemiological risk factors[5], natural histories[6], and response to systemic and local therapies[7], especially in medical treatment[4]. These differences indicate that the management of breast cancer should consider individually within the various distinct subtypes in order to properly assess the relevant evidence and arrive at appropriate therapeutic effect.

A choice of tamoxifen or toremifene for hormone receptor-positive breast cancer is still controversial due to their similar efficacy and safety [8-10]. Consequently, tamoxifen or toremifene was selected in terms of clinician preference and clinical experience, which lacks of an objective evaluation. Traditional serum tumor markers (STM), like CEA and CA 15-3, have been widely investigated in predicting outcome of breast cancer for their relation with tumor burden and tumorigenesis, no matter in metastatic[11] or early breast cancer[12-13]. Nevertheless, whether preoperative CA 15-3 and CEA levels can forecast patient prognosis of various luminal subtype operable breast cancer treated with toremifene and be candidate predictor for selecting medication remain unknown. Therefore, in this study, we retrospectively investigated the relationship between CA 15-3 and CEA and clinicopathological parameters and patient outcomes in these group patients.

1 Materials and Methods

1.1 Patients

We conducted a large-scale retrospective study by searching the breast cancer database at Sun Yat-sen University Cancer Center, Guangzhou, China. Patients were considered eligible if they met the following criterions: conducting mastectomy or lumpectomy in our hospital between January 2000 and August 2009, hormone-sensitive invasive ductal cancer with histopathologic confirmation, and received toremifene as adjuvant endocrine treatment after surgery, irrespective of adjuvant chemotherapy or radiotherapy had been given. Clinical and pathological data were extracted from medical records. Baseline data included age at diagnosis, tumor size, axillary lymph node status, histologic grade, TNM stage, estrogen receptor (ER), progesterone receptor (PR), HER2 status, available preoperative CA15-3 and CEA concentration, and follow-up records. Tumor staging was based on the 7th edition of the American Joint Committee on Cancer criteria. Patients were excluded when they met one of the following criteria: non-infiltrating ductal carcinoma, contralateral breast cancer, stage IV diseases with distant metastases at diagnosis, a prior history of malignancies or second primary malignancies, medical records were incomplete. This study was approved by the institutional review board of Sun Yat-sen University

Cancer Center, and all patients who were enrolled in the study had given written informed consent for the use of their medical records for research purposed.

1.2 Measurement of STM

Serum CEA and CA 15-3 levels were measured using a commercially automatic electrochemistry luminescence immunoassay system (ROCHE E170; Roche Diagnostics, Germany). The cut-off points of CEA and CA 15-3 were 5.0 ng/mL and 25 U/mL, respectively, which was also the value of upper limit of normal of our hospital. Patients with STMs higher or lower than the cut-off point were considered elevated-level or normal-level, respectively.

1.3 Immunohistochemical Test of Receptors

Expression of ER, PR, HER2 and Ki67 was demonstrated by IHC according to the Envision method using the primary monoclonal antibodies NCL-ER-6F11 for ER, NCL-PgR-312 for PR, CB11 for HER2 (Novocastra Laboratories, Newcastle On-Tyne, UK) and MIB 1 for Ki67 (Immunotech, Westbrook, ME). Positive for ER and PR were considered if there were at least 1% positive tumor nuclei in the sample on testing. Positive for HER2 was either IHC 3+ (defined as uniform intense membrane staining of >30% of invasive tumor cells) or fluorescence in situ hybridization amplified (ratio of HER2/CEP17 >2.0 or average HER2 gene copy number > 6 signals/nucleus) for those test systems without an internal control probe. A Ki-67 cut-off point of 15% was used to stratify patients into high-level and low-level group. Since Ki67 was not advocated for routine test, patients before 2005 had rarely an available Ki67 value.

1.4 Subgroup Definitions

In terms of the St Gallen 2011 recommendations, we defined three breast cancer subtypes in our patients based on ER, PR, HER2 and Ki67 as luminal A-like, luminal B-like (HER2-negative), luminal B-like (HER2-positive). If Ki67 labeling index assessment is not available, histological grade was used to make this definition. Therefore, three molecular subtypes were defined as follow: Luminal A-like (ER and/or PR-positive, HER2-negative, Ki67 < 14% or grade I/II [if a Ki67 index is not available]); Luminal B-like (HER2-negative), ER and/or PR-positive, HER2-negative, Ki67 ≥14% or grade III [if a Ki67 index is not available]); Luminal B-like (HER2-positive), ER and/or PR-positive, HER2-positive, any Ki67.

1.5 Statistics methods

The primary endpoints included disease-free survival (DFS) and overall survival (OS). DFS was calculated as the time from date of surgery until date of first recurrence, metastasis, or death, whichever came first, or to the date of last follow-up (in patients without recurrence or metastasis). OS was calculated as the time from date of surgery until date of death due to cancer, or to the date of last follow-up (if death did not occur). The association of CEA and CA 15-3 with individual clinicopathological characteristics, including patient age, tumor size, axillary lymph node status, histological grade, TNM stage, cancer embolus, ER/PR status, HER2 status, Ki67, subtype, and treatment received, were compared by chi-square test or Fisher exact test when appropriate. DFS and OS curves were constructed using the Kaplan-Meier method, and the log-rank test was used to evaluate the statistical significance of differences. Preoperative CEA and CA 15-3 value and common clinical factors were included in univariate Cox proportional hazards regression analysis for both DFS and OS. Factors that were significant in the univariate analysis were subjected to a multivariate Cox proportional hazards regression analysis and stratified analysis (forward stepwise selection [Wald] method; $P = 0.05$ was selected for entry into the model, and $P = 0.10$ was selected for removal) to identify the most significant independent prognostic factors affecting survival. A two-tail P value of ≤0.05 was considered statistically significant in all cases. All statistical analysis was performed using Statistical Package for the Social Sciences for Windows version 19.0 (IBM SPSS Inc., Chicago, IL, USA).

2 Results

2.1 Descriptive analysis of clinicopathological characteristics and STM

Finally, 368 patients were enrolled in the present study. All patients were female. The median follow-up duration was 85 months (range, 16 ~ 170 months). Patients was grouped into two arms in terms of the CEA and CA 15-3 concentrations. Patients with STM higher or lower than the cut-off value (5 μg/mL for CEA, 25 U/mL for CA 15-3) were classified into an elevated-level or normal-level group, respectively. The demographic data are summarized in Table 1. Elevated CEA level was associated with tumor size, axillary lymph node status, TNM stage, ER status, HER2 status, subtype, and adjuvant radiotherapy. No significant difference in distribution was observed for age, histologic grade, cancer embolus, PR status, Ki67, and adjuvant chemotherapy. Elevated CA 15-3 level was associated with young age, axillary lymph node status, HER2 status, and adjuvant radiotherapy. No significant difference in distribution was observed for tumor size, histologic grade, TNM stage, cancer embolus, ER status, PR status, Ki67, subtype, and adjuvant chemotherapy.

A total of 345 (93.8%) patients underwent modified radical mastectomy, and 23(6.2%) received breast-conserving surgery. Of 336 patients who received chemotherapy, 20 received a regimen consisting of cyclophosphamide, methotrexate, and 5-fluorouracil (CMF); 148 received regimens contain anthracycline; 146 received regimens contain both anthracycline and taxane; 21 received other regimens. 32 patients (8.7%) received neoadjuvant chemotherapy with anthracycline and/or taxane. A total of 88 patients received radiotherapy within 6 months after surgery. None of patients with HER2-positive treated with trastuzumab that target HER2 receptor.

2.2 Kaplan-Meier OS and DFS curve analysis

According to the results of survival analysis, the 5-year DFS rates were 81.4% and 51.7% in patients with normal-level and high-level CEA ($P < 0.001$), 82.3% and 56.8% in patients with normal-level and high-level CA 15-3 ($P < 0.001$), respectively. The 5-year OS rates were 89.7% and 67.2% in patients with normal-level and high-level CEA ($P = 0.001$), 90.0% and 73.7% in patients with normal-level and high-level CA 15-3 ($P = 0.002$), respectively.

The prognosis of the 368 patients who stratified into three subgroups according to subtype was studied. As shown in Figure 1 and 2, we found the prognosis of the patients with higher CEA and CA 15-3 concentrations were worse than that of those with STM normal. And according to the results of Kaplan-Meier analysis and the log-rank test, a significant difference was observed in DFS between elevated-level CEA and normal-level CEA in luminal A-like and luminal B-like (HER2-negative) group ($P = 0.003$ and $P = 0.006$, respectively). Similar results were also observed in DFS between elevated-level CA 15-3 and normal-level CA 15-3 in luminal A-like and luminal B-like (HER2-negative) group ($P = 0.001$ and $P = 0.002$, respectively). Only luminal A group has a statistically difference in OS between elevated and normal in both CEA and CA 15-3 ($P = 0.022$ and $P = 0.002$, respectively).

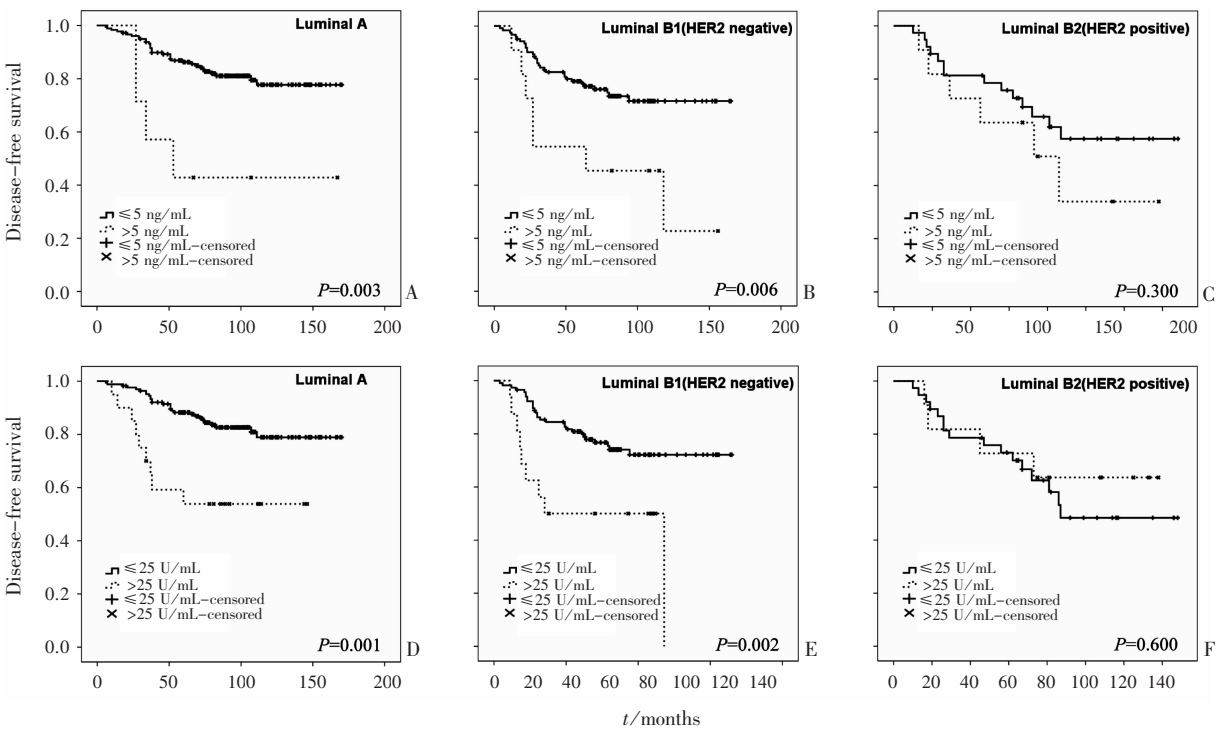
2.3 Univariate and multivariate cox analysis and stratified analysis

In univariate analysis, both high-level CEA and CA 15-3 were associated with poor DEF and OS ($P < 0.001$ and $P < 0.001$, $P = 0.002$ and $P = 0.003$, respectively; Table 2). In multivariate Cox survival analysis, the results indicated that high-level CA 15-3 ($P = 0.010$, hazard ratio [HR] =1.91; 95% confident interval [CI], 1.16-3.13), high-level CEA ($P = 0.013$, HR = 2.01; 95%CI, 1.16 ~ 3.48), TNM stage ($P < 0.001$, HR = 8.01; 95%*CI*, 2.51 ~ 25.55), nodal status ($P < 0.001$, HR = 2.25; 95%CI, 1.46 ~ 3.47) were independent prognostic factors for DFS, high-level CA 15-3 ($P = 0.030$, HR = 2.04; 95%CI, 1.07 ~ 3.86), histologic grade ($P = 0.038$, HR = 1.85; 95%CI, 1.04 ~ 3.32), TNM stage ($P = 0.027$, HR = 5.07; 95%*CI*, 1.20 ~ 21.37), nodal status ($P = 0.002$, HR = 2.59; 95%CI, 1.43 -

Table 1 Baseline characteristics grouped by CEA and CA15-3 level *n*(%)

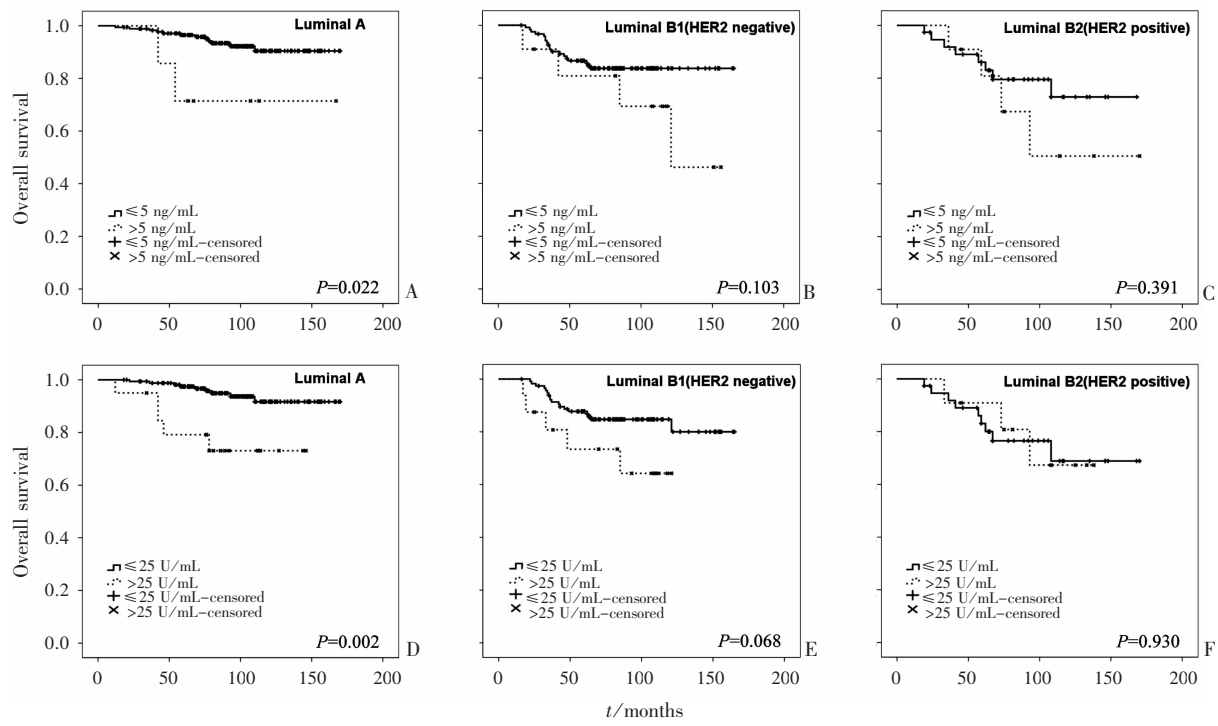
Characteristics	*n*	CEA			CA15-3		
		Normal	Elevated	*P*	Normal	Elevated	*P*
Age							
⩽ 35 years	60	52(86.7)	8(13.3)	0.147	47(78.3)	13(21.7)	0.024[1)]
> 35 years	308	287(93.2)	21(6.8)		274(89.0)	34(11.0)	
Size							
⩽ 20 mm	132	128(97.0)	4(3.0)	0.010[1)]	121(91.7)	11(8.3)	0.056
> 20 mm	236	211(89.4)	25(10.6)		200(84.7)	36(15.3)	
Nodal Status							
0	199	192(96.5)	7(3.5)	0.001[1)]	183(92.0)	16(8.0)	0.012[1)]
1-3	99	89(89.9)	10(10.1)		80(80.8)	19(19.2)	
⩾4	70	58(82.9)	12(17.1)		58(82.9)	12(17.1)	
HG							
Ⅰ	46	44(95.7)	2(4.3)	0.068	41(89.1)	5(10.9)	0.228
Ⅱ	191	180(94.2)	11(5.8)		171(89.5)	20(10.5)	
Ⅲ	131	115(87.8)	16(12.2)		109(83.2)	22(16.8)	
TNM Stage							
Stage Ⅰ	85	83(97.6)	2(2.4)	0.003[1)]	80(94.1)	5(5.9)	0.063
Stage Ⅱ	204	190(93.1)	14(6.9)		176(86.3)	28(13.7)	
Stage Ⅲ	79	66(83.5)	13(16.5)		65(82.3)	14(17.7)	
Cancer Embolus							
Yes	356	328(92.1)	28(7.9)	1.000[2)]	312(87.6)	44(12.4)	0.395
None	12	11(91.7)	1(8.3)		9(75.0)	3(25.0)	
ER Status							
Positive	303	284(93.7)	19(6.3)	0.013[1)]	263(86.8)	40(13.2)	0.594
Negative	65	55(84.6)	10(15.4)		58(89.2)	7(10.8)	
PR Status							
Positive	342	315(92.1)	27(7.9)	1.000	299(87.4)	43(12.6)	0.913
Negative	31	24(92.3)	2(7.7)		22(84.6)	4(15.4)	
HER2 Status							
Positive	49	38(77.6)	11(22.4)	<0.001[1)]	38(77.6)	11(22.4)	0.029[1)]
Negative	319	301(94.4)	18(5.6)		283(88.7)	36(11.3)	
Ki67							
< 14%	138	130(94.2)	8(5.8)	0.993	120(87)	18(13)	0.725
⩾ 14%	104	98(94.2)	6(5.8)		92(88.5)	12(11.5)	
Unknown	126						
Subtype [3)]							
Luminal A	186	179(96.2)	7(3.8)	<0.001[1)]	166(89.2)	20(10.8)	0.088
Luminal B1	133	122(91.7)	11(8.3)		117(88.0)	16(12.0)	
Luminal B2	49	38(77.6)	11(22.4)		38(77.6)	11(22.4)	
Adjuvant Chemotherapy							
Yes	334	308(92.2)	26(7.8)	1.000	290(86.8)	44(13.2)	0.650
None	352	31(91.2)	3(8.8)		31(91.2)	3(8.8)	
Adjuvant Radiotherapy							
Yes	88	76(86.4)	12(13.6)	0.022[1)]	69(78.4)	19(21.6)	0.004[1)]
None	280	263(93.9)	17(6.1)		252(90.0)	28(10.0)	

CEA, carcinoembryonic antigen; CA15-3, cancer antigen 15-3; HG, histologic grade; ER, estrogen receptor; PR, progesterone receptor; HER2, human epidermal growth factor receptor 2. 1) P<0.05 was considered statistically significant. 2) Fisher exact test. 3) Luminal A, ER and/or PR-positive, HER2-negative, Ki67<14% or grade I/II(if a Ki67 index is not available, grade will instead of Ki67); Luminal B1, ER and/or PR-positive, HER2-negative, Ki67⩾14% or grade III (if a Ki67 index is not available, grade will instead of Ki67); Luminal B2, ER and/or PR-positive, HER2-positive, any Ki67.



Bold line represents patients with normal level and dotted line represents patients with elevated levels of STM.

Fig.1　Survival curves of disease-free survival according to preoperative CEA level in luminal A (A), luminal B1 (B), luminal B2 (C) and CA 15-3 level in luminal A (D), luminal B1 (E), luminal B2 (F)



Bold line represents patients with normal level and dotted line represents patients with elevated levels of STM.

Fig.2　Survival curves of overall survival according to preoperative CEA level in luminal A (A), luminal B1 (B), luminal B2 (C) and CA 15-3 level in luminal A (D), luminal B1 (E), luminal B2 (F)

Table 2 Univariate analysis of prognostic factors related to DFS and OS in patients with breast cancer

variable	*n*	Disease-free survival		Overall survival	
		HR(95% CI)	*P* value[1)]	HR(95% CI)	*P* value[1)]
Age					
≤ 35(reference)	60	1.00		1.00	
> 35	308	0.63(0.39-1.03)	0.065	0.59(0.31-1.12)	0.108
Size					
≤ 20 mm(reference)	132	1.00		1.00	
> 20 mm	236	3.74(2.12-6.59)	<0.001	3.11(1.50-6.64)	0.003
Nodal Status					
< 4(reference)	398	1.00		1.00	
≥ 4	70	3.32(2.17-5.08)	<0.001	3.97(2.23-7.06)	<0.001
TNM Stage					
Ⅰ(reference)	85	1.00		1.00	
Ⅱ/Ⅲ	281	10.84(3.43-34.25)	<0.001	7.86(1.91-32.36)	0.004
HG					
Ⅰ/Ⅱ(reference)	237	1.00		1.00	
Ⅲ	131	1.95(1.31-2.92)	0.001	2.51(1.42-4.41)	0.001
Cancer Embolus					
Negative(reference)	356	1.00		1.00	
Positive	12	2.83(1.23-6.48)	0.014	3.07(1.10-8.56)	0.032
CEA					
≤ 5 ng/mL(reference)	339	1.00		1.00	
> 5 ng/mL	29	3.14(1.86-5.31)	<0.001	3.07(1.53-6.19)	0.002
CA 15-3					
≤ 25 U/mL(reference)	321	1.00		1.00	
> 25 U/mL	47	2.50(1.55-4.02)	<0.001	2.61(1.38-4.91)	0.003
Subtype [2)]					
Luminal A(reference)	186	1.00	0.006	1.00	0.003
Luminal B1	133	1.60(1.02-2.51)	0.043	2.53(1.30-4.93)	0.006
Luminal B2	49	2.39(1.38-4.11)	0.002	3.59(1.66-7.76)	0.001

HR, hazard ratio; 95% CI, 95% confident interval; HG, histologic grade; CEA, carcinoembryonic antigen; CA15-3, cancer antigen 15-3. 1) $P < 0.05$ was considered statistically significant. 2) Luminal A, ER and/or PR-positive, HER2-negative, Ki67<14% or grade I/II(if a Ki67 index is not available, grade will instead of Ki67); Luminal B1, ER and/or PR-positive, HER2-negative, Ki67≥14% or grade III (if a Ki67 index is not available, grade will instead of Ki67); Luminal B2, ER and/or PR-positive, HER2-positive, any Ki67.

4.69) were independent prognostic factors for OS in operable breast cancer patients treated with toremifene (Table 3).

Table 4 shows the results of multivariate analysis of various factors for DFS and OS stratified by subtype. In luminal A-like and Luminal B-like (HER2-negative) group, CA 15-3 was an independent factor for predicting disease-free survival ($P = 0.001$, HR = 3.74, 95%CI = 1.75 ~ 8.00 and $P = 0.009$, HR = 2.76, 95%CI = 1.30 ~ 5.87, respectively). Additionally, in luminal A-like group, CA 15-3 also an independent factor for predicting overall survival ($P = 0.003$, HR = 5.27, 95%CI = 1.74 ~ 15.94). The prognostic value of CEA was just enough significant for predicting disease-free survival ($P = 0.050$, HR = 2.87, 95%CI = 1.00 ~ 8.25) in luminal A-like patients exclusively.

Table 3 Multivariate analysis of prognostic factors related to DFS and OS in patients with breast cancer

Variable	Disease-free survival		Overall survival	
	HR(95% CI)	*P* value[1)]	HR(95% CI)	*P* value[1)]
Nodal Status				
< 4(reference)	1.00		1.00	
⩾ 4	2.25(1.46 ~ 3.47)	<0.001	2.59(1.43 ~ 4.69)	0.002
TNM Stage				
Ⅰ(reference)	1.00		1.00	
Ⅱ/Ⅲ	8.01(2.51 ~ 25.55)	<0.001	5.07(1.20 ~ 21.37)	0.027
HG				
Ⅰ/Ⅱ(reference)	NA	NS	1.00	
Ⅲ			1.85(1.04 ~ 3.32)	0.038
CEA				
⩽ 5 ng/mL(reference)	1.00		NA	NS
> 5 ng/mL	2.01(1.16 ~ 3.48)	0.013		
CA 15-3				
⩽ 25 U/mL(reference)	1.00		1.00	
> 25 U/mL	1.91(1.16 ~ 3.13)	0.010	2.04(1.07 ~ 3.86)	0.030

HR, hazard ratio; 95% CI, 95% confident interval; HG, histologic grade; CEA, carcinoembryonic antigen; CA15-3, cancer antigen 15-3; NA, not available; NS, not significant. 1) $P < 0.05$ was considered statistically significant.

3 Discussion

Even when multidisciplinary therapy has been performed, disease relapse occurs in nearly 20% of all patients with breast cancer within a few years. Relapse probably arises from the growth of occult micrometastasis that has already become established by the time of surgery and cannot be detected with conventional staging procedures. Therefore, identification of specific indicators for metastatic potential of primary tumors would allow a better prognostic stratification of patients and thus more effective treatment. The results of Table 3 further confirm the predictive value of CA 15-3 in DFS and OS in patients with primary operable breast cancer. Furthermore, during stratified analysis, our study demonstrated that CA 15-3 was independent factor of predicting outcome in luminal A-like and luminal B-like (HER2-negative), except luminal B-like (HER2-positive) subtype breast cancer with toremifene-therapy after surgery. Concentrations of CA 15-3 can be a predictor probably because of its association with the potential for micrometastasis of cancer cells[14].

The results of this study are similar to those reported in earlier studies. Study by Wu et al[13] found that both CEA and CA 15-3 were independent prognostic factors for DFS, and may differ in breast cancer molecular subtypes. Li et al[15] have reported that serum tumor marker CA 15-3 was exclusively prognostic in luminal A-like group but not in other subtypes patients. However, they did not distinguish luminal B-like (HER2-negative) from luminal B-like (HER2-positive) subtype. Consistent results were observed when we combined these two groups of patients in our study. Nevertheless, to our knowledge, luminal B-like (HER2-negative) and luminal B-like (HER2-positive) have difference in biological characteristic [16-19], we have reason to classify them into two groups. Fortunately, our results demonstrated our hypothesis. The most likely explanation that why elevated CA 15-3 concentrations cannot forecast the prognosis of luminal B (HER2-positive) disease is that HER2-positive breast cancer is relatively resistant to selective estrogen receptor modulator. This

Table 4 Multivariate analysis of prognostic factors related to the DFS and OS in patients with breast cancer, grouped by molecular subtype

Subtype [2)]	Variable	Disease-free survival		Overall survival	
		HR(95% CI)	*P* value[1)]	HR(95% CI)	*P* value[1)]
Luminal A	CA 15-3				
	≤ 25 U/mL(reference)	1.00		1.00	
	> 25 U/mL	3.74(1.75 ~ 8.00)	0.001	5.27(1.74 ~ 15.94)	0.003
	Nodal Status				
	< 4(reference)	1.00		1.00	
	≥ 4	4.16(2.06 ~ 8.40)	<0.001	10.43(3.50 ~ 31.08)	<0.001
	TNM Stage				
	Ⅰ(reference)	1.00		NA	NS
	Ⅱ/Ⅲ	11.60(1.57 ~ 85.89)	0.016		
	CEA				
	≤ 5 ng/mL(reference)	1.00		NA	NS
	> 5 ng/mL	2.87(1.00 ~ 8.25)	0.050		
Luminal B1	CA 15-3				
	≤ 25 U/mL(reference)	1.00		NA	NS
	> 25 U/mL	2.76(1.30 ~ 5.87)	0.009		
	Nodal Status				
	< 4(reference)	1.00	0.002	NA	NS
	≥ 4	2.85(1.49 ~ 5.47)			
	Cancer embolus				
	Negative(reference)	NA	NS	1.00	
	Positive			4.38(1.29 ~ 14.80)	0.018
Luminal B2	Size				
	≤ 20 mm(reference)	1.00		NA	NS
	> 20 mm	7.27(0.97 ~ 54.39)	0.053		

HR, hazard ratio; 95% CI, 95% confident interval; HG, histologic grade; CEA, carcinoembryonic antigen; CA15-3, cancer antigen 15-3; NA, not available; NS, not significant. 1) $P < 0.05$ was considered statistically significant. 2) Luminal A, ER and/or PR-positive, HER2-negative, Ki67 < 14% or grade Ⅰ/Ⅱ (if a Ki67 index is not available, grade will instead of Ki67); Luminal B1, ER and/or PR-positive, HER2-negative, Ki67 ≥ 14% or grade Ⅲ (if a Ki67 index is not available, grade will instead of Ki67); Luminal B2, ER and/or PR-positive, HER2-positive, any Ki67.

subtype is quite sensitive to anti-HER2 target therapy, but all the patients included in our study hadn't receive target medicine. Approximately half of HER2-positive breast cancers would express the steroid hormone receptors for estrogen, progesterone, or both. Nevertheless, in these tumors, the levels of steroid hormone receptors are typically lower than in HER2-negative, hormone-receptor-positive tumors[19].

As well as preoperative CA 15-3 concentrations, lymph node status similarly acts as a predictor for DFS and OS in luminal A-like, and DFS in luminal B-like (HER2-negative) in present study, which was never reported in other studies based on our knowledge. Our results revealed that traditional predictors had different independent predictive ability among different molecular subtypes. Moreover, lymph node status as a classical pathological marker is the golden standard for prediction, which can verify the reasonability of our study with a stratified analysis[20].

In our study, we applied the 2011 St Gallen recommendation using the tumor grade instead of Ki67 when the Ki67 value was non-available[4]. However, the 2013 St Gallen Panel did not recommend grade 3 as a substitute for high Ki67

to distinguish luminal A-like and luminal B-like (HER2-negative) subtype[21]. Moreover, the 2015 St Gallen Panel[22] further classified the ER-positive/HER2-negative disease into different risk subgroup in terms of clinicopathological factors. Nevertheless, the classification seems subjective and controversial. Several studies had demonstrated prognostic value when divided ER-positive/HER2-negative disease into luminal A-like and luminal B-like subtype. Brouckaert et al[20] had ever conducted a study included 4318 patients, to establish the outcome of breast cancer patients with primary operable tumor by applying the 2011 St Gallen surrogate definition for breast cancer subclassification using tumor grade instead of Ki67. In that study, cases that classified as luminal A-like cancers were all grades 1 and 2; classified as luminal B-like (HER2-negative) cancers were all grade 3. The results showed that surrogate phenotypes present with significant differences in disease-free interval, distant metastasis-free interval, locoregional relapse-free interval, breast cancer-specific survival and OS. And luminal A-like tumors presented with the best outcome parameters, though the effect weakened at longer follow-up. Similar definition and results were also adopted and observed in study of Dawood et al [23], which were comparable with the study of Cheang et al[3] by using Ki67 to distinguish luminal A-like and luminal B-like (HER2-negative). Hence, we believed that replacing Ki67 by tumor grade was appropriate.

This study has several limitations. First, this is a retrospective study, with an unavoidable bias in subject selection. Second, the sample size is relatively small in our study, especially in stratified analyses of various subtypes. Consequently, the study was possibly not sufficiently powerful to demonstrate the importance of preoperative CA 15-3 level. Third, the patients in present study all received toremifene as adjuvant endocrine therapy, while tamoxifen was more recommended in clinical practice. Our study cannot illustrate the prognostic value of STM in patients with treatment of tamoxifen. Fourth, none of the HER2-positive patients treated with trastuzumab, which may contribute the worst prognosis to patients with luminal B-like (HER2-positive) subtype. Whether preoperative CA 15-3 and CEA level can predict prognosis of luminal B-like (HER2-positive) patients receiving trastuzumab is unknown. Further investigating is necessary in this field.

In conclusion, our study illustrated the predictive value of CA 15-3 in DFS and OS in patients with primary operable breast cancer. Furthermore, our results demonstrated that CA 15-3 provide independent prognostic value in luminal A-like and luminal B-like (HER2-negative), except luminal B-like (HER2-positive) subtype breast cancer with toremifene-therapy after surgery. Further studies are required to investigate the prognostic role of the STM in patients with tamoxifen-treated and prospective study are needed to provide further support for our findings.

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