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Research Article

TO EVALUATE THE VASCULARITY OF LYMPHNODE BY DOPPLER USG FLOWMETRY AND HISTOLOGICAL VASCULAR QUANTITATION IN BREAST CANCER

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Abstract: ***Introduction:*** Axillary lymph-node involvement is a major determinant of staging, prognosis and treatment planning in breast carcinoma. Tumour-induced angiogenesis alters intranodal vascular architecture and haemodynamics, which may be assessed non-invasively by Doppler ultrasonography and quantified microscopically through histological microvessel density. **Objectives:** To evaluate axillary lymph-node vascularity using colour Doppler flowmetry and histological vascular quantitation, assess their correlation, determine associations with established prognostic factors and examine vascular changes following neoadjuvant chemotherapy. **Materials and Methods:** This prospective observational cohort study included 30 women with histopathologically confirmed invasive breast carcinoma. Preoperative colour duplex Doppler assessment recorded vascular pattern, resistive index, pulsatility index and systolic/diastolic ratio. Excised axillary nodes underwent immunohistochemical vascular quantitation using microvessel density. Serial Doppler measurements were compared before and after neoadjuvant chemotherapy. Data were analysed using SPSS version 26, with $p<0.05$ considered significant. **Results:** Mixed vascularity was most frequent (43.3%), followed by peripheral (30.0%) and hilar patterns (26.7%). Mean RI, PI and S/D ratio were 0.75±0.10, 1.57±0.37 and 5.12±2.19, respectively. Mean MVD was 49.97±14.12 vessels/HPF. Post-NACT reductions in RI, PI and S/D ratio were significant (all $p<0.001$). Higher RI and PI were associated with tumour size >4 cm ($p=0.03$) and nodal positivity ($p=0.01$). MVD increased with tumour grade ($p<0.001$) and was higher in node-positive cases ($p=0.002$). RI, PI and S/D ratio correlated positively with MVD ($r=0.62$, 0.58 and 0.49, respectively).**Conclusion:** Colour Doppler indices reflected histological angiogenic activity and adverse prognostic characteristics in axillary lymph nodes. Serial Doppler evaluation may also provide a useful non-invasive method for monitoring vascular response to neoadjuvant chemotherapy.

Keywords: Breast carcinoma; axillary lymph node; colour Doppler; angiogenesis; microvessel density; neoadjuvant chemotherapy.

INTRODUCTION

Breast cancer is a major malignancy among women, and hypoxia-driven angiogenesis supports tumour growth, invasion and metastatic dissemination.[1] Axillary lymph nodes form the principal regional staging basin in breast carcinoma. Conventional ultrasonography assesses nodal morphology, while power Doppler and strain elastography add information on vascularity and stiffness, improving differentiation of benign and malignant superficial nodes.[2] Superb microvascular imaging detects more low-flow signals than conventional Doppler and has shown greater diagnostic performance in cervical lymph-node assessment.[3] Colour-Doppler vascular patterns have likewise demonstrated useful sensitivity, specificity and accuracy for distinguishing metastatic from benign cervical nodes against pathological diagnosis.[4]

The relationship between imaging and microscopic angiogenesis is supported by research in papillary thyroid carcinoma, where contrast-enhanced ultrasound features were evaluated with microvessel density and microvessel area in relation to nodal metastasis.[5] In a metastatic mouse lymph-node model, high-frequency contrast-enhanced ultrasound demonstrated microcirculatory focal defects corresponding to disrupted architecture and cellular heterogeneity on histology.[6] In breast cancer, multiparametric axillary ultrasound showed that mixed or capsular-perforating vascular patterns were more frequent in metastatic nodes and that combined assessment outperformed isolated parameters.[7] Contrast-free quantitative microvascular imaging has also shown potential for classifying axillary metastasis through morphometric analysis of nodal microvessels.[8] Doppler assessment of breast masses further indicates that vascular characteristics add diagnostic information to B-mode findings.[9]

In India, breast cancer has been reported to constitute approximately 30% of cancers and 20% of cancer-related deaths among women, with age-adjusted incidence and mortality rates of 25.8 and 12.7 per 100,000, respectively. Indian histopathological evidence also demonstrates significant associations of CD34-based microvessel density with tumour grade, vascular invasion, nodal positivity and Nottingham Prognostic Index.[10] Quantitative microvascular ultrasound may detect angiogenic changes during neoadjuvant chemotherapy and assist early response assessment.[11] However, direct correlation of axillary nodal Doppler flowmetry with histological vascular quantitation, including treatment-related change, remains insufficiently studied in Indian tertiary-care populations. This study at Subharti Medical College, Meerut, was therefore designed to address this clinically relevant gap.

MATERIALS AND METHODS

Study Design: Prospective observational cohort study evaluating axillary lymph-node vascularity by colour Doppler ultrasonography and correlating it with histological vascular quantitation in invasive breast carcinoma.

Study Setting: Departments of Surgery, Radiology and Pathology, Subharti Medical College, Meerut.

Study Population: The study included adult female patients with histopathologically confirmed invasive carcinoma of the breast who presented to the Department of Surgery and fulfilled the predefined eligibility criteria. Eligible participants were enrolled consecutively during the study period.

Sample Size: A total of 30 patients were included. Consecutive sampling was used, whereby all eligible patients presenting during the study period were recruited until the required sample size was achieved.

Inclusion Criteria: Female patients with histopathologically confirmed invasive breast carcinoma who provided written informed consent.

Exclusion Criteria: Patients with metastatic breast carcinoma, those unwilling to participate, or those who did not provide signed consent.

Data Collection and Investigations: Age, menopausal status, tumour size, tumour location and clinical axillary nodal status were recorded. Preoperative colour duplex Doppler ultrasonography of the breast lesion and axillary lymph nodes was performed. Doppler parameters included peak systolic velocity, mean diastolic frequency and minimum diastolic frequency. Patients underwent sentinel lymph-node biopsy or axillary lymph-node dissection as clinically indicated. Excised lymph nodes were stained with endothelial markers such as CD31, CD34 or factor VIII. Microvessel density and percentage vessel area were calculated in vascular hotspots under high-power microscopy.

Ethical Consideration: The study was conducted after obtaining approval from the Institutional Ethics Committee of Subharti Medical College. Written informed consent was obtained from every participant before enrolment. Patient confidentiality was maintained throughout data collection, analysis and reporting. The Institutional Ethics Committee approval number and date should be inserted in the final manuscript.

Statistical Analysis: Data were analysed using SPSS version 26. Continuous variables were expressed as mean ± standard deviation and categorical variables as frequencies and percentages. Normality was assessed using the Kolmogorov–Smirnov test. Pearson's correlation coefficient evaluated the relationship between Doppler and histological vascular parameters. A p-value <0.05 was considered statistically significant.

RESULTS

Table 1: Baseline (Pre-NACT) Doppler USG Colour Flowmetry Parameters of Axillary Lymph Nodes

Doppler Parameter	Result
Vascular Pattern	
Hilar	8 (26.7%)
Peripheral	9 (30%)
Mixed	13 (43.3%)
Resistive Index (RI)	0.75 (SD = 0.10)
Pulsatility Index (PI)	1.57 (SD = 0.37)
S/D Ratio	5.12 (SD = 2.19)

Table 1 demonstrates that mixed vascularity was the predominant pre-NACT Doppler pattern, observed in 13 patients (43.3%), followed by peripheral and hilar patterns in 30.0% and 26.7%, respectively. Mean RI, PI and S/D ratio were 0.75 ± 0.10, 1.57 ± 0.37 and 5.12 ± 2.19.

Table 2: Histological Vascular Quantitation of Axillary Lymph Nodes

Parameter	Result
Micro-vessel Density (MVD)	49.97 (SD = 14.12) vessels/HPF
Vascular Grade	
Low (<40 vessels/HPF)	8 (26.7%)
Moderate (40–60 vessels/HPF)	13 (43.3%)
High (>60 vessels/HPF)	9 (30%)

Table 2 shows a mean microvessel density of 49.97 ± 14.12 vessels/HPF. Moderate vascularity was most frequent, observed in 13 patients (43.3%), followed by high vascularity in 9 (30.0%) and low vascularity in 8 patients (26.7%), indicating substantial histological vascular proliferation.

Table 3: Effect of Neo-Adjuvant Chemotherapy on Doppler Parameters

Parameter	Pre-NACT Mean (SD)	Post-NACT Mean (SD)	p value
RI	0.75 (0.10)	0.68 (0.09)	<0.001
PI	1.57 (0.37)	1.36 (0.31)	<0.001
S/D Ratio	5.12 (2.19)	4.17 (1.70)	<0.001

Table 3 demonstrates significant reductions in all Doppler indices following neoadjuvant chemotherapy. Mean RI declined from 0.75 ± 0.10 to 0.68 ± 0.09, PI from 1.57 ± 0.37 to 1.36 ± 0.31, and S/D ratio from 5.12 ± 2.19 to 4.17 ± 1.70 (all p < 0.001).

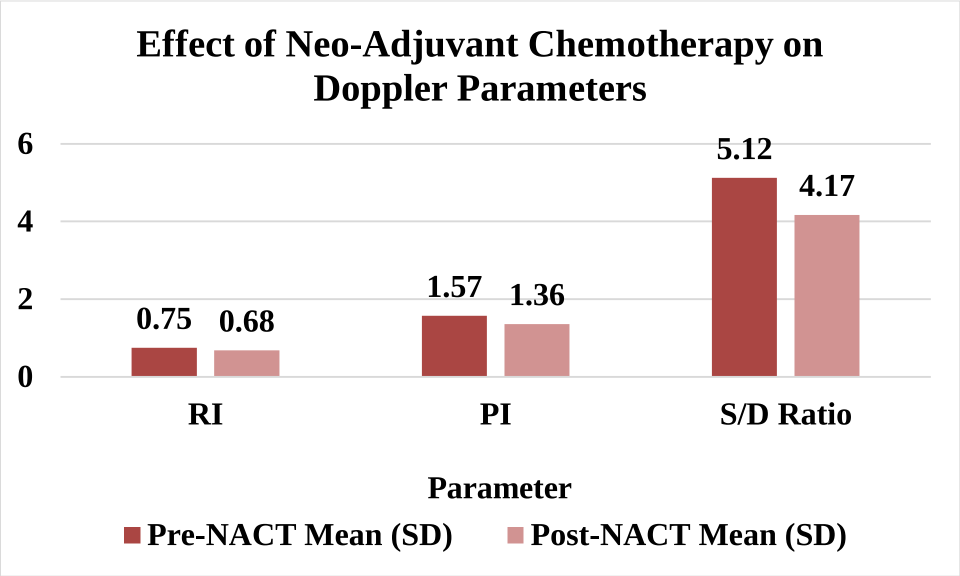

Figure1: Bar Chart Showing Distribution of Histological Vascular Grades

Table 4: Association Between Pre-NACT Doppler Parameters and Prognostic Factors

Prognostic Factor	Mean RI	Mean PI	p value
Tumour size >4 cm	0.79	1.68	0.03
Tumour size ≤4 cm	0.71	1.49	
Node positive	0.78	1.65	0.01
Node negative	0.69	1.41	

Table 4 shows that tumours measuring >4 cm had higher mean RI and PI than tumours ≤4 cm (0.79 vs 0.71 and 1.68 vs 1.49; p = 0.03). Node-positive patients also demonstrated higher indices than node-negative patients (RI: 0.78 vs 0.69; PI: 1.65 vs 1.41; p = 0.01).

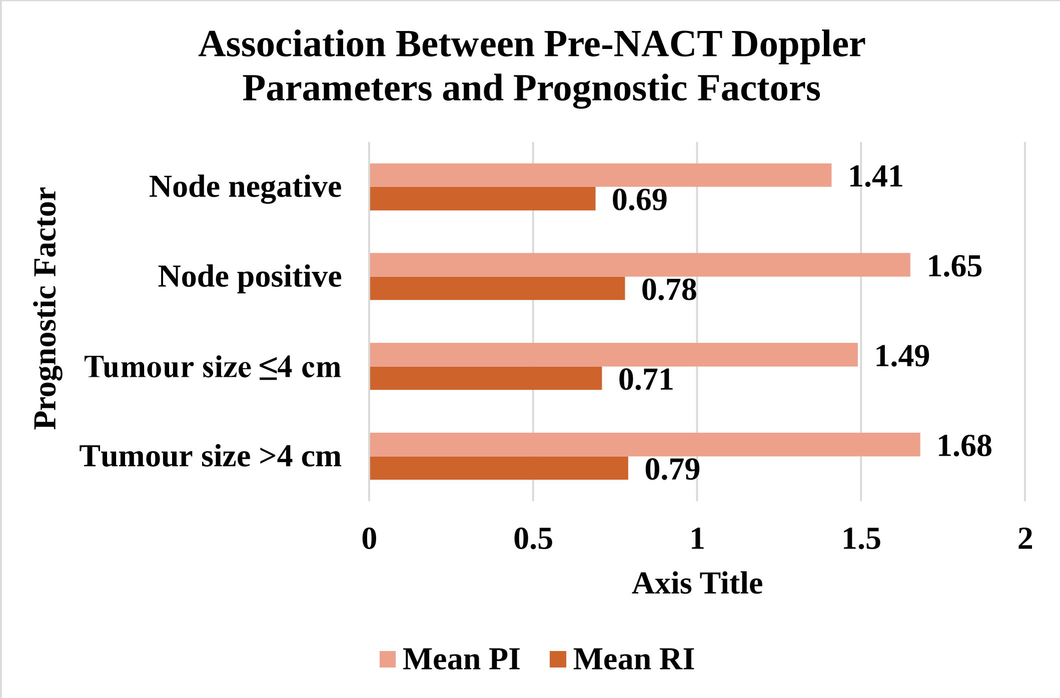

Figure 2: Bar Chart Showing Mean RI/PI Across Different Prognostic Categories

Table 5: Association Between Histological Vascular Quantitation and Prognostic Parameters

Parameter	Mean MVD (SD)	p value
Tumour Grade I	38 (8)	
Tumour Grade II	49 (10)	
Tumour Grade III	63 (11)	**<0.001**
Node Positive	57 (12)	
Node Negative	39 (9)	**0.002**

Table 5 demonstrates a progressive increase in mean microvessel density across tumour grades, from 38 ± 8 vessels/HPF in Grade I to 63 ± 11 vessels/HPF in Grade III ($p < 0.001$). Node-positive cases also showed significantly greater MVD than node-negative cases (57 ± 12 vs 39 ± 9 vessels/HPF; $p = 0.002$).

Table 6: Correlation Between Doppler Indices and Histological Micro-vessel Density

Parameter	Correlation Coefficient (r)	p value
RI vs MVD	0.62	<0.001
PI vs MVD	0.58	<0.001
S/D vs MVD	0.49	0.003

Table 6 demonstrates significant positive correlations between Doppler indices and histological microvessel density. RI showed the strongest association with MVD ($r = 0.62$, $p < 0.001$), followed by PI ($r = 0.58$, $p < 0.001$) and S/D ratio ($r = 0.49$, $p = 0.003$).

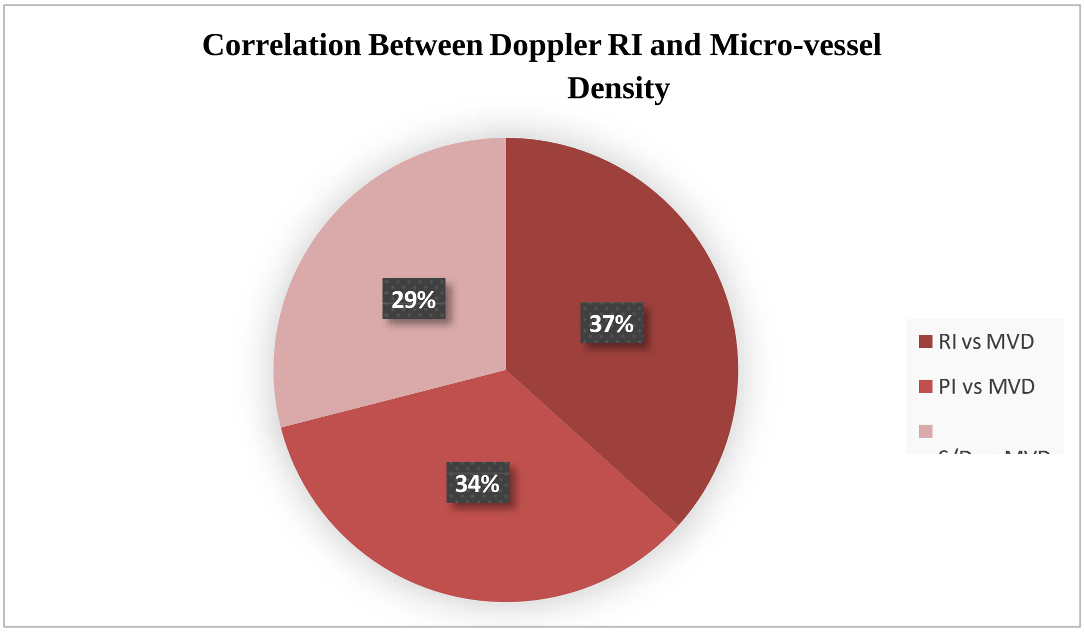

Figure 3: Scatter Plot with Regression Line Showing Correlation Between Doppler RI and Micro-vessel Density

Discussion

Mixed vascularity was the predominant pre-NACT pattern, occurring in 43.3%, followed by peripheral vascularity in 30.0% and hilar flow in 26.7%; mean RI, PI and S/D ratio were 0.75 ± 0.10, 1.57 ± 0.37 and 5.12 ± 2.19. Abdelgawad et al. (2020)[4] similarly found colour-Doppler vascular patterns useful for metastatic nodal differentiation, with 91.7% sensitivity, 80.8% specificity and 88.6% accuracy. Dobruch-Sobczak et al. (2024)[7] Combining vascular and structural assessment improved axillary-node discrimination (AUC= 0.823). Ferroni et al, (2023) [8] AUC = 0.90, sensitivity = 90% and specificity = 88% Conversely, a standard evaluation of breast masses (Mehdikhani et al., 2024)[9] rather than axillary nodes was reported with higher RI and PI in malignant lesions.

Mean histological microvessel density was 49.97 ± 14.12 vessels/HPF, with moderate vascularity in 43.3%, high vascularity in 30.0% and low vascularity in 26.7%. Similarly Goyal et al (2023)[10] reported significant correlation between increased CD34 derived microvessel density and increased tumour grade, vascular invasion and lymph-node positivity but their mean was lower at 24.16 ± 6.77 vessels/HPF. Liu et al (2022)[5] also reported significantly increased microvessel density and microvessel area in node positive compared with node negative patients. 11/15 microvascular imaging biomarkers significantly differed between reactive and metastatic nodes (Ferroni et al., 2023)[8]. Elayat et al. (2024)[1] reported biological evidence of angiogenesis associated with invasion and metastatic dissemination, but they did not report patient-level values for MVD.

Neoadjuvant chemotherapy produced significant reductions in RI from 0.75 ± 0.10 to 0.68 ± 0.09, PI from 1.57 ± 0.37 to 1.36 ± 0.31 and S/D ratio from 5.12 ± 2.19 to 4.17 ± 1.70, with all $p < 0.001$. Sabeti et al. (2025)[11] reported treatment-related vascular changes in 32 responders (60%) of 53 patients with significant changes in vessel density ($p = 0.023$) and vessel diameter and fractal dimension each with p = 0.002.3 Yang et al (2024)[3] have proposed that low flow can be detected with microvascular imaging. Maeda et al. (2025) demonstrated the heterogeneity of the microcirculation of metastatic nodes.[9] Mehdikhani et al (2024)[6] reported raised RI and PI in malignant lesions. However, RI, PI and S/D ratio were not reported by Sabeti et al. (2025)11. Tumours >4 cm had a higher mean RI and PI than tumours ≤4 cm (0.79 vs 0.71 and 1.68 vs 1.49; $p = 0.03$). Node-positive cases had higher values than node-negative cases (RI: 0.78 vs 0.69; PI: 1.65 vs 1.41; $p = 0.01$). Similarly, much higher RI and PI in malignant lesions were demonstrated by Mehdikhani et al. (2024)[9] with AUC of 0.863 and 0.882 respectively. Node positive disease was associated with larger tumor size and vascular parameters (Liu et al 2022)5. Ferroni et al (2023)[8] obtained an AUC of 0.90 for the prediction of metastatic nodes and Dobruch-Sobczak et al (2024)[7] obtained an AUC of 0.823 with nodal features included. Microvessel density increased progressively from Grade I at 38 ± 8 vessels/HPF to Grade II at 49 ± 10 and Grade III at 63 ± 11 vessels/HPF ($p < 0.001$). Node-positive cases also had higher MVD than node-negative cases (57 ± 12 vs 39 ± 9 vessels/HPF; $p = 0.002$). Goyal et al. (2023)[10] also reported significant correlation of CD34 based MVD with Bloom–Richardson grade and lymph-node positivity but the overall mean MVD was on the lower side (24.16 ± 6.77 vessels/HPF). Liu et al (2022)[5] reported that the MVD and microvessel area were significantly increased in the patients with metastasis positive. Angiogenesis is associated with tumor progression and metastasis (Elayat et al., 2024)[1]. Of the 15 microvascular biomarkers, 11 of the 15 patients had different values between metastatic and reactive nodes (Ferroni et al, 2023)[8]. Histological MVD was not reported according to tumour grade by Ferroni et al., unlike the present grading analysis.

RI, PI and S/D ratio showed significant positive correlations with histological MVD, with coefficients of $r = 0.62$ ($p < 0.001$), $r = 0.58$ ($p < 0.001$) and $r = 0.49$ ($p = 0.003$), respectively. Likewise, Liu et al. (2022)[5] demonstrated imaging derived vascular parameters microvessel area and MVD were significantly increased in node positive disease. Ferroni et al . (2023)[8] demonstrated significant differences in 11 of 15 microvascular imaging biomarkers between metastatic and reactive nodes, with 10 of these showing $p < 0.01$. Sensitivity, specificity and accuracy of Doppler vascular patterns were reported to be 91.7%, 80.8% and 88.6% respectively by Abdelgawad et al. 2020[4], and AUCs of 0.863 and 0.882 for RI and PI respectively by Mehdikhani et al. 2024[9]. However, none of these studies reported direct Pearson correlations between RI, PI, S/D ratio and histological MVD.

Conclusion

In this cohort of 30 women with invasive breast carcinoma, axillary lymph-node vascularity assessed by colour Doppler closely reflected histological angiogenic activity and adverse tumour characteristics. Mixed vascularity was the dominant pre-NACT pattern, while mean RI, PI and S/D ratio indicated increased intranodal vascular resistance. Histological examination demonstrated a mean MVD of 49.97 ± 14.12 vessels/HPF, with moderate-to-high vascular grades in most nodes. Higher RI and PI were significantly associated with tumour size >4 cm and nodal positivity, whereas MVD rose progressively with tumour grade and was markedly greater in node-positive disease. The positive correlations between RI, PI, S/D ratio and MVD confirm that Doppler indices can serve as non-invasive surrogates of microscopic vascular proliferation. Significant post-NACT reductions in RI, PI and S/D ratio further support serial Doppler assessment for treatment-response monitoring. These findings endorse integrated Doppler and histological vascular evaluation for nodal risk stratification in breast cancer.

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