Lymphatic and Blood Vessels in Male Breast Cancer

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Abstract. *Background: It is assumed that lymphatic vessels are responsible for breast cancer dissemination. Patients and Methods: In 32 male breast carcinomas we evaluated the correlation between: (i) lymphatic vessel density (LVD), distribution of podoplanin-immunostained vessels (DPV), blood vessel density (BVD), infiltration of immune cells and (ii) known clinicopathological parameters. Results: Lymphatic and blood vessels were found in 77.8% and 100% of breast carcinomas, respectively. Double-negative estrogen and progesterone receptor tumors (ER^-/PR^-) presented significantly higher LVD than ER/PR positive cases, while high-grade tumors exhibited significantly higher DPV than low-grade carcinomas. We detected significantly higher frequency of vascular invasion in high-grade and double-negative carcinomas than in low-grade and ER/PR-positive ones, respectively. Conclusion: The relationship between high number of lymphatic vessels and high tumor grade or steroid receptor negativity might confirm the hypothesis regarding the influence of lymphangiogenesis on the formation of a more aggressive phenotype in male breast cancer.*

Male breast cancer is a very rare form of malignant carcinoma and, hence, has been a subject of limited research (1, 2). Compared to female breast cancer (3), carcinomas diagnosed in males are characterised by: (i) more advanced stages of the disease, (ii) lower grade (G1 or G2), (iii) ductal histology, (iv) higher frequency of steroid receptor positivity and (v) lower frequency of human epidermal growth factor receptor 2 (HER2) over-expression (4-6).

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It is assumed that lymphatic vessels are responsible for breast cancer dissemination and formation of metastases (7). Currently, several markers of lymphatic vessels are tested. Among the ones most extensively studied are: podoplanin, vascular endothelial growth factor receptor-3 (VEGFR-3), Prox-1 and desmoplakin (8, 9). The above-mentioned molecules play multiple functions in the process of lymphangiogenesis and are not exclusively specific for endothelium of lymphatic vessels (8, 9). For example, in mammary gland, podoplanin expression is observed not only in the luminal surface of lymphatic endothelial cells but also in myoepithelial cells surrounding normal ducts (10) or myofibroblasts of tumor stroma (11-13).

In female breast cancer, correlations between known clinicopathological parameters (grade, steroid receptors expression, nodal status *etc*.) and (i) lymphatic vessel density, (ii) lymphovascular invasion (on the basis of podoplanin expression) and (iii) blood vessel density (14-23) have been tested. To the best of our knowledge, the aforementioned parameters and relations have not been studied in male breast cancer, so far.

Therefore, the aim of the present study was to assess: (i) lymphatic vessel density (LVD), distribution of podoplanin-stained vessels (DPV), blood vessels density (BVD), infiltration of immune cells and (ii) correlation between the above-mentioned markers and known clinicopathological parameters in male breast carcinomas.

Patients and Methods

Patients. Initially, 81 male patients with breast cancer who were treated at the Centre of Oncology in Krakow between 1950 and 2010 were included into the study. These cases represented 0.5% of all (17,320) patients treated for breast cancer in this period. The aforementioned group was presented by Sas-Korczynska *et al*. (24, 25). The immunohistochemical analysis was performed on tissue samples from 32 patients (treated between 1976 and 2010), which were adequate for this staining procedure (adequate amount of tissue in paraffin block, good quality of material).

The consent to perform the study was given by the Bioethics Committee of the Regional Medical Chamber in Krakow.

The mean age of the 32 patients was 62.7±12.5 (±standard error (SE)) years (range=34-84; median=63 years). To all patients surgery was applied: mastectomy in 31/32 (96.8%), tumorectomy with axillary lymphadenectomy in 1/32 (3.2%). Clinicopathological characteristics of the above-mentioned group is presented in Table I.

Materials. Archival tumor specimens were obtained from the Department of Tumor Pathology and were reviewed by a pathologist (AHL) to confirm histological diagnosis and tumor grade (according to Elston-Ellis modified version of the Boom-Richardson scale).

Immunohistochemistry (IHC). Immunohistochemistry was performed on formalin-fixed, paraffin-embedded tissue sections, which were cut at 4 μm, mounted on SuperFrost slides (Menzel-Gläser, Braunschweig, Germany). Sections were dewaxed in xylene and rehydrated through a series of alcohols. Table II summarizes all IHC procedures. Before incubation with properly diluted antibody (overnight incubation at 4˚C) and visualization system (Table II) we applied: (1) antigen retrieval - Target Retrieval Solution (TRS, pH=6.0, 96˚C for 50 min, DakoCytomation Denmark A/S, Glostrup, Denmark); (2) quenching the activity of endogenous peroxidases; 30 min in 0.3% H_2O_2 in 100% methanol; (3) blocking of unspecific antibody binding; 2.5% normal horse serum (20 min, room temperature, Vector Laboratories, Burnligame, CA, USA). Eventually, slides were counterstained with Mayer's hematoxylin. In the double staining procedure, VIP was used as peroxidase substrate (Vector Laboratories) for visualization of CD34 (violet color), while DAB for podoplanin (brown color).

Evaluation of immunohistochemistry. The percentage of Ki-67 immunopositive cells was calculated in 500-1,000 cells (at ×400 magnification, 5-10 high power fields) and expressed as the MIB-1 labeling index (MIB-1LI).

Distribution of podoplanin-positive vessels (DPV) was assessed in the whole examined tumor, in more than 3 fields. In each field, the absence or presence of lymphatics (at least one brown-stained lymphatic vessel; Figure 1a and b - grey arrow) was recorded. The large lymphatic vessels with lumen, the small ones without lumen and lymphatics with emboli were included into DPV assessment. Based on the aforementioned assessment, each field was classified as positive (with at least one podoplanin-positive vessel) or negative (without lymphatics). Eventually, the percentage of fields with at least one lymphatic vessel was calculated.

The blood and lymphatic vessel density (BVD and LVD), respectively, was assessed applying CD34 as a marker of blood vessels (Figure 1a, b and c – violet color) and podoplanin as a marker of lymphatic vessels (Figure 1 a and b – brown color); using the modified method described by Weidner (9, 11, 12). More than 3 most intensively vascularized intratumoral fields were acquired (x10 objective magnification, area 1.46 mm^2 of the specimen field). In each field, vessels were marked manually and then counted automatically. The analysis was performed with digital image analysis using a BX41 microscope, DP71 camera and Cell D software (Olympus Europa GmbH, Hamburg, Germany). Eventually, MVD and LVD were calculated as the number of vessels in the most vascularized field per 1 mm^2.

Vascular invasion (VI), encompassing both lymphovascular invasion (LVI) (Figure 1 b and d - black arrow) and blood vascular invasion (BVI) (Figure 1d - grey arrow), was considered evident if at least one tumor cell cluster was clearly visible inside the podoplanin-stained or CD34-stained vascular space.

Based on hematoxylin and eosin staining, we indicated five different patterns of immune cell infiltration: L0, lack of infiltration; L1, infiltration of peritumoral area; L2, infiltration of tumor stroma (between nests of cancer cells); L3, infiltration of peritumoral area and concurrently tumor stroma; L4, infiltration of peritumoral area, tumor stroma and additionally immune cells dispersed between tumor cells.

The expression of estrogen (ERα) and progesterone receptors (PR) was evaluated in the invasive component of the tumors and was considered positive if more than 1% of tumor cells showed nuclear staining.

Only tumors with continuous strong membranous HER2 reaction (3+) were considered HER2-immunopositive.

Table I. *Clinicopathological characteristics of 32 male breast cancer patients*.

Parameter	N	%
Tumour stage		
1	4	12.5
2	4	12.5
3	1	3.1
4	12	37.5
Unknown	11	34.4
Nodal status (pN)		
0	15	46.9
1	17	53.1
Grade		
1	8	25.0
2	17	53.1
3	5	15.6
Unknown	2	6.3
Adjuvant therapy		
Not administered	7	21.9
RT only	4	12.5
HT only	9	28.1
CT only	3	9.4
RT+HT	5	15.6
CT+HT	1	3.1
CT+HT+RT	3	9.4
Radiotherapy		
0	20	62.5
1	12	37.5
Chemotherapy		
Not administered	22	68.8
Adjuvant (CMF; FAC; AC)	7 (3; 1; 3)	21.9 (9.4; 3.1; 9.4)
Neoadjuvant (CTX; FAC; AT)	3 (1; 1; 1)	9.4 (3.1; 3.1; 3.1)
Hormonotherapy		
Not administered	14	43.8
Stilbestrol	1	3.1
Tamoxyfen	17	53.1

RT, Radiotherapy; HT, hormonotherapy; CT, chemotherapy; CMF, cyclophosphamide, methotrexate, fluorouracil; FAC, fluorouracil, doxorubicin, cyclophosphamide; AC, doxorubicin and cyclophosphamide; CTX, cyclophosphamide; AT, doxorubicin and paclitaxel or docetaxel.

Table II. *Immunohistochemical procedures used for visualization of markers studied.*

Antigen	Clone	Manufacturer	Dilution	Visualization system	Peroxidase substrate	Number of stained cases/ number of positively stained cases
Ki-67	MIB-1	DAKO[1]	1:75	BrightVision[4]	DAB[5]	24/24
ERα	6F11	Leica Biosystems[2]	1:100	(30 min, room		27/25
PR	PGR/2		1:200	temperature)		
HER2	-	DAKO[1]	1:250			25/1
Podoplanin	D2-40	CellMarque[3]	1:100			DPV: 17/13
						LVD: 18/14
CD34	QBEnd 10	DAKO[1]	1:50		VIP[5]	BVD: 19/19

DPV, Distribution of podoplanin-stained vessels; LVD, lymphatic vessel density; BVD, blood vessel density; DAB - 3,3'-Diaminobenzidine. [1]DakoCytomation Denmark A/S, Glostrup, Denmark, [2]Leica Biosystems Newcastle Ltd, Newcastle, UK, [3]CellMarque, Rocklin, CA, USA, [4]Immunologic, Duiven, the Netherlands, [5]Vector Laboratories, Burnligame, CA, USA.

Table III. *Relationship between analyzed parameters by the Mann–Whitney U test.*

		Total (N) (%)	MIB-1LI (N) mean±SD	LVD (N) mean±SD	DPV (N) mean±SD	BVD (N) mean±SD
Total			(24) 35.3±15.4	(18) 21.3±37.5	(17) 56.2±40.8	(19) 106.9±33.8
Nodal status (PN)	Negative	15 (46.9)	(9) 35.1±18.6	(7) 40.0±56.3	(6) 73.6±38.9	(7) 108.1±33.1
	Positive	17 (53.1)	(15) 35.3±13.8	(11) 8.0±9.9	(11) 46.7±40.3	(12) 106.2±35.7
Grade	1+2	25 (83.3)	(17) 29.9±7.8	(14) 19.6±42.4	(13) 44.7±39.5	(15) 112.6±33.2
	3	5 (16.7)	(5) 59.4±10.7[a]	(3) 27.6±19.9	(3) 91.7±14.4[b]	(3) 87.9±37.0
ER/PR	ER– and PR–	2 (7.4)	(2) 64.0±10.0	(2) 38.7±7.3	(2) 100.0±0.00	(2) 76.4±44.07
	ER+ or PR+	25 (92.6)	(22) 32.6±13.0[c]	(15) 19.4±40.7[d]	(14) 49.2±41.12	(16) 109.8±33.07
Vascular invasion	Absent	16 (80.0)	(15) 33.6±8.8	(14) 19.4±42.4	(13) 47.8±40.3	(15) 110.0±31.76
	Present	4 (20.0)	(4) 44.9±23.5	(4) 24.3±17.8	(4) 83.5±33.0	(4) 95.2±43.80
Infiltration of immune cells	L0, L1, L2	13 (72.2)	(12) 36.6±14.8	(11) 9.8±14.9	(11) 48.9±40.7	(13) 103.1±30.8
	L3, L4	5 (27.8)	(5) 30.7±5.7	(5) 47.9±64.9	(4) 60.7±48.6	(4) 125.2±47.9

DPV, Density of podoplanin-stained vessels; LVD, lymphatic vessel density; BVD, blood vessel density; L0, lack of infiltration; L1, infiltration of peritumoral area; L2, infiltration of tumor stroma (between nests of cancer cells); L3, infiltration of peritumoral area and concurrently tumor stroma; L4, infiltration of peritumoral area, tumor stroma and additionally immune cells dispersed between tumor cells. a, p=0.001; b, p=0.074; c, p=0.041; d, p=0.085.

Statistical analysis. The STATISTICA v.10 software (StatSoft, Inc. Tulsa, OK, USA) was used for all calculations. A p-value <0.05 was considered significant. Differences between two groups were estimated using the Mann–Whitney *U*-test. The Pearson's correlation coefficient was used in testing correlations between the two continuous variables. The Pearson's chi-squared test (χ^2) was applied to test independence of categorical variables expressed in a cross-tab.

Results

Podoplanin and CD34 expression in tumor, stroma and non-malignant tissue structures. Podoplanin expression was found in lymphatic vessels (Figure 1a and b - brown color, grey arrow), cancer-associated, stromal fibroblasts (Figure 1b - brown colour, asterisk), myoepithelial cells surrounding non-atypical ducts (Figure 1e - brown colour, arrowhead) or *in situ* carcinomas, as well as in basal cells of skin epidermis (Figure 1f - brown colour, arrowhead). Lymphatic vessels were found in 14/18 (77.8%) of male breast cancers.

CD34 expression was found in endothelial cells of blood vessels (Figure 1a, b and c - violet color) and myofibroblasts surrounding normal ducts and lobules (Figure 1d and e - asterisk). In all studied carcinomas (100%), CD34-stained vessels were present.

Correlation between parameters used for assessment of angio- and lymphangiogenesis and clinicopathological variables. A significant positive correlation was found between LVD (number of vessels in one hot-spot) and DPV (distribution of podoplanin-positive vessels) (R=0.689, p=0.005). Additionally, we observed a significantly negative correlation between LVD and BVD, as well as between DPV and BVD (R=–0.533, p=0.041 and R=–0.543, p=0.036, respectively). Moreover, MIB-1LI was significantly positively related to DPV (R=0.618, p=0.014).

MIB-1LI was significantly higher in ER/PR immuno-negative carcinomas and in high-grade (G3) carcinomas than

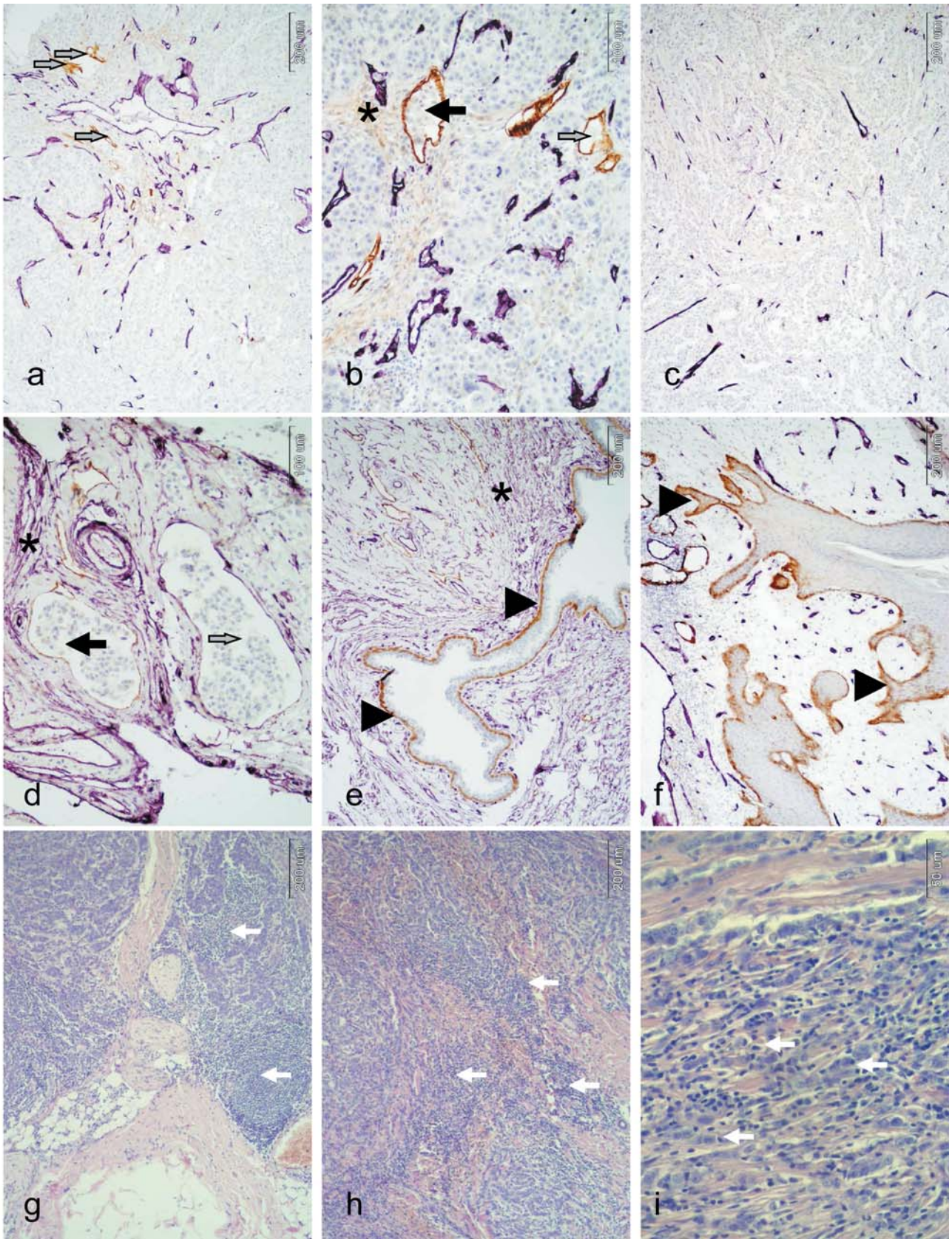
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Table IV. *Relationship between analyzed parameters by the Pearson's chi-squared test.*

		Vascular invasion		Infiltration of immune cells	
		Absent N (%*)	Present N (%*)	L0, L1, L2 N (%*)	L3, L4 N (%)
Nodal status (pN)	0	7 (87.5)	1 (12.5)	3 (42.9)	4 (57.1)
	1	9 (75.0)	3 (25.0)	10 (90.9)	1 (9.1)[a]
Grade	1+2	14 (87.5)	2 (12.5)	10 (66.7)	5 (33.3)
	3	1 (33.3)	2 (66.7)[b]	2 (100.0)	0 (0.0)
ER/PR	ER– and PR–	0 (0.0)	2 (100.0)	2 (100.0)	0 (0.0)
	ER+ or PR+	15 (88.2)	2 (11.8)[c]	10 (66.7)	5 (33.3)

ER, Estrogen receptor; PR, progesterone receptor; L0, lack of infiltration; L1, infiltration of peritumoral area; L2, infiltration of tumor stroma (between nests of cancer cells); L3, infiltration of peritumoral area and concurrently tumor stroma; L4, infiltration of peritumoral area, tumor stroma and additionally immune cells dispersed between tumor cells. *Percentage from the row; a, p=0.025; b, p=0.035; c, p=0.004.

in ER/PR-positive and low-grade ones, respectively (Table III). Moreover double negative tumors (ER–/PR–) presented higher number of lymphatic vessels in one "hot spot" (LVD) than in ER/PR positive cases (Table III). Additionally, high-grade tumors were characterized by higher distribution of podoplanin-positive vessels (DPV) than low-grade carcinomas (Table III).

We observed a significantly higher frequency of vascular invasion in high grade and double negative carcinomas than in low grade and ER/PR positive ones, respectively (Table IV). Furthermore, patients with lymph node involvement presented less pronounced or no infiltration of lymphatic cells (Table IV).

We did not study the correlation between HER2 and other parameters because over-expression of this marker was noted only in 1/25 cases.

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Figure 1. *(a-b) Blood (violet color) and lymphatic (brown color, grey asterisk) vessels in tumor; (b) lymphatic vessel invasion (black arrow), podoplanin immuno-positivity of cancer-associated stromal fibroblasts (asterisk); (c) blood vessels (violet color) presence and absence of lymphatic vessels; (d) blood (grey arrow) and lymphatic vessel invasion (black arrow) at the margin of the tumor (close to normal tissue), CD-34 stained myofibroblasts (asterisk) next to blood and lymphatic vessels; (e) podoplanin expression in normal myoepithelial cells (arrowhead), CD-34 stained myofibroblasts (asterisk) next to blood and lymphatic vessels; (f) podoplanin expression in basal layer of skin epithelium (arrowhead) next to blood and lymphatic vessels; (g) infiltration of immune cell in the peritumoral area (white arrows); (h) infiltration of immune cell in tumor stroma (between nests of cancer cells) (white arrows); (i) immune cells disperse between tumor cells (white arrows). Microphotographs (a) and (c-h) were taken at ×10 (objective), microphotograph (b) at ×20 (objective), while microphotograph (i) at ×40 (objective).*

Discussion

In male breast carcinomas, expression of podoplanin was found in lymphatic vessels, myoepithelial cells and in stromal cancer-associated fibroblasts, which is in agreement with other (10-23) and our own studies on female breast cancer (26-28). Moreover, in this rare form of malignancy, intratumoral lymphatic vessels (vessels between tumor cells or in tumor stroma) were presented in 77% of cases similarly as in our previous study on female ductal breast cancer (79%) and within the range of other authors' results (100% (15), 50% (18)).

In male breast carcinomas analyzed in the present study, high LVD was significantly related to steroid receptor negativity. Such relation was reported by some researchers in female breast carcinomas (15, 21, 26, 27) but others failed to detect the aforementioned relationship (14, 17, 18, 20, 22, 23) (Table V). The significant correlation between high number of lymphatics (in our study DPV – distribution of podoplanin stained vessels) and grade, found for male carcinomas, has also been reported for female malignancies (14, 18, 21) (Table V). Moreover, similarly to other authors (14, 18), lack of vascular invasion was found more frequently in low-grade and steroid receptor-positive carcinomas (Table V). These results confirm the hypothesis concerning the involvement of lymphangiogenesis in the formation of a more aggressive breast cancer phenotype, which, interestingly, holds true for both male and female breast cancer.

The discrepancies between the results (shown above) might be due to the effect of the methodological aspects of LVD assessment. Some authors, similarly to us, assessed LVD in one hot spot (14, 18, 20), while others in more than one hot spot (15, 17, 19, 22) or in randomly selected fields (16, 21). Moreover, some researchers counted vessels in the intratumoral and peritumoral areas of the tumor (16, 18, 21). In the present, as well as previous studies, we have tested the

Table V. *Main characteristics, methodological aspects and results of studies evaluating LVD using podoplanin.*

First author, year of publication	No. of patients included into the study (Percentage of: N+; G3; ER+; PR+; HER2+; invasive ductal carcinoma)	Method of LVD assessment	Correlation between high LVD and:	Lack of correlation between LVD and:	Method of BVD assessment	Correlation between high BVD and:	Lack of correlation between BVD and:	% of cases with vascular invasion	Correlation between VI and:
Schoppmann et al. 2004 (14)	374 (43.3; 40.4; 77.0; ?; ?; 87.4)	1 "hot spot"/ field	↓age, ↑G, LVI+, menopausal status -	N, pT, histology, ER				28.1	↑G, N+, ↑LVD
Nakamura et al. 2005 (15)	113, M0 (49.6; 33.6; 59.3; 55.8; 28.3; 92.0)	5 "hot spots"/ field	↑T, N+, ER–, HER2+, LVI+, VEGF-C+	Age, histology, PR, p53, G				50.4	↑LVD, N+
van der Schaft 2007 (16)	121 (42.1; 16.5; 53.7; 43.8; ?; 100.0)	4 randomly selected fields/mm^2, in IT and ST area	none	Both stromal and intratumoral: age, ER, PR, T, G, N					↑VEGF-D
Mylona et al. 2007 (17)	146, LVD was assessed only in 111, without preCT, preRT (59.6; 24.3; 55.5; 48.2; ?; 80.0)	5 "hot spots"/ mm^2	none	TNM, G, T, N, ER, PR,topoIIα, VEGF-C, VEGF-D	CD34, 5 "hot spots"/ mm^2	none	TNM, G, T, N, ER, PR, topoIIα, VEGF-C, VEGF-D		
El-Gohary et al. 2008 (18)	48, without preCT, preRT (50.0; 18.8; 70.8; 70.8; 29.2; 56.3)	1 "hot spot" assessed in IT and PT area	Intrat and perit LVD: N+, ↑T, ↑TNM, ↑G, LVI+, BVI+ Perit LVD : HER2+,	Intrat and Perit LVD: histology, ER, PR, VI	CD31, 1 "hot spot"	↑LVD, N+, ↑TNM, ↑G, BVI+	T, histology, ER, PR, HER, VI	38	↑N, ↑T, ↑TNM, ER–, PR–, HER2+
Zhang 2008 (19)	70, unilateral, M0, without preCT, preRT (45.7; 41.4; 77.1; 65.7; 18.6; 84.3)	5 "hot spot"/ field	↑VEGF-C and ↑COX-2	Other correlation no studied				35.7	↑VEGF-C, ↑LVD
Gu et al. 2008 (20)	61, M0, without preCT, preRT (52.5; 9.8; 59.0; 65.6; 37.7; 85.2)	1 "hot spot"/field	N+, ↑TNM	Histology, age, ER, PR, HER2					
Mohammed et al. 2009 (21)	177, without preCT, preRT (29.0; 29.4; 66.7; 56.4; ?; ?)	all vessels in whole section/ mm^2	↑PTNM, ER, PR–,↑G, N+, LVI+	Age, T, histology, MVD	CD34, Chalkley counting	↑T, ↑G, N+, ER/PR–	LVD	31.6	↑LVD
Tsutsui et al. 2010 (22)	242, M0, (43.3; ?; 43.8; ?; ?; 242)	3 "hot spots"/ field	N+, ↑MIB-1, ↑VEGF-A, ↑MVD, ↑Akt	T, ER	FVIII, 3 "hot spots". field	↑LVD, ↑Akt	?		
Mohammed et al. 2011 (23)	(i) 397=197: basal (CK5/6+ and/or CK14+), <1.5 cm, N0 + 200: non-basal, N0 (ii) 99/397: TNP (0; 50.3; 54.4; 41.6; ?; ?)	all vessels in whole section/ mm^2	none	Basal, T, G, ER, PR (in basal and TNP)	CD34, Chalkley counting	Basal+, TNP+, T, ↑G, ER–, PR–	T, G, ER, PR (in basal and TNP)	TNP: 26 Non-TNP: 26	↑T

N, nodal status; G, grade; T, tumor size; TNM, stage; ER, estrogen receptor; PR, progesterone receptor; HER2, epidermal growth factor receptor 2; TNP, triple negative phenotype (ER-/PR-/HER2-); ?, undetermined; LVD, lymphatic vessel density; BVD, blood vessel density, LVI, lymphatic vessel invasion, BVI, blood vessel invasion, VI, vessel invasion from haematoxylin and eosin staining; ST, stromal area; IT, intratumoural area; PT, peritumoural area; preCT, pre-operative chemotherapy; HT, hormonotherapy; poRT, preoperative radiotherapy; ↓, low value of the parameter; ↑, high value of the parameter; +, presence of particular feature; -, absence of particular feature.

clinical utility of DPV (distribution of podoplanin stained vessels) and, based on the obtained results, we can recommend it as alternative for assessment of lymphatic vessels in hot spots.

Blood vessels were found in 100% of cases, both in male and female breast carcinomas (27). Unlike other authors (18, 21, 23) we (both in male and in female breast cancer) did not find a significant correlation between BVD and clinicopathological parameters (Table V).

We observed a significant negative correlation between blood and lymphatic vessel density. On the contrary, other researchers, in female breast carcinomas, reported an inverted relationship (22). However, the different methods applied for both lymphatic and blood vessel density might lead to different results. Moreover, one cannot exclude the possibility of different governing mechanisms involved in lymph- and angiogenesis in male and female breast cancer.

In the present study conducted on male breast cancer, we failed to confirm the significant relation between intensive leukocyte infiltration and high number of lymphatic vessels, which was observed in females with this type of malignancy (27) and in cervical cancer patients (28). In the present study, only a tendency towards statistical significance was observed. Interestingly, the presence of inflammatory cells in tumor might be associated with both angiogenesis and lymphangiogenesis. In growing tumors, expression and secretion of vascular endothelial growth factor (VEGF) – the most important factor switching the aforementioned processes – might be accomplished not only by hypoxic tumor cells but also by activated leukocytes (7). On the other hand, VEGF is responsible for chemotaxis of monocytes and macrophages, as well as increased leukocyte trans-endothelial migration (7). Therefore, the relation between high number of immune cells and high number of lymphatics is not surprising. The question that arises concerns the role of immune cells for breast cancer progression. To some extent, an explanation of this issue comes from the results obtained in the present study since, in patients with lymph node involvement, we observed less pronounced or no infiltration of lymphatic cells, a finding that may suggest a potential role of immune cells in the prevention of male breast cancer cell spreading.

Conclusion

(i) In most male breast carcinomas (77.8%), lymphatic vessels are present within the tumor. (ii) The relationship between high number of lymphatic vessels and high tumor grade or steroid receptor negativity might confirm the hypothesis regarding the influence of lymphangiogenesis in the formation of a more aggressive phenotype in male breast cancer.

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