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Original Article

Prognostic significance of CD4/CD8 ratio in patients with breast cancer

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Abstract: Increase of CD4/CD8 ratio has been observed in patients with various cancers. Our research was to assess the correlation between CD4/CD8 ratio and clinical variables, and determine whether CD4/CD8 ratio could be used as a prognostic factor in patients with breast cancer. The patients with breast cancer underwent surgery from January 2005 to June 2006. Prognostic parameters and survival rates were analyzed by the Kaplan-Meier method, log-rank test and multivariate Cox proportional hazards model. Eighty patients met the inclusion criteria and were included in the analysis. CD4/CD8 ratio was associated with tumor depths (P<0.001), metastatic lymph node ratio (MLNR, P<0.001), distant metastasis-free survival (DMFS, P<0.001) and progression-free survival (PFS, P<0.001). Multivariate analysis revealed the tumor depths, CD4/CD8 ratio and MLNR were independent predictors of DMFS (P<0.001; P=0.003; P<0.001, respectively) and PFS (P<0.001; P=0.002; P<0.001, respectively). Our data demonstrated that patients with an increased CD4/CD8 ratio (≥1.7) had significantly poor DMFS and PFS (log-rank P<0.0001). An elevated ratio of CD4/CD8 was found to associate with tumor progression and poor survival in patients with breast cancer.

Keywords: Breast cancer, CD4/CD8 ratio, metastatic lymph node ratio, prognosis

Introduction

Breast cancer is the most generally disease among women and distant metastasis is the primary cause associated death. Many efforts have been done to improve survival rate by early diagnosis and multiple therapies [1]. Consequently, we should develop additional prognostic factors to permit individual prognosis. Such factors will improve clinical decision-making, and may help provide the individualized treatment.

Tumor cells are frequently surrounded by inflammatory cells, particularly lymphocytes. It has been evidenced that cells of the adaptive immune system perform surveillance and can eliminate nascent tumors [2, 3]. T and B lymphocytes, as immune cells, play an important role in the immunological surveillance and can help in the elimination of tumor cells [4-6]. It has been reported that higher levels of particular lymphocyte subpopulations in the circulating blood were correlated to tumor development and poor prognosis in breast patients [7-10]. However, these associations, the influence of CD4/CD8 ratio on outcome in patients with breast cancer are unclear.

The relationship between CD4/CD8 ratio and breast cancer prognosis merits further exploration. In the present study, we aimed to evaluate the correlation between CD4/CD8 ratio and clinical variables in order to design a convenient and effective prognostic model predicting outcome in breast cancer patients.

Patients and methods

Patients

In this study, a series of 80 patients with breast cancer who underwent surgery at Shanghai Jiao Tong University Affiliated Sixth People's Hospital from January 2005 to June 2006, were included in the analysis. Written informed

Table 1. Clinicopathologic characteristics

Characteristics	Cases (n, range, %)
Median age, years (range)	51 (32-74)
Pathologic type	
Ductal	76 (95.0%)
Others	4 (5.0%)
Depth	
T1 (≤2 cm)	36 (45.0%)
T2 (>2 and ≤5 cm)	22 (27.5%)
T3 (>5 cm)	22 (27.5%)
Number of metastatic lymph nodes	
0	29 (36.25%)
1	13 (16.25%)
2-5	31 (38.75%)
>5	7 (8.75%)
Metastatic lymph node ratio	
<0.2	49 (61.25%)
≥0.2	31 (38.75%)
Cut-off value of CD4/CD8 ratio	1.7
ER	
Negative (0-1+)	43 (53.75%)
Overexpressed (2-3+)	37 (46.25%)
PR	
Negative (0-1+)	38 (47.5%)
Overexpressed (2-3+)	42 (52.5%)
HER-2	
Negative (0-1+)	54 (67.5%)
Overexpressed (2-3+)	26 (32.5%)
Treatment regimen	
Surgery only	2 (2.5%)
Surgery plus postoperative C	16 (20.0%)
Surgery plus postoperative R	7 (8.75%)
Surgery plus postoperative CRT	55 (68.75%)
Radiotherapy	
Yes	62 (77.5%)
No	18 (22.5%)

Abbreviations: R, radiotherapy; C, chemotherapy; CRT, chemo-radiotherapy.

consents were provided by the patients. The patients who received preoperative chemotherapy or radiotherapy and could not be contacted during follow-ups were excluded. We obtained the clinicopathological, adjuvant therapy and tumor-specific data from the patients' medical recording system of the hospital. Ratios of CD4/CD8 were measured within 1 week before initiation of therapy in all patients. Two pathologists independently analyzed the patients' histological documentation, and the same conclusions were obtained.

Follow-up

After finish of therapy, patients were examined every 3 months in the first year, and every 6 months during the second and third years, and once a year for up to 10 years or until death. Median follow-up was 97.3 months (range, 17-120 months). No patients were lost to follow-up. The following end points were assessed: distant metastasis-free survival (DMFS) and progression-free survival (PFS).

Statistical analysis

The metastatic lymph node ratio (MLNR) was defined as the ratio of metastatic lymph nodes to total lymph nodes harvested. The optimal cutoff point for the number of the lymph node ratio as predictors of survival was checked by the log-rank test statistic [11]. Calculation and plotting of the DMFS and PFS curves of the patients groups were done using the Kaplan-Meier method. The log-rank test was used for the comparison of the survival curves and Cox proportional hazards model was used to investigate the relative importance of the features. All comparisons were two tailed. A P value <0.05 was considered as statistical significance. All statistical analyses were performed using SPSS 18.0 software package (SPSS, Inc., Chicago, IL, USA).

Results

Clinicopathological features

Eighty patients with breast cancer were included in the analysis. The median age at diagnosis was 51 years (range, 32-74). Median total lymph nodes harvested were 7 (range: 1-35 nodes). MLNR cutoff was designed as 0.2 by the cut-point survival analysis. Clinicopathologic characteristics were shown in **Table 1**. All patients were staged according to the 7th edition of the AJCC staging system for breast cancer [12].

Prognostic factors for tumor progression

The median DMFS of this cohort was 91.8 months, and DMFS rate was 47.5%. The median PFS of this cohort was 80.0 months, and PFS rate was 43.8%. The optimal cut-off value of CD4/CD8 ratio based on the ROC analysis was 1.7, with sensitivity of 64.7% and specificity of 69.2%. Univariate analysis identified tumor depths (P<0.001), MLNR (P<0.001), and CD4/

Table 2. Univariate analysis of factors associated with DMFS and PFS

	Cases (n)	DMFS		PFS	
		Percent	*P*-value	Percent	*P*-value
Age (years)					
<50	42	57.1%	0.055	52.9%	0.079
≥50	38	36.8%		34.2%	
Depth					
T1	36	88.9%	<0.001	88.9%	<0.001
T2	22	27.35		15.8%	
T3	22	0.0%		0.0%	
MLNR					
<0.2	49	75.5%	<0.001	69.45	<0.001
≥0.2	31	3.2%		3.2%	
CD4/CD8 ratio					
≥1.7	42	9.55	<0.001	4.8%	<0.001
<1.7	38	89.5%		86.8%	
ER					
Negative (0-1+)	43	39.5%	0.094	37.2%	0.148
Overexpressed (2-3+)	37	56.8%		51.4%	
PR					
Negative (0-1+)	38	55.3%	0.136	50.0%	0.008
Overexpressed (2-3+)	42	40.5%		38.1%	
HER-2					
Negative (0-1+)	54	53.7%	0.086	50.0%	0.082
Overexpressed (2-3+)	26	34.6%		30.8%	
Radiotherapy					
Yes	62	82.3%	0.013	79.0%	0.007
No	18	33.3%		22.2%	

Abbreviations: DMFS, distant metastasis-free survival; PFS, progression-free survival; MLNR, Metastatic lymph node ratio.

CD8 ratio (*P*<0.001) as significant prognostic factors for DMFS and PFS times. The radiotherapy were also significantly associated with DMFS and PFS (*P*=0.013; *P*=0.007, respectively, **Table 2**). As shown in **Table 3**, strong correlations were observed between CD4/CD8 ratio and tumor depths (*P*<0.001), MLNR (*P*<0.001), DMFS rate (*P*<0.001) and PFS rate (*P*<0.001). The Kaplan-Meier curves for DMFS and PFS are shown in **Figure 1**.

Multivariate analyses for DMFS and PFS were performed using Cox proportional hazards regression to adjust for various prognostic factors (**Table 4**). Tumor depths, CD4/CD8 ratio, and MLNR were validated as independent prognostic factors for DMFS and PFS in patients with breast cancer (*P*<0.001, *P*=0.003, *P*<0.001; *P*<0.001, *P*=0.002, *P*<0.001, respectively).

Prognostic model for DMFS and PFS in breast cancer

We constructed a prognostic model for DMFS and PFS in breast cancer. Patients were divided into three subgroups according to the tumor depths (>2 cm), CD4/CD8 ratio (≥1.7) and MLNR (≥0.2) as follows: 1) patients with 0 risk factors (30 patients); 2) patients with 1 risk factor (9 patients); and 3) patients with 2-3 risk factors (41 patients). The DMFS rates for groups 1, 2 and 3 were 100%, 90.3% and 67.2% (*P*<0.001) and the PFS rates were 100%, 85.7% and 60.5%, respectively (*P*<0.001). The DMFS and PFS curves for the three groups clearly separated from each other (**Figure 2**).

Discussion

As the lymphocytes play an important role in immunity, especially anti-tumor immunity, the percentage of lymphocyte subsets may be a prognostic factor of breast cancer outcomes [13]. In our data, we found that CD4/CD8 ratio ≥1.7 was associated with a worse significant DMFS and PFS in patients with breast cancer than CD4/CD8 ratio <1.7. CD4/CD8 ratio was suggested to be an independent factor in breast cancer patients. Simultaneously, other pathologic risk factors such as the primary tumor depth and MLNR were found to significantly associate with DMFS and PFS. Consistent with historical data, adjuvant radiotherapy in our data was associated with effective DMFS and PFS.

Breast cancer cells are often infiltrated by inflammatory cells, especially T lymphocytes [10]. Previous reports have shown that the ratio of CD4/CD8 T cells may independently predict all-cause mortality [14]. Indeed, high CD4/CD8 ratio in tumors has been correlated with lymph nodes metastasis and reduced patient survival in breast cancer [9, 15]. The mechanisms by which higher CD4/CD8 ratio negatively influences DMFS and PFS in breast cancer patients

Table 3. Relationship between CD4/CD8 ratio and the clinicopathological variables

	Cases (n)	CD4/CD8 ratio		*P*-value
		<1.7	≥1.7	
Age (years)				
<50	42	23	19	0.126
≥50	38	15	23	
Depth				
T1	36	33	3	<0.001
T2	22	4	18	
T3	22	1	21	
MLNR				
<0.2	49	34	15	<0.001
≥0.2	31	4	27	
DMFS				
Yes	38	34	4	<0.001
No	42	4	38	
PFS				
Yes	35	33	2	<0.001
No	45	5	40	
ER				
Negative (0-1+)	43	18	25	0.194
Overexpressed (2-3+)	37	20	17	
PR				
Negative (0-1+)	38	18	20	0.580
Overexpressed (2-3+)	42	20	22	
HER-2				
Negative (0-1+)	54	30	24	0.052
Overexpressed (2-3+)	26	8	18	
Radiotherapy				
Yes	18	8	10	0.490
No	62	30	32	

Abbreviations: DMFS, distant metastasis-free survival; PFS, progression-free survival; MLNR, Metastatic lymph node ratio.

may be explained by the following factors. Firstly, antecedent studies showed higher levels of CD4 T lymphocytes associated to lymph node metastasis [7]. Patients who had high T CD4 lymphocytes and positive lymph nodes had a worse outcome [16, 17]. Secondly, CD8 T cell is a major constituent of the immune system who represents a candidate biomarker of the immune response associated with cancer. It has been reported that CD8 T lymphocytes are associated with better outcome in breast cancer [9, 10, 18]. Moreover, an increase of CD8 T cells has been associated with favorable prognosis in ovarian cancer [10], renal cell carcinoma [19], lung cancer [20]. Similarly, our study shows that the CD4/CD8 ratio is an effective predictor of outcome in breast cancer patients.

Breast cancer is a clinically staged disease, and the ratio of metastatic lymph node is not included in the AJCC staging system. MLNR is the ratio of positive nodes to the number of total retrieved nodes. And it was defined to describe the lymph node status of patients more accurately which was used to estimate outcome in breast cancer. In antecedent studies, the association between low MLNR and improvement survival has been showed in cervical cancer [21], gastric cancer [22], and colorectal cancer [23]. More, MLNR was also devised to be an independent prognostic factor in gallbladder cancer [24] and esophageal cancer [25]. We evaluated the prognostic value of MLNR in breast cancer. We then divided the patients into two groups based on their MLNR. We performed that patients with MLNR≥0.2 had a worse DMFS and PFS than patients with MLNR<0.2. Our results were consistent with many other studies, which suggest the importance of MLNR in outcome of patients with breast cancer.

In this study, we have found that CD4/CD8 ratio, MLNR and the depth of the primary tumor were associated to DMFS and PFS in breast cancer. In spite of the data are based on a limited number of patients, our views are supported by previous data and could contribute importantly to comprehend the mechanisms which regulate the interactions between lymphocytes and breast cancer.

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Disclosure of conflict of interest

None.

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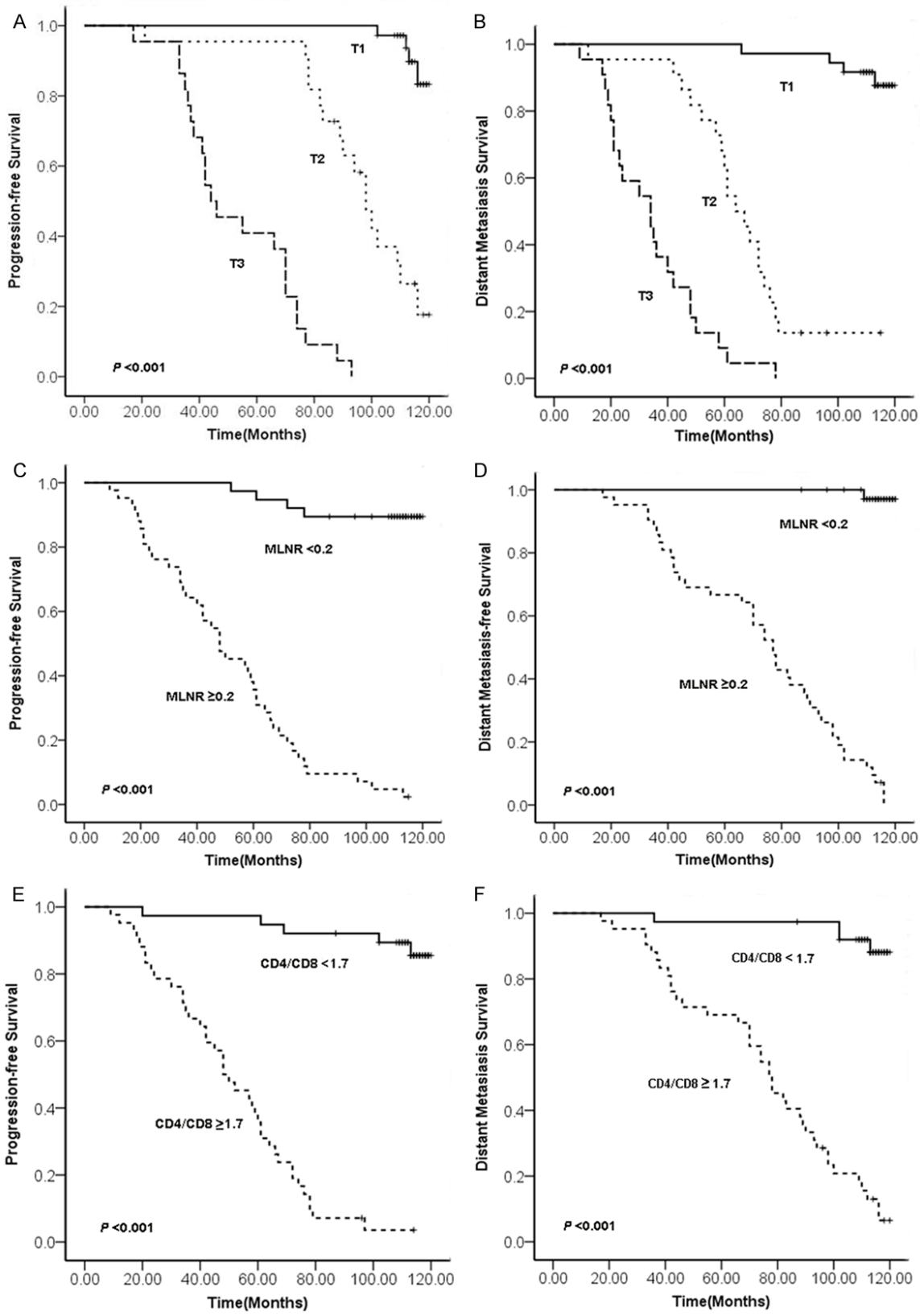

Figure 1. Factors related outcome regarding DMFS and PFS. Kaplan-Meier progression-free survival (PFS) and distant metastasis-free survival (DMFS) curves for patients with breast cancer stratified by depths of tumor (A, B), metastatic lymph node ratio (MLNR, C, D), and CD4/CD8 ratio (E, F).

Table 4. Multivariate analysis of factors associated with DMFS and PFS

	DMFS			PFS		
	P-value	HR	95% CI	*P*-value	HR	95% CI
Age	0.078	1.728	0.937-3.188	0.076	1.703	0.944-3.070
Pathologic type	0.559	1.397	0.431-4.536	0.312	1.745	0.541-5.629
Depth	<0.001	12.120	6.422-22.874	<0.001	7.735	4.786-12.501
CD4/CD8 ratio	0.003	0.364	0.188-0.704	0.002	0.394	0.211-0.734
MLNR	<0.001	8.817	4.452-17.461	<0.001	6.849	3.624-12.944
ER	0.051	0.499	0.266-0.934	0.056	0.527	0.289-0.960
PR	0.065	1.867	1.001-3.482	0.193	1.481	0.820-2.678
HER-2	0.236	1.453	0.783-2.695	0.215	1.462	0.802-2.663
Radiotherapy	0.017	1.989	1.472-3.072	0.012	2.147	1.553-3.382

Abbreviations: DMFS, distant metastasis-free survival; PFS, progression-free survival; MLNR, Metastatic lymph node ratio.

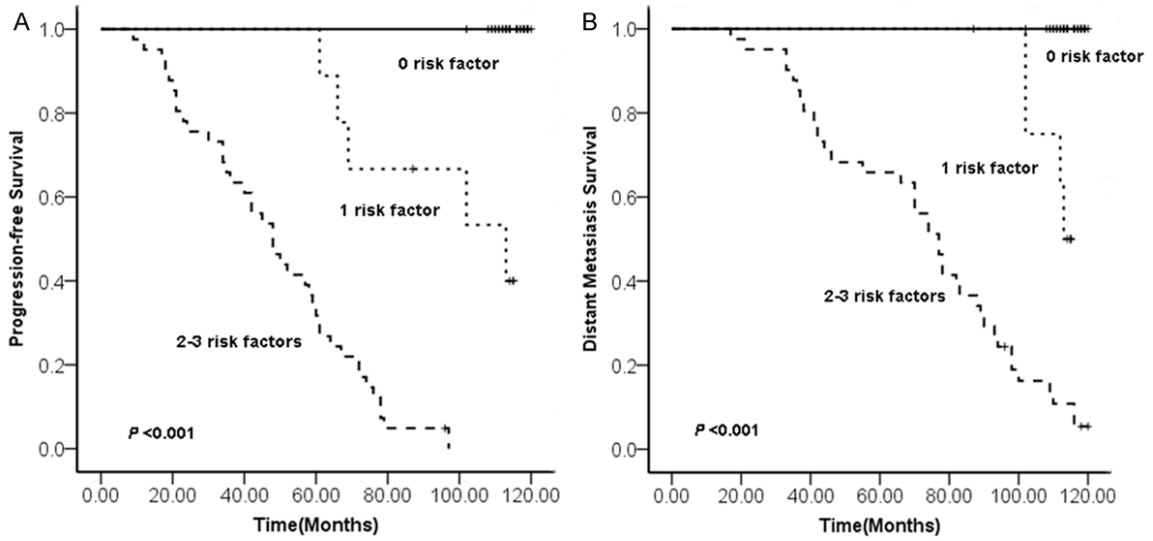

Figure 2. Prognostic model for DMFS and PFS in breast cancer. Kaplan-Meier progression-free survival (PFS) curves (A) and distant metastasis-free survival (DMFS) curves (B) for patients with breast cancer stratified using the prognostic score model based on depths of tumor, metastatic lymph node ratio (MLNR) and CD4/CD8 ratio.

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