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Original Research

Predictive Value of VEGF-C and D Combined with Ultrasound Pathological Features for Nonsentinel Lymph Node Metastasis in SLN-Positive Early-Stage Breast Cancer

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Abstract

Background: To explore the predictive value of vascular endothelial growth factor (VEGF)-C and D combined with ultrasonic pathological features for nonsentinel lymph node (NSLN) metastasis in positive sentinel lymph nodes (SLNs) early-stage breast cancer. **Methods**: To review the clinical data of 170 SLN-positive early breast cancer patients. We examined VEGF-C and D positive expression in cancerous and paraneoplastic tissues and counted ultrasound and pathological features. **Results**: The rate of VEGF-C and D positivity in cancer tissues was higher than that in paracancerous tissues ($p < 0.05$). The rates of VEGF-C and D positivity in the cancer tissues with vascular infiltration, number of SLN positives >2, proportion of SLN positives >0.5, burr sign on ultrasound, and NSLN metastasis were higher than those of patients without vascular infiltration, number of SLN positives ≤2, proportion of SLN positives ≤0.5, no burr sign, and no NSLN metastasis, respectively ($p < 0.05$). The results also showed that the presence of vascular infiltration and burr sign, a high number of SLN positivity, the percentage of SLN positivity >0.5, VEGF-C and D positivity were all NSLN metastasis independent risk factors for metastasis ($p < 0.05$). Receiver operating characteristic (ROC) curve analysis showed that the area under the ROC curve (AUC) for VEGF-C and D combined with ultrasound and pathological features to predict NSLN metastasis was the highest. **Conclusions**: The ultrasound and pathological features of SLN-positive early breast cancer patients, such as vascular infiltration, VEGF-C and D positivity, were all independent risk factors for NSLN metastasis, and VEGF-C and D combined with ultrasound and pathological features had high predictive efficacy for NSLN metastasis. It provides reliable indicators to screen for NSLN metastasis in a high-risk group from SLN-positive patients with early-stage breast cancer.

Keywords: positive anterior lymph nodes; early breast cancer; vascular endothelial growth factor; nonposterior lymph node metastasis

1. Introduction

As the sentinel lymph node (SLN) is the primary site of metastasis from the primary cancer lesion, assessing its metastatic status helps in the clinical selection of the optimal treatment strategy to maximize the survival benefit for patients [1]. Lymph node metastases play a key role in patient treatment failure and shortened survival. A phase 3 clinical trial noted that SLN-negative patients who underwent SLN resection alone without axillary lymph node dissection (ALND) had similar overall survival and disease-free survival to those who underwent ALND [2]. Subsequently, Donker *et al*. [3] reached similar conclusions. From this, it can be assumed that not all SLN-positive individuals will benefit from ALND. Currently, there is still some controversy regarding the management of SLN in early-stage breast cancer patients with negative axillary lymph nodes, but pathology suggests the presence of one to two SLN-positive lesions, and patients may not receive ALND for those with postoperative adjuvant radiotherapy or other combination treatment options [4,5]. Therefore, finding reliable protocols to screen for nonsentinel lymph node (NSLN) metastases at high risk from SLN-positive patients effectively prevents patients from receiving unnecessary ALND.

Vascular endothelial growth factor (VEGF) promotes the production of blood vessels and lymphatic vessels. It also has various functions, such as promoting epithelial cell division and regulating vascular permeability [6]. VEGF-C and -D both belong to the VEGF family, which is widely expressed in early embryos and various organs and tissues. In recent years, studies related to malignant tumours such as oesophageal cancer [7] and squamous lung cancer [8] have pointed to the association of VEGF-C and D with lymph node metastasis. However, their roles in lymph node generation and metastasis of breast cancer are not well defined. This study aimed to investigate the predictive value of VEGF-C and D combined with ultrasound and pathological features for NSLN metastasis to guide the choice of clinical ALND treatment.

2. Methods

2.1 Patients

We reviewed the clinical data of 170 SLN-positive early breast cancer patients who were sourced from con-

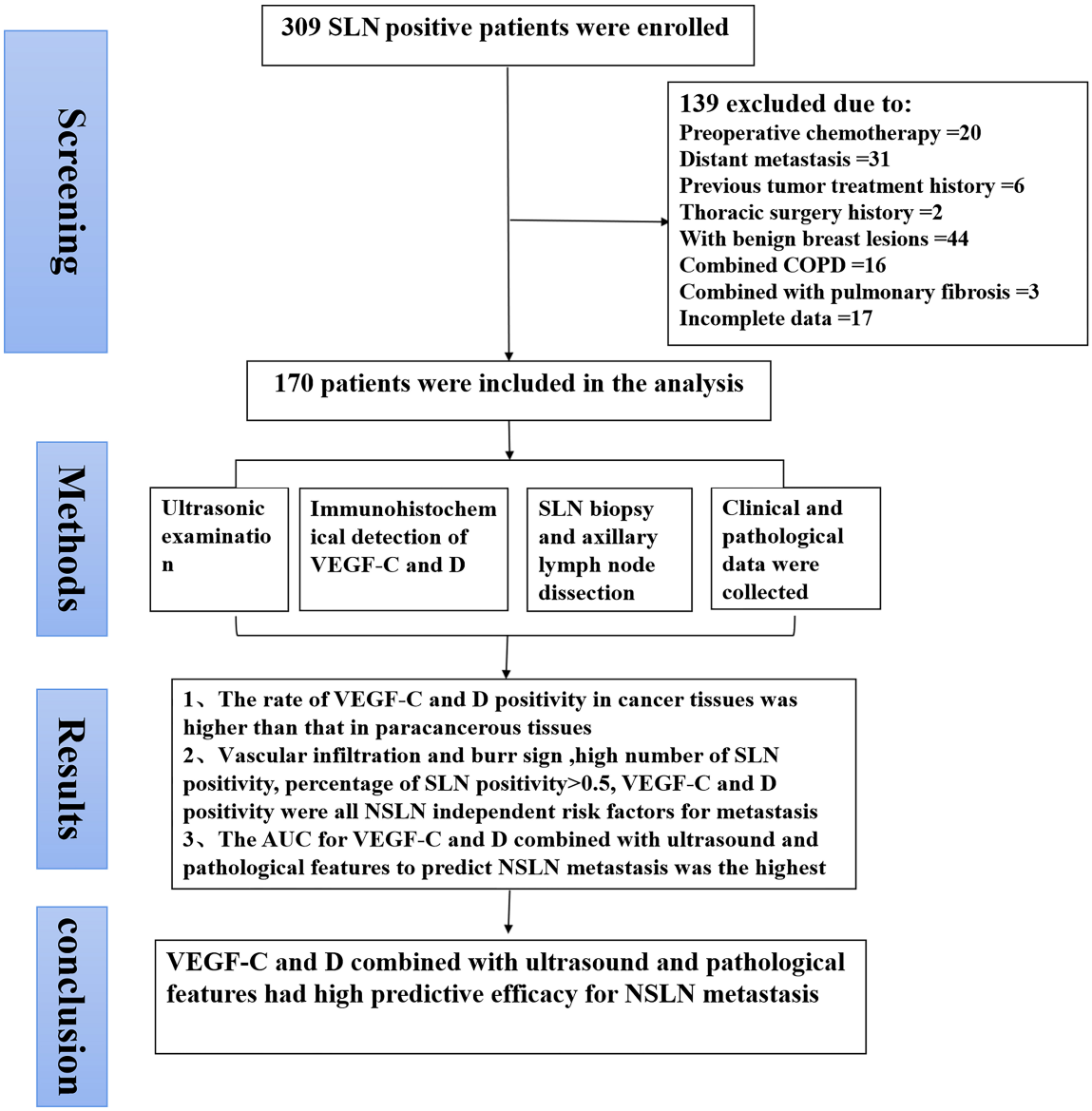

Fig. 1. Flowchart of the study. SLN, sentinel lymph node; COPD, chronic obstructive pulmonary disease; VEGF, vascular endothelial growth factor; NSLN,nonsentinel lymph node; AUC, area under the receiver operating characteristic curve.

firmed patients admitted to Nanchang Third Hospital from February 2014 to January 2018. All were female, aged 26–67 years, with a mean age of 48.75 ± 9.21 years. Flowchart of the study (Fig. 1).

2.2 Inclusion Criteria

(1) Pathological examination confirmed the diagnosis of invasive breast cancer; (2) Female, aged >18 years, with unilateral onset; (3) Preoperative pathological examination confirmed the diagnosis of early invasive breast cancer; (4) Tumour diameter <5 cm; (5) No signs of metastasis were found on SLN imaging; (6) SLN biopsy was positive; (7) None had received radiotherapy before surgery; (8) Received ultrasound examination with clear images within 1–2 weeks before surgery; (9) Complete clinical information.

2.3 Exclusion Criteria

(1) Distant metastasis of breast cancer lesions; (2) other malignant tumours or history of previous tumour treatment; (3) history of previous chest surgery; (4) combined with mastitis or other benign breast lesions; (5) severe liver and kidney dysfunction, haematological system, autoimmune system diseases; and (6) chronic obstructive pulmonary disease, pulmonary fibrosis and other diseases that may affect the abnormal expression of VEGF.

2.4 Ultrasound Examination

GE Voluson E9 (General Electric, Fairfield, CT, USA), Super Sonic Imagine Aixplorer (SuperSonic Imagine, aix en Provence, France) diagnostic ultrasound instrument with a probe frequency of 4 to 15 MHz was used for the examination. The patient's hands were both above the

Table 1. The positive rates of VEGF-C and D in different tissues (n/%).

Group	n	VEGF-C	VEGF-D
Cancer tissues	170	133 (78.24%)	105 (61.76%)
Adjacent tissues	170	62 (36.47%)	32 (18.82%)
χ^2		59.028	65.149
p		<0.001	<0.001

VEGF, vascular endothelial growth factor.

head, and the breast and axillary area were scanned longitudinally, transversely and radially. Senior sonographers analysed nodal features based on Breast Imaging (BI) Reporting and Data System (RADS) [9], including size (maximum tumour diameter), margins (burr sign, smooth, faint, *etc*.), presence or absence of calcification and type of calcification (intranodal, extranodal, intraductal), and location (external superior, external inferior, internal superior, internal inferior, areolar area), using the Adler semiquantitative method for blood flow grading (0, I, II, III).

2.5 Immunohistochemical Detection

After intraoperative sampling of cancerous and paracancerous tissue for paraffin sectioning, VEGF-C and D were detected by the immunohistochemistry streptavidin-perosidase (S-P) method, and the required antibodies were purchased from Abcam (item number: ab106512, ab137368, Cambridge, UK). The sections were operated according to the product instructions, and known positive sections and phosphate buffered saline (PBS) were used as positive and negative controls instead of primary antibodies, respectively. The cell pulp was brown, and tan staining was used as positive cells. Cell pulp staining was made according to the 400 $\times$ field of view. A positive cell count $>$10% of the total number of cells in the field of view was defined as positive, and $\leq$10% was defined as negative. Positive rate (%) = number of positive cases/total number of cases $\times$ 100%.

2.6 SLN Biopsy and Axillary Lymph Node Dissection

Patients were generally anaesthetized and detected by the metabotropic dye tracer method, with 2 mL of metabotropic dye injected into the edge of the areola above the outer breast and fully massaged for approximately 10 minutes. A 3–4 cm axillary incision was made, and all blue-stained lymph nodes were removed. Axillary lymph node dissection was conducted for SLN-positive patients by rapid frozen section and postoperative pathology, including regional lymph nodes I and II [10]. The presence or absence of metastases in NSLN metastasis was clarified on the basis of postoperative pathological findings.

2.7 Clinical and Pathological Data Collection

Patient records were collected, and patients' age ($\leq$60 years/$>$60 years), clinical stage (T1/T2), pathological type (International Classification of Diseases (ICD) classification /other typologies), histological grading (1/2/3), molecular typing (Luminal A/B1/B2/human epidermal growth factor receptor (HER)-2+/Triple-negative), vascular infiltration (Yes/No), SLN number ($\leq$2/$>$2), SLN positive number ($\leq$2/$>$2), SLN positive percentage ($\leq$0.5/$>$0.5), NSLN (metastatic/nometastatic).

2.8 Data Analysis

SPSS 26.0 (Version 26.0, International Business Machines Corporation, Armonk, NY, USA) was used to analyse the data, and the count data were expressed as a number or %, the χ^2 test was used for comparison between groups, and the nonparametric test was used to analyse the rank count data. Analysis of factors affecting NSLN metastasis was conducted using binary logistic regression analysis. The predictive efficacy of NSLN metastasis was assessed using subject operating characteristic (ROC) curves. VEGF-C and D alone was diagnosed by fitting the ROC curve using software, reading the maximum point of the Jorden index and calculate the corresponding cutoff value, sensitivity, specificity and area under the ROC curve (AUC). Combined application was to fit VEGF-C and D to ultrasound features (Burr) or pathological features (SLN positive number, SLN positive proportion $>$0.5) in LogP mode and then perform ROC analysis. $p < 0.05$ was considered statistically significant.

3. Results

3.1 VEGF-C and D were Positively Expressed in Cancer Tissues and Adjacent Tissues

The positive rates of VEGF-C and D in breast cancer tissues were higher than those in adjacent tissues ($p < 0.05$) (Table 1).

3.2 Relationship Between VEGF-C, D and Clinicopathologic and Ultrasound Features

The rates of VEGF-C and D positivity in cancer tissues of SLN-positive patients with vascular infiltration, number of SLN positives $>$2, percentage of SLN positives $>$0.5, burr sign on ultrasound, and NSLN metastasis were higher than those of patients without vascular infiltration, number of SLN positives $\leq$2, percentage of SLN positives $\leq$0.5, no burr sign, and no NSLN metastasis, respectively ($p < 0.05$) (Tables 2,3).

3.3 NSLN Metastasis in Patients with Different Expression Levels of VEGF-C and D

NSLN metastases occurred in 74 of 170 patients (74/170, 43.53%), with 307 metastases. The NSLN metastasis rates of VEGF-C- and D-positive patients (52.63%, 63.81%) were higher than those of negative patients (10.81%, 10.77%) ($p < 0.05$) (Table 4).

There were 70 NSLN metastases in VEGF-C-positive patients (52.63%), and the number of metastases was

Table 2. Clinicopathological characteristics of different VEGF-C, D expression (n/%).

Clinicopathological features	*n*	VEGF-C positive (*n* = 133)	χ^2	*p*	VEGF-D positive (*n* = 105)	χ^2	*p*
Age (years)			1.582	0.208		0.205	0.651
≤60	152	121 (79.61%)			93 (61.18%)		
>60	18	12 (66.67%)			12 (66.67%)		
Clinical Staging			0.250	0.617		1.229	0.268
T1	72	55 (76.39%)			41 (56.94%)		
T2	98	78 (79.59%)			64 (65.31%)		
Pathology Type			0.014	0.907		0.037	0.846
IDC	148	116 (78.38%)			91 (61.49%)		
Other	22	17 (77.27%)			14 (63.64%)		
Histology grade			0.873	0.646		0.119	0.942
1	3	3 (100.00%)			2 (66.67%)		
2	102	79 (77.45%)			62 (60.78%)		
3	65	51 (21.54%)			41 (63.08%)		
Vascular infiltration			10.485	0.001		38.225	<0.001
No	114	81 (71.05%)			52 (45.61%)		
Yes	56	52 (92.86%)			53 (94.64%)		
SLN number			0.631	0.427		0.239	0.625
≤2	36	27 (72.97%)			24 (64.86%)		
>2	134	106 (79.10%)			81 (60.45%)		
SLN positive number			10.134	0.001		22.551	<0.001
≤2	140	103 (73.57%)			75 (53.57%)		
>2	30	30 (100.00%)			30 (100.00%)		
SLN positive percentage			5.201	0.023		23.298	<0.001
≤0.5	111	81 (72.97%)			54 (48.65%)		
>0.5	59	52 (88.14%)			51 (86.44%)		
Molecular type			1.558	0.816		1.437	0.838
Luminal A	30	22 (73.33%)			18 (60.00%)		
Luminal B1	84	69 (82.14%)			55 (65.48%)		
Luminal B2	19	14 (73.68%)			11 (57.89%)		
HER-2+	21	16 (76.19%)			11 (52.38%)		
Triple-negative	16	12 (75.00%)			10 (62.50%)		
NSLN			20.596	<0.001		45.947	<0.001
Metastatic	74	70 (94.59%)			67 (90.54%)		
Nonmetastatic	96	63 (65.63%)			38 (39.58%)		

IDC, International Classification of Diseases; SLN, sentinel lymph node; NSLN, nonsentinel lymph node; VEGF, vascular endothelial growth factor; HER-2, human epidermal growth factor receptor 2.

12.03% (16/133), 15.04% (20/133), 7.52% (10/133), 6.02% (8/133), and 13.53% (18/133) for 1, 2, 3, 4, and ≥5 metastases, respectively. VEGF-C-negative patients had 4 NSLN metastases (10.81%), all with 1 metastasis. There were 67 (63.81%) NSLN metastases in VEGF-D-positive patients, and the number of metastases was 1, 2, 3, 4 and ≥5 in 15.24% (16/105), 16.19% (17/105), 9.52% (10/105), 7.62% (8/105) and 15.24% (16/105), respectively. There were 7 (10.77%) NSLN metastases in VEGF-D-negative patients, and the number of metastases was 1 and 2 in 6.15% (4/65) and 4.62% (3/65) of patients, respectively (Figs. 2,3).

3.4 Binary Logistic Regression Analysis

A total of 170 SLN-positive patients were taken as samples, and whether the patients had NLSN metastasis (metastasis = 1, nonmetastasis = 0) was taken as the dependent variable. The clinicopathological features and ultrasound features of the patients were used as independent variables to establish a binary logistic regression analysis model. The stepwise regression method was adopted, with $\alpha_{\text{elimination}} = 0.10$ and $\alpha_{\text{inclusion}} = 0.05$. The results showed that the presence of vascular infiltration [odds ratio (OR) (95% confidence interval (CI)) = 3.332 (1.150 to 9.654), $p = 0.027$], a high number of positive SLNs [OR (95% CI) = 5.372 (1.892 to 15.247), $p = 0.002$], a percentage of positive SLNs >0.5 [OR (95% CI) = 6.363 (1.317 to 26.874), $p = 0.035$], a burr sign [OR (95% CI) = 3.724 (1.240 to 11.180), $p = 0.019$], VEGF-C positivity [OR (95% CI) = 5.464 (1.239 to 24.091), $p = 0.025$], and VEGF-D positivity [OR (95% CI) = 4.604 (1.356 to 15.625), $p = 0.014$] were all independent risk factors for NSLN metastasis (Table 5 and Fig. 4).

Table 3. Relationship between VEGF-C, D and ultrasonic characteristics (n/%).

Ultrasonic characteristics	n	VEGF-C positive (n = 133)	χ^2	p	VEGF-D positive (n = 105)	χ^2	p
Tumour size			0.019	0.889		0.327	0.568
<2 cm	66	52 (78.79%)			39 (59.09%)		
≥2 cm	104	81 (77.88%)			66 (63.46%)		
Burr			6.690	0.010		4.857	0.028
No	97	69 (71.13%)			53 (54.64%)		
Yes	73	64 (87.67%)			52 (71.23%)		
Calcifications			1.272	0.259		0.334	0.564
No	78	58 (74.36%)			50 (64.10%)		
Yes	92	75 (81.52%)			55 (59.78%)		
Blood flow signal grade			4.930	0.177		4.014	0.260
0	11	8 (72.73%)			4 (36.36%)		
I	48	41 (85.42%)			33 (68.75%)		
II	83	66 (79.52%)			51 (61.45%)		
III	28	18 (64.29%)			17 (60.71%)		
Location			0.699	0.951		3.529	0.273
Above outside	59	46 (77.97%)			32 (54.24%)		
Below outside	17	14 (82.35%)			12 (70.59%)		
Inside and above	10	8 (80.00%)			7 (70.00%)		
Inside and below	13	11 (84.62%)			10 (76.92%)		
Areola region	71	54 (76.06%)			44 (61.97%)		

VEGF, vascular endothelial growth factor.

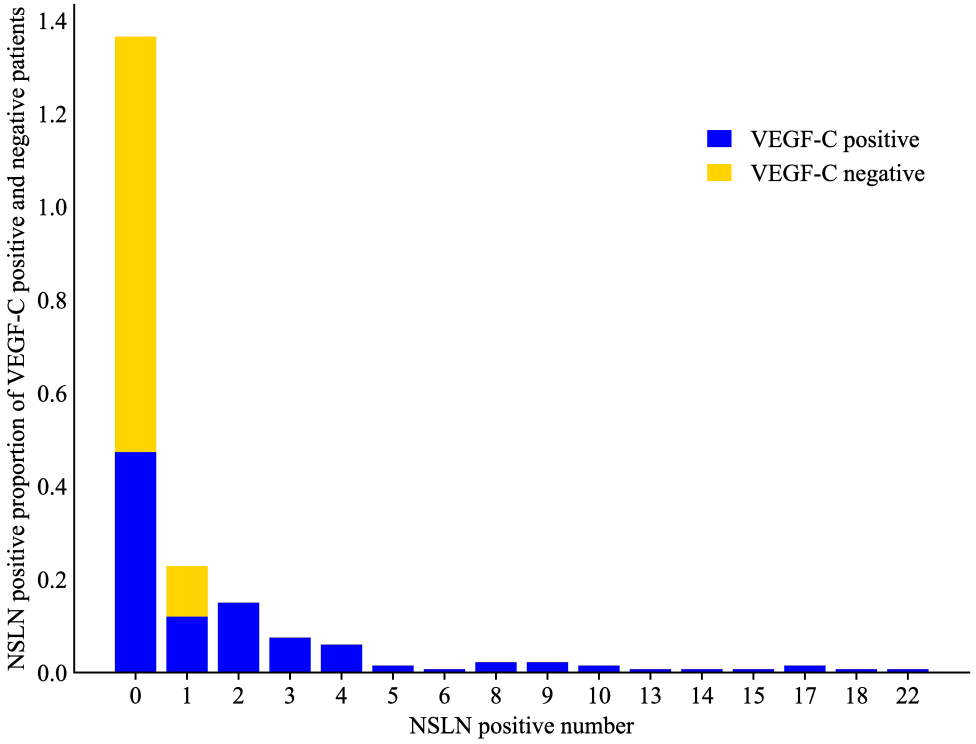

Fig. 2. Proportion of positive NSLN in VEGF-C-positive and -negative patients. VEGF, vascular endothelial growth factor; NSLN, nonsentinel lymph node.

3.5 Predictive Value of VEGF-C, D Combined with Ultrasound and Pathological Features for NSLN Metastasis

ROC diagnostic models were developed with NSLN metastasis as positive and NSLN nonmetastasis as negative. ROC analysis showed that the AUC (95% CI) of VEGF-C and VEGF-D applied alone to predict NSLN metastasis was 0.645 (0.568–0.717) and 0.755 (0.683–0.817), respectively, which was lower than the predictive efficacy of Model 2 (VEGF-C + VEGF-D + Burr) ($Z = 6.005$, $p < 0.001$; $Z = 3.386$, $p = 0.001$) and lower than the predictive efficacy of Model 3 (VEGF-C + VEGF-D + Vascular infiltration +

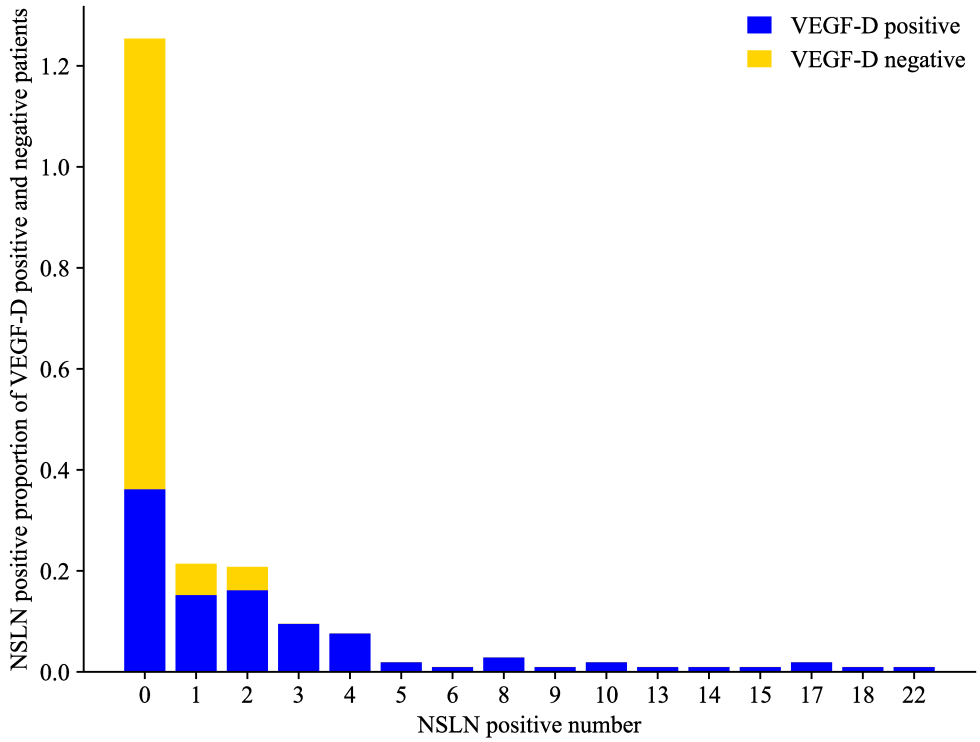

Fig. 3. Proportion of NSLN-positive patients among VEGF-D-positive and -negative patients. VEGF, vascular endothelial growth factor; NSLN, nonsentinel lymph node.

Table 4. Comparison of NSLN metastasis in different expression levels of VEGF-C and D (n/%).

Group		*n*	NSLN Nonmetastatic	NSLN Metastatic	χ^2	*p*
VEGF-C	Positive	133	63 (47.37%)	70 (52.63%)	20.596	<0.001
	Negative	37	33 (89.19%)	4 (10.81%)		
VEGF-D	Positive	105	38 (36.19%)	67 (63.81%)	45.947	<0.001
	Negative	65	58 (89.23%)	7 (10.77%)		

VEGF, vascular endothelial growth factor; NSLN, nonsentinel lymph node.

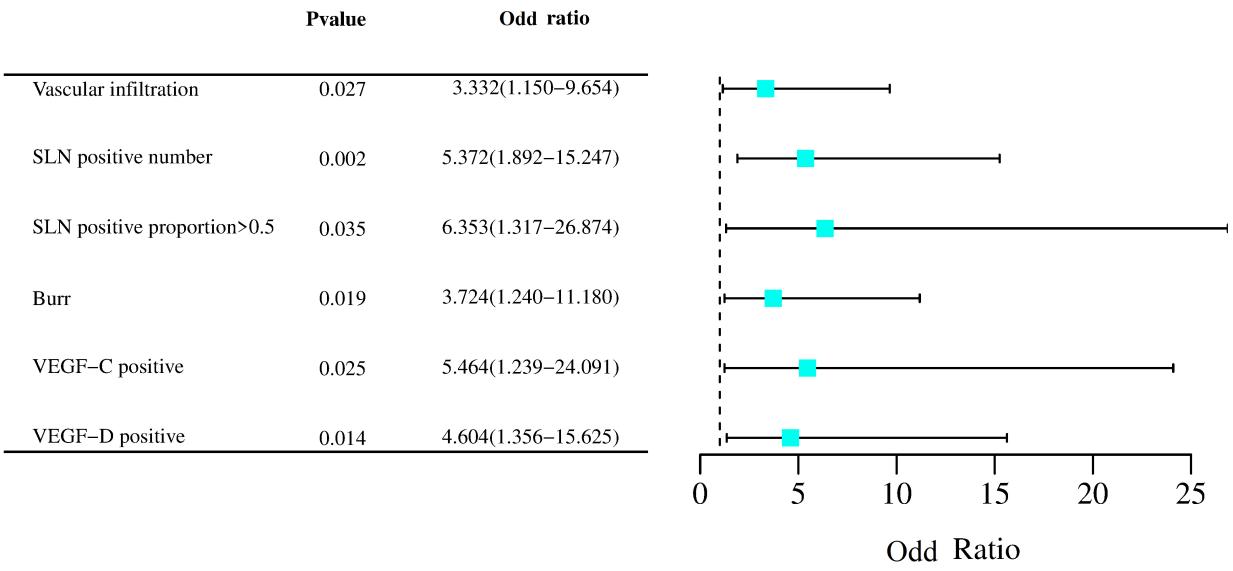

Fig. 4. Forest plot of risk factors for NSLN metastasis. SLN, sentinel lymph nodes; VEGF, vascular endothelial growth factor.

Table 5. Results of binary logistic regression analysis.

Factors	Assignment	β	*SE*	Wald χ^2	*p*	OR	95% CI
Vascular infiltration	Yes = 1, No = 0	1.203	0.543	4.915	0.027	3.332	1.150~9.654
SLN positive number	Continuous input	1.681	0.532	9.975	0.002	5.372	1.892~15.247
SLN positive percentage >0.5	Yes = 1, No = 0	1.849	0.876	4.456	0.035	6.353	1.317~26.874
Burr	Yes = 1, No = 0	1.315	0.561	5.495	0.019	3.724	1.240~11.180
VEGF-C positive	Yes = 1, No = 0	1.698	0.757	5.032	0.025	5.464	1.239~24.091
VEGF-D positive	Yes = 1, No = 0	1.527	0.624	5.997	0.014	4.604	1.356~15.625

SLN, sentinel lymph nodes; VEGF, vascular endothelial growth factor; OR, odds ratio; CI, confidence interval; SE, standard error.

Table 6. Predictive value of VEGF-C and D combined with ultrasound and pathological features for NSLN metastasis.

Indicators	Cut-off	Sensitivity% (n/N)	Specificity% (n/N)	Youden	AUC (95% CI)
Model 1	>0.516*	81.08% (60/74)	78.12% (75/96)	0.5921	0.859 (0.796~0.907)
Model 2	>0.566*	86.49% (64/74)	65.62% (63/96)	0.5211	0.822 (0.756~0.877)
Model 3	>0.235*	90.54% (67/74)	62.50% (60/96)	0.5304	0.823 (0.757~0.877)
VEGF-C	Positive	94.59% (70/74)	34.38% (33/96)	0.2897	0.645 (0.568~0.717)
VEGF-D	Positive	90.54% (67/74)	60.42% (58/96)	0.5096	0.755 (0.683~0.817)

Note: The joint application was fitted with the LogP model. Model 1: VEGF-C, VEGF-D, vascular infiltration, SLN positive number, SLN positive proportion >0.5, Burr for overall LogP model fitting diagnosis. Model 2: VEGF-C and VEGF-D combined with ultrasound features (Burr) for LogP model fitting diagnosis. Model 3: VEGF-C and VEGF-D combined with pathological features (SLN positive number, SLN positive proportion >0.5) for LogP model fitting diagnosis. *Cut-off values of joint Models 1, 2 and 3 are dummy indicators calculated based on this Log(P/1-P) model and have no practical significance. VEGF, vascular endothelial growth factor; CI, confidence interval; AUC, area under the ROC curve.

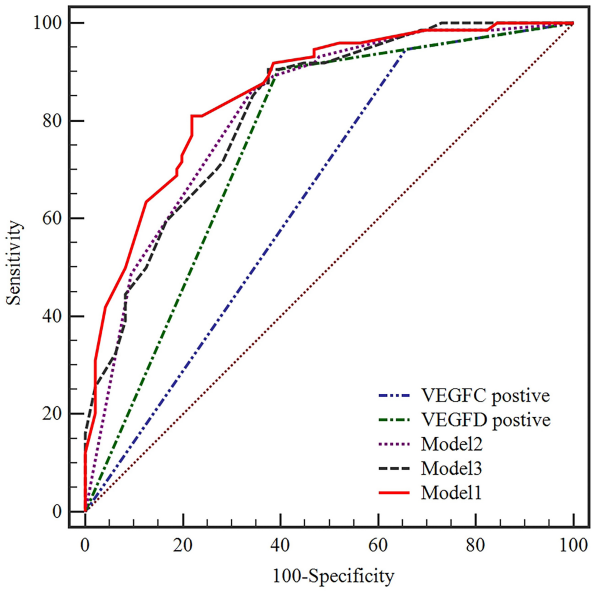

Fig. 5. ROC curve of the combination of VEGF-C and D ultrasound and pathological features to predict NSLN metastasis. ROC, receiver operating characteristic; VEGF, vascular endothelial growth factor; SLN, sentinel lymph nodes.

SLN positive number + SLN positive proportion >0.5) (Z = 5.850, $p < 0.001$; Z = 3.359, $p = 0.001$). The AUC of Model 1 (overall association) was 0.859 (0.796–0.907), which was higher than the predictive efficacy of Model 2 versus Model 3 (Z = 2.210, $p = 0.027$; Z = 2.060, $p = 0.039$) (Table 6 and Fig. 5).

4. Discussion

The VEGF-C- and D-positive rates in breast cancer tissues were 78.24% and 61.76%, respectively, which were higher than the positive rates in paraneoplastic tissues (36.47% and 18.82%), indicating that VEGF-C and D in breast cancer lesions were abnormally highly expressed, while they were expressed at low levels in paraneoplastic tissues. The VEGF-C- and D-positive rates showed differences in the presence or absence of vascular infiltration, the number of positive SLNs >2 or not, and the percentage of positive SLNs >0.5, suggesting that the positive expression of both proteins was related to the above pathological features, which may be related to the proangiogenic function of the VEGF family [11]. Recent studies have found that VEGF-C and VEGF-D can induce lymphangiogenesis, and both can activate signalling pathways related to endothelial cell migration and tubulogenesis by binding to VEGF receptor 3 (VEGFR-3) in the endothelium [12,13]. Lymphatic vessels are important conduits for distant metastasis of tumour cells, and VEGF-C and VEGF-D facilitate lymphatic vessel expansion.

In this study, the logistic analysis revealed that the risk of NSLN metastasis increased 2.332-fold in those with vas-

cular infiltration compared to those without vascular infiltration, and the risk of NSLN metastasis increased 4.372-fold for each increase in the number of positive SLNs. SLN positive percentage >0.5 increased 5.353-fold compared to ≤0.5, suggesting a strong association between vascular infiltration, the number of SNL positivity >2 and its proportion >0.5 and NSLN metastases. Independent risk factors for metastasis, consistent with the findings of several previous studies [14–16]. SLN is the first drainage area of primary tumour metastasis. Toberer *et al*. [17] found that VEGF and its receptors 2 and 3 were expressed more intensely in SLN-positive cutaneous melanoma patients than in SLN-negative patients, and VEGFR-3 was confirmed to be associated with SNL status. The burr sign is a typical imaging signs of malignant lesions. Previous study has shown that the marginal burr sign is more likely to be found in triple-negative breast cancer patients and can predict prognostic regression [18]. This study revealed that NSLN metastasis risk was increased 2.724-fold in patients with burr signs compared to those without, suggesting that burr signs increase the risk of NSLN metastasis.

In this study, the NSLN metastasis rate in VEGF-C- and D-positive patients was higher than that in VEGF-negative patients, and logistic analysis showed that VEGF-C- and D-positive patients increased 4.464- and 3.604-fold, respectively, compared with negative patients, indicating that both positive proteins were risk factors for NSLN metastasis. Li *et al*. [19] concluded that VEGF-C and its receptor 3 can promote lymph node metastasis in renal cell carcinoma. One study noted that VEGF-C and D transcript and protein expression levels were increased in different grades of endometrial cancer [20], suggesting that high expression of both increases the metastatic intensity of endometrial cancer. This result suggested that the possible mechanism by which VEGF-C and D promote lymphatic metastasis is that VEGF-C and D can bind to VEGFR-3 in lymphatic vessel endothelial cells and activate multiple signalling pathways, such as phosphatidylinositol 3-kinase regulatory subunit 1 (PIK3R1) [21] and wingless-type mouse mammary tumor virus (MMTV) integration site family (WNT5A) [22], to promote lymphatic vessel expansion, thus increasing lymph node metastasis risk. In addition, this study showed by ROC analysis that the AUC (95% CI) of VEGF-C and D combined with ultrasound and pathological features to predict NSLN metastasis was 0.859 (0.796–0.907), which was higher than the predictive efficacy of VEGF-C and D alone and their combined ultrasound or pathological features, respectively, indicating that these two proteins alone and in combination with ultrasound and pathological features help to improve the early identification of NSLN metastasis and reduce the risk of unnecessary surgery in people with a low risk of NSLN metastasis.

The analysis of this study as a single-centre retrospective study still has some limitations, the sample size that can be included in this time is limited, and the measurements of VEGF-C and VEGF-D have some differences between different testing institutes, which still needs to be improved in the future work, and can be explored by multicentre prospective large-sample data analysis implementation.

5. Conclusions

The ultrasound and pathological features of SLN-positive breast cancer, such as vascular infiltration, a high number of positive SLNs, an SLN positivity ratio >0.5, burr sign, and VEGF-C and D positivity, are independent risk factors for NSLN metastasis, and VEGF-C and D alone and in combination with the above ultrasound and pathological features have high predictive efficacy for NSLN metastasis. This study can be used as a reference for whether SLN-positive patients should receive ALND. It's unclear whether the study's findings are applicable to other types of cancer or patient groups. Further research may be needed to generalize the findings.

Availability of Data and Materials

The simulation experiment data used to support the findings of this study are available from the corresponding author upon request.

Author Contributions

All the authors have contributed to the document retrieval, conception and design of this study. Material preparation, data collection and analysis, and patient follow-up were conducted by JC, QL, WL, XT, ZW and LX. The first draft of the manuscript was written by JC and QL, and all authors commented on the first few versions of the manuscript. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work to take public responsibility for appropriate portions of the content and agreed to be accountable for all aspects of the work in ensuring that questions related to its accuracy or integrity.

Ethics Approval and Consent to Participate

All subjects gave their informed consent for inclusion before they participated in the study. The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Ethics Committee of The Third Hospital of Nanchang (approval number: 201526).

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Conflict of Interest

The authors declare no conflict of interest.

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