Featured Article

High LYVE-1–Positive Lymphatic Vessel Numbers Are Associated with Poor Outcome in Breast Cancer

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ABSTRACT

Purpose: The clinical significance of intratumoral or peritumoral lymph vessel density is not known. LYVE-1, a lymphatic endothelium-specific hyaluronan receptor, is a novel lymphatic vessel marker that is expressed on lymph vessel endothelial cells of both normal and neoplastic tissues.

Experimental Design: We investigated expression of LYVE-1 by immunohistochemistry in 180 unilateral, invasive ductal breast carcinomas and assessed the presence and density of lymph vessels within the tumor and at the tumor periphery.

Results: A minority (12%) of breast carcinomas had intratumoral lymph vessels, whereas peritumoral lymph vessels were identified in almost all cases (94%). No substantial association was found between the number of LYVE-1–positive vessels and the number of CD31 or vascular endothelial growth factor receptor-3–positive vessels, or vascular endothelial growth factor-C expression. The number of metastatic axillary lymph nodes increased in parallel with increasing lymph vessel counts \( (P = 0.033) \). A higher than the median lymph vessel count at the tumor periphery was significantly associated with unfavorable distant disease-free survival and overall survival. Women with high peritumoral lymph vessel density had only 58% (95% confidence interval, 46–70%) 5-year distant disease-free survival as compared with 74% (66–83%) among those with a low peritumoral lymph vessel density \( (P = 0.0088) \). In contrast, the presence of intratumoral lymph vessels was associated with neither axillary nodal status nor survival. Lymph vessel density was not an independent prognostic factor in a multivariate survival analysis.

Conclusions: A high peritumoral lymph vessel density is associated with a poor outcome in ductal breast cancer.

INTRODUCTION

Lymphatic vessel endothelium expresses several lymphatic marker molecules (reviewed in refs. 1 and 2). These include LYVE-1, the lymphatic vessel endothelial hyaluronan receptor-1 (3); Prox1, a homeobox transcription factor involved in the sprouting of lymphatic vessels from embryonic veins during development (4); podoplanin, glomerular podocyte membrane mucoprotein expressed on the endothelium of lymphatic capillaries but not on blood vascular endothelium (5); the \( \beta \)-chemokine receptor D6 (6); and the macrophage mannose receptor (7). Vascular endothelial growth factor receptor (VEGFR)-3 is expressed on the lymphatic vessels of the normal tissues, but it lacks lymphatic vessel specificity in human cancer, because tumor blood vessels may also express VEGFR-3 (8). LYVE-1, a hyaluronan receptor related to CD44, is expressed on lymph vessel endothelial cells of both normal and neoplastic tissues and on both the luminal and abluminal surfaces of the lymphatic endothelial cells (3, 9). The precise function of LYVE-1 remains unknown. However, it is likely to play a role in either hyaluronan homeostasis or in the regulation of cellular trafficking to the lymph nodes (10).

A primary tumor may give rise to lymph node metastases via several mechanisms. These include cancer invasion into intratumoral lymph vessels, invasion of cancer into pre-existing lymphatics located at the tumor periphery, or tumor cell-induced growth of new lymphatics (1, 2). The relative importance of these individual mechanisms is not known, and it may vary in different histologic types of cancer (1, 2). The presence of proliferating intratumoral lymphatics has been reported recently in head and neck cancer, in melanoma, and in papillary thyroid carcinoma (11–14), and it was associated with a poor outcome in these cancers (13, 15). The “functionality” of intratumoral lymph vessels has been disputed, because they fail to take up injected fluorescent dyes. Such arguments have given rise to the view that peritumoral lymphatic vessels may be of a greater importance in the genesis of regional lymph node metastases than intratumoral lymph vessels (16).

Regional lymph node metastases are common even in small breast cancers. In rodent breast cancer models, VEGF-C overexpressing breast cancers showed increased intratumoral lymphangiogenesis after orthotopic transplantation into nude mice. In these models, a high density of lymph vessels expressing LYVE-1 is associated with a high frequency of regional lymph node metastases (17, 18). A recent study on human breast cancer found no evidence for lymphangiogenesis, and the tu-
mors seemed to invade and destroy lymphatic vessels rather than promote their proliferation. Furthermore, the majority of lymph vessels were located at the tumor periphery and only few, if any, invasive breast carcinomas had intratumoral lymph vessels (19). In the present study, we investigated a series of early breast cancer for the presence of both intratumoral and peritumoral lymphatics and report the clinical and prognostic significance of both vessel types.

MATERIALS AND METHODS

Patients. Women with histopathologically diagnosed unilateral, invasive ductal breast cancer treated at the Department of Surgery and Oncology, Helsinki University Hospital, from January 1987 to December 1990 were included in the study. We required a histologic specimen of ductal breast carcinoma to be available for study inclusion. We excluded patients with in situ carcinoma, distant metastases at the time of the diagnosis, synchronous or metachronous bilateral breast cancer, malignancy other than breast cancer in history (except for basal cell carcinoma or cervical carcinoma in situ), and women who did not undergo breast surgery, which left 180 patients for the analysis. The median age at the time of the diagnosis was 57 years (range, 34–89 years).

Breast surgery consisted of level II and III axillary nodal dissection in all cases. Most (72%) patients received postoperative radiotherapy, which was given to all patients treated with a breast sparing surgical procedure. Adjuvant systemic therapy was not given for women with axillary node-negative disease, whereas 36 (49%) of the patients with node-positive disease (n = 73) were treated with adjuvant antiestrogen therapy, and 14 (19%) were treated with adjuvant chemotherapy (of whom two patients also received endocrine therapy). The patients were followed up at 6-month intervals in an outpatient department for the first 5 years after the diagnosis and then annually. The median duration of follow-up of the patients still alive was 10.1 years.

Immunostaining for LYVE-1, CD31, VEGFR-3, and Vessel Counts. Rabbit polyclonal antibera specific for human LYVE-1 was prepared and purified as described elsewhere (3). In brief, anti-LYVE-1 antibody was generated against a soluble recombinant LYVE-1 construct that comprised the extracellular domain fused to the hinge, CH2 and CH3 domains of human recombinant LYVE-1 construct that comprised the extracellular domain fused to the hinge, CH2 and CH3 domains of human IgG1, and affinity-purified on columns containing recombinant human LYVE-1. The anti-LYVE-1 antibody detects lymphatic vessels in histologically normal tissues and tumor tissues as shown elsewhere (3, 11, 13). Five micrometer sections were cut from paraffin-embedded tissue blocks containing tumor and were deparaffinized in an ethanol series, after which antigen retrieval was carried out by heating the sections in Antigen Retrieval Buffer (DAKO, Copenhagen, Denmark) for 20 minutes at 90°C. The sections were incubated for 30 minutes in 0.6% H2O2 to quench the endogenous peroxidase activity. The slides were incubated overnight with the anti-LYVE-1 antibody at the concentration of 1 μg/mL at 4°C, followed by a biotin-streptavidin-horseradish peroxidase reaction (Vectastain Elite ABC kit; Vector Laboratories, Inc., Burlingame, CA). Diaminobenzidine was used as the chromogen. The slides were counterstained with H&E and mounted. The staining method was reproducible when repeated several times on eight of the samples.

A mouse CD31 monoclonal antibody (Novocastra Laboratories Ltd., Newcastle upon Tyne, United Kingdom) was used as a pan-endothelial marker. The Vectastain ABC kit was used to do immunostaining at the antibody concentration of 20 μg/mL. VEGFR-3 was stained with the 9D9 antibody (a mouse monoclonal antibody against the extracellular domain of human VEGFR-3; a kind gift from professor Kari Alitalo, Helsinki, Finland) at the concentration of 10 μg/mL as described in detail previously (20). The standardization and reproducibility of the anti-VEGFR-3 antibody is described elsewhere (21). The immunostaining results were reproducible when repeated several times on 10 randomly selected samples.

The number of LYVE-1, CD31, and VEGFR-3 stained vessels was assessed by two of the authors (P. B. and V-M. W.) independently from all 180 tumor sections. The immunostained sections were first scanned at a low magnification, and the areas with the greatest number of microvessels (vessel “hot spots”) were selected for further evaluation. The microvessel count was then determined by counting all immunostained vessels in two separate hot spots at a magnification of 200× using a 0.25 mm2 microscope ocular grid. The average number of LYVE-1–positive vessels in the two selected vessel hot spots was then calculated. Whenever the average number was higher than seven (the median number of LYVE-1–positive vessels) in the vessel hot spots, the cancer was considered to have a high lymphatic vessel density (n = 87), otherwise a low lymphatic vessel density (n = 93). The two investigators agreed on classification in 93% of the cases for LYVE-1 staining (κ-coefficient 0.89). Cases with disagreement in classification were jointly reviewed with a multihedead microscope, and the consensus classification was used in further analyses.

In immunostainings for CD31 and VEGFR-3, any positive cell clusters were considered as endothelial cells and countable microvessels. The two investigators agreed on classification of VEGFR-3–receptor staining (higher than the median versus lower than the median) in 93% of the cases (κ-coefficient 0.91), and in 79% of the cases in evaluation for CD31 expression (κ = 0.84). All vessel counts were assessed without knowledge of patient outcome or other clinical data.

Assessment of Tumor VEGF-C Expression. An anti-VEGF-C antibody (Santa Cruz Biotechnology, Santa Cruz, CA) was used to stain VEGF-C with immunohistochemistry at a concentration of 1 μg/mL, followed by a biotin-streptavidin-horseradish peroxidase reaction (Vectastain Elite ABC kit; Vector Laboratories). Diaminobenzidine was used as the chromogen. Antigen Retrieval Buffer (DAKO) was used to carry out antigen retrieval.

VEGF-C expression could be assessed in 128 (71%) of the 180 cases (tissue material was exhausted in the rest of the cases). Tissue macrophages express VEGF-C and were used as a positive internal control. Carcinoma VEGF-C expression was classified either as positive (n = 78, ≥10% of the carcinoma cells expressed VEGF-C) or negative (n = 50, absent expression or expression in <10% of the carcinoma cells).

Statistical Analysis. The χ2 test (2 by 2 tables) or the χ2 test for a linear trend (2 by 3 tables) was used to analyze contingency tables. Correlations between CD31, VEGFR-3, and
the vessel numbers as continuous variables were used to assess LYVE-1–positive vessel counts with the Spearman rank correlation test. Life tables were computed according to the Kaplan-Meier method. Distant disease-free survival was calculated from the date of the diagnosis to the first occurrence of distant metastases. All patients who were considered to have died from breast cancer had distant metastases diagnosed before death. Overall survival was calculated from the date of the diagnosis to the date of death. Survival curves were compared with the log-rank test. Multivariate survival analysis was done with the Cox proportional hazards model entering the following covariates: the number of axillary nodal metastases (0 versus 1–3 versus ≥3), the primary tumor size (≤20 mm versus >20 mm), the lymphatic vessel count (low versus high), and histologic grade (well differentiated versus moderately to poorly differentiated). All \( P \) values are two-tailed.

RESULTS

LYVE-1, CD34, VEGFR-3, and VEGF-C Expression in Breast Cancer. LYVE-1 expression was mainly present in thin-walled (lymphatic) structures and in tissue macrophages. Most of the LYVE-1–positive vessels were located in the peritumoral area or at the tumor edge. LYVE-1–positive vessels were detectable within the tumor in 21 (12%) of the 180 carcinomas investigated, suggesting that intratumoral lymphatic vessels may be present in breast cancer (Fig. 1). In contrast to LYVE-1–positive vessels, CD31- and VEGFR-3–positive vessels were found intratumorally, at the tumor edge, and in the peritumoral rim in the great majority of cases. These additional structures most likely represent tumor blood vessels.

The median number of LYVE-1–positive vessels was 7 (range, from 0–50), and only 12 (6%) tumors were devoid of LYVE-1–positive vessels. The median number of CD31 and VEGFR-3–positive vessels was 47 (range, 20–178) and 24 (range, 0–80), respectively. No substantial association was found between the number of LYVE-1–positive vessels and CD31 or VEGFR-3–positive vessels (\( n = 50 \); the Spearman rank correlation coefficient 0.05, \( P = 0.77 \); 0.072, \( P = 0.65 \), respectively). In contrast, high counts of VEGFR-3–positive vessels were strongly associated with high CD31-positive vessel counts supporting the hypothesis that most of the VEGFR-3 vessels are blood vessels and not lymph vessels (coefficient 0.42, \( P = 0.0084 \)).

A majority (69%) of the tumors expressed VEGF-C protein. VEGF-C protein was expressed in carcinoma cells and

![Fig. 1 Examples of immunostaining for LYVE-1 and VEGF-C proteins in invasive ductal carcinoma. A, a few LYVE-1–positive lymphatic vessels are present at the tumor periphery. B, several CD31-positive vessels are found in the same tumor. C, a section containing a lymph vessel that stains for LYVE-1 and that is surrounded by tumor cells and appears as an intratumoral structure (arrows). The endothelium of the lymph vessel is intact, and no invading cancer cells are present in the vessel lumen. D, LYVE-1–positive vessels located at the tumor (T) periphery. E, VEGF-C expression in carcinoma cells. F, negative immunostaining for VEGF-C. Magnification ×100 (A, B), ×200 (E, F), and ×400 (C, D).](https://clincancerres.aacrjournals.org/issue)
(In many samples VEGF-C was expressed only at the tumor periphery. In cases where coexistent ductal carcinoma in situ was present, the intraductal component frequently expressed VEGF-C. No association was found between tumor VEGF-C and LYVE-1 expression (P = 0.71).

**Associations between the LYVE-1–Expressing Vessel Counts, Tumor VEGF-C Expression, and Clinicopathological Parameters.** The number of metastatic axillary lymph nodes increased with an increasing LYVE-1–positive vessel density at the tumor periphery (P = 0.033; Spearman rank correlation coefficient 0.16). When both the LYVE-1–expressing vessel count and the number of axillary lymph node metastases were treated as categorical variables (low versus high, and 0 versus 1–3 versus >3 metastases, respectively), 59% of the women with at least four positive axillary lymph nodes had a high peritumoral LYVE-1–positive vessel count as compared with 54% of those with 1 to 3 metastatic axillary nodes and 43% of those with no metastatic nodes (P for a linear trend of 0.081). Similarly, patients with axillary node-positive cancer (pN+) tended to have more often high peritumoral lymphatic vessel density than those with node-negative disease (pN0, P = 0.083).

No association was found between the lymph vessel density and age at diagnosis, the primary tumor size, histologic grade, hormone receptor status, HER-2 expression, Ki-67 antigen expression, or p53 expression (P > 0.1 for all analyses, data not shown). Intratumoral LYVE-1 lymph vessel positivity was associated with a poor histologic grade of differentiation (grade III; P = 0.010) but not with any other of the factors listed above (P > 0.1 for all analyses). Tumor VEGF-C expression was associated with a poor histologic grade of differentiation (P = 0.0044) but not significantly with the axillary lymph node status (P = 0.15) or any of the other factors listed above (P > 0.10 for all analyses).

**Survival.** A high LYVE-1–positive vessel count was associated with unfavorable distant disease-free and overall survival (P = 0.0088 and 0.013, respectively). The 5-year distant disease-free survival was 58% [95% confidence interval (CI), 46–70%] among women who had a breast cancer that had a high tumor lymph vessel density, and 74% (95% CI, 65–83%) when the density was low (Fig. 2). In addition to a high lymph vessel density, the presence of axillary nodal metastases, a large primary tumor size, and a poor histologic grade of differentiation were associated with unfavorable 5-year distant disease-free survival in a univariate analysis (P = 0.0003, 0.0016, and 0.049, respectively), whereas the estrogen or progesterone receptor status, Ki-67 antigen expression, age at diagnosis, and p53 expression were not (P > 0.30 for each analysis). The presence of intratumoral lymph vessels as determined by immunostaining for LYVE-1 expression was not associated with unfavorable distant disease-free or overall survival (P = 0.39 and 0.25, respectively). In subgroup analyses, a high tumor lymph vessel density was associated with a poor outcome in axillary nodal–positive cancer and also in several other subgroups investigated (Fig. 2, Table 1). In the subgroup of patients treated with adjuvant systemic therapy (n = 48), women with cancer with a higher than the median lymph vessel density had poorer distant disease-free survival than those with a lower than the median density (5-year distant disease-free survival 21% versus 65%, respectively, P = 0.0001), but no significant difference in disease-free survival was found among those who did not receive adjuvant therapy (n = 132, 5-year distant disease-free survival 72% versus 77%, P = 0.38).

To investigate whether a high LYVE-1 vessel count has independent influence on distant disease-free survival, it was
entered into a multivariate survival analysis together with the factors that were significantly associated with outcome in univariate survival analyses. In the multivariate analysis, a large primary tumor size (risk ratio = 1.85; 95% CI 1.12–3.06; P = 0.016) and a high number of axillary nodal metastases (risk ratio = 1.99; 95% CI 1.20–3.29; P = 0.0079) were independently associated with unfavorable outcome, whereas histologic grade (risk ratio = 1.37; 95% CI 0.60 to 3.14; P = 0.45) and the lymph vessel density (risk ratio = 1.23; 95% CI 0.76 to 1.98; P = 0.40) were not retained in this model. The number of axillary lymph node metastases remained as the only independent prognostic factor when overall survival was used as the end point instead of distant disease-free survival (data not shown).

**DISCUSSION**

Breast cancer commonly gives rise to lymph node metastases, and the degree of locoregional lymph node involvement is one of the most important prognostic factors in this disease. The present results show that intratumoral lymph vessels may be present in early ductal breast cancer, but the density of peritumoral lymph vessels may have a far greater clinical significance. High peritumoral lymph vessel counts were associated with a high number of metastatic axillary lymph nodes at the time of the diagnosis and with poor distant disease-free and overall survival. The effect on survival was, however, limited to the subgroup of axillary node-positive breast cancer in the present series (Table 1). Intratumoral lymph vessels, in turn, had no association with the number of axillary lymph node metastases or survival, although intratumoral lymph vessels were more commonly present in poorly differentiated ductal breast cancers than in well-differentiated ones. These findings are in line with experimental data. In mice transplanted with breast cancer, cancer VEGF-C overexpression may induce intratumoral lymphangiogenesis and promote genesis of both regional lymph node metastases and distant metastases (17, 22).

A variety of putative lymphatic vessel markers as assessed from the primary tumor have shown few associations with the presence of lymphatic metastases in various histologic types of human cancer (23). Only a few studies have addressed the clinical significance of intra- and peritumoral lymph vessels in breast cancer, and no data have been available on the influence of the lymph vessel density on survival. One study found a high density of peritumoral vessels immunopositive for podoplanin to be associated with the presence of axillary lymph node metastases in a series of 45 breast carcinomas, but no such vessels were found within the tumors (24). In contrast, in another series consisting of 75 breast carcinomas, no association was found between the LYVE-1–positive vessel count and the primary tumor size, stage, or the axillary nodal status (19). In this study too, virtually no LYVE-1–positive intratumoral lymph vessels were found within the breast carcinomas. Jacqz-mier et al. (25) used a VEGFR-3–specific antibody in immunostaining of 60 breast carcinomas but found no association between the lymph vessel count and the presence of axillary lymph node metastases. However, antibodies against the VEGFR-3 tyrosine kinase discriminate poorly between the blood and lymphatic vessels in human neoplasias including breast cancer (8, 26).

The presence of intratumoral lymphatics in human breast cancer has been hotly debated (27). We observed intratumoral lymphatic structures in 12% of the breast carcinomas investigated. This figure might be viewed as a minimum proportion, because no attempt was made to section serially the tumors with no intratumoral LYVE-1–positive vessels. On the other hand, some of the peritumoral lymphatic vessels may have become trapped between infiltrating sheets of cancer cells and appear as intratumoral structures. However, intratumoral lymphatics appeared to be located randomly within the tumors suggesting the presence of true intratumoral lymph vessels. Moreover, multiple lumina containing lymph vessels that were not confined to islands of stroma formed by invaginations of normal tissue were found within the tumors. It is not known whether the intratumoral LYVE-1–positive structures are functional lymph vessels. Many of the intratumoral vessels had a lower LYVE-1–staining intensity than the peritumoral ones in the same patient suggesting that LYVE-1 expression may be down-regulated in the intratumoral lymph vessels. We did not find tumor cell emboli within the intratumoral LYVE-1–positive vessels, although carcinoma cells were occasionally found within the

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**Table 1 Five-year distant disease-free survival according to breast tumor lymphatic vessel density**

<table>
<thead>
<tr>
<th>Subgroup</th>
<th>Lymphatic vessel density</th>
<th>N (%)</th>
<th>5-year distant disease-free survival % (95% CI)</th>
<th>P</th>
</tr>
</thead>
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<tr>
<td>Node negative</td>
<td>Low 61 (57)</td>
<td>77 (66–87)</td>
<td></td>
<td>0.66</td>
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<tr>
<td></td>
<td>High 46 (43)</td>
<td>76 (64–88)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Node positive</td>
<td>Low 32 (44)</td>
<td>67 (50–84)</td>
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<td>0.050</td>
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<tr>
<td></td>
<td>High 41 (56)</td>
<td>46 (31–62)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tumor size ≤ 20 mm</td>
<td>Low 50 (52)</td>
<td>83 (72–94)</td>
<td></td>
<td>0.046</td>
</tr>
<tr>
<td></td>
<td>High 46 (48)</td>
<td>67 (53–80)</td>
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<tr>
<td>Tumor size &gt; 20 mm</td>
<td>Low 43 (51)</td>
<td>63 (48–77)</td>
<td></td>
<td>0.34</td>
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<tr>
<td></td>
<td>High 41 (49)</td>
<td>58 (42–73)</td>
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<tr>
<td>Histological grade I</td>
<td>Low 16 (53)</td>
<td>82 (62–104)</td>
<td></td>
<td>0.83</td>
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<tr>
<td></td>
<td>High 14 (47)</td>
<td>86 (67–104)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Histological grade II/III</td>
<td>Low 88 (59)</td>
<td>72 (63–81)</td>
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<td>0.026</td>
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<tr>
<td></td>
<td>High 62 (41)</td>
<td>53 (41–66)</td>
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<tr>
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<td>Low 64 (56)</td>
<td>64 (52–76)</td>
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<td></td>
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<td>65 (52–78)</td>
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<td>ER negative</td>
<td>Low 26 (48)</td>
<td>92 (83–103)</td>
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<td></td>
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<td>50 (31–69)</td>
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<td>PgR positive</td>
<td>Low 45 (54)</td>
<td>62 (48–77)</td>
<td></td>
<td>0.83</td>
</tr>
<tr>
<td></td>
<td>High 38 (46)</td>
<td>66 (51–81)</td>
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</tr>
<tr>
<td>PgR negative</td>
<td>Low 28 (48)</td>
<td>81 (67–96)</td>
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<td></td>
<td>High 30 (52)</td>
<td>52 (34–70)</td>
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<tr>
<td>Ki-67 ≤ 20%</td>
<td>Low 31 (53)</td>
<td>68 (51–85)</td>
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<tr>
<td></td>
<td>High 27 (47)</td>
<td>73 (56–90)</td>
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</tr>
<tr>
<td>Ki-67 ≥ 20%</td>
<td>Low 51 (49)</td>
<td>75 (63–87)</td>
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<td>0.021</td>
</tr>
<tr>
<td></td>
<td>High 53 (51)</td>
<td>56 (42–69)</td>
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<td></td>
</tr>
<tr>
<td>Age ≤ 50</td>
<td>Low 28 (56)</td>
<td>76 (60–93)</td>
<td></td>
<td>0.39</td>
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<tr>
<td></td>
<td>High 22 (44)</td>
<td>62 (41–83)</td>
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</tr>
<tr>
<td>Age ≥ 50</td>
<td>Low 65 (50)</td>
<td>72 (61–83)</td>
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<td></td>
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<td>63 (50–74)</td>
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<tr>
<td>p53 high</td>
<td>Low 20 (63)</td>
<td>83 (66–101)</td>
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<tr>
<td>HER-2 negative</td>
<td>Low 70 (54)</td>
<td>79 (69–89)</td>
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<td>Low 13 (46)</td>
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<tr>
<td></td>
<td>High 15 (54)</td>
<td>64 (39–89)</td>
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peritumoral vessels, which might reflect the rarity of intratumoral lymph vessels or their slower lymph flow. Lack of association between the presence of intratumoral lymphatic structures and the axillary nodal status or survival suggest that intratumoral lymph vessels may have little clinical significance in ductal breast cancer and that they may not be fully functional. However, the small number of patients with intratumoral lymph vessels prohibits making firm conclusions on their clinical significance from the present data.

The reasons why the prognostic significance of the peritumoral lymph vessel density was confined mainly to the subgroup of women with metastatic axillary lymph nodes remain speculative. Hypothetically, breast carcinomas that have a high peritumoral lymphatic network might produce high concentrations of VEGF-C and other growth factors, which might promote angiogenesis and lymphangiogenesis of distant metastases, enhancing further dissemination and growth of cancer. The present results provide, however, little support to this hypothesis, because no association was found between carcinoma VEGF-C expression and LYVE-1–positive vessel counts.

We conclude that both intratumoral and peritumoral LYVE-1–positive vessel structures may be present in ductal breast cancer. The clinical significance of the intratumoral lymph vessels seems small as compared with the peritumoral ones. A high peritumoral lymph vessel density is associated with an increased risk for distant metastases and unfavorable outcome as compared with cancers with a low density.

ACKNOWLEDGMENTS

We thank Professor Kari Alitalo, Helsinki, Finland, for kindly providing VEGFR-3 antibody, and Onerva Leivimaki for technical help.

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