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Original Article

The relationship between vitamin D, ratio of neutrophil to lymphocyte, and ratio of lymphocyte to monocyte in preoperative serum and prognosis of patients with breast conserving surgery in breast cancer

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Abstract: Objective: To explore the relationship between vitamin D, ratio of neutrophil to lymphocyte (NLR), and ratio of lymphocyte to monocyte (LMR) in preoperative serum and prognosis of patients with breast conserving surgery in breast cancer. Methods: 206 cases of breast conserving surgery in breast cancer were prospectively analyzed. Peripheral venous blood was collected 1-7 days before operation. Enzyme-linked immunosorbent assay (ELISA) was used to detect serum 25-hydroxyvitamin D3 (25(OH)D) levels, neutrophils, lymphocytes, monocytes, NLR and LMR levels in the peripheral blood and were detected by hematology analyzer. According to the levels of 25(OH)D, NLR and LMR in the patients' peripheral blood, the receiver operating characteristic curve (ROC) for NLR and LMR was used to predict the overall survival (OS) in patients with breast cancer. Kaplan-Meier and Cox regression models were used for survival analysis of patients. Results: There were significant differences in the proportion of adjuvant radiotherapy and proportion of TNM staging between the high 25(OH)D group and the low 25(OH)D group (P<0.05). The median PFS and OS of the high 25(OH)D group were significantly longer than those of the low 25(OH)D group (P<0.05). The median PFS and OS of the low NLR group and LMR group were significantly longer than those of the high NLR group and low LMR group, respectively (P<0.05). Cox regression single factor analysis has shown that TNM staging, adjuvant radiotherapy, molecular typing, 25(OH)D, NLR and LMR were related factors affecting the prognosis of breast cancer patients (P<0.05). The optimal cutoff value for preoperative 25(OH)D, NLR and LMR to evaluate the prognosis of patients was 21.03 ng/mL (AUC=0.720, Sensitivity =58.82%, Specificity =78.16%), 2.76 (AUC=0.737, Sensitivity =64.71%, Specificity =82.76%) and 5.26 (AUC=0.718, Sensitivity =65.71%, Specificity =79.62%), respectively. High grading of the cancer, no adjuvant radiotherapy, different molecular typing, 25(OH)D<21.03 ng/mL, NLR≥2.76 and LMR<5.26 were independent prognostic factors affecting breast cancer (P<0.05). Conclusion: High grading of the cancer, no adjuvant radiotherapy, different molecular typing and 25(OH)D<21.03 ng/mL, NLR≥2.76, LMR<5.26 in preoperative peripheral blood are independent prognostic factors affecting OS in patients with breast cancer.

Keywords: Vitamin D, ratio of neutrophil to lymphocyte (NLR), ratio of lymphocyte to monocyte (LMR), breast cancer, breast conserving surgery, prognosis

Introduction

5%-7% of women suffer from breast cancer and are diagnosed before 40 years old [1, 2]. Advancements in medical diagnostics has been reducing the mortality of patients with breast cancer year by year. Some patients still experience recurrence and metastasis as well as treatment failure [3]. It is particularly crucial to find biological markers closely related to the occurrence, metastasis, recurrence and prognosis of breast cancer.

Vitamin D can regulate immune function and is closely related to the occurrence and development of various cancers [4]. Previous studies have shown that the prevalence rate of breast cancer was lower in people with higher vitamin

D levels [5, 6]. Also the cancer microenvironment was considered as one factor in tumor growth, invasion and metastasis [7]. Cancer-related inflammation contributed to the proliferation of tumor cells, promoted angiogenesis and metastasis of breast cancer, destroyed the adaptive immune response and changed the effect of chemotherapeutics [8]. Emerging evidences suggested that elevated ratios of neutrophil to lymphocyte (NLR) are an independent prognostic index of malignant tumors and is associated with low survival rates of various cancers, such as colon, ovarian, esophageal and gastric cancers [9, 10]. Elevated NLR is a useful prognostic factor for predicting poor prognosis in patients with stage III and IV rectal cancer in the study of Simanjuntak et al. [11]. Nomelini et al. [12], showed that NLR and platelet counts could be used as prognostic factors for ovarian malignant tumors. Lymphocytes can eliminate tumor cells by inhibiting cell-induced cytotoxicity, proliferation and migration, and tumor-related macrophages. Lymphocytes and monocytes are closely related to the prognosis of tumors [13]. Current studies have shown that the ratio of lymphocyte to monocyte (LMR) is an independent prognostic factor for some solid tumors, such as hematological malignancies and gastrointestinal tumors [14, 15].

Previous studies have shown that vitamin D, NLR and LMR were important prognostic factors to determine various tumors [16-18]. However, there are few studies on the relationship between vitamin D, NLR, LMR in preoperative peripheral blood and the prognosis of breast conserving surgery in breast cancer patients. This study aimed to explore the relationship between vitamin D, NLR, LMR and the prognosis of breast cancer, and to evaluate whether vitamin D, NLR and LMR can be used as predictors of the prognosis of breast conserving surgery in breast cancer patients, so as to provide reference for clinical treatment.

Materials and methods

General data

206 patients received breast conserving surgery in our hospital from April 2009 to August 2012 and were prospectively analyzed. Inclusion criteria: breast conserving surgery was performed in our hospital, and the pathological diagnosis was breast cancer [19]; breast cancer TNM staging was in accordance with UICC and AJCC [20]; estrogen receptor status (ER), progesterone receptor status (PR) and Her-2 conformed to immunohistochemical detection guidelines [21]; Karnofsky (KPS) score of functional status [22] ≥80; hematology, liver and kidney function were normal; the subjects and their family members have been informed and signed a fully informed consent; the clinical medical records are complete (past, present, family and personal history). This study has been approved by the ethics committee of our hospital. Exclusion criteria: Preoperative history of chemotherapy, radiotherapy and immunotherapy; preoperative use of drugs affecting vitamin D, neutrophils, lymphocytes and monocytes; existence of stress response and hematological diseases; associated connective tissue diseases, endocrine and metabolic diseases, neurological diseases, immune diseases and other tumors; past psychosis and family history of psychosis; not treated according to doctor's advice.

The age of the patients was 27-74 years old, with an average age of (46.18±9.73) years. Among them, 95 patients were menopausal and 111 patients were non-menopausal; ER: 106 patients were positive and 100 patients were negative; PR: 119 patients were positive and 87 patients were negative; Her-2: 70 patients were positive and 136 patients were negative; TNM staging: stage I 60 cases, stage II A 77 cases, stage II B 58 cases and stage III 11 cases; adjuvant radiotherapy 182 cases, and no adjuvant radiotherapy 24 cases; lymph node metastasis 78 patients, and no lymph node metastasis 12 patients; tumor size: <2 91 cases, and >2 115 cases; molecular typing: 54 cases of Luminal A, 54 cases of Luminal B, 39 cases of Her-2 over-expression, 59 cases of triple negative.

Detection of vitamin D, ratio of neutrophil to lymphocyte, and ratio of lymphocyte to monocyte in preoperative serum

Peripheral venous blood was collected on an empty stomach before operation, and serum was separated by centrifugation. The centrifugation speed was 670.8 (*g) at 20~25°C, and the centrifugation time was 10 minutes. According to the instructions of the 25(OH)D ELISA detection kit (Shanghai Huzhen Biotechnology Co., Ltd., China), the expression of

25-hydroxyvitamin D3 (25(OH)D) in serum was detected by enzyme-linked immunosorbent assay (ELISA) [23]. Samples and kits were taken out from the refrigerator 45 minutes in advance and placed at room temperature. Sample wells standard wells and blank wells were set up. No reagents were added to the blank well, and 50 μl of sample or standard dilution by different multiples were added to the remaining wells. Each well was covered with film, and incubated at 37°C for 30 minutes. The liquids in each well were discarded, wells were dried and washed three times. 50 μl of the antibodies labeled with biotin were added to each well. Each well was covered with film, and incubated at 37°C for 1 hour. The liquids in each well were discarded, wells were dried and washed three times. Each well was mixed with 80 μl streptavidin and incubated at 37°C for 30 minutes. The liquids in each well were discarded, wells were dried and washed three times. Each well was mixed with 50 μl of substrate A and B liquids, and incubated at 37°C for 10 minutes. The wells were colored at room temperature, away from light. The OD value of each pore was measured at 450 nm wavelength by Elx-800 enzyme-linked immunometric meter (BioTek, Winooski, VT, USA), and the 25(OH)D level was calculated.

Peripheral venous blood was collected 1-7 days before operation. The neutrophils, lymphocytes, monocytes, NLR and LMR in the peripheral blood were measured by DxH 800 blood cell analyzer (Beckman Coulter, Chaska, MN, USA).

Follow-up

Follow-up was conducted by telephone communication. According to NCCN Guidelines [24], follow-up was carried out every 3 months for 1-2 years and then every 6 months for 2-5 years. The follow-up time was 5 years and the final follow-up was 31 August 2017. Progressive free survival time (PFS) was the time of distant metastasis, local recurrence or death after operation. Overall survival time (OS) was the time from the first day of operation to the last follow-up or death.

Statistical method

SPSS 19.0 (IBM Corp, Armonk, NY, USA) was used for statistical analysis. GraphPad Prism 7 was used to illustrate the data in pictures. The counting data was expressed by the number of cases/percentage [n (%)]. The chi-square test was used to compare the counting data between groups. The optimal cut-off values of 25(OH)D, NLR and LMR were determined by ROC to predict the OS of patients with breast cancer. PFS and OS of breast cancer patients were mapped by the Kaplan-Meier method and compared by Log-rank test. Cox regression model was used for single and multiple factor analysis. $P<0.05$ indicated that there was a statistically significant difference.

Results

Optimal cutoff values of 25(OH)D, NLR and LMR

The 25(OH)D concentration range in preoperative serum was 18.62-24.17 (ng/mL) in 204 patients with breast cancer, with an average of (19.28±2.87) ng/mL. The peripheral blood NLR ranged from 0.97 to 3.16, with an average of (2.03±0.93) and the peripheral blood LMR ranged from 1.46 to 12.37, with an average of (6.11±1.94). The 25(OH)D, NLR and LMR in peripheral blood were used to evaluate the ROC curve of prognosis in patients with breast cancer (5-year OS), and the optimal cutoff value was determined, taking into account sensitivity and specificity. The AUC value was 0.720, the sensitivity was 58.82%, the specificity was 78.16%, and the optimal cutoff value was 21.03 ng/mL by using 25(OH)D to evaluate the prognosis of patients. According to preoperative 25(OH)D level =21.03 (ng/mL), 124 cases were in the high 25(OH)D group (25(OH)D≥ 21.03 ng/mL), and 82 cases were in the low 25(OH)D group (25(OH)D<21.03 ng/mL). The AUC value was 0.737, the sensitivity was 64.71%, the specificity was 82.76%, and the optimal cutoff value was 2.76 by using NLR to evaluate the prognosis of patients. According to preoperative NLR ratio =2.76, 97 cases were in the high NLR group (NLR≥2.76), and 109 cases were in the low NLR (NLR<2.76).

The AUC value was 0.718, the sensitivity was 65.71%, the specificity was 79.62%, and the optimum cut-off value was 5.26 by using LMR to evaluate prognosis of patients. According to preoperative LMR ratio =5.26, 116 cases were in the high LMR group (LMR≥5.26), and 90

Table 1. ROC results of peripheral 25(OH)D, NLR, LMR to assess prognosis in breast cancer patients

Diagnostic indicator	AUC	95% CI	Standard error	Cut-off	Sensitivity (%)	Specificity (%)
25(OH)D	0.720	0.600-0.821	0.062	21.03 ng/mL	58.82	78.16
NLR	0.737	0.617-0.856	0.061	2.76	64.71	82.76
LMR	0.718	0.594-0.839	0.064	5.26	65.71	79.62

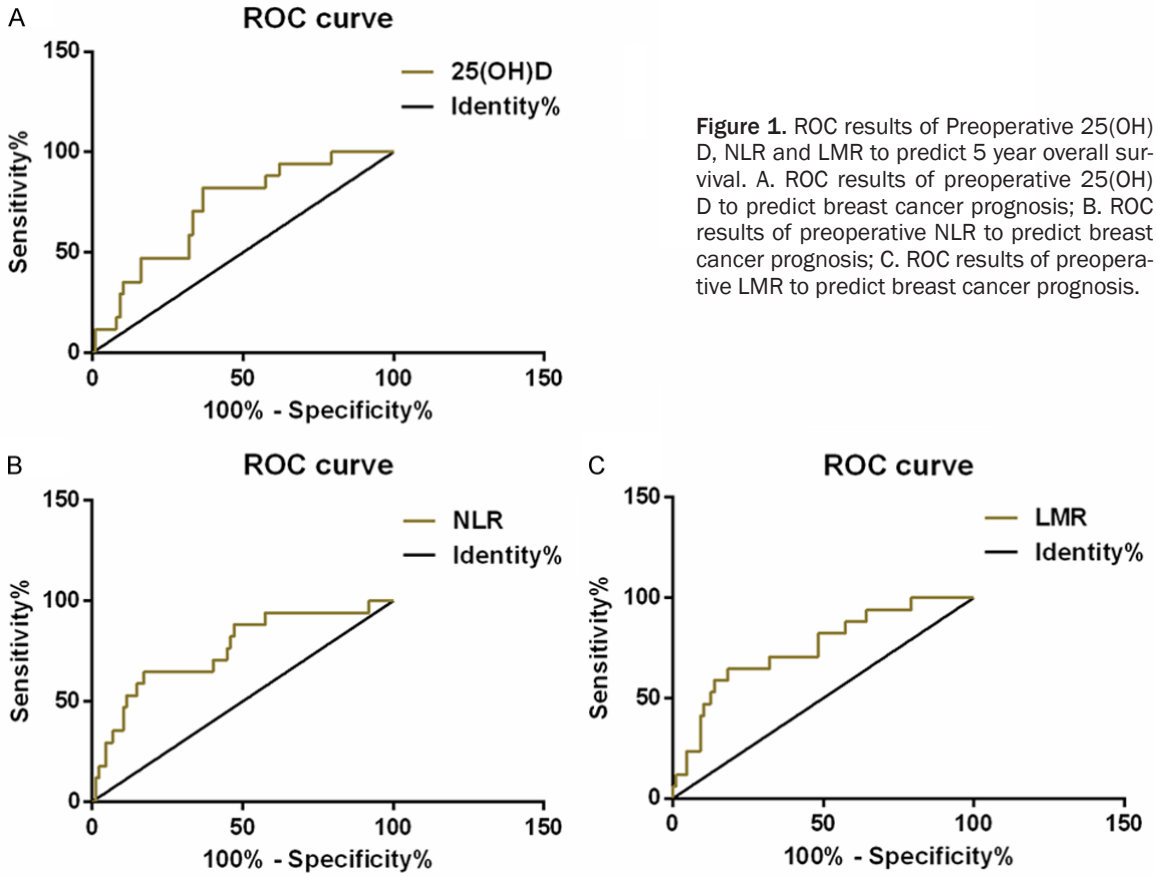

Figure 1. ROC results of Preoperative 25(OH)D, NLR and LMR to predict 5 year overall survival. A. ROC results of preoperative 25(OH)D to predict breast cancer prognosis; B. ROC results of preoperative NLR to predict breast cancer prognosis; C. ROC results of preoperative LMR to predict breast cancer prognosis.

cases were in the low LMR (LMR<5.26) (**Table 1** and **Figure 1**).

The relationship between 25(OH)D, NLR, LMR and clinical pathological features of breast cancer

There were no significant differences in age, menopausal status, ER, PR, Her-2, TNM staging, lymph node metastasis, tumor size, nor molecular typing between the preoperative high 25(OH)D group and the low 25(OH)D group (P>0.05), while there was a significant difference in adjuvant radiotherapy (P<0.05). There were no significant differences in age, menopausal status, ER, PR, Her-2, adjuvant radiotherapy, lymph node metastasis, tumor size, and molecular typing between the preoperative high NLR group and the low NLR group (P>0.05), while there was a significant difference in TNM staging (P<0.05). There were no significant differences in age, menopausal status, ER, PR, Her-2, TNM staging, adjuvant radiotherapy, lymph node metastasis, tumor size, and molecular typing between the preoperative high LMR group and the low LMR group (P>0.05) (**Tables 2-4**).

The relationship between 25(OH)D, NLR, LMR in preoperative peripheral blood and postoperative PFS and OS in patients with breast cancer

The 5-year survival rate of the 206 patients with breast cancer was 91.26% (188/206). The OS of the high 25(OH)D group was 94.35%

Table 2. Clinicopathological features of preoperative high 25(OH)D group and low 25(OH)D group in breast cancer [n (%)]

Clinical pathological features	n	High 25(OH)D group (n=124)	Low 25(OH)D group (n=82)	χ^2	P
Age (year)				0.216	0.642
≤48	114	67 (54.03)	47 (57.32)		
>48	92	57 (45.97)	35 (42.68)		
Menopausal state				1.187	0.276
Yes	95	61 (49.19)	34 (41.46)		
No	111	63 (50.81)	48 (58.54)		
ER				2.943	0.067
Positive	106	56 (45.16)	50 (60.98)		
Negative	100	68 (54.84)	32 (39.02)		
PR				3.651	0.056
Positive	119	65 (52.42)	54 (65.85)		
Negative	87	59 (47.58)	28 (34.15)		
Her-2				2.382	0.123
Positive	70	37 (29.84)	33 (40.24)		
Negative	136	87 (70.16)	49 (59.76)		
TNM staging				2.628	0.105
Phase I	60	43 (34.68)	17 (20.73)		
II A period	77	41 (33.06)	36 (43.90)		
II B period	58	35 (28.23)	23 (28.05)		
Phase III	11	5 (4.03)	6 (7.32)		
Auxiliary radiotherapy				6.070	0.014
Yes	182	104 (83.87)	78 (95.12)		
No	24	20 (16.13)	4 (4.88)		
Lymph node metastasis				2.111	0.146
Yes	78	42 (33.87)	36 (43.90)		
No	128	82 (66.13)	46 (56.10)		
Tumor size (cm)				0.633	0.426
≤2	91	52 (41.94)	39 (47.56)		
>2	115	72 (58.06)	43 (52.44)		
Molecular typing				1.820	0.177
Luminal A	54	36 (29.03)	18 (21.95)		
Luminal B	54	34 (27.42)	20 (24.39)		
Her-2 enriched	39	21 (16.94)	18 (21.95)		
Triple-negative	59	33 (26.61)	26 (31.71)		

(117/124), and the OS of the low 25(OH)D group was 86.59% (71/82). The OS of the high NLR group was 85.57% (83/97), and the OS of the low NLR group was 96.33% (105/109). The OS of the high LMR group was 96.55% (112/116), and the OS of the low LMR group was 84.44% (76/90). Kaplan-meier survival curve analysis has shown that the OS of the high 25(OH)D group was significantly longer than that of the low 25(OH)D group (P<0.05), the OS of the low NLR group was significantly longer than those of the high NLR group (P< 0.05), and the OS of the high LMR group was significantly longer than those of the low LMR group (P< 0.05). The median PFS of the high 25(OH)D group was 56.4 months, which was significantly higher than that of the low 25(OH)D group (46.2 months) (P< 0.05). The median PFS of the low NLR group was 57.6 months, which was significantly higher than that of the low NLR group (44.9 months) (P<0.05). The median PFS of high LMR group was 53.4 months, which was significantly higher than that of low LMR group (46.8 months) (P<0.05) (**Figure 2**).

Cox regression analysis of factors influencing breast cancer survival

Cox regression single factor analysis has shown that high TNM staging, namely higher grading of the cancer, no adjuvant radiotherapy, different molecular typing, 25(OH)D<21.03 ng/mL, NLR≥2.76 and LMR <5.26 were related factors affecting breast cancer prognosis (P<0.05), while age, menopausal status, ER, PR, Her-2, lymph node metastasis and tumor size had no significant effect on breast cancer prognosis (P>0.05). Further Cox regression multivariate analysis has shown that higher grading of the cancer, no adjuvant radiotherapy, different molecular typing, 25(OH)D<21.03 ng/mL, NLR≥2.76 and LMR<5.26 were independent prognostic factors affecting breast cancer survival (P<0.05) (**Tables 5**, **6**).

Table 3. Clinicopathological features of preoperative high NLR group and low NLR group in breast cancer [n (%)]

Clinical pathological features	n	High NLR group (n=97)	Low NLR group (n=109)	χ^2	P
Age				2.232	0.135
≤48	114	59 (60.82)	55 (50.46)		
>48	92	38 (39.18)	54 (49.54)		
Menopausal state				2.577	0.108
Yes	95	39 (40.21)	56 (51.38)		
No	111	58 (59.79)	53 (48.62)		
ER				2.019	0.155
Positive	106	55 (56.70)	51 (46.79)		
Negative	100	42 (43.30)	58 (53.21)		
PR				1.969	0.160
Positive	119	61 (62.89)	58 (53.21)		
Negative	87	36 (37.11)	51 (46.79)		
Her-2				3.167	0.075
Positive	70	39 (40.21)	31 (28.44)		
Negative	136	58 (59.79)	78 (71.56)		
TNM staging				3.970	0.046
Phase I	60	21 (21.65)	39 (35.78)		
II A period	77	42 (43.30)	35 (32.11)		
II B period	58	25 (25.77)	33 (30.28)		
Phase III	11	9 (9.28)	2 (1.83)		
Auxiliary radiotherapy				3.148	0.056
Yes	182	80 (82.47)	102 (93.58)		
No	24	17 (17.53)	7 (6.42)		
Lymph node metastasis				0.247	0.619
Yes	78	35 (36.08)	43 (39.45)		
No	128	62 (63.92)	66 (60.55)		
Tumor size (cm)				1.858	0.173
≤2	91	38 (39.18)	53 (48.62)		
>2	115	59 (60.82)	56 (51.38)		
Molecular typing				0.611	0.434
Luminal A	54	23 (23.71)	31 (28.44)		
Luminal B	54	24 (24.74)	30 (27.52)		
Her-2 enriched	39	22 (22.68)	17 (15.60)		
Triple-negative	59	28 (28.87)	31 (28.44)		

Discussion

Metastasis of breast cancer is a difficult problem after surgery [25, 26]. Recurrence and metastasis are the main factors leading to treatment failure for breast cancer patients, which seriously affects the OS of breast cancer patients [27].

The results of this study have shown that there was a significant difference in the proportion of adjuvant radiotherapy between the high 25(OH)D group and low 25(OH)D group before operation. In the study of Vrieling et al. [28], the level of 25(OH)D was affected by chemotherapy. Adjuvant chemotherapy or radiotherapy may induce nausea and vomiting, affect the patient's diet and physical activity subsequently, and indirectly affect the level of 25(OH)D. Therefore, radiation therapy may affect the level of 25(OH)D. By observing the relationship between 25(OH)D and survival and the prognosis of breast cancer, the median PFS and OS of the high 25(OH)D group were significantly longer than those of the low 25(OH)D group, and 25(OH)D was an independent prognostic factor of breast cancer. It was suggested that breast cancer patients with high 25(OH)D levels have better PFS and OS, which is similar to the study of Goodwin et al. [29]. The lack of 25(OH)D may be may be related to poor prognosis of early breast cancer patients.

Inflammatory cells are important participants in the development of malignant tumors. Inflammatory media can cause DNA mutation and oxidative damage of cells, thereby changing the microenvironment of tumors and promoting the proliferation and migration of tumor cells [30]. In the human body, the level of neutrophils in the peripheral blood often reflects the inflammatory reaction of the body [31, 36]. Previous studies have shown that inflammatory cells in peripheral blood circulation were closely related to the prognosis of tumors, including neutrophils, lymphocytes and monocytes [32]. Previous studies have shown that NLR and LMR in preoperative

Table 4. Clinicopathological features of preoperative high LMR group and low LMR group in breast cancer [n (%)]

Clinical pathological features	n	High LMR group (n=116)	Low LMR group (n=90)	χ^2	P
Age				0.815	0.367
≤48	114	61 (52.59)	53 (58.89)		
>48	92	55 (47.41)	37 (41.11)		
Menopausal state				2.406	0.121
Yes	95	59 (50.86)	36 (40.00)		
No	111	57 (49.14)	54 (60.00)		
ER				0.225	0.635
Positive	106	58 (50.00)	48 (53.33)		
Negative	100	58 (50.00)	42 (46.67)		
PR				0.732	0.392
Positive	119	64 (55.17)	55 (61.11)		
Negative	87	52 (44.83)	35 (38.89)		
Her-2				2.581	0.108
Positive	70	34 (29.31)	36 (40.00)		
Negative	136	82 (70.69)	54 (60.00)		
TNM staging				3.467	0.087
Phase I	60	41 (35.34)	19 (21.11)		
II A period	77	39 (33.62)	38 (42.22)		
II B period	58	33 (28.45)	25 (27.78)		
Phase III	11	3 (2.59)	8 (8.89)		
Auxiliary radiotherapy				2.368	0.124
Yes	182	106 (91.38)	76 (84.44)		
No	24	10 (8.62)	14 (15.56)		
Lymph node metastasis				0.794	0.373
Yes	78	47 (40.52)	31 (34.44)		
No	128	69 (59.48)	59 (65.56)		
Tumor size (cm)				1.130	0.288
≤2	91	55 (47.41)	36 (40.00)		
>2	115	61 (52.59)	54 (60.00)		
Molecular typing				0.366	0.545
Luminal A	54	32 (27.59)	22 (24.44)		
Luminal B	54	33 (28.45)	21 (23.34)		
Her-2 enriched	39	17 (14.66)	22 (24.44)		
Triple-negative	59	34 (29.31)	25 (27.78)		

peripheral blood could better predict the prognosis of cancer patients, both of which are considered adverse prognostic factors of various tumors [33]. Studies have shown that the increase of NLR is associated with the decrease of PFS and OS in patients with solid tumors [34]. Nishijima et al. [35], examined PubMed and online databases, and found a total of 11197 patients that were enrolled for meta-analysis. The results showed that the decrease of LMR level often predicted a poor prognosis of cancer patients. Results have shown that the median PFS and OS of the low NLR group were significantly longer than those of the high NLR group, and the median PFS and OS of the high LMR group were significantly longer than those of the low LMR group. NLR and LMR were independent prognostic factors for breast cancer patients. Lower NLR and higher LMR indicated better PFS and OS. Azab et al. [36], confirmed that high NLR was an independent prognostic factor for short-term and long-term mortality in breast cancer, and high NLR predicted poor prognosis of breast cancer patients. In the study of Ni et al. [37], high LMR in peripheral blood was beneficial to the prognosis of patients with locally advanced breast cancer. It was similar to our research conclusion. However, the cut-off values of NLR and LMR in the studies of Azab and Ni, were different from those in our studies, which may be related to the different disease stages, surgical methods and treatment strategies.

Postoperative TNM staging, molecular typing and adjuvant radiotherapy were the main factors affecting the prognosis of breast cancer [38-40]. Our study also confirmed that high grading of the cancer, no adjuvant radiotherapy, different molecular typing, 25(OH)D<21.03 ng/mL, NLR≥2.76 and LMR<5.26 were independent risk factors of breast cancer patients after surgery. Although TNM staging and molecular typing could be used as prognostic factors for breast cancer patients, these indicators depended on samples obtained by

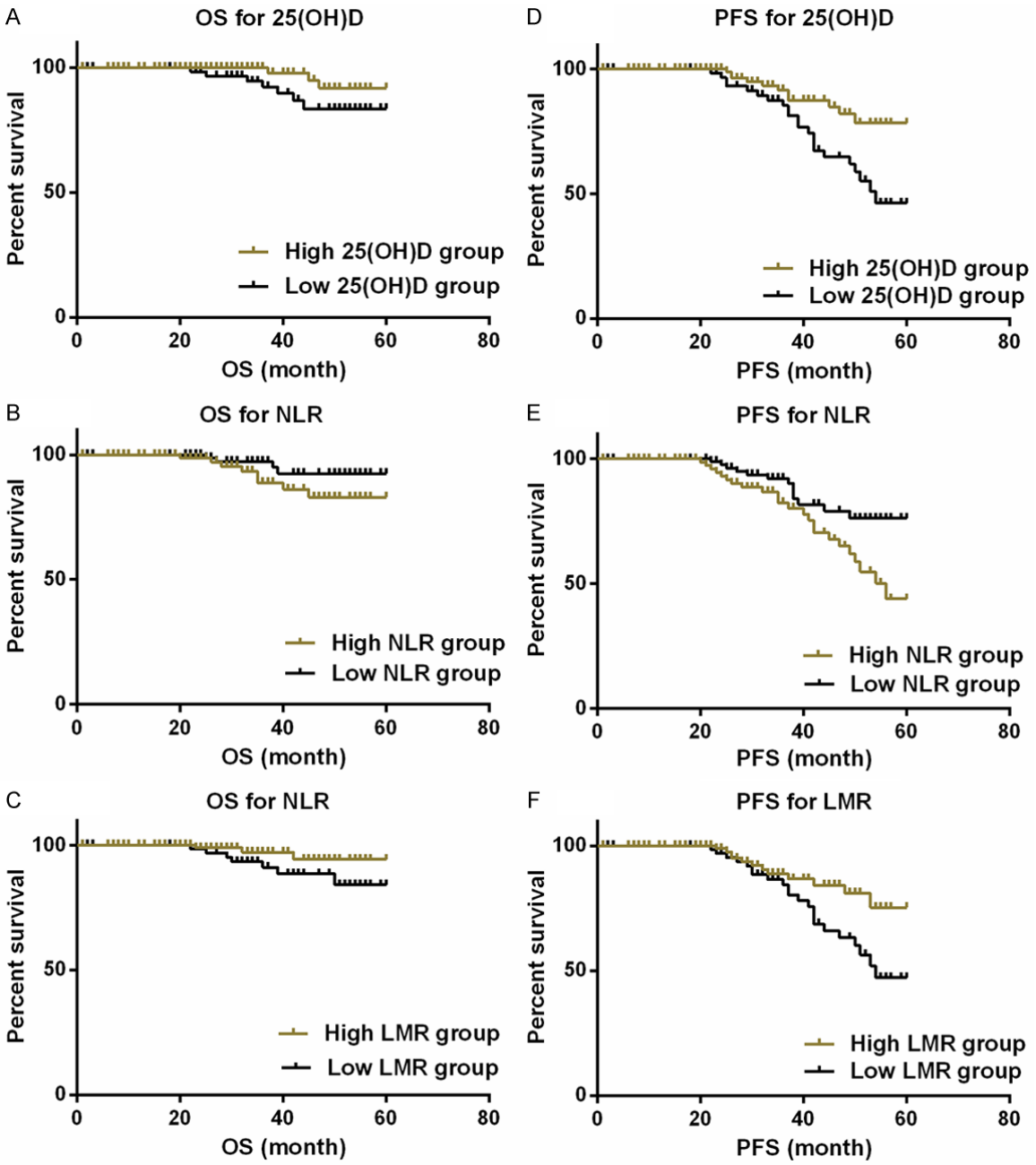

Figure 2. The relationship between 25(OH)D, NLR, LMR in preoperative peripheral blood and postoperative PFS, OS in breast cancer patients. The relationship between 25(OH)D in preoperative peripheral blood and postoperative OS in breast cancer patients (A); the relationship between NLR in preoperative peripheral blood and postoperative OS in breast cancer patients (B); the relationship between LMR in preoperative peripheral blood and postoperative OS in breast cancer patients (C); the relationship between 25(OH)D in preoperative peripheral blood and postoperative median PFS in breast cancer patients (D); the relationship between NLR in preoperative peripheral blood and postoperative median PFS in breast cancer patients (E); the relationship between LMR in preoperative peripheral blood and postoperative median PFS in breast cancer patients (F).

pathological puncture or resection of primary tumors. Therefore, biopsy and surgical excision limited the clinical application of these indicators. The results of this study confirmed that 25(OH)D, NLR and LMR in the peripheral blood can be used as predictors of the prognosis of breast cancer, but there ware still some shortcomings. First, there was no long-term follow-

Table 5. Variable assignment of Cox regression analysis

Factor	Variable	Assignment
Age	X1	≤48=1, >48=2
Menopausal state	X2	Yes =1, No =2
ER	X3	Positive =1, Negative =2
PR	X4	Positive =1, Negative =2
Her-2	X5	Positive =1, Negative =2
TNM staging	X6	I stage =1, II A stage =2, II B stage =3, III stage =4
Auxiliary radiotherapy	X7	Yes =1, No =2
Lymph node metastasis	X8	Yes =1, No =2
Tumor size	X9	≤2=1, >1=2
Molecular typing	X10	Luminal A=1, Luminal B=2, Her-2 enriched =3, Triple-negative =4
25(OH)D	X11	≥21.03=1, <21.03=2
NLR	X12	≥2.76=1, <2.76=2
LMR	X13	≥5.26=1, <5.26=2

Table 6. Univariate and multivariate analysis of OS prognosis in breast cancer

Variable	Univariate analysis		multi-factor analysis	
	HR (95% CI)	P	HR (95% CI)	P
Age	1.170 (0.703-2.018)	0.526		
Menopausal state	1.301 (0.746-2.235)	0.337		
ER	0.624 (0.293-1.276)	0.193		
PR	1.286 (0.376-4.449)	0.673		
Her-2	1.174 (0.317-4.290)	0.787		
TNM staging	3.036 (1.556-5.913)	0.001	3.670 (1.748-7.271)	0.001
Auxiliary radiotherapy	2.370 (1.302-4.374)	0.003	2.581 (1.371-4.780)	0.002
Lymph node metastasis	1.732 (0.841-3.512)	0.094		
Tumor size	1.146 (0.276-1.674)	0.797		
Molecular typing	3.044 (1.378-6.430)	0.005	2.487 (1.130-5.675)	0.020
25(OH)D	1.893 (1.217-2.895)	0.008	1.648 (1.045-2.696)	0.034
NLR	1.802 (1.056-3.024)	0.001	1.968 (1.169-3.324)	0.021
LMR	2.749 (1.467-5.212)	0.023	2.186 (1.136-4.226)	0.011

up for breast cancer patients. Secondly, PFS and OS in different molecular typing of breast cancer have not been classified and observed. Thirdly, the study period should be prolonged and the frequency of sampling should be shortened in further study to minimize the bias.

In conclusion, 25(OH)D<21.03 ng/mL, NLR≥ 2.76, LMR<5.26 are independent prognostic factors affecting OS in breast cancer patients. Peripheral blood 25(OH)D, NLR, LMR levels showed predictive value for prognosis in breast cancer patients who have undergone breast-conserving surgery.

Disclosure of conflict of interest

None.

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