Nonmetastatic Axillary Lymph Nodes Have Distinct Morphology and Immunophenotype in Obese Patients with Breast Cancer at Risk for Metastasis

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Obese patients with breast cancer have worse outcomes than their normal weight counterparts, with a 50% to 80% increased rate of axillary nodal metastasis. Recent studies suggest a link between increased lymph node adipose tissue and breast cancer nodal metastasis. Further investigation into potential mechanisms underlying this link may reveal potential prognostic utility of fat-enlarged lymph nodes in patients with breast cancer. This study uses a deep learning model to identify morphologic differences in nonmetastatic axillary nodes between obese, node-positive, and node-negative patients with breast cancer. The model was developed using nested cross-validation on 180 cases and achieved an area under the receiver operator characteristic curve of 0.67 in differentiating patients using hematoxylin and eosin-stained whole slide images. The top predictive patches from the slides according to the model were reviewed by a pathologist, and their morphologic differences were quantified. This analysis showed an increased average adipocyte size ($P = 0.004$), increased white space between lymphocytes ($P < 0.0001$), and increased red blood cells ($P < 0.001$) in nonmetastatic lymph nodes of node-positive patients. Preliminary immunohistochemistry analysis on a subset of 30 patients showed a trend of decreased CD3 expression and increased leptin expression in fat-replaced axillary lymph nodes of obese, node-positive patients. These findings suggest a novel direction to further investigate the interaction between lymph node adiposity, lymphatic dysfunction, and breast cancer nodal metastases, highlighting a possible prognostic tool for obese patients with breast cancer. (Am J Pathol 2023, 1:1–11; https://doi.org/10.1016/j.ajpath.2023.11.005)

Breast cancer is the most prevalent cancer in women worldwide, and it is one of the leading causes of death in women.1,2 Obesity, currently affecting >30% of women in the United States,3 significantly increases the incidence and worsens the prognosis of patients with breast cancer, among all breast cancer subtypes.4,5 Specifically, obese women are 50% to 80% more likely to develop axillary metastasis4–6 and have higher breast cancer–specific mortality than normal weight women.7

The interaction between obesity, immunity, and breast cancer progression is complex, and the understanding of this link is an evolving field of research. Studies have demonstrated an increased risk of poor cancer prognosis among obese patients with ectopic fat within organs such as liver, muscle, and heart.8–10 Enlarged adipocytes within ectopic fatty depots lead to the secretion of proinflammatory cytokines and metabolic dysregulation that generates a tumor-permissive microenvironment.6 In particular, leptin has been identified as an adipokine that is increased in patients with breast cancer.11,12 The adipocyte-rich environment can also provide local fatty acids to fuel tumor growth.13,14 With
the prevalence of obesity rapidly increasing in almost all countries,15 further investigation of the underlying mechanisms that put obese patients at an increased risk for axillary nodal metastases is crucial. Findings could support the evaluation of lymph node fat content in the workup of patients with breast cancer and may inform prognosis, personalized treatment strategies, and future targeted therapies.

As was recently shown by Almekinders et al,16 peripectoral breast hyperadiposity results in local steroid hormone biosynthesis and endocrine dysregulation, and it is a potentially strong prognostic biomarker for invasive breast cancer in patients with ductal carcinoma in situ. Prior studies have shown that nonmetastatic axillary lymph nodes may be enlarged by excess hilar fat deposition and are more commonly seen in women with obesity.17,18 Fat-enlarged nonmetastatic axillary lymph nodes identified on mammography and breast magnetic resonance imaging are associated with a high risk of axillary metastasis in obese patients with invasive breast cancer, and this association is maintained when adjusting for patient and tumor characteristics.19 This study hypothesized that hyperadiposity and micro-immune dysregulation may also occur in axillary nodal adiposity, generating a premetastatic niche for nodal metastasis in women with invasive breast cancer. This study aimed to investigate whether there are changes in the morphology and immunophenotype of nodal hyperadiposity and the immune microenvironment in nonmetastatic axillary lymph nodes that are associated with lymph node metastasis in breast cancer.

Materials and Methods

A deep learning (DL) framework was developed and trained to identify differences in morphologic patterns in nonmetastatic axillary lymph nodes from node-positive and node-negative patients. The DL model evaluated scanned whole slide images (WSIs) of hematoxylin and eosin–stained slides using a histologic image feature extractor to learn interpretable morphologic patterns that may shed light on underlying structural changes that contribute to the increased risk of nodal metastases in obese patients. Possible alterations of lymphatic, lipid, and metabolic-related protein expression related to nodal metastasis were accessed through an immunohistochemistry (IHC) analysis for a limited number of cases.

Table 1  Study Cohort Characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Overall</th>
<th>Node-negative patients</th>
<th>Node-positive patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total, N</td>
<td>180</td>
<td>92</td>
<td>88</td>
</tr>
<tr>
<td>Age, mean (SD), years</td>
<td>60.9 (11.0)</td>
<td>62.1 (9.9)</td>
<td>59.6 (11.9)</td>
</tr>
<tr>
<td>BMI, mean (SD), kg/m²</td>
<td>36.4 (5.8)</td>
<td>36.5 (6.1)</td>
<td>36.3 (5.6)</td>
</tr>
<tr>
<td>Tumor grade, n (%)</td>
<td>1</td>
<td>26 (14.9)</td>
<td>17 (19.5)</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>94 (53.7)</td>
<td>43 (49.4)</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>55 (31.4)</td>
<td>27 (31.0)</td>
</tr>
<tr>
<td>Tumor size, mean (SD), mm</td>
<td>28.5 (23.6)</td>
<td>24.1 (19.6)</td>
<td>32.8 (26.4)</td>
</tr>
<tr>
<td>Molecular subtypes, n (%)</td>
<td>151 (84.8)</td>
<td>74 (82.2)</td>
<td>77 (87.5)</td>
</tr>
<tr>
<td></td>
<td>11 (6.2)</td>
<td>3 (3.3)</td>
<td>8 (9.1)</td>
</tr>
<tr>
<td></td>
<td>16 (9.0)</td>
<td>13 (14.4)</td>
<td>3 (3.4)</td>
</tr>
<tr>
<td>LVI, n (%)</td>
<td>96 (55.5)</td>
<td>66 (76.7)</td>
<td>30 (34.5)</td>
</tr>
<tr>
<td></td>
<td>77 (44.5)</td>
<td>20 (23.3)</td>
<td>57 (65.5)</td>
</tr>
</tbody>
</table>

BMI, body mass index; ER, estrogen receptor; HER2, human epidermal growth factor receptor 2; LVI, lymphovascular invasion; PR, progesterone receptor; TNBC, triple-negative breast cancer.

Study Population and Data Collection

This study and the use of human participant data in this project were approved by the Dartmouth-Health Institutional Review Board with a waiver of informed consent. The study included obese patients (body mass index, >30 kg/m²) with histologically confirmed invasive breast cancer who underwent sentinel lymph node excision or axillary lymph node dissection at Dartmouth-Hitchcock Medical Center (Lebanon, NH). A total of 180 breast cancer cases were included, including 88 patients with axillary nodal metastasis (labeled as node positive) and 92 patients without nodal metastasis (labeled as node negative). Patients' demographics, clinical data, and pathologic information, including age and body mass index at the time of cancer diagnosis, tumor size, tumor grade, hormone receptor and human epidermal growth factor receptor 2 status, and the presence of lymphovascular invasion, were collected from electronic medical records (Table 1). All patients included in this study did not receive neo-adjuvant treatment, including chemotherapy, before surgery. The detailed data collection workflow is illustrated in Supplemental Figure S1.
Figure 1  Summary of study design. A: Pathologist’s annotation of axillary lymph nodes (circled in blue) and patch extraction from whole slide image (WSI) for use in model training. B: A convolutional neural network (CNN) was used to extract image features from randomly sampled patches from a WSI. The resultant feature vectors were then averaged and fed into a fully connected neural network (NN) to predict the node-positive probability of the WSI. C: The trained model was applied to each patch to classify its nodal status. Then, the patches were ranked by their model probability score within their label groups.
Axillary Lymph Node Histology Images

Patients with breast cancer underwent sentinel lymph node biopsy or axillary lymph node dissection at the time of their breast cancer surgery (partial or full mastectomy). Lymph nodes received from patients with breast cancer were grossed according to standard procedures; all nodes were dissected longitudinally into 2-mm increments and submitted completely for histologic evaluation of metastatic disease. Tissue is formalin fixed and embedded into paraffin blocks, sliced into sections (5 μm thick), and stained with hematoxylin and eosin for microscopic evaluation. The lymph node slides were retrieved and reviewed by a board-certified anatomic pathologist who specializes in breast pathology (K.E.M.). For both study sets (node-positive and node-negative groups), only the nonmetastatic lymph nodes were chosen for evaluation. Lymph nodes with metastatic foci of cancer were excluded from the data set. The slides were scanned using the Leica Aperio-AT2 scanner (Leica Biosystems, IL) at ×20 magnification (0.5 μm/pixel) and stored in SVS image format. A total of 636 WSIs (303 node-positive and 333 node-negative) were collected.

Lymph Node Annotation and Patch Extraction from Whole Slide Images

The data preparation pipeline is illustrated in Figure 1A. Regions of axillary lymph nodes containing lymphoid tissue and intranodal adipocytes were manually annotated on WSIs with ASAP software version 1.9 (the Netherlands; 2018). Patches of 224 × 224 pixels were extracted from the pathologist-annotated regions at ×10 magnification level (1 μm/pixel) to reduce the number of resultant patches. A total of 575,906 patches were extracted from the annotated regions from the hematoxylin and eosin–stained WSIs (251,341 from node-positive patients and 324,565 from node-negative patients).

Deep Learning Framework for Histologic Feature Extraction

A DL framework was developed on the basis of previous work, including the studies of Wulczyn et al.20 and Jiang et al.21 to classify the patients’ breast cancer nodal status using the nonmetastatic lymph node histopathology. Given that the number of WSIs per patient varied, the patients’ nodal status was assigned to each WSI as the label, and the model was trained to classify each WSI separately. To start, randomly selected patches from each WSI were fed into a convolutional neural network with a ResNet-18 architecture and with ImageNet-pretrained weights.22,23 The patches were converted to patch-level feature vectors of size 512, which were then averaged into a WSI-level feature vector. The resulting vector was fed into a two-layer fully connected neural network, with a hidden layer of 128 neurons and an output size of 2, indicating predictions of node-positive and node-negative classes. In the end, a SoftMax normalization was applied to the model output to generate a probability score. The WSI was classified as node positive if the resulting probability score was >0.5, or as node negative otherwise. The patient-level classification was determined by averaging the probability scores of all WSIs from the patient, and then converting the average score to a binary outcome using a threshold of 0.5.

The model training and hyperparameter tuning was performed with five-fold nested cross-validation stratified by patients’ nodal status. The data set was split into five outer splits at the patient level to avoid information leak across different slides of the same patient. Each split was used as the held-out test once, whereas the rest of the patients were randomly split into 80% training set and 20% validation set for hyperparameter selection (inner loop). For each inner loop, the model hyperparameters tuned included batch size, number of patches sampled during training, and initial learning rate for the ResNet-18 backbone and the fully connected layers. Image augmentation was applied to patches including random horizontal and vertical flip, random 90-degree rotation, and random color jittering during training. The detailed hyperparameter configurations are shown in Supplemental Table S1. The models were trained with an Adam optimizer and a cosine annealing learning rate scheduler for 300 epochs. The model with the lowest validation cross-entropy loss during training was used for model evaluation. All models were trained using Nvidia Titan Xp graphic processing units with 12-GB memory. The model was evaluated and trained with Python version 3.824 and PyTorch version 1.13.25

For evaluation, the predictions of all outer test splits were aggregated and evaluated using overall accuracy, precision, recall, sensitivity, F1-score, and the area under the receiver operator characteristic curve. The CIs of the evaluation metrics were generated from 2000 bootstrap samples. The model pipeline is illustrated in Figure 1B. The detailed model training and evaluation pipeline is described in Model Visualization and Statistical Analysis of Morphologic Features.

Model Visualization and Statistical Analysis of Morphologic Features

Figure 1C illustrates the process of identifying morphologic features from top model-selected patches, as well as the quantification and statistical analysis of these features. Specifically, the trained model was applied to all patches from each patient to calculate a patch-level probability
score. The 64 most-predictive patches from each patient were selected and grouped by patients’ labels for pathologist review. A pathologist (L.M.H.), blinded to the patients’ labels and nodal status, reviewed the selected patches to search for any distinct histologic patterns between these two groups. The visual findings of morphologic patterns were subsequently quantified on the entire image data set across all patches of the lymph nodes using image processing techniques described in Quantification of Lymph Node Morphologic Features. The statistical significance of the difference in the quantified patterns between the two classes was determined by the U-test, with statistical significance considered at $P < 0.05$.

### Quantification of Lymph Node Morphologic Features

#### Adipocyte

The adipocyte quantification method in this study is similar to that described by Osman et al.\textsuperscript{26} The image was transformed into the HSV format. A binary threshold of five was applied to the saturation channel of the image to eliminate background noise. An opening operation using a disk-shaped kernel was performed on the image, followed by a dilation operation using the same kernel. Finally, an opening operator using a disk-shaped kernel was applied to the inverse of the previous image to generate the mask for adipocytes. The contour-finding algorithm was applied to the adipocyte mask to identify the area of individual adipocytes. Areas that were $<500$ pixels (ie, small tissue gaps) or $>20,000$ pixels (ie, background or large tissue gaps) were removed from the adipocyte areas.

#### Lymphoid White Space

The image was converted to HSV format. A binary threshold of 30 was applied to the saturation channel of the image to eliminate background noise and generate a binary tissue mask. The tissue mask was then inverted to generate a mask for all white spaces. The edges of the white space mask were filled in to eliminate intensities caused by the background. All connected components (objects) that had $>200$ pixels were removed from the white space mask. This step eliminated white spaces resulting from adipocytes and tissue gaps caused by broken tissues during laboratory procedures. The remaining mask is referred to as the lymphoid white space.

#### Lymphoid Erythrocyte

The image was transformed into the HSV format. Pixels within the intensity range of 0 to 10 and 160 to 180 (red color) from the hue channel were considered as erythrocytes.

### IHC of Selected Markers

A total of 30 lymph node samples, 15 node positives and 15 node negatives, were selected for IHC analysis. The goal in this preliminary IHC analysis was to shed light on the interactions between lymph node adiposity, lymphatic dysfunction, and breast cancer nodal metastases by selecting a combination of immunohistochemical stains to evaluate potential differences in functional pathways, including T-cell (CD3) and B-cell (CD20) proportions, fatty acid metabolism [fatty acid synthase, lipoprotein lipase, Spot14, and fatty acid translocase (CD36)], and adipose inflammation (leptin, adiponectin, tumor necrosis factor-$\alpha$, and IL-6). For this data set, a radiologist selected lymph node samples based on the extent of fat replacement within the lymph nodes using radiologic studies, with 15 samples obtained from patients who had positive lymph nodes and 15 samples obtained from patients who had negative lymph nodes. Specifically, the 15 node-positive samples had high levels of fat replacement, and the 15 node-negative samples had low levels of fat replacement. For these samples, slides were deparaffinized and rehydrated. Primary antibodies against CD3 (Leica; catalog number PA0553; clone LN10), CD20 (Leica; catalog number NCL-L-CD20-L26; clone L26), fatty acid synthase (Abcam, Cambridge, UK; catalog number ab22759; polyclonal), lipoprotein lipase,\textsuperscript{27} Spot14,\textsuperscript{28} CD36 (Abcam; catalog number ab133625; clone EPR6573), leptin (Abcam; catalog number ab16227; polyclonal), adiponectin (Abcam; catalog number ab75989; clone EPR3217), tumor necrosis factor (Abcam; catalog number ab220210; clone TNFA/1172), and IL-6 (Abcam; catalog number ab9324; clone 1.2-2B11-2G10) were processed and stained according to manufacturers’ protocols. The expression level of the IHC stains was digitally quantified. The detailed image analysis method is described in Quantification of IHC Stains.

### Quantification of IHC Stains

IHC stains were quantified in the lymphoid tissue region, adipose tissue region, or both, based on the location of expression of each stain. From the whole slide image of the stained lymph node, all patches from areas containing stained tissue were extracted. Each patch was classified as adipose rich or lymphocyte rich using the adipocyte detector

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**Table 2** Slide and Patient-Level DL Model Performance

<table>
<thead>
<tr>
<th>Level</th>
<th>AUC</th>
<th>Accuracy</th>
<th>F1-score</th>
<th>Precision</th>
<th>Recall</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Slide level</td>
<td>0.69 (0.65–0.73)</td>
<td>0.64 (0.60–0.67)</td>
<td>0.63 (0.59–0.67)</td>
<td>0.62 (0.56–0.67)</td>
<td>0.65 (0.59–0.70)</td>
<td>0.63 (0.58–0.68)</td>
</tr>
<tr>
<td>Patient level</td>
<td>0.67 (0.59–0.75)</td>
<td>0.67 (0.60–0.73)</td>
<td>0.63 (0.54–0.71)</td>
<td>0.69 (0.58–0.79)</td>
<td>0.58 (0.48–0.68)</td>
<td>0.75 (0.66–0.84)</td>
</tr>
</tbody>
</table>

The 95% CIs are included in parentheses.

AUC, area under the receiver operator characteristic curve; DL, deep learning.
described above. The hematoxylin channel was separated from the patches using the Scikit-image package version 0.19.2 with methods outlined by Ruifrok and Johnson.29

### Results

**Deep Learning—Aided Interpretation of Distinct Lymph Node Morphologic Features**

As described above, the DL model evaluated through nested cross-validation on patches from WSIs. On the basis of this evaluation, the model achieved an area under the receiver operator characteristic curve of 0.67 (95% CI, 0.59—0.75), and the detailed model performance at both slide and patient levels is shown in Table 2.

Through examination of the top predictive patches by the DL model, pathologists identified notable variations in histologic characteristics between node-positive and node-negative cases. These differences were confirmed with the quantified metrics using an in-house image analysis pipeline. The histologic features that are considered and quantified in this study include the following: the number and

![Figure 2](image-url) Distinct morphologic characteristics of axillary lymph nodes in node-positive and node-negative patients. **A** Left panels: Example quantification of lymph node adipocyte size in fat-enlarged lymph nodes of node-positive and node-negative patients. The largest adipocyte from each patch was highlighted with orange or blue. Right panels: Box-and-whisker plot of the average size of adipocyte and the SD of the adipocyte size in node-positive and node-negative samples. **B** Left panels: Example quantification of lymphoid white space defined by white space/lymphoid (W/L) ratio. Right panel: Box-and-whisker plot of the W/L ratio in node-positive and node-negative samples. **C** Left panels: Example quantification of red blood cells in lymph node tissue defined by erythrocyte/lymphoid (E/L) ratio. Right panel: Box-and-whisker plot of the E/L ratio in node-positive and node-negative samples. *P < 0.05, **P < 0.01, ***P < 0.001, and ****P < 0.0001. Scale bars = 50 μm (A–C).
size of adipocytes, the proportion of white space between the lymphocytes (defined by white space/lymphoid ratio), and the proportion of red blood cells (defined by erythrocyte/lymphoid ratio). The results and some examples of these measurements and analysis are illustrated in Figure 2.

This analysis showed that node-positive patients had significantly larger intranodal adipocytes, with more variation in adipocyte size, compared with node-negative patients (Figure 2A). In addition, there was a significantly increased proportion of white space between lymphocytes and red blood cells in the lymph node parenchyma of node-positive patients (Figure 2, B and C). These morphologic differences were also significantly distinct between the model-assigned labels (Supplemental Figure S2). In addition, a positive correlation was observed between the increase of average size of adipocytes and higher white space/lymphoid ratio,
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with a Pearson correlation coefficient of 0.26 (P < 0.001) (Supplemental Figure S3).

IHC Stain Expression Patterns in Lymph Node Lymphoid and Adipose Tissue

The sample size in this preliminary IHC analysis study (N = 30) is too small to determine statistically significant findings. However, the visual analysis of distribution of IHC stains revealed that fat-enlarged lymph nodes from node-positive patients displayed a lower ratio between CD3<sup>+</sup>/CD20<sup>+</sup> cells because of decreased CD3 expression (Figure 3Ai). Specifically, the intensity of CD3 and CD20 is significantly correlated in nonfatty lymph nodes compared with node-negative samples (Pearson correlation coefficient = 0.83; P < 0.001), whereas no significant correlation was found in fat-replaced lymph nodes from node-positive samples (Pearson correlation coefficient = 0.28; P = 0.30) (Figure 3Aii). Figure 3Aii and Aiv show examples of a fat-replaced node from a node-positive patient with low CD3<sup>+</sup>/CD20<sup>+</sup> ratio and a nonfat-replaced node from a node-negative patient with high CD3<sup>+</sup>/CD20<sup>+</sup> ratio, respectively. In addition, node-positive patients exhibited a slightly elevated expression of leptin in intranodal and perinodal adipose tissue compared with node-negative patients, as demonstrated in Figure 3B.

Discussion

This study investigated the morphologic and immunophenotypic characteristics of nonmetastatic axillary lymph nodes in relation to the risk of breast cancer nodal metastasis among obese women. Using a cohort of 88 node-positive and 92 node-negative patients, a DL model was trained that identified several morphologic features that were distinct in axillary lymph nodes from node-positive patients, including significantly increased average size of adipocytes, a higher proportion of white spaces within lymphoid tissue, and a higher number of erythrocytes within lymphoid tissue. In addition, the preliminary IHC analysis of 30 axillary lymph node samples showed some visual trends, such as decreased CD3 staining, lower CD3<sup>+</sup>/CD20<sup>+</sup> ratios, and elevated leptin expression around nodal adipocytes in the fat-enlarged lymph nodes from node-positive patients, compared with those of the normal lymph nodes from node-negative patients. Together, these study findings suggest a link between several histologic characteristics of fat-enlarged axillary lymph nodes and nodal metastases in patients with breast cancer and obesity.

Patients with obesity have poor breast cancer outcomes that are not fully explained by body mass index. Understanding the factors that contribute to variable breast cancer outcomes among obese women is essential for improving prognosis and identifying potential therapeutic targets, ultimately leading to better outcomes for patients. Previous studies have found that adipose cells can contribute to cancer progression through the release of signaling molecules, extracellular proteins, lipids, and metabolites that support tumor growth and invasiveness. Although there have been many studies examining ectopic adipose located in and around organs, there is limited research investigating the impact of ectopic adipose within lymph nodes on cancer outcomes. A recent study found that the presence of enlarged axillary lymph nodes due to fat infiltration, as seen on mammography and breast magnetic resonance imaging, is correlated with an increased risk of nodal metastasis in obese patients when adjusting for patient and tumor characteristics. The current study identified distinct histologic features that could contribute to a tumor-promoting adipose microenvironment. These findings support the prior radiology study, which has shown a strong correlation between fat-enlarged lymph nodes and nodal metastases.

A greater average adipocyte size was observed within the axillary lymph nodes of node-positive patients than those of the node-negative patients. Previous research has established a link between adipocyte hypertrophy in obesity and impaired metabolic regulation that promotes cancer progression, partly due to hypoxia of hypertrophic adipocytes that leads to altered adipokine secretion and ensuing inflammation. The results align with those of Almendingers et al, who found a correlation between larger adipocyte size and an elevated risk of invasive breast cancer following ductal carcinoma in situ. This underlines the importance of further research on the role of ectopic nodal adiposity in the development breast cancer nodal metastases.

Metastatic sites are selectively primed by the primary tumor even before the initiation of metastases occurs, resulting in a premetastatic niche that is devoid of cancer cells. The hematoxylin and eosin analysis revealed an increased proportion of lymphoid white spaces in lymph nodes from node-positive patients and a positive correlation between lymphoid white spaces and average adipocyte size of the lymph node. Possible theories to account for the increased white space include interstitial fluid caused by impaired lymphatic drainage, which promotes migration of tumor cells. Another potential explanation is the production of soluble factors, such as protein ligands or extracellular matrix-modifying proteins, that are secreted to induce remodeling of metastatic sites to facilitate seeding, as explained by Folllain et al. Dissemination of tumor-related factors, such as cytokines, chemokines, growth factors, matrix metalloproteinases, and extracellular vesicles, has been shown to promote dissemination of tumor cells through stimulation of inflammatory cells and angiogenesis and leads to remodeling of the extracellular matrix. Of note, there was an increased amount of extravasated red blood cells in the nodal tissue in the node-positive group. Although speculation, one theory could be linked to the increased shear fluid forces in vessels with circulating tumor cells, whereas endothelial cell remodeling to promote tumor

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cell extravasation can provide an explanation for the leaky vasculature. Vascular leakage and immunosuppression, among others, are changes that have been described in the premetastatic niche. The finding postulates that the increased white space and extravasated red blood cells could represent morphologic evidence of preconditioning the premetastatic niche in axillary lymph nodes in obese patients.

The preliminary downstream IHC analysis identified changes in immune cell populations and adipokine expression, although these results were not statistically significant because of the limited statistical power of the small sample size. CD3 intensity was lower in fat-enlarged nodes, which may reflect decreased immune function in fatty nodes, a finding that has been reported in lymph nodes of obese mice. Furthermore, nonmetastatic fat-enlarged nodes of patients with nodal metastases also showed increased leptin expression. Leptin has been shown to stimulate proliferation, angiogenesis, migration, and metastasis in breast cancer cell lines by activating the oncogenic pathways, and high serum leptin is associated with a twofold to fivefold increase in breast cancer risk. Therefore, increased leptin expression in fat-enlarged nodes may also be associated with a premetastatic niche and requires further evaluation through large-scale IHC analysis.

This study is a trailblazing effort that examined premetastatic morphologic changes in lymph nodes in a cohort of obese patients with a focus on fat-enlarged lymph nodes. There are several limitations to this study. This study has a relatively small sample size; in particular, the IHC analysis was restricted to a small number of patients because of limited resources. The data used in this study are from a single institution, which may introduce bias and chance of overfitting, and thereby limit the generalization of the findings to a larger population. Further validation of the findings is necessary through large-scale, multicenter studies that include a diverse patient population and external validation. This study is also limited by its retrospective design.

Histology images of axillary lymph nodes obtained via sentinel lymph node biopsy or axillary lymph node dissection were only available at the time of the cancer diagnosis. This temporal limitation makes it challenging to establish a causal relationship between the identified lymph node features and the development of nodal metastasis. However, future studies could investigate the relationship between lymph node characteristics related to nodal hyperadiposity and prospective outcomes, including cancer recurrence, distant metastasis, and long-term patient prognosis, to further investigate the clinical importance of the identified morphologic features.

In conclusion, this study identified several histopathologic and immunohistochemical features of nonmetastatic axillary lymph nodes in relation to breast cancer nodal metastases in obese patients with breast cancer. These findings suggest that nodal hyperadiposity and alterations in the immune microenvironment may play a role in forming the premetastatic niche. These results emphasize the need for further research into increased lymph node adiposity.

Further investigation into features of the fat-enlarged nodes that account for an increased likelihood of nodal metastases is warranted. If confirmed with larger studies, fatty axillary lymph nodes may serve as a prognostic imaging marker that can be readily assessed in all patients with breast cancer. This information may inform personalized treatment strategies and targeted therapies in obese patients with breast cancer.

**Author Contributions**

All authors contributed to the design of the study: K.E.M. was responsible for collecting the histologic and immunohistochemistry image data; L.M.H. annotated the histology images; R.M.d.-A. collected the clinical data; L.M.H. and K.E.M. analyzed and interpreted the histologic findings; Q.S. cleaned and preprocessed the data, implemented the methods, conducted statistical analysis, and wrote the first draft of the manuscript; all authors revised the manuscript and verified the presented results; and S.H. supervised the study.

**Disclosure Statement**

None declared.

**Supplemental Data**

Supplemental material for this article can be found at http://doi.org/10.1016/j.ajpath.2023.11.005.

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