The Role of Lymph Node Metastasis in the Systemic Dissemination of Breast Cancer

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ABSTRACT

Background. Lymphatic invasion is necessary for regional lymph node (RLN) metastasis in breast cancer (BC), while systemic metastasis requires blood vessel (BV) invasion. The site of BV invasion could be at the primary BC site or through lymphovascular anastomoses. The vague pathologic term “lymphovascular invasion” (LVI) encourages the belief that peri/intratumoral BV invasion may be common. We investigated the relative contribution of RLN metastasis to systemic metastasis by studying the relationship among LVI and RLN and/or systemic metastasis in a population-based cohort of breast cancer patients.

Methods. Fisher’s exact test was done to assess global associations among LVI and RLN and/or systemic metastasis in a prospective database of breast cancer patients undergoing RLN biopsy. Logistic regression was used to determine multivariable contributions of LVI to metastasis when controlling for available demographic, radiologic, and pathologic variables.

Results. Of 1668 patients evaluated 25.4% were RLN positive and 10.4% had LVI. RLN metastasis was predicted by tumor size ($P < .0001$), HER-2/neu overexpression ($P = .0022$) and the interaction between LVI positive and HER-2/neu positive tumors ($< .0001$). Patients with LVI/HER-2-neu positive were 3 times as likely to have positive RLNs compared with patients LVI/HER-2-neu negative.

Systemic metastasis was significantly ($P = .0013$) associated with LVI/ RLN positive, but not with LVI positive/ RLN negative patients ($P = .137$).

Conclusions. LVI predicted RLN metastasis. LVI also significantly predicted systemic metastasis, but only when the RLN was also positive. Since RLN requires lymphatic invasion, these data support the hypothesis that primary breast cancers often invade lymphatics to gain access to the systemic circulation.

The systemic metastasis cascade, a concept theorized, confirmed at autopsy by pathologists, clinically and in animal models, and studied in numerous laboratory experiments, suggests that tumor-induced angiogenesis promotes systemic breast cancer (BC) metastases because of direct peritumoral and/or intratumoral invasion of tumor cells into blood vessel capillaries (BVCs).^{1–3} A possible alternative route to the systemic circulation occurs via lymphatic capillary (LC) invasion, passage through peripheral lymphatic trunks, progressing anatomically through the regional lymph nodes (RLNs), the large lymphatic ducts, and the existing lymphaticovenous anastomoses (LVA) in the neck.⁴ Direct local tumoral or peritumoral blood capillary invasion, allowing tumor cells to bypass the RLNs, supports the current surgical paradigm established by Fisher in BC, which led to the belief that BC is often a systemic disease from its origins and that the proper role for complete axillary lymphadenectomy (ALND) is for staging; the therapeutic value of this procedure still vigorously debated.⁵ Systemic metastases established primarily through LC invasion and metastasis to the RLNs would support the Halstedian hypothesis, with a predominant pattern of orderly lymphatic and lymph node progression; systemic metastasis would follow in some patients.⁶ RLN metastasis treated by ALND and/or

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locoregional radiotherapy may produce long-term survival without systemic metastasis, suggesting a stage when tumor cells are confined to the breast and the RLNs and have not yet metastasized to visceral sites.⁷

Pathologists currently allude to the process of peritumoral and/or intratumoral invasion into capillaries as “lymphovascular invasion” (LVI) or use the synonymous term angiolymphatic invasion, electing to avoid the more direct terminology “lymphatic invasion,” or “blood vessel invasion,” because it is not easy to assuredly distinguish microscopically between LC and BVC using standard morphological criteria after hematoxylin and eosin staining. LVI is associated with a greater likelihood of RLN and systemic metastases, but it is not clear whether systemic metastases in the presence of LVI are always associated with invasion of LCs with an orderly anatomic progression through lymphatic trunks to the RLNs or whether direct local BVC invasion is the predominant method by which BC cells gain access to the systemic circulation.⁸ Descriptions and diagrams of the anatomic pathways of systemic metastasis in textbooks, scientific articles, and reviews demonstrate direct invasion of blood vessels and suggest that angiogenesis not only produces more blood vessels (BVs) that provide greater blood flow to the tumor, but that there is also a greater likelihood of a larger surface “window” of BV endothelium for tumor cells to invade and metastasize.⁹

Preclinical and clinical studies of RLN metastasis and identification of LVI using specific immunohistochemical stains that distinguish lymphatic endothelial cells (LECs) from blood vessel endothelial cells (BECs) strongly suggest that lymphatic invasion is the preferred method by which tumor cells metastasize to the RLNs, but fail to also conclude that this initial lymphatic invasion is also the predominant method by which tumor cells gain access to the systemic circulation.^{10–13} We interrogated our breast cancer sentinel lymph node (SLN) database, providing a 14-year follow-up of our patients, to determine whether the relationships among LVI, RLN, and systemic metastases support direct peritumoral and/or intratumoral BVC or LC invasion as the predominant pattern of metastasis.

METHODS

Patients

All the patients in this study were diagnosed and managed by a multidisciplinary team of breast cancer physicians and ancillary staff in a vertically integrated academic health system. Invasive breast cancer (BC) was diagnosed by image- or palpation-guided core needle biopsy of clinical or radiologic breast abnormalities in most

cases, while a tiny proportion of the patients were diagnosed by excision biopsy of a breast lesion in the operating room. All the patients were referred initially to a breast surgeon and preoperatively or postoperatively to specialists in radiation and medical oncology, and occasionally to a plastic surgeon, either by individually arranged office visits, or through a Multidisciplinary Breast Tumor Clinic manned by specialist physicians, breast cancer nurses, social workers, and American Cancer Society volunteers.¹⁴ The Breast Cancer Multidisciplinary Tumor Board met weekly to discuss each of these patients, reviewing their clinical presentation, radiologic imaging studies, and pathology. This study captures only those breast cancer patients in whom there was no preoperative clinical, radiologic, or pathologic evidence of axillary lymph node metastasis.¹⁵ The patients were offered an operative sentinel node biopsy (SLNB) by appropriately trained and credentialed surgeons as part of their breast cancer management.¹⁶ The first few hundred patients undergoing SLNB also underwent a complete axillary lymphadenectomy.¹⁶

We present here a retrospective cohort study of 1668 clinically node negative patients undergoing 1548 successful SLNBs from April 1995 through April 2009.

The techniques used in the operating room have been reported previously, documenting SLN biopsy, incorporating preoperative intradermal radiocolloid injection and/or intraoperative injection of 5 mL of 1% isosulfan blue or dilute methylene blue into the breast parenchyma adjacent to the breast cancer and into the subareolar lymphatic plexus in the same “clock” position as the tumor.¹⁶ In all cases where a SLN was not identified, or the patients in whom both procedures were planned and completed as part of the quality assurance, or where the sentinel node(s) contained tumor metastasis a complete axillary lymph node dissection (ALND) was performed.^{15,16} The primary breast tumor was excised by mastectomy or breast-conserving surgery using standard techniques. The terms lumpectomy, partial mastectomy, quadrantectomy, and wide excision were used interchangeably.

All patients were seen by surgical, medical, and radiation oncologists and standard National Comprehensive Cancer Network guidelines used to advise patients regarding the use of these three major modalities of BC treatment.

The collection of data and the study was approved by the Institutional Review Board.

Pathologic Evaluation

All primary tumors and axillary lymph nodes were subjected to expert pathologic evaluation by a team of breast pathologists as previously reported.^{15,16} The data were abstracted from pathology reports. In some cases (see

below) re-review of the tissue sections were done to obtain more LVI information.

Tissue sections were processed using standard pathologic protocols.¹⁶ A minimum of three sections were submitted from the primary tumor including adjacent uninvolved breast tissue to assess patterns of invasion, distance to the closest surgical margins, and LVI. Other primary breast cancer characteristics were assigned by consensus to each tumor by a team of pathologists, meeting every day to look at each new case with a multiheaded microscope, and the use of a checklist and reported electronically. The checklist included: pTNM stage, anatomic part(s) of the breast involved, histopathologic type, histologic grade (Nottingham), maximum dimension (centimeters), invasive margins (positive or negative), DCIS margins (positive or negative), extensive intraductal component (yes or no), DCIS size, (when feasible), lymph nodes (number with metastasis/number examined), largest node metastasis (centimeters), extracapsular extension, lymphovascular invasion (present or absent, focal or extensive), dermal lymphatic invasion (yes or no), breast tumor markers (estrogen, progesterone, and HER-2-neu receptors; positive or negative), and distant metastasis (assessable or not, yes or no).

Lymphovascular invasion (LVI) was assessed at the periphery of the tumor and intratumoral regions as a standard pathological evaluation. Attention was given to separate true LVI from retraction artifact, often overcalled as LVI. Special immunostain markers to identify endothelial cells were not performed as part of the routine pathologic evaluation. LVI was scored as positive or negative in tumor sections stained with hematoxylin and eosin. Only those patients with unmistakable clusters of tumor cells in the lumen of an endothelial-lined vascular space within or close to the edge of the BC were scored unequivocally positive. Patients with inflammatory breast cancer and dermal lymphatic emboli were excluded. In a small proportion of cases where either of these conclusions could not be conclusively reached, and the pathologist recorded the finding as “suspicious,” we elected to classify the patients as negative for purposes of analysis. LVI information was not available initially in 49 patients, and we found, processed, evaluated, and recorded the microscopic tissue slides in 40, but there was no tissue available for re-review for the other 9 patients.

Lymph nodes were fixed in 10% buffered formalin and embedded in paraffin. The SLN was serially sectioned along the short axis into 2-mm slices, and 4–6 sections, 5-micron thick, cut at various depths, and standard hematoxylin and eosin staining on glass slides performed. Cytokeratin immunohistochemistry (IHC) was deliberately avoided except to confirm nanometastases in the node, particularly with primary lobular carcinomas. An axillary

node was classified as positive (containing metastatic tumor cells) according to standard pathologic criteria and current American Joint Commission on Cancer guidelines.

Patient Follow-Up

Our large electronic medical record system captured all patients in a longitudinal study, providing accurate details regarding recurrence, and of any deaths. Only a tiny proportion of patients migrated to other health systems, requiring telephone calls and letters by the institutional tumor registry to obtain updated recurrence information. We also accessed the National Death Index Retrieval Program, US Department of Health and Human Services, searching for patients who may have died and whose information was otherwise incomplete.

Data Retrieval, Collection, and Abstracting

All patient information was available on an electronic medical record system, updated whenever a clinical event occurred. Demographic, clinical, operative, radiological, and pathological information was carefully abstracted by the trained staff in the Department of Biostatistics and Research Epidemiology (BRE) in consultation with the principal investigator (SDN) and recorded in an Excel database. Regular meetings were conducted to discuss data input. The database was periodically updated and searched for errors that were corrected. The database was used for analysis by a biostatistician (AK) and epidemiologist (SAH) in consultation with the senior author.

Statistical Methods

Logistic regression models were used for all statistical analyses.¹⁷ Eleven patients had two SLN biopsies, one on each side. If a patient had surgery on both sides on the same date, the side with the positive SLN or with SLN data collected (if no positive SLN found) was used for purposes of length of follow-up analysis. If a patient had 2 surgeries on different dates, data from the second surgery were used. Patients with pathology of “DCIS only,” and with negative SLNs, included in our sentinel node data base, were excluded from the analysis ($N = 61$). A total of 1668 patients were included in the initial analysis.

We first tested each predictor of interest in a univariate GEE model. Individual predictors with $P < .10$ were candidates for multivariable modeling. The backward model selection method was used to determine the final multivariable model. Odds ratios (OR) and 95% confidence intervals (95% CI) were calculated for the univariate models and the multivariable model. An OR of 1.0 indicates no association between the predictor and RLN

positive. An OR >1 indicates a positive association between RLN positive and the predictor variable, whereas an OR <1 indicates a negative relationship between RLN positive and the predictor. Model fit was assessed using the c-statistic.

The association between RLN positive, LVI, tumor size, grade, HER-2-neu, ER, PR, and age with systemic metastasis was assessed. First, the individual association between each parameter and metastasis was tested using chi-square tests. All significant parameters ($P < .05$) were then tested in a multivariable logistic regression model. All possible interactions were tested at $P < .10$.

In an additional analysis, the association between LVI, systemic metastasis, and RLN metastasis was tested using Fisher's exact test.

Kaplan-Meier curves were constructed to depict graphically the relationships among LVI, RLN positive, and systemic metastasis.

RESULTS

Figure 1 shows the design of the study. A total of 1668 newly diagnosed and previously untreated invasive BC patients with clinically, radiologically, and pathologically negative ipsilateral axillary lymph nodes were evaluated and offered a SLN biopsy as part of their surgical treatment. The median length of follow-up was 3.62 years (mean = 4.15). The range was 0–14 years.

The results of this study are conclusions based on the 174 of 1659 patients (10.4%) in whom a definitive pathology report showed invasion of vessels related to the primary tumor (LVI). All patients with RLN data were included in the initial analysis ($N = 1668$).

Table 1 shows the demographics of the study. There were 424 of 1668 patients (25.4%) with RLN metastasis; 34 of 120 patients (28.3%) whose SLN was not found and

who had a complete axillary dissection had metastases in the axillary nodes. There were 9 patients in whom LVI data could not be obtained, and 8 patients in this group with RLN metastases were not included, leaving 407 patients with complete data included.

The first part of the study evaluated predictors of axillary RLN metastasis. Table 2 presents the results from the univariate logistic regression models. Individual predictors of RLN positive included HER-2-neu ($P = .004$), tumor size ($P < .0001$), tumor grade ($P < .0001$), age ($P = .0099$), and LVI ($P < .0001$), and these were considered in the multivariable model.

Multivariate analysis identified tumor size ($P < .0001$), ER ($P = .0048$), and an interaction between LVI and HER-2-neu ($<.0001$) as significant predictors of RLN metastasis (Table 3). Patients with tumor size 1–2 cm (group 2) were 4.5 times more likely to have RLN positive (aOR: 4.52; 95% CI: 3.54, 5.78). The odds increased to 8.75 for patients with tumor size ≥ 2 cm (group 3) (aOR: 8.75; 95% CI: 6.65, 11.5) compared with those with tumors <1 cm. Patients who were ER positive were 1.3 times more likely to have RLN positive. Patients who were LVI positive were 14 times more likely than LVI negative patients to have positive RLNs if they had HER-2-neu overexpression (aOR: 14.0; 95% CI: 1.36, 12.3). The odds of RLN positive for LVI positive vs LVI negative patients was reduced to 4.7 if a patient was HER-2-neu negative (aOR: 4.72; 95% CI: 3.67, 6.08). The c-statistic for this model was 0.739, indicating the model has good predictive ability.

The number of patients with systemic metastasis predicted by univariate analysis is presented in Table 4. The final multivariate model included RLN positive by LVI, tumor grade, and tumor size. Results are presented in Table 5. The overall P value for grade was $P = .002$; for tumor size: $P = .012$; for RLN: $P = .011$; and for LVI:

FIG. 1 Study design

TABLE 1 Demographic information of the patients studied

Description	N	%
Total cases	1668	100
SLN found	1548	92.8
No. SLNs removed 1.87 (1.49) (mean $\pm$ SD)		
RLN tumor positive ^a	424	25.4
Tumor size (cm) 1.66 (1.13) (mean $\pm$ SD)		
<1	444	26.6
1–2	785	47.1
2–5	411	24.6
>5	21	1.3
Unmeasurable	7	0.4
Tumor grade		
1	402	24.1
2	759	45.5
3	493	29.6
Not graded	14	0.8
Hormone receptors		
ER positive	1301	78.0
PR positive	1207	72.3
HER2-neu		
Positive	205	12.3
Negative	1187	71.0
Not performed	278	16.7
Surgery		
Mastectomy	352	23.7
Full axillary dissection	671	40.2
Chemotherapy	714	42.8
Metastases	89	5.3
Lymphovascular invasion	174	10.4
IDCA	1481	88.8
ILCA	196	11.8

^a Includes all patients with positive axillary nodes, SLN positive, and those in whom the SLN was not found and a complete axillary dissection performed

IDCA invasive ductal carcinoma, ILCA invasive lobular carcinoma, SLN sentinel lymph node, RLN regional lymph nodes

$P = .024$. Results from the model indicated the odds of systemic metastasis were 2.2 times higher among patients who were RLN positive and who had LVI compared with those with negative RLNs and LVI positive. For tumor grade, grade 3 was used as the reference group since only 1 patient with a grade 1 tumor had systemic metastasis. The odds of systemic metastasis among patients with grade 1 tumors were only 0.045 (95% CI: 0.006, 0.337). Patients with grade 2 tumors were approximately 59% less likely to have systemic metastasis compared with patients with grade 3 tumors. There was a significant difference in the odds of having systemic metastasis between patients with tumors >2 cm versus tumors <1 cm (aOR: 2.5; 95% CI:

TABLE 2 Univariate logistic regression results for RLN positive models

Variable	RLN positive (N = 424)	RLN negative (N = 1244)	Odds ratio (95% CI)	P value
HER-2-neu				
Positive	64 (19%)	141 (13%)	1.52 (1.14, 2.03)	.004
Negative	272 (81%)	913 (87%)		
ER				
Positive	326 (77%)	975 (79%)	0.92 (0.79, 1.06)	.235
Negative	95 (23%)	260 (21%)		
PR				
Positive	302 (72%)	905 (73%)	0.92 (0.75, 1.13)	.408
Negative	120 (28%)	330 (27%)		
Tumor size				
<1	32 (8%)	412 (33%)	Reference	
1–2	201 (48%)	584 (47%)	4.41 (2.97, 6.54)	<.0001
≥ 2	188 (45%)	244 (20%)	9.97 (6.69, 14.86)	<.0001
Grade				
1	53 (13%)	349 (28%)	Reference	
2	205 (49%)	554 (45%)	2.44 (1.56, 3.80)	<.0001
3	161 (38%)	332 (27%)	3.19 (2.46, 4.14)	<.0001
Age				
<50	102 (24%)	227 (18%)	0.71 (0.54, 0.92)	.0099
≥ 50	322 (76%)	1014 (82%)		
Lymphovascular invasion				
Positive	114 (27%)	60 (5%)	7.20 (6.01, 8.63)	<.0001
Negative	310 (73%)	1174 (95%)		

ER estrogen receptor, PR progesterone receptor, RLN regional lymph nodes

TABLE 3 Multivariable model with tumor size, ER, and HER-2-neu by LVI interaction and the relationship to the RLN

Variable	Odds ratio (95% CI)	P value
Tumor size		
<1	Reference group	
1–2	4.52 (3.54, 5.78)	<.0001
≥ 2	8.75 (6.65, 11.5)	<.0001
ER: positive vs. negative	1.28 (1.08, 1.53)	.0048
HER-2-neu*Lymphatic invasion		
HER2 ⁺ vs. HER2 ⁻ : LVI ⁺	4.09 (1.36, 12.3)	.012
HER2 ⁺ vs. HER2 ⁻ : LVI ⁻	1.38 (1.03, 1.83)	.029
LVI ⁺ vs. LVI ⁻ : HERer2 ⁺	14.0 (4.63, 42.6)	<.0001
LVI ⁺ vs. LVI ⁻ : HER2 ⁻	4.72 (3.67, 6.08)	<.0001

1.00, 6.32; $P = .05$). Patients with tumor size 1–2 cm were approximately 53% less likely to have metastasis compared with patients with tumors >2 cm (OR: 0.47; 95% CI: 0.28, 0.81; $P = .007$). The c-statistic for this model (Table 5) is 0.796.

TABLE 4 Univariate logistic regression results for prediction of systemic metastasis

Variable	No. metastasis (<i>N</i> = 1576)	Metastasis (<i>N</i> = 89)	<i>P</i> value
RLN positive	377 (24%)	46 (52%)	<.001
HER-2-neu positive	190 (14%)	15 (22%)	.086
ER positive	1246 (80%)	52 (59%)	<.001
PR positive	1159 (74%)	46 (52%)	<.001
Grade			
1	400 (26%)	2 (2%)	<.001
2	722 (46%)	34 (38%)	
3	440 (28%)	53 (60%)	
Age ≥50	1268 (81%)	66 (74%)	.142
LVI	147 (9%)	26 (30%)	<.001
Tumor size			
<1	433 (28%)	8 (9%)	<.001
1–2	748 (48%)	29 (33%)	
>2	375 (24%)	51 (58%)	

TABLE 5 Final logistic regression model for prediction of systemic metastasis

Comparison	OR	95% CI lower bound	95% CI upper bound	<i>P</i> value
RLN	1.89	1.16	3.08	.011
LVI	1.87	1.08	3.22	.024
Grade 1 vs. 3	0.078	0.018	0.33	.005
Grade 2 vs. 3	0.479	0.30	0.76	.002
Tumor group 2 vs. 1	1.21	0.54	2.74	.645
Tumor group 3 vs. 1	2.82	1.26	6.32	.012

The association between LVI, RLN positive, and systemic metastasis was tested using Fisher's exact test (Tables 6, 7). There was no association between LVI and systemic metastases for RLN negative patients ($P = .137$). Among RLN positive patients, systemic metastasis was significantly ($P = .0013$) higher among patients with LVI (19%) compared with LVI negative patients (6.8%). We included 423 RLN positive for this analysis because we had 1 RLN positive patients with missing LVI data.

Figures 2, 3, 4, 5 show Kaplan-Meier constructs of systemic disease and the interaction of LVI and RLN. Figure 2 shows a significant ($P < .0001$) increase in systemic metastases in patients with both LVI and RLN positive compared with those with neither LVI or RLN metastases. Figure 3 shows insignificant (.246) differences in systemic metastasis for those patients who had negative RLNs, whether or not their tumors showed LVI. Figure 4 shows patients whose tumors exhibited LVI; those with positive RLNs had significantly ($P = .023$) more systemic

TABLE 6 LVI and systemic metastases for RLN negative patients

Lymphatic invasion	Metastases		Total	<i>P</i> value
	No	Yes		
No	1137 (96.8)	38 (3.2)	1175	.137
Yes	55 (93.2)	4 (6.8)	59	

TABLE 7 LVI and systemic metastases for RLN positive patients

Lymphatic invasion	Metastases		Total	<i>P</i> value
	No	Yes		
No	285 (92.2)	24 (7.8)	309	.0013
Yes	92 (80.7)	22 (19.3)	114	

Probability of Metastasis **FIG. 2** Kaplan-Meier plots of systemic metastases comparing RLN negative/LVI negative to RLN positive/LVI positive ($P < .0001$)**Probability of Metastasis** **FIG. 3** Kaplan-Meier plots of systemic metastases comparing RLN negative/LVI negative to RLN negative/LVI positive ($P = .246$)

FIG. 4 Kaplan-Meier plots of systemic metastases comparing RLN positive/LVI positive to RLN negative/LVI positive ($P = .023$)

FIG. 5 Kaplan-Meier plots of systemic metastases comparing RLN positive/LVI negative to RLN positive/LVI positive ($P = .0018$)

metastases compared with those with negative RLNs. Figure 5 compares those patients with RLN metastases in which one group had LVI and the other did not, showing a significantly ($P = .0018$) higher incidence of systemic metastases in those with both RLN and LVI positive.

DISCUSSION

Tumor invasion into endothelial-lined vascular spaces, called lymphovascular invasion (LVI) by pathologists, was significantly associated with both regional lymph node (RLN) and systemic metastases in this study of 1668 clinically N0 breast cancer patients offered sentinel lymph node biopsy and/or complete ALND and followed for 1–14 years (median 3.62 years). Analysis of the data also showed that systemic metastasis in the presence of LVI required metastasis to the RLNs for the association to be

true, supporting the likelihood that the invaded vessels were lymphatics rather than blood vessels.

Systemic breast cancer metastasis has been extensively studied preclinically, clinically, and in vitro.^{1,4,10} Extensive work in the metastatic cascade yielded much information about the key steps in the process.^{1,18} Tumor cells invade breast parenchymal tissue, a process requiring proteolysis, adhesion receptors, and directional motility, but little is known about the initial steps whereby the cells interact with tumoral or peritumoral vessels and gain access to the systemic circulation.^{19,20}

While tumors can enter the blood directly by intravasating into blood vessel capillaries or indirectly via lymphatics the relative contributions of both pathways as routes of egress from the primary site is still debated.⁴ Direct observation by videomicroscopy in an animal tumor model of preferential tumor cell invasion into peritumoral lymphatics has yet to be demonstrated in human breast cancer.²¹ Highly specific lymphatic endothelial immunohistochemical stains allowed direct pathological observation that BC invaded lymphatics in close to 100% of cases when LVI was present.¹³ Other clinical and pre-clinical studies showed a correlation between sentinel node metastasis and lymphangiogenesis, stimulated by VEGF-A, C, and D in the tumor microenvironment, suggesting a higher likelihood of lymphatic invasion with a larger surface area of lymphatic endothelium.^{10,22–24} Clinical and animal epithelium-derived tumors usually invaded lymphatics and metastasized first to the RLNs.^{10,24–26}

Physiologic and pathophysiologic studies provide further indirect evidence that tumor cells are more likely to invade peritumoral lymphatics than blood vessels. Lymphatic capillaries lack interendothelial tight junctions, pericytes, and basement membranes seen in blood vessels.^{27,28} High intratumoral interstitial fluid pressure (IFP), caused by abnormal structure and function of blood and lymphatic vessels in tumors, results in significant fluid convection near the tumor margin, creating peritumoral edema, transport of tumor-derived molecules and cells that generate lymphangiogenesis and angiogenesis, and increased lymphatic flow.^{21,27–31} Interstitial hypertension compresses blood vessels but, because of anchoring filaments that tether LECs to surrounding stromal tissues, and overlapping endothelial cells that open up gaps in the lymphatic capillary wall, lymphatic lumina may remain open despite the increased pressure and mechanically enhance the intravasation of fluid and tumor cells.^{28,32} The low-shear lymphatic system is also less likely to cause mechanical destruction of tumor cells than the high-shear blood circulation.¹⁸

Some of the earlier studies in the mechanisms involved in lymph node metastasis focused heavily on the physiology and pathophysiology of the RLN. The possible

“barrier function” of RLNs in cancer was addressed more than 40 years ago, showing a short transit time of cells through nodes and a suggestion that there was a very close association between lymphatics and blood vessels.³³ It seemed likely that the majority of tumor cells traversing the lymph nodes either entered the efferent lymphatic or the venous system through lymphaticovenous communications. Some studies showed direct lymphaticovenous connection in lymph nodes using air or bacteria, radioactive chromium, and radio-opaque contrast material in patients.^{34–36} Willis, upon careful pathological evaluation of patients, suggested that hematologic dissemination of tumors occurred via lymphatic tributaries to veins in RLNs.³⁷ Other studies failed to show such a connection using radiolabeled erythrocytes.³⁸ Recent experiments demonstrate tumor cytokine-induced sentinel lymph node angiogenesis and lymphangiogenesis, suggesting an anatomic pathway for tumor cells in the lymph node to migrate from lymphatics through adjacent dilated high-endothelial venules, although this pathway has not yet been documented directly.^{23,39}

A direct association between lymphatic and hematogenous metastasis has been noted in many clinical studies.^{40–42} Although it is difficult to conclude the order of events with certainty, the common observation in all these studies points to the likelihood that RLN metastasis occurs before hematogenous metastasis. The orderly progression of common cancers through the lymphatic system to the blood stream has also been described in animal tumor models.^{10,25}

Intramammary lymphatic tumor emboli were strongly correlated with RLN and systemic metastasis in breast cancer patients when regular sections were stained with hematoxylin and eosin.⁸ The incidence of blood vessel invasion was reported in 4.7%, while others reported this phenomenon in 71% of patients.^{19,43} The conclusions from a number of investigators in the 1960s and 1970s clearly demonstrate a strong belief that systemic metastases occurred when intramammary blood vessels were invaded; invasion of intramammary lymphatics was thought to be associated with RLN metastasis but not necessarily with systemic metastases.^{44,45} Modern studies acknowledge the difficulty in distinguishing blood and lymph vessels. The availability of immunohistochemical techniques that specifically stain lymphatics enabled the pathologists in one study to confidently identify vascular invasion to be lymphatic in 96.4% of cases; they also found a significant correlation with RLN metastasis and lymphatic invasion.¹³ Our study, while it did not pathologically identify LVI as lymphatic, certainly found the same clinical association with presumed lymphatic invasion and both RLN and systemic metastases.

It is reasonable to assume that all the RLN-positive patients had LVI, but in those patients we only found 174

of 423 (41%) with LVI. This probably represents a sampling error; if every micron of the tumor were evaluated LVI would likely be found in a much higher number. Similarly, in those patients whose primary tumors exhibited LVI but failed to show RLN metastasis we could expect that a higher proportion of positive nodes would be found if more than a small fraction of the node were evaluated. Routine histologic examination of axillary lymph nodes in patients with breast cancer significantly underestimates lymph node metastasis.⁴⁶ Some of the RLNs were likely truly negative since intramammary lymphatic tumor emboli likely take time in transit and may not reach the RLNs.

Hematogenous dissemination may occur without identifiable RLN metastasis. Direct blood vessel invasion may occur in some patients in whom no lymphatic invasion occurs. Patients harboring bone marrow micrometastases in the absence of RLN metastases have been reported in 20%–40% of carcinomas.⁴⁷ Experimental and clinical studies in other tumor types have shown mostly apoptotic or dead tumor cells shedding into the venous effluent of tumors.^{9,48} Proof of truly independent RLN and hematogenous metastases requires a genomic approach that has not yet been reported.

The analysis presented in this paper does not include patients with clinically and/or radiologically identified axillary lymph node positive cases diagnosed preoperatively. Patients with more advanced locoregional disease than the cases studied here are more likely to have systemic metastases.^{5,12,26} Because such advanced cases would have progressed through the same stages as the patients presented in this paper, it is unlikely that the addition of those patients would alter our conclusions.

Our analysis does not directly contradict the paradigms currently used for surgical treatment of breast cancer. The continued debate about the therapeutic efficacy of complete axillary lymphadenectomy for sentinel node positive breast cancer can only be resolved by carefully planned and executed randomized prospective clinical studies such as the elegantly designed but incomplete ACOSOG Z0011 study.

In summary, our study clearly supports the Nottingham group who provided direct histologic evidence in human breast cancer that LVI is predominantly (>95% of cases) lymphatic invasion.¹³ Our data showed that LVI is a predictor of both RLN and systemic metastasis. The likelihood of systemic metastasis was extremely small and statistically insignificant when LVI was observed and where no RLN metastasis was found. RLN metastasis was an independent predictor of systemic metastasis and the likelihood of metastasis was higher and statistically significant when both LVI and RLN metastasis occurred together, particularly when the tumor overexpressed HER-2-neu. This

information could prove valuable as a preoperative predictor of systemic disease in patients with breast cancer who also have RLN metastasis.

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