Lymphatic Vascularization in Prostate Adenocarcinoma: Correlation with Tumor Grade, Androgen Withdrawal and Prognosis

MARIA R. AMBROSIO[1], BRUNO J. ROCCA[1,2], AURORA BARONE[1], ALESSANDRO GINORI[1], FILIPPO CRIVELLI[2], LUIGI PIRTOLI[3] and MARIA T. DEL VECCHIO[3]

[1]*Department of Medical Biotechnologies, Section of Pathology, University of Siena, Siena, Italy;*
[2]*Pathology Unit, Ospedale di Circolo di Busto Arsizio, Presidio Ospedaliero di Saronno, Saronno, Italy;*
[3]*Department of Medicine, Surgery and Neurosciences, Section of Pathology and Radiological Sciences, University of Siena, Siena, Italy*

Abstract. *Background/Aim: The lymphatic system plays an active role in the metastatic process by directly facilitating recruitment of cancer cells into the vessels. The present study aimed to assess the lymphatic vessel area and the lymphatic vessel density in prostate adenocarcinoma and to correlate these parameters with patients prognosis and outcome. Patients and Methods: The lymphatic vessel area and the lymphatic vessel density were evaluated using the D2-40 monoclonal antibody in 153 patients with prostate adenocarcinoma who had been treated by radical prostatectomy, in comparison to 152 non-neoplastic controls. We also estimated the lymphatic vessel area in a set of 139 patients who had undergone radical prostatectomy after neoadjuvant treatment with combined androgen blockade. Results: Lymphatic vessel area was higher in periglandular than in interglandular stroma, inversely correlated with tumor differentiation (in untreated patients) and was influenced by hormonal treatment. Lymphatic vessel density was not significantly different between the non-tumoral and the high-grade prostate intraepithelial neoplasm compartment, whereas it was higher in tumoral than in non-tumoral compartments, mainly in periglandular stroma. In addition, it increased in parallel to the tumor grade progression and positively correlated with all the main prognostic factors of prostate adenocarcinoma. Conclusion: The evaluation of lymphatic vessel density on radical prostatectomy with positive nodes may help to discriminate those patients at higher risk of developing an aggressive disease, which may need early androgen deprivation therapy to delay the worsening of clinical disease.*

Correspondence to: Maria Raffaella Ambrosio, Department of Medical Biotechnologies, Section of Pathology – University of Siena, via delle Scotte 6, 53100 Siena, Italy. Fax: +39 0577586579, e-mail: maradot@libero.it

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Most deaths from cancer are the result of metastasis (1). Metastatic tumor spread occurs, at least in the early stages, through the lymphatic path by lymphoangiogenesis (2,3). Lymphatic vessels are characterized by poorly developed junctions and large inter-endothelial gaps, thus being susceptible to tumor cell invasion (4, 5). Patients with lymph node metastasis exhibit significantly decreased disease-specific and biochemical recurrence-free survival rates (1, 8). Organ-confined prostate adenocarcinoma (PAC) is curable in most cases by surgery or radiation therapy, and the prognosis for these patients is excellent (6). Advanced and metastatic disease are associated with high morbidity and mortality (7) due to the development of castration resistance. A number of morphological studies have examined the relationship between lymphangiogenesis and PAC (9, 10). However, these studies were carried out on small series of patients and produced conflicting results regarding whether lymphatic vessels are involved in tumor progression.

The present study aimed at clarifying lymphatic vascularization in PAC samples, analyzing the effect of androgens and of androgen inhibition on prostate vascularization, and at assessing the correlation between lymphatic vessel density (LVD) and the main clinicopathological factors of PAC. Increased knowledge on PAC pathogenesis may give rise to new therapeutic targets and prognostic markers.

Patients and Methods

Prostate tissue samples. Ethics approval for this study was obtained from the Institutional Review Board at the University of Siena (Italy). The study was performed on 292 radical prostatectomy specimens from patients with PAC, collected between January 1999 and December 2003. Of these patients, 153 had been treated by

radical prostatectomy (hereafter referred to as untreated patients) and 139 had undergone radical prostatectomy after neoadjuvant treatment with combined androgen blockade therapy (CAB) for three months (hereafter referred to as treated patients). The non-neoplastic controls were represented by 102 specimens from peripheral zone of the prostate of patients undergoing cysto-prostatectomy for bladder cancer but without cancer in the prostate gland. The mean age of the patients at the time of surgery was 69 years (range=55 to 79 years). The following biochemical and pathological parameters were recorded: total prostate specific antigen (tPSA) level, Gleason score (only on surgical specimens from untreated patients), surgical margins infiltration, extra-prostatic extension, seminal vesicles invasion, lymph node metastasis, TNM stage (according to the AJCC Cancer Staging Manual, Seventh Edition (11). The clinicopathological features are summarized in Table I.

Histology. Tissues had been fixed in 10% buffered formalin, embedded in paraffin and processed according to the standard procedures as previously described (12). Untreated adenocarcinomas were graded according to the updated Gleason grading system by combining the different Gleason pattern (13, 14). Treated adenocarcinomas were classified as good, moderate or poor responders (12, 15).

Immunohistochemistry. The immunohistochemical stainings were performed as previously described (11) using the monoclonal antibody D2-40 (dilution: 1:40; Dako, Milan, Italy). Quantification of LVD was assessed by applying the counting method of Weidner *et al*. (16). In untreated PAC, LVA and LVD were evaluated separately for each Gleason pattern, whereas in treated PAC, they were assessed depending on the response to treatment.

Morphometric analysis. In each of the neoplastic and non-neoplastic sample, five fields with maximum LVA at a magnification of ×200 were identified using the program LUCIA G (Nikon, Tokyo, Japan).

Statistical analysis. Both univariate (Kolmogorov-Smirnov test, Fisher exact test and χ^2 test), and multivariate analysis, were performed by computing the hazard ratio (HR) and its 95% confidence interval for each prognostic factor, and considering a *p*-value of 0.05 or less as statistically significant.

Results

Evaluation of LVA. LVA was higher in the periglandular than in the interglandular stroma in all the neoplastic and non-neoplastic compartments examined ($p<0.001$). Specifically, periglandular LVA (mean±SD) was higher in non-neoplastic than in high-grade prostate intraepithelial neoplasia (HGPIN) and neoplastic specimens (7784±664 μm^2 *vs*. 5447±664 μm^2 *vs*. 2138±526 μm^2 in untreated patients; 4092±507 μm^2 *vs*. 1535±292 μm^2 *vs*. 1626±268 μm^2 in treated patients) ($p<0.05$). LVA inversely correlated with Gleason pattern (Gleason 3=3828±609 μm^2, Gleason 4=1512±591 μm^2, Gleason 5=1075±378 μm^2; $p<0.01$) in untreated patients, and with response to treatment (2108±285 μm^2 in poor, 1657±339 μm^2 in moderate, 1113±180 μm^2 in good responders) in treated patients ($p<0.05$). Interglandular LVA significantly decreased in the tumoral compartment in comparison with non-neoplastic and HGPIN compartments ($p<0.001$ for untreated patients, and $p<0.05$ for treated patients) and inversely correlated with the Gleason pattern (1266±225 μm^2 in Gleason 3, 248±80 μm^2 in Gleason 4, not measurable in Gleason 5) in untreated patients ($p<0.001$), but was not correlated with the response to treatment in treated patients ($p=0.4$) (Figures 1-3). A graphic representation of the results is shown in Figure 4.

Table I. *Clinicopathological features of the patients*.

Variable	Group	N	%
Preoperative PSA,	<4	6	(2%)
	4-10	95	(32.5%)
	>10	191	(65.5%)
Surgical margin infiltration	Positive	162	(55.5%)
	Negative	130	(44.5%)
Extra-prostatic extension	Positive	129	(44.2%)
	Negative	163	(55.8%)
Seminal vesicle invasion	Positive	49	(16.8%)
	Negative	243	(83.2%)
Lymph node metastasis	Positive	95	(33%)
	Negative	197	(67%)
Prostate cancer stage	I	29	(9.9%)
	II	128	(43.9%)
	III	101	(34.6%)
	IV	95	(33%)

Evaluation of LVD. LVD was not significantly different between the non-tumoral and the HGPIN compartment [2.01 *vs*. 1.40/high-power field (HPF); $p=0.11$], whereas it was higher in tumoral than in non-tumoral compartments (3.59 *vs*. 2.01/HPF; $p<0.005$). In tumoral compartments, interglandular LVD was lower than the periglandular ($p<0.001$). Periglandular LVD increased with tumor pattern (Gleason pattern 3=2.33; Gleason pattern 4=2.6; Gleason pattern 5=3.6/HPF; $p<0.001$). In lymph node-positive cases, LVD was significantly higher than in node-negative cases (mean 3.5 *vs*. 2.4/HPF; $p<0.001$) (Figure 5).

Association of LVD with the prognostic factors of prostatic carcinoma. LVD was significantly associated with higher stage ($p<0.001$) and Gleason score ($p<0.001$); surgical margin infiltration ($p<0.001$), extra-prostatic extension ($p<0.001$), seminal vesicle invasion ($p<0.001$), and lymph node metastases ($p<0.001$). No correlation was identified with the age of the patients ($p=0.62$) and the preoperative PSA ($p=0.570$). The patients with higher LVD had a shorter DFS [mean=35.61 months (95% confidence interval=1-99 months) *vs*. 69.28 months (reference=13-144 months); log-rank test, $p<0.001$]. Univariate Cox analysis showed that all the prognostic factors of PAC statistically correlated with

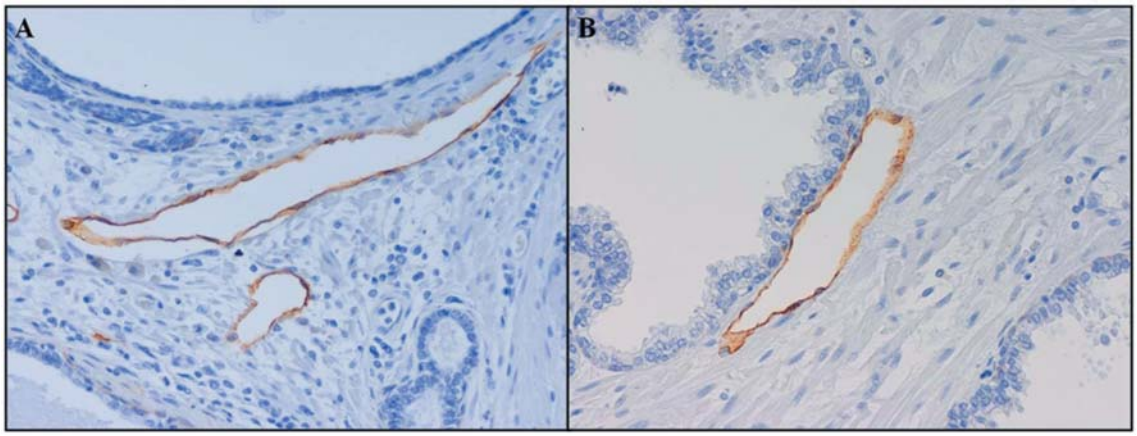

Figure 1. *Lymphatic vessel area in non-neoplastic (A) and high-grade prostatic intraepithelial neoplasm (B) samples. No significant difference between specimens is apparent. D2-40 stain. Original magnification: ×20.*

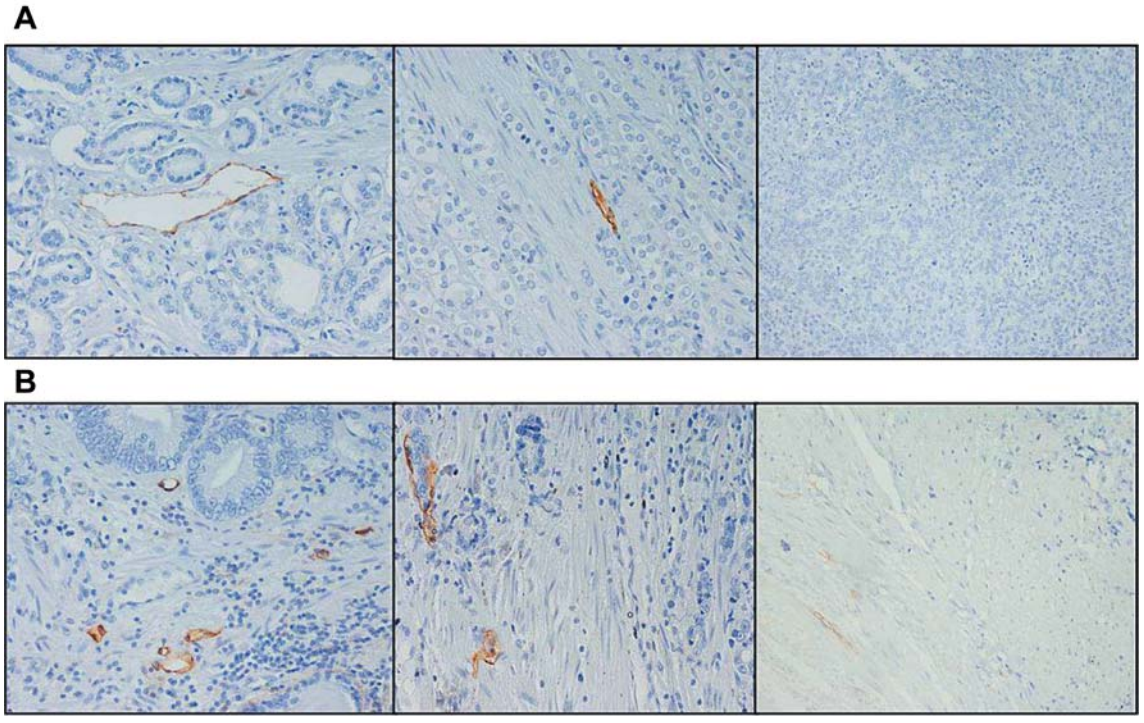

Figure 2. *Lymphatic vessel area in untreated patients with prostate adenocarcinoma. A: In interglandular stroma, lymphatic vessel area significantly decreases from Gleason pattern 3 (left), to Gleason 4 (middle) and 5 (right) ($p<0.001$ for Gleason 3 vs Gleason 4 and 5; $p<0.01$ for Gleason 4 vs. Gleason 5). B: In periglandular stroma, lymphatic vessel area decreases as Gleason pattern increases; in Gleason pattern 5, small lymphatic vessels are still detectable (lower right) ($p<0.01$). D2-40 stain. Original magnification: ×10.*

disease-free survival ($p<0.001$) except for age ($p=0.73$). Stepwise multivariate analysis enrolling the above parameters and the LVD identified Gleason score, extra-prostatic extension and LVD as the three major discriminant prognostic factors in PAC (HRs of 2.06, 22.7 and 57.2, respectively; $p<0.001$).

Discussion

Lymphatic vessels are fundamental components of the immune system and provide interstitial drainage; they are also a route of tumor cell dissemination (5), thanks to their thin walls and discontinuous basement membrane (5). Previous studies carried

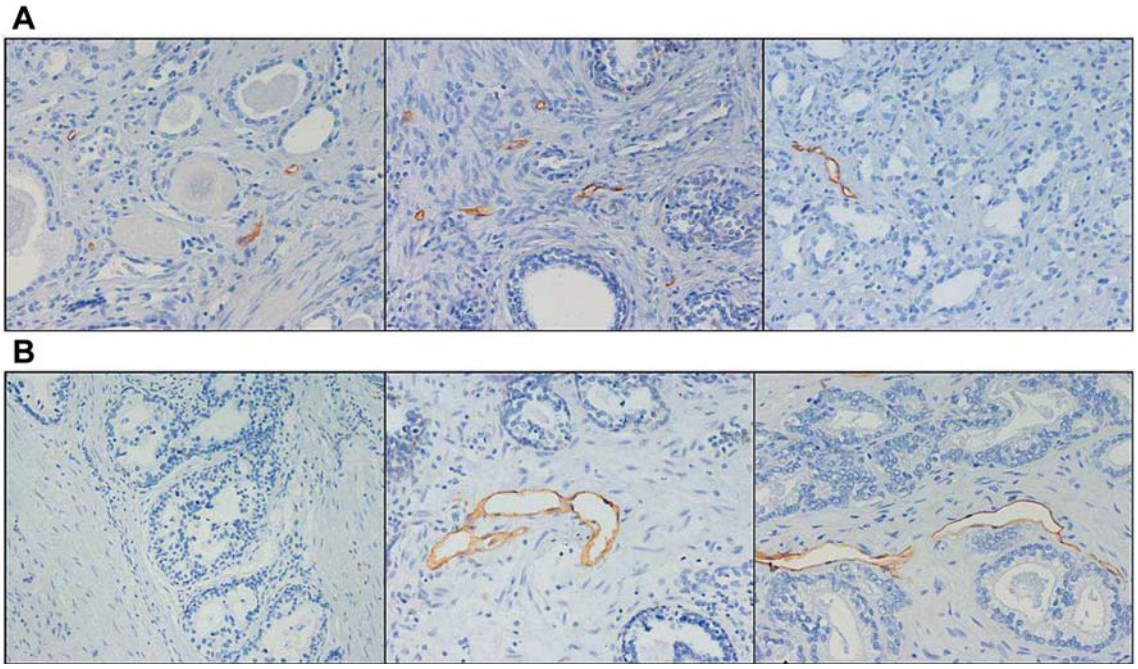

Figure 3. *Lymphatic vessel area in treated patients with prostate adenocarcinoma. A: In interglandular stroma, lymphatic vessel area is lower in the specimens from good responders (left), than in those from moderate (middle) and poor responders (right) (p=0.4). B: In periglandular stroma, lymphatic vessel area progressively increases from the specimens from good (left), to moderate (middle), and poor (right) responders (p<0.05). D2-40 stain. Original magnification: ×10.*

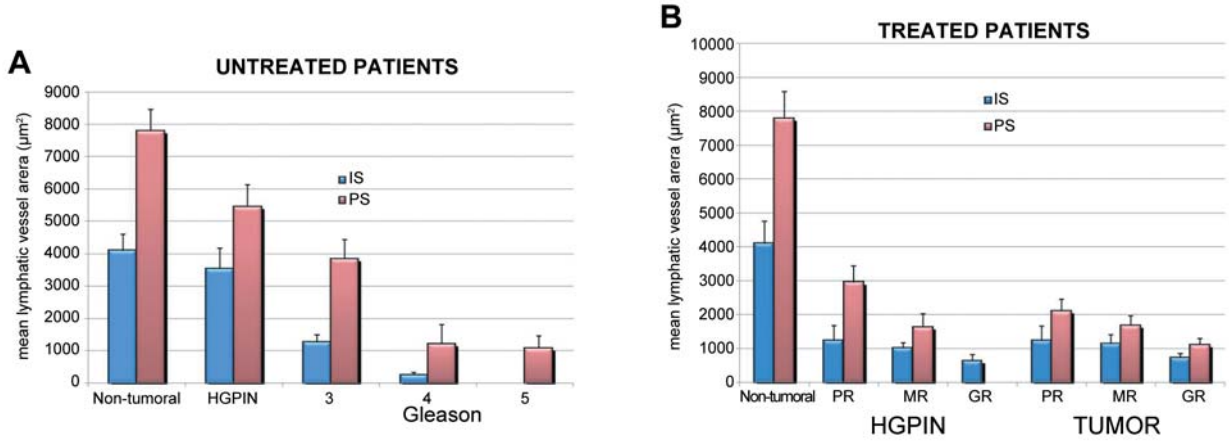

Figure 4. *Lymphatic vessel area in untreated and treated patients. A: Lymphatic vessel area in non-tumoral, high-grade prostatic intraepithelial neoplasia (HGPIN) and Gleason pattern (GL) 3 to 5 specimens of untreated patients is shown. B: Lymphatic vessel area in non-tumoral, HGPIN and neoplastic samples of poor (PR), moderate (MR) and good (GR) responders is represented. IS: Interglandular stroma; PS: periglandular stroma.*

out on lymphatic vascularization in PAC gave conflicting results (17-22). They had been performed on small series of cases, used different stains for lymphatic vessels and provided information limited to the LVD. In our study of 292 radical prostatectomy specimens and 102 non-neoplastic controls, we evaluated not only the LVD but also the LVA. In samples from untreated patients, we found that LVA and LVD were higher in the periglandular than in the interglandular stroma, supporting the view that metastatic spread occurs mainly via peritumoral lymphatic vessels. The decrease of LVA in neoplastic compared with non-neoplastic and HGPIN specimens may be related to the outgrowth of stromal cells, which is a feature of PAC and,

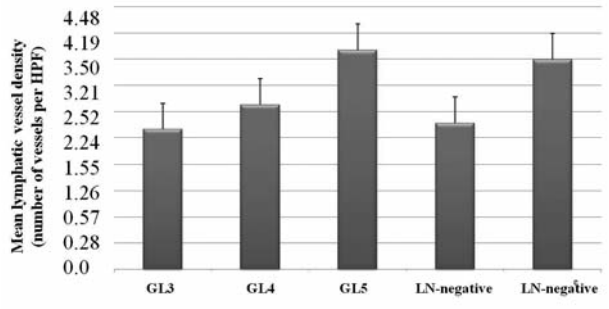

Figure 5. *Lymphatic vessel density in untreated patients with prostate adenocarcinoma. Lymphatic vessel density increases as Gleason pattern (GL) progresses and is higher in lymph nodes (LN)-positive cases than in those with negative LNs (p<0.005).*

thus, may influence the shape of the lymphatic vessels walk. In samples from treated patients, we found that LVA was lower in the specimens from good responders than in the samples from poor or moderate responders. It could be postulated that androgen withdrawal inhibits tumor growth by also reducing lymphangiogenesis.

We also observed that LVD positively correlated with the main clinico-pathological parameters involved in PAC prognosis, and with disease-free survival and lymph node. Based on these finding, one may need to consider aggressive post-surgical management of patients with a high LVD in the PAC surgical specimen (23, 24).

In conclusion, a better understanding of the mechanisms leading to invasiveness and lymphatic metastasis of PAC (25, 26) represents a challenge for tumor vascular biology. Since we have suggested that androgens regulate the prostatic vasculature and that vascularization affects patients outcome, a better-timed and aggressive therapeutic approach by combining early androgen targeted therapy, lymphagiogenesis inhibitors (27), radiotherapy and chemotherapy may inhibit the metastatic spread of neoplastic cells. However, the scenario is even more complicated when considering the tumor heterogeneity for a single prostate cancer patient, which would result in an intro-patient variation.

Conflicts of Interest

None.

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