LYMPHATIC AND SMALL BLOOD VESSEL DENSITY IN THE TUMOR AND PERITUMORAL TISSUE IN INVASIVE BREAST CARCINOMA OF NO SPECIAL TYPE

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Summary

Background: Peritumoral clefts are noticed in carcinomas of many organs, and were thought to be artifacts due to fixation and preparation of slides. Today they are considered to be additional marker of malignancy and the reflection of epithelial-stromal interaction. The hypothesis was that peritumoral clefts in breast carcinoma reflect changes in stromal composition and characteristics, and are related to lymphangiogenesis in tumor tissue. Materials and methods: One hunderd (59% with axillary lymph nodes metastases) invasive ductal breast carcinomas of no special type were analyzed for the presence and abundance of peritumoral clefts and immunohistochemically for CD34, vimentin, smooth muscle actin (SMA) and D2-40 (podoplanin) in tumor stroma. Results: Peritumoral clefts were found in 92% of invasive carcinomatous tissue and were absent in surrounding healthy tissue and around ducts of in situ component (with 5% do 100%. The threshold of 30% of peritumoral clefts was determined by receiver operating characteristic (ROC) analysis. The density of small lymphatic vessels in the stroma of the tumor, and outside the tumor tissue did not show significant correlation with the threshold value. In patients with axillary metastases, peritumoral lymphovascular invasion (LVI) was found twice and intratumoral LVI five times more often than in patients without axillary metastases. Neither peritumoral nor intratumoral LVI were significantly correlated with the threshold value of 30%. In patients with axillary lymph node metasases, peritumoral lymphovascular invasion (LVI) was found twice as often, and intratumoral LVI 5 times as often than in patients without axillary lymph node metastases. Conclusion: Peritumoral clefts in invasive breast carcinoma reflect stromal changes of fibroblasts and are not associated with lymphangiogenesis, that is probably caused by some other pathogenesis.

KEY WORDS: *breast, invasive ductal carcinoma, lymphangiogenesis, angiogenesis*

GUSTOĆA LIMFNIH I MALIH KRVNIH ŽILA U STROMI TUMORA I PERITUMORALNOG TKIVA U INVAZIVNOM KARCINOMU DOJKE BEZ POSEBNIH OBILJEŽJA

Sažetak

Uvod: Peritumoralne retrakcijske pukotine više se ne smatraju samo artefaktom, već dodatnim pokazateljem ponaša-nja invazivnih karcinoma mnogih organa. One predstavljaju morfološki odraz promjena u građi i sastavu tumorske strome, a možda i jedan od stadija limfangiogeneze tumora. Hipoteza istraživanja bila je da su u tkivu invazivnog duktalnog karci-noma dojke peritumoralne retrakcijske pukotine povezane s povećanom gustoćom limfnih žila što doprinosi lakšem rasapu tumorskih stanica i lošijoj prognozi. Materijal i metode: Analizirano je 100 invazivnih duktalnih karcinoma dojke u žena, 41% bez, a 59% s metastazama u limfne čvorove aksile. Osim rutinski, analizirana je imunohistokemijska izraženost CD34, vimentina, glatkomišićnog aktina (SMA) i CD2-40 (podoplanina). Rezultati: Peritumoralne pukotine su nađene u 92% kar-cinoma, a u okolnom zdravom tkivu i oko žarišta duktalnog karcinoma in situ nisu nađene. Proširenost pukotina bila je 5%

do 100%. Gustoća malih limfnih žila unutar tumora i peritumoralno nije pokazala statistički značajnu povezanost s apsolutnim postotkom peritumoralnih pukotina, kao niti s izraženošću peritumoralnih pukotina s obzirom na graničnu vrijednost od 30%. U bolesnica s metastazama peritumoralna limfovaskularna invazija (LVI) je nađena 2 puta češće, a intratumoralna LVI skoro 5 puta češće nego u bolesnica bez metastaza, što je statistički značajno različito. Ni peritumoralna, niti intratumoralna LVI nisu pokazale statistički značajnu povezanost s apsolutnim postotkom peritumoralnih pukotina, kao niti s izraženošću peritumoralnih pukotina s obzirom na graničnu vrijednost od 30%. Zaključak: U invazivnom duktalnom karcinomu dojke peritumoralne pukotine predstavljaju morfološki odraz promijenjenih osobina tumorske strome, ali nisu povezane s gustoćom malih krvnih žila kao odrazom limfangiogeneze, čija je patogeneza vjerojatno drugačija.

KLJUČNE RIJEČI: *dojka, invazivni duktalni karcinom, limfangiogeneza, angiogeneza*

INTRODUCTION

Peritumoral clefts were first described in the tissue of prostatic adenocarcinoma, but their importance has long been neglected (1,2). Some studies resulted in the discovery of an association between clefting and the more agressive tumor phenotype, as well as with shorter periods of biochemical relapse of the disease and survival in prostatic adenocarcinoma, so the authors proposed that the percentage of peritumoral clefting in prostatic adenocarcinoma tissue should be included into the diagnostic criteria (5,6). Except in prostatic adenocarcinoma, they were also noticed in other malignant tumors of the skin, breast, colon, urinary bladder and other organs where they are considered to be an additional marker of malignancy (3-6). The studies of breast carcinomas have shown that different degree of peritumoral clefts can be observed in about 60% of them, in association with the tumor diameter, histological type and grade, lymphovascular invasion, axillary lymph node metastases, worse prognosis and shorter survival. Malignant epithelial tumors are characterized by the interaction between the epithelial component and surrounding tumor stroma that has an important role in the tumor development and progression (7). The amount of peritumoral clefts also showed significant statistical correlation with lymphangiogenesis assessed by lymph vessel density, and vascular endothelial growth factor C (VEGF-C) expression in the center and periphery of the tumor (8,9). Some authors think that peritumoral clefts represent an unrecognized way of of lymphatic spread through the complex network of stromal (pre-lymphatic) channels connected to the main lymphatic system of the breast, with the stromal retraction that enhances the transition od mesenchymal cells into endothelial cells, so that peritumoral clefts might represent an early, unfinished stage of lymphocapillary invasion (8,9). In this study we examined intratumoral and peritumoral lymphatic and small blood vessel density (LVD and BVD, respectively), tumor cell invasion of lymphatic and small blood vessels and their correlation with peritumoral clefts.

MATERIAL AND METHODS

We investigated 100 samples of invasive breast carcinoma diagnosed after surgery in the period from 1998. to 2007. The tumor tissue was retrieved from the archive of Clinical Department of Pathology and Cytology 'Ljudevit Jurak' of the Sestre milosrdnice University Hospital in Zagreb, Croatia. Axillary lymph nodes metastases were diagnosed in 59% (59/100) of the cases. Besides the analysis of the original tumor samples, immunohistochemical analysis was done on routinely fixed and paraffin embedded tissue. The presence of fibroblasts and myofibroblasts was assesed using monoclonal antibodies to vimentin (monoclonal mouse; clone V9; code M 0725; dilution 1:50; Dako Epos, Danska), CD34 (monoclonal mouse; clone QBEnd 10; code M 7165; dilution 1:50; Dako Epos, Danska) and smooth muscle actin –SMA (monoclonal mouse; clone 1A4; code M 0851; dilution 1:50; Dako Epos, Danska) and lymphatic vessels were assessed with D2-40 (monoclonal mouse; clone D2-40; code M 3619; dilution 1:100; Dako Epos, Danska). Expression of CD34 was also used to verify the blood vessels, because the endothelium expresses CD34 antibody. The results were visualized by LSAB method on Dako TechMate TM automatized system for immunohistochemical staining using streptavidin immunoperoxidase protocol MSIP. Peritumoral clefts were considered clear spaces without endothelial lining

that separated tumor cells, glands and solid nests from the surrounding stroma. Any intensity of endothelial reaction of vascular structures to D2-40 was considered positive, even if the D2-40 positive cells were found only in clusters, without the obvious formation of the lumen. The number of lymphatic vessels, lymphovascular density (LVD) and the number of small blood vessels, blood vessel density (BVD) was determined in the 'hot spot' area inside the tumor tissue (intratumoral) and in the surrounding tissue (peritumoral). Intratumoral lymphatic and blood vessels were considered those localized in the stroma, between tumor cell nests, while peritumoral lymphatic channels were considered those localized in the narrow area of the normal breast tissue, up to 2 mm outside the tumor tissue. In order to determine 'hot spot' areas with greatest LVD and BVD, tissue samples were examined under the 40x magnification. The density of vessels was assessed by counting them at 5 high power fields at 400x magnification, and noted as the absolute number. Lymphovascular invasion (LVI) was defined as the findings of at least one tumor cell cluster inside the vascular channel showing undoubted both D2-40 and CD34 positivity. Vascular invasion (BVI)was defined as the findings of at least one tumor cell cluster inside the vascular channel showing only CD34 positivity. LVI and BVI was assessed on the whole surface of the tumor tissue, on slides at 40x magnification and noted as the absolute number of lymphocapillary and small blood vessel invasive foci inside the tumor and in the peritumoral area. After the assessment of peritumoral clefts, ROC (receiver operating characteristic) curve was calculated, dividing the patients into two prognostically different groups (those with and without metastases) and determined the predictive, cut-off value for the percentage of peritumoral clefts, which was 30%. According to the cut-off value the patients were grouped in 2 groups: 1 – with peritumoral clefts in ≤ 30% of the tumor surface, and 2 – with peritumoral clefts in > 30% of tumor surface (the latter was considered to be extensive findings). Except for intratumoral and peritumoral LVD and LVI, histopathological chacteristics compared with peritumoral clefts were pattern of growth (expansive vs. infiltrative), tumor diameter in milimeters, pT stage, histological grade (according to Elston and Ellis modification of Nottingham classification) (10), axillary lymph node status (pN stage), the ratio of positive and negative lymph nodes, hormone receptor and HER2/neu status assesed by immunohistochemistry, and the age of the patients. For statistical analysis Spearman coefficient of correlation, Mann-Whitney U test and Wilcoxon tests were used. For the analysis of categories of variables c2 test was used. ROC analysis was used for determination of statistically significant cut off value of the percentage of peritumoral clefts. All p values < 0,05 were considered significant. Program support IBM SPSS statistics 19.0.0.1 was used.

RESULTS

Peritumoral clefts were found in 92% (92/100) of invasive ductal carcinoma of no special type, in the range from 5% to 100%. In patients whose tumors showed extensive peritumoral clefts significant correlation with larger diameter of the tumor (p=0.009), pT stage of disease (p=0.018), presence of metastases in axillary lymph nodes (p<0.001) and the ratio of positive vs. negative lymph nodes (p<0.001), but the age of the patients, hormonal status, tumor grade, LVD in the tumor and the peritumoral area, lymphovascular and vascular invasion in the tumor and in the peritumoral area did not show correlation with the expression of peritumoral clefts. Immunohistochemical reaction to D2-40, showed intratumoral lymphatic vessels in 56% (56/100) of carcinomas, while 100% of carcinomas contained peritumoral lymphatic vessels. Intratumoral lymphatic vessels were small, linear and narrow, while peritumoral lymphatic vessels showed larger diameter and wider lumen (Figure 1). Median number of intratumoral lymphatic vessels was 2.5 vessels/5 high power fileds (HPF). Median number of peritumoral lymphatic vessels was 15 vessels/5HPF (Wilcoxon test, p<0,001) (Table 1). No significant differences were found when medians of LVD and small BVD were compared between the group of tumors containing smaller and larger number of myofibroblasts, respectively (Table 2). Lymphovascular invasion (LVI) was found inside the tumor tissue in 13% (13/100) of the cases, while peritumoral LVI was found in 43% (43/100) of the cases (Wilcoxon test, p<0.001). In the tumor stroma the number of lymphatic vessels containing tumor thrombi ranged from 1 to 30, and outside the tumor the number of vessels con-
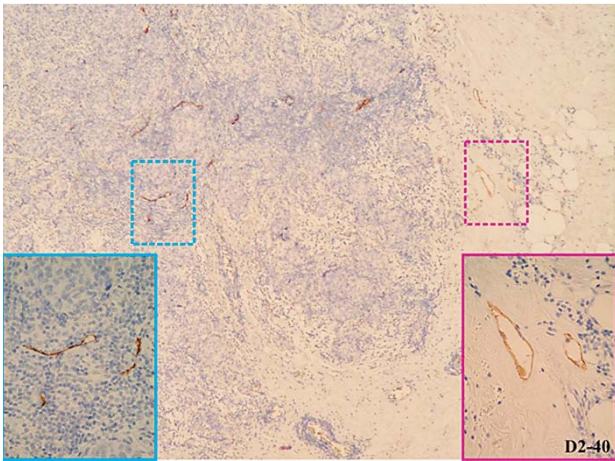
Figure 1. Intratumoral lymphatic vessels are mostly small and narrow (bottom left, D2-40 x 200), while peritumoral lymphatic vessels show larger diameter and widely opened lumen (bottom right, D2-40 x 200).

taining tumor thrombi ranged from 1 to 37 (Figure 2). (Wilcoxon test, p<0.001). Out of 13 patients with intratumoral LVI, 11 had metastases in axillary lymph nodes. Out of 43 patients with peritumoral LVI, 32 had metastases in axillary lymph nodes. In 41 patients axillary metastases intratumoral LVI was found in 4% (2/41), and peritumoral in 27% (11/41). In 59 patients with axillary metastases intratumoral LVI was found in 19% (11/59), and peritumoral in 54% (32/59) of breast carcinomas (p<0.001).

Small blood vessels highlighted by CD34 expression were found both in the intra and peritumoral stroma in all 100 cases (100%) (Figure 3). The median of the number of small blood vessels was 89 vessels/5HPF, and in the peritumoral area, median of BVD was 38.5 vessels/5HPF (Wilcoxon test, p<0.001) (Table 3). Vascular invasion was found in only 1/100 cases in the tumor tissue, and in 3/100 cases in the peritumoral area. In tumors
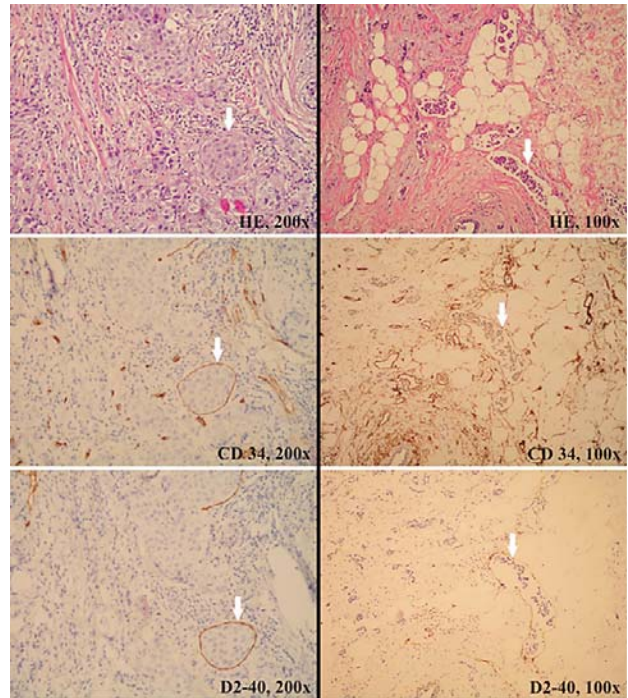

Figure 2. Lymphovascular invasion (tumor thrombi). White arrows point towards the tumor thrombus inside the lymphatic vessels that show positivity for CD34 and D2-40. Left intratumoral; right peritumoral (from top to bottom: HE, CD34, D2-40)
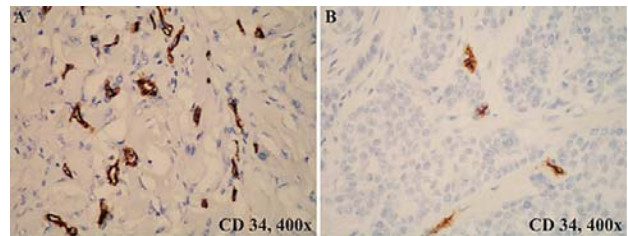

Figure 3. Comparison of two 'hot spots' in invasive ductal breast carcinoma, left with higher CD34 positive blood vessel density, and right with lower CD34 positive blood vessel density.

Table 1.

NUMBER OF SMALL INTRATUMORAL AND PERITUMORAL LYMPHATIC VESSELS IN 100 INVASIVE DUCTAL BREAST CARCINOMAS OF NO SPECIAL TYPE

Quantitative parameter	N	Min	Max	Percentile		
				25.	Median	75.
Intratumoral small lymphatic vessel density (on 5 HPF)	100	0.00	31.00	0,00	2.50	8.00
Peritumoral small lymphatic vessel density (on 5 HPF)	100	3.00	38.00	9,00	15.00*	2000

*Wilcoxon test, p<0.001

Table 2.

DENSITY OF INTRATUMORAL AND PERITUMORAL SMALL LYMPHATIC AND BLOOD VESSELS, AND LYMPHOVASCULAR AND VASCULAR INVASION IN RELATION TO THE INTENSITY OF SMA EXPRESSION IN THE STROMA OF 100 INVASIVE DUCTAL BREAST CARCINOMAS OF NO SPECIAL TYPE (N.S.)

Quantitative parameter	Intesity of stromal SMA expression	N	Min	Max	Percentile		
					25.	Median	75.
Intratumoral small lymph vessel density (on 5 HPF)	Weak	43	0	31	0.00	2.00	7.00
	Strong	57	0	22	0.00	3.00	8.00
Peritumoral small lymph vessel density (on 5 HPF)	Weak	43	3	38	9.00	17.00	21.00
	Strong	57	3	28	10.50	15.00	20.00
Intratumoral lymphovascular invasion	Weak	43	0	30	0.00	0.00	0.00
	Strong	57	0	3	0.00	0.00	0.00
Peritumoral lymphovascular invasion	Weak	43	0	20	0.00	0.00	1.00
	Strong	57	0	37	0.00	0.00	2.00
Intratumoral small blood vessel density (on 5 HPF)	Weak	43	32	239	56.00	84.00	109.00
	Strong	57	34	208	61.50	92.00	127.00
Peritumoral small blood vessel density (on 5 HPF)	Weak	43	15	107	27.00	35.00	47.00
	Strong	57	14	86	31.50	41.00	52.00
Intratumoral vascular invasion	Weak	43	0	1	0.00	0.00	0.00
	Strong	57	0	0	0.00	0.00	0.00
Peritumoral vascular invasion	Weak	43	0	5	0.00	0.00	0.00
	Strong	57	0	1	0.00	0.00	0.00

Table 3.

THE NUMBER OF SMALL INTRATUMORAL AND PERITUMORAL BLOOD VESSELS IN 100 INVASIVE DUCTAL BREAST CARCINOMAS OF NO SPECIAL TYPE.

Quantitative parameter	N	Min	Max	Percentile		
				25.	Median	75.
Intratumoral small blood vessel density (on 5 HPF)	100	32.00	239.00	60.00	**89.00***	122.50
Peritumoral small blood vessel density (on 5 HPF)	100	14.00	107.00	29.25	**38.50**	49.75

* Wilcoxon test p>0.001

with expansile pattern of growth (39%) median number of intratumoral lymphatic vessels vas found to be 1/5 HPF, while in tumors with infiltrative pattern of growth (61%) this number was 4/5HPF (p<0,001).

DISCUSSION

The increase of small blood vessel density due to neoangiogenesis in breast carcinoma tissue is clearly related to the development of distant metastases (11-13). So far the studies have not shown the correlation of neoangiogenesis with regional lymph node metastases, and lymphatic vessels are still considered to be the primary way

of carcinoma cells spreading into the regional lymph nodes (11-13).

Until approximately a decade ago, there were no reliable markers to differentiate between small blood and small lymphatic vessels, resulting in relatively small number of studies of lymphangiogenesis. The discovery od specific lymph vessel marker such as D2-40 (podoplanin) facilitated the differentiation between small lymphatic and small blood vessels (14,15). Earlier studies showed that the intratumoral BVD is a reliable sign of tumor induced angiogenesis, so it was expected that the quantitative analysis of intratumoral and peritumoral LVD in comparison to LVD in the healthy breast tissue might shed some light on the questions about lymphangiogenesis in breast carcino-

ma (15). However, the results of the studies are different. Agarwal and co. used D2-40 to study the density of intra and peritumoral lymphatic vessels and described significantly smaller intra and peritumoral LVD in breast carcinoma than in the healthy breast tissue, benign breast lesions and carcinoma in situ (16). Other authors described that the intratumoral LVD determined by D2-40 did not differ significantly from the density in the healthy breast tissue (17). Several authors noticed lymphatic vessels only in the vicinity of the preexisting ducts and lobules, and thought that they were the remains of normal lymphatic vessels entrapped within the tumor tissue (16, 17). Because the majority of small blood vessels within the tumor were not accompanied by lymphatic vessels, it was accepted that the invasion by tumor cells destroys lymphatic vessels and that the peritumoral blood vessels suffice for the development of lymphogenous metastases (18). More recent studies are more in favor of the process of lymphangiogenesis, describing the possible mechanism of its development (19-21).

Lymphangiogenic factors, secreted by malignant epithelial, but also stromal cells, like vascular endothelial growth factor-C (VEGF-C) and vascular endothelial growth factor D (VEGF-D) were discovered (22-24). The pathogenesis of their action is thought to be the activation of vascular endothelial growth factor receptor-3 (VEGFR-3) (22,23). Some tried to resolve the question of active formation of new lymphatic vessels by double immunohistochemical staining for D2-40 and proliferation marker Ki-67, again with the conclusion that there is no lymphangiogenesis in breast carcinoma tissue (16). The use of D2-40 enabled some authors to visualize intratumoral lymphatic vessels in 80 to 85% of carcinomas, while van der Schaft and co. found them in only 12 % of carcinomas (25-26). The differences between the number of lymphatic vessels that were found in different studies might be explained by the different methodologies of counting, like different locations where they were counted within the tumor tissue and different magnifications at counting. In this study the endothelium of lymphatic vessels was also visualised with D2-40 antibody (podoplanin) and they were found in intratumoral stroma in 56% of invasive ductal breast carcinomas. Intratumoral lymphatic vessels were mostly small and linear with the narrow lumen. They appeared to be squeezed from the outside by the malignant epithelial cells, and on the routinely H-E stained sections they were inconspicuous. The median of intratumoral lymphatic vessels in this study was 2,5 vessels/5HPF, while in some other studies the range was very wide: from 0,3 to 9,7 (16,17,26). In spite of the diagnostic value and usefullness of D2-40 as a lymphatic vessel marker, the identification of the relatively small number of intratumoral lymphatic vessels is not easy and their distribution within the tumor tissue is heterogeneous, so many of them might not be present at the very slide containing the sample of tumor tissue used for immunohistochemical analysis. Van der Auwera and co. stress that the intratumoral LVD depends upon the tumor pattern of growth, because they found less lymphatic vessels in carcinomas showing expansile pattern of growth than in carcinoma with infiltrative pattern of growth. Carcinomas with expansile growth are relatively well circumscribed, made of malignant epithelial cell and desmoplastic tumor stroma and do not contain normal breast tissue within the tumor mass, while in carcinomas with infiltrative growth rows of tumor cells infiltrate between the preexisting breast structures that contain also the lymphatic vessels. In their opinion, the findings of lymphatic vessels in carcinomas with expansile pattern confirm strongly the process of intratumoral lymphangiogenesis (27). The results of this study are similar to the results of the study by Van der Auera and co. with the median of intratumoral LVD in carcinomas with expansile growth being significantly higher than in carcinomas with infiltrative growth pattern (27). This difference is in favor of the opinion that breast carcinomas contain intratumoral lymphatic vessels that are more numerous in tumors showing infiltrative pattern of growth, but can also be found in tumors with expansile growth, in the desmoplastic stroma that shows different phenotypic characteristics than the normal breast stroma.

According to some studies intratumoral LVD is not correlated with the diameter and grade of the carcinoma, nor with the lymph node status, but other studies have found their correlation with lymphovascular invasion and metastases to axillary lymph nodes, carcinoma diameter, grade and stage of the disease (17,18). The results of this study show that intratumoral lymphatic vessels are present in some breast carcinomas, but be-

cause of their appearance, small number that depends upon the pattern of tumor growth and uncertain correlation with clinical parameters, their role in the development of regional lymph node metastases is not clear.

In this study, lymphatic vessels were found in the peritumoral tissue in all 100 cases of breast carcinoma. They were large, with widely opened lumens, and could be easily found on routinely stained slides. This finding confirms findings of other studies (17,26). The median of their density was significantly higher than inside the tumor stroma and some of them contained tumor emboli, similar to the findings of other authors (17, 18, 28). Although the opinions about the prognostic significance of intratumoral LVD differ, there seems to be an agreement about the role of peritumoral lymphatic vessels that might be sufficient for the tumor cells to reach the regional lymph nodes even in the absence of intratumoral lymphatic vessels (17-19,28,29). There is no straightforward answer to the question whether peritumoral lymphatic vessels are preexistant or are newly formed. The results of the studies that included the expression of proliferation markers are in favor of the active lymphangiogenesis, but it seems that some tumors still do not succeed in induction of new lymphatic vessels at their periphery (30-33).

Whether in preexistant or newly formed lymphatic vessels, lymphovascular invasion is defined as the presence of the tumor thrombus inside the vascular space lined by endothelium (34). In the routine H-E stained sections, LVI can easily be mistaken for a cluster of epithelial cells surrounded by a peripheral retraction cleft, and blood vessel is sometimes difficult to differentiate from the lymphatic vessel, so the main characteristic for their differentiation is the erythrocyte content in the blood vessels. Tumor thrombus can also completely fill the vessel lumen, making it difficult to discern it from the surrounding tumor cells, so LVI is lately also asessed immunohistochemically with D2-40. In their stuy, De Mascarel and co. compared the location and number of tumor thrombi in carcinomas without axillary lymph node metastases in tumor sections stained by H-E and D2-40. In sections stained by H-E tumor thrombi were found in 15% of carcinomas, away from the main tumor mass, while in the tissue stained by D2-40 they were found in 41%, they were multiple, widespread and localized nearer to the main tumor mass (34). In their study, only the presence of peritumoral thrombi had prognostic significance, the same as in other studies that showed the significant correlation of peritumoral LVI with lymph node metastases (34-36). In this study, peritumoral LVI was found twice as often in patients who had lymph node metastases than in the patients who had not, similar to the results from the literature, and stressing the importance of peritumoral LVI in tumor spreading. Intratumoral LVI was found in 4% of the tumors without lymph node metastases and in 19% of the tumors that spread to the lymph nodes, which is significant. These findings point out to the importance LVI findings with regard to the possibility of lymph node metastases and, accordingly, increased risk of recurrence and shorter survival. This finding can have a role in screening of the patients with negative axillary lymph nodes, who might benefit from adjuvant chemotherapy.

Pathophysiology of blood vessels is entirely different. Because blood vessels provide tumor tissue with oxygen and nutrients, they are associated with growth promotion and hematogenous spread. Japanese authors showed that small BVD is the independent determinant of the duration of remission and total survival (12, 37, 38). Meta analysis of 43 independent studies showed significant correlation of high density of small blood vessels with shorter survival in breast carcinoma patients, especially those with negative lymph nodes (12). As with counting of the lymphatic vessels, different results of the studies reflect difficulties and differences in counting of small blood vessels, but most authors agree that angiogenesis in breast carcinoma exists. El-Gendi and co. found the median number of small blood vessels in the tumor tissue to be 17.9 vessels/1 HPF (29). The results of this study are similar, with median number of small blood vessels in "hot spot" being 8 vessels/5 HPF. When compared with significantly smaller BVD in the peritumoral area that was 38.5/5HPF, this finding is an obvious sign of angiogenesis. The results by El-Gendi and co. do not show the significant correlation od BVD with carcinoma diameter and grade, LVI and lymph node metastases, but they showed significant correlation of BVD inside the tumor with LVD, suggesting the common developmental pathway for both angiogenesis and lymphangiogenesis (29). Although small BVD is cer-

tainly important for tumor cells survival, the clinical implications of aforementioned findings remain uncertain. As opposed to LVI, BVI with tumor cells is rarely found. In one of the earliest and largest studies of this feature, tumor invasion into the blood vessels was found in 4,2% while a more recent study reported blood vessel invasion in 1.1% of the cases (38,39). In this study, intratumoral blood vessel invasion was found in only 1/100 cases, and peritumoral blood vessel invasion was found in 3/100 cases. In some studies, both LVI and BVI are considered a bad prognostic feature, but the majority of studies showed only the correlation of LVI with shorter survival (14, 28, 36, 39,40). Acs and co. found the significant correlation of the presence of extensive peritumoral clefts with lymphangiogenesis, as measured by LVD and VEGF-C expression. (9, 41). In this study peritumoral clefts were found in 92% of tumors, with immunohistochemically proven changes of stromal cells phenotype. These changes are in accordance with the role of CAFs in tumor spread and their role in tumor stroma infiltration and metastasis. As far as is known today, this role seems to be one of the most important for tumor agressiveness (41-43). Therefore, it seems plausible that the growth factors as well as others, still not comply understood soluble mediators produced and excreted by CAFs may have a role in both angio and lymphangiogenesis in breast carcinoma.

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