Prospective Study of Prostate Tumor Angiogenesis and Cancer-Specific Mortality in the Health Professionals Follow-Up Study

Lorelei A. Mucci, Anna Powolny, Edward Giovannucci, Zhiming Liao, Stacey A. Kenfield, Rulong Shen, Meir J. Stampfer, and Steven K. Clinton

From the Channing Laboratory, Brigham and Women's Hospital, Harvard Medical School; Departments of Epidemiology and Nutrition, Harvard School of Public Health, Boston MA; Department of Pathology, Division of Hematology and Oncology, and Department of Medicine, Ohio State University Medical Center and Comprehensive Cancer Center, Columbus, OH.

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Corresponding author: Lorelei A. Mucci, ScD, MPH, Channing Laboratory, Brigham and Women's Hospital and Harvard Medical School, 181 Longwood Ave, 3rd floor, Boston, MA 02115; e-mail: lmucci@hsph.harvard.edu.

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ABSTRACT

Purpose

Tumor growth requires the development of independent vascular networks that are often primitive in morphology and function. We examined whether microvessel morphology contributes to the considerable biologic heterogeneity of prostate cancer.

Methods

We evaluated microvessel morphology as a predictor of prostate cancer mortality among 572 men in the Health Professionals Follow-Up Study diagnosed with cancer during 1986 to 2000. We immunostained prostatectomy tumor block sections for endothelial marker CD34 and assessed microvessel density, vessel size (area and diameter), and irregularity of vessel lumen using image analysis. Proportional hazards models were used to assess microvessel density and morphology in relation to lethal prostate cancer.

Results

Poorly differentiated tumors exhibited greater microvessel density, greater irregularity of the vessel lumen, and smaller vessels. During 20 years of follow-up, 44 men developed bone metastases or died of cancer. Men with tumors exhibiting the smallest vessel diameter, based on quartiles, were 6.0 times more likely (95% CI, 1.8 to 20.0) to develop lethal prostate cancer. Men with the most irregularly shaped vessels were 17.1 times more likely (95% CI, 2.3 to 128) to develop lethal disease. Adjusting for Gleason grade and prostate-specific antigen levels did not qualitatively change the results. Microvessel density was not linked to cancer-specific mortality after adjusting for clinical factors.

Conclusion

Aggressive tumors form vessels that are primitive in morphology and function, with consequences for metastases. Vascular size and irregularity reflect the angiogenic potential of prostate cancer and may serve as biomarkers to predict prostate cancer mortality several years after diagnosis.

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INTRODUCTION

Prostate cancer is biologically heterogeneous. Many localized cancers are slow-growing, even in the absence of therapy,^{1,2} whereas some men with apparently organ-confined disease develop metastases despite local therapy.^{3,4} An understanding of the key molecular events associated with prostate cancer progression remains to be elucidated.

Angiogenesis is one potential pathway that contributes to prostate cancer heterogeneity. The establishment of a vascular supply plays a pivotal role in the development of cancer by providing for the distribution of growth factors, nutrients, and oxygen critical for growth of solid tumors.⁵ In addition, angiogenesis can facilitate metastases by sup-

plying tumor cells direct access to vasculature. Although angiogenesis begins with integration of existing host vasculature, continued tumor growth requires new vessel networks that are often primitive in organization, function, and morphology.⁶

The extent of neoangiogenesis, as estimated by microvessel density, is related to the tumor's aggressive potential^{7,8} and is inversely related to cancer survival for several malignancies.⁹ In prostate cancer, increased tumor microvessel density predicts poorly differentiated tumors¹⁰ and biochemical failure after treatment.^{11,12} Prospective studies of angiogenesis and prostate cancer death, a more relevant clinical outcome, are limited. Moreover, no study to date has comprehensively assessed morphologic aspects of angiogenesis, such as vessel size

and shape, in relation to prostate cancer outcomes. Quantitative assessment of angiogenesis could have potential for the classification of prostate cancer into biologic subtypes.

We investigated microvessel density and morphologic measurements of angiogenesis in relation to tumor characteristics and lethal prostate cancer among 572 men treated with prostatectomy in the Health Professionals Follow-Up Study (HPFS). The research was approved by the institutional review boards at the Harvard School of Public Health and Partners Healthcare.

METHODS

Study Population

The HPFS prostatectomy cohort is a community sample of men with clinically localized prostate cancer (T1/T2) diagnosed from 1986 to 2000 and who elected prostatectomy as curative therapy. The men were participants in the ongoing prospective HPFS study of 51,529 male dentists, optometrists, osteopaths, podiatrists, pharmacists, and veterinarians in the United States. They completed biennial questionnaires to collect lifestyle and medical information. Incident prostate cancer was initially identified through self-report or, rarely, when prostate cancer was mentioned on death certificates and was confirmed by review of medical records, pathology reports and death certificates.

Hospital pathology departments where cohort members underwent surgery were contacted to retrieve archival formalin-fixed paraffin-embedded prostatectomy specimens. Of 1,593 men who underwent prostatectomy, we retrieved blocks for 64% ($n = 1,023$). Most hospitals sent blocks for the entire case, although some selected blocks containing tumor. The current analysis is based among 572 men for whom the first batch of morphologic assessment of angiogenesis was completed. The clinical characteristics for these men are similar to the entire HPFS prostatectomy cohort.

Clinical and Follow-Up Data

Information on clinical and pathologic stage, age, and prostate-specific antigen (PSA) at diagnosis was abstracted from medical records. Study pathologists reviewed hematoxylin and eosin slides to provide uniform Gleason grading.¹³ Information on race/ethnicity was collected on the baseline HPFS questionnaire in 1986, and body mass index was calculated using height and weight reported on questionnaires immediately preceding the cancer diagnosis.

Men were observed prospectively for clinical course through March 2008. Death certificates and relevant medical records were reviewed to confirm the cause; follow-up is more than 98% complete. Beginning in 2000, we also collected information on development of distant metastases abstracted from medical records.

Biomarkers of Angiogenesis

Morphologic assessment of angiogenesis was determined on serial sections of the prostatectomy blocks. The study pathologist identified all blocks containing cancer and evaluated between one and nine blocks with cancer per case. Protein expression for endothelial cell marker CD34 was ascertained by immunohistochemistry on 5- μ m sections using primary mouse monoclonal antibody anti-CD34 (QBEND10; Biogenex, San Ramon, CA) at 1:200 dilution for 30 minutes after incubation with peroxidase-blocking reagent (Dual Endogenous Enzyme Block, Dako, Carpinteria, CA). Detection was accomplished by a peroxidase-labeled polymer conjugated to antimouse immunoglobulin G, liquid 3,3'-diaminobenzidine+Substrate Chromogen System (K3468; Dako), and EnVision+ Dual Link System (Dako). Immunohistochemistry was performed in an OptiMax Automated Cell-Staining System (BioGenex). Slides were counterstained with hematoxylin (Sigma-Aldrich, St Louis, MO).

Semiautomated image analysis was accomplished under supervision of the pathologist using Image ProPlus 4.5 software (Media Cybernetics, Bethesda, MD). Slide images under high-powered fields ($\times 200$) were captured using a Spot RT Slider Camera (Diagnostic Instruments, Sterling Heights, MI)

mounted on a Nikon Eclipse E800 microscope (Nikon Instruments, Melville, NY). The pathologist guided the imaging and directed the system to disregard vessels that were transected multiple times. Images were obtained from each focus of cancer. For blocks with large cancer volume, multiple nonoverlapping images from the focus were obtained. Microvessel density was calculated as the number of vascular structures in a high-powered field. Vessel size was determined as the average vessel diameter (in micrometers) and area comprised by a vessel (in square micrometers). Irregularity of the vessel lumen was calculated as $\text{perimeter}^2/4 \cdot \pi \times \text{area}$, with a value of 1.0 indicating a perfect circle and values greater than 1.0 indicating increasing irregularity. Vessels that were incompletely captured on the section were included in the calculation of microvessel density, but not size or shape. Greater microvessel density, smaller vessel size, and more irregularly shaped vessels are hypothesized to be characteristic of cancers with greater angiogenic potential^{14,15} (Table 1). The laboratory remained blinded to lethal outcome status during the immunohistochemistry and morphologic evaluation.

Statistical Analysis

For each individual, there were up to nine measurements, and minimum, mean, and maximum values were calculated across each angiogenesis measure. Because the findings were qualitatively similar, we present only the results using the mean values.

We compared angiogenesis measures by age, PSA, and Gleason grade. We used unconditional logistic regression models to calculate odds ratios and 95% CIs to evaluate the associations of the vascular parameters (quartiles) to predict extraprostatic disease (T3/T4/N1) at prostatectomy. We used Cox proportional hazards models to evaluate the angiogenesis biomarkers and time to development of lethal prostate cancer. Person-time was calculated from date of diagnosis to development of metastases, cancer death, or censored at time of death from other causes or end of follow-up (January 1, 2007). Hazard ratios (95% CI) were used as effect measures. We adjusted for age at diagnosis (years, continuous), Gleason grade (≤ 6 , 7, 8 to 10), and PSA at diagnosis (< 4.1 , 4.1 to 9.9, ≥ 10 ng/mL) and further adjusted for pathologic stage. We calculated C statistics to assess the ability of the angiogenesis measures to predict lethal prostate cancer, comparing the clinical variables (age, Gleason, PSA at diagnosis, stage) to that including clinical variables and the angiogenesis measures (quartiles). Analyses were undertaken using SAS Statistical Analysis (version 9.1; SAS Institute, Cary, NC).

Table 1. Histopathologic Measures of Microvessel Density and Morphology: HPFS Prostatectomy Cohort, 1986 to 2000

Measure	Microvessel Density*	Vessel Area	Diameter of Blood Vessels	Irregularity of Vessel Lumen†
What is more angiogenic?	Greater No.	Smaller size	Smaller diameter	Irregular shape
Mean	76 vessels	542 μm^2	25.2 μm	4.0
Range	13-491	104-2795	12.9-55.6	1.6-7.9
Correlations‡				
Microvessel density	1			
Vessel area	-0.15	1		
<i>P</i>	.0003			
Diameter	-0.10	+0.91	1	
<i>P</i>	.02	< .0001		
Irregularity	-0.07	-0.31	-0.26	1
<i>P</i>	.08	< .0001	< .0001	

Abbreviation: HPFS, Health Professionals Follow-Up Study.

*Microvessel density is the number of vessels per high-powered field.

†A score of 1.0 indicates a perfect circle.

‡Age-adjusted Pearson correlation coefficients.

RESULTS

The HPFS prostatectomy cohort included 572 men with incident prostate cancer diagnosed from 1986 to 2000, with the majority diagnosed in the era since introduction of PSA screening (Table 2). The median age at diagnosis was 66.3 years, and 95% of men were white.

We calculated age-adjusted Pearson coefficients for within-tumor correlations between the parameters defining vascular density and structure (Table 1). Men whose tumors had a greater microvessel density also tended to have smaller vessel diameter. There was a weak correlation between microvessel density and irregularity of vessel lumen. In contrast, vessel area and diameter were strongly correlated. There was a strong inverse correlation between vessel size and shape, such that tumors with smaller vessel diameter and smaller vessel area exhibited greater irregularity of the vessel lumen, although this correlation would be expected based on the method for calculating vessel irregularity.

Table 2. Clinical and Demographic Characteristics of the HPFS Prostatectomy Cohort (N = 572), 1986 to 2000*

Characteristic	No.	%
Total, N	572	
Age at diagnosis, years		
Median	66.3	
Range	47.0-79.8	
Race		
White	519	95.6
African American	9	1.7
Asian	9	1.7
Other	6	1.1
Pathologic tumor stage		
T2	398	70.2
T3a†	98	17.3
T3b‡	41	7.2
T4/N1	30	5.3
Gleason grade		
2-5	27	4.7
6	128	22.4
7	333	58.2
3 + 4	199	34.7
4 + 3	134	23.5
8-10	84	14.7
Year of diagnosis		
1986-1989, pre-PSA era	42	7.3
1990-1993, peri-PSA era	174	30.4
1994-2000, PSA era	356	62.2
PSA at diagnosis, ng/mL		
Median	7.0	
Range	0.4-77	
Follow-up time, years		
Median	9.9	
Range	0.8-19.9	
Body mass index		
Median	25.1	
Range	17.8-38.3	

Abbreviations: HPFS, Health Professionals Follow-Up Study; PSA, prostate-specific antigen.

*Numbers do not add up to 572 if data are missing.

†T3a indicates penetration through capsule wall.

‡T3b indicates involvement of the seminal vesicles.

PSA levels at diagnosis were unrelated to microvessel density, but were significantly related to vessel diameter and irregularity (Fig 1). Compared with men with low PSA (< 4.0 ng/mL), those with PSA greater than 10.0 had microvessels that were on average smaller diameter ($P = .07$) and more irregularly shaped ($P = .001$). PSA was less correlated with microvessel area ($P = .18$). Gleason grade was strongly related to microvessel density, with Gleason 7 ($P < .0001$) and Gleason 8 to 10 ($P < .0001$) tumors exhibiting increased microvessel density compared with low-grade tumors. Compared with low-grade tumors, Gleason 8 to 10 tumors had vessels that were smaller in diameter ($P < .0001$) and area ($P = .0007$) and displayed greater irregularity in the vessel lumen ($P = .052$). Among Gleason 7 tumors, those with a dominant 4 (4 + 3) tended to also exhibit greater microvessel density and have vessels that comprised smaller area compared with dominant pattern 3 (3 + 4) tumors, although the differences were not statistically significant. When we focused specifically on the pattern in the high-powered field, the associations were considerably stronger. Compared with fields of Gleason pattern 3, those with pattern 4 had vessels that were smaller diameter (20.3 v 23.7 μm ; $P < .001$), smaller area (323 v 488 μm^2), were more irregularly shaped (4.5 v 4.0 ; $P < .001$), and greater density (82 v 61 vessels; $P < .001$).

On prostatectomy, 29.8% of men had extraprostatic disease (Table 2). Seven percent had tumors involving the seminal vesicles, whereas 5% involved the bladder wall or extended to the regional lymph nodes. In Table 3, we examined the angiogenesis biomarkers to predict presence of extraprostatic disease. Among men with tumors with the greatest microvessel density based on quartiles, 39% had extraprostatic disease, compared with 21% of those with the smallest microvessel density. The odds of having extraprostatic disease was 2.5 (95% CI, 1.5 to 4.2) times higher comparing extreme quartiles based on microvessel density. Adjusting for Gleason and PSA somewhat attenuated the relation, although microvessel density remained a significant predictor. Vessel size was also associated with extraprostatic extension. Both smaller vessel area (odds ratio comparing extreme quartiles, 1.9; 95% CI, 1.1 to 3.2) and smaller diameter (odds ratio, 2.0; 95% CI, 1.2 to 3.5) were associated with a greater likelihood of extension outside the prostate. The relation between irregularity of the vessel lumen and extraprostatic extension was more modest and was not significant after adjusting for clinical variables. Associations between the angiogenesis measures and extraprostatic disease were similar when we excluded T3a tumors.

During up to 20 years of follow-up (median, 9.9 years), 35 men died of prostate cancer, nine men were alive with bone metastases, and 94 men died of other causes (and never developed metastases). In contrast to its relation with extraprostatic extension, microvessel density was not a significant predictor of lethal disease (Table 4). Vessel size and shape, however, provided good discrimination of lethal prostate cancer. Comparing extreme quartiles, 23 of 143 cases were lethal among men with the smallest vessel area, compared with three among 143 cases with the largest vessel area. Adjusting for age, the hazard ratio for lethal disease in relation to vessel area was 6.6 (95% CI, 2.0 to 21.9) comparing the smallest and largest area. Smaller vessel area and smaller diameter remained independent predictors after adjusting for Gleason grade, PSA, and age at diagnosis. The strongest association was noted for irregularity of the vessel lumen. Among men with the greatest lumen irregularity, 22 died of prostate cancer or developed metastases, compared with only one man in the quartile with the

Fig 1. Microvessel density (MVD) and architecture in prostate tumors according to prostate-specific antigen (PSA) at diagnosis and Gleason grade, Health Professionals Follow-Up Study prostatectomy cohort, 1986 to 2000. Mean and 95% CIs comparing MVD, vessel area and diameter, and irregularity, stratified by PSA levels at diagnosis (< 4.1, 4.1 to 10, ≥ 10 ng/mL) and pathologic Gleason grade (2 to 6, 7, 8 to 10), are shown.

most regularly shaped lumens. The age-adjusted hazard ratio comparing extreme quartiles was 17.1 (95% CI, 2.3 to 128). Additionally adjusting for clinical characteristics, tumors with greater vessel irregularity experienced a 10-fold greater risk of developing lethal disease compared with those with the most regularly shaped vessels. In secondary analyses, adjusting for pathologic stage, vessel irregularity remained a strong predictor of outcome, although the effect was somewhat attenuated (*P* for trend approximately .013). Vessel area and diameter were no longer statistically significant.

In secondary analyses, we considered whether the angiogenesis measures predicted lethal outcomes among men with low to moderate risk prostate cancer, defined by Gleason grade ≤ 7. Although the number of outcomes was small (*n* = 19), the data suggested that vessel irregularity and smaller vessel size predict outcome even among men with low to moderate grade tumors.

The discrimination of models that included the clinical factors was compared with the model also including the angiogenesis biomarkers using lethal prostate cancer as an end point. The addition of angiogenesis markers led to marked improvement in prediction of lethal prostate cancer. The C statistics were 0.82 (95% CI, 0.76 to 0.87) for the model including age, Gleason grade (re-review), and PSA at diagnosis and 0.87 (95% CI, 0.82 to 0.92) for the model also including vessel area, diameter, and vessel irregularity. When pathologic stage

was also included in the model, the C statistics were 0.88 (95% CI, 0.84 to 0.93) for the clinical model and 0.92 (95% CI, 0.89 to 0.96) for the model also including vessel size and shape.

DISCUSSION

In this large United States prostatectomy cohort, we demonstrate a strong link between quantitative morphologic measures of angiogenesis in relation to prostate cancer progression. Compared with low Gleason grade tumors, poorly differentiated tumors exhibited significantly greater microvessel density and smaller vessel size on the basis of both diameter of the vessel and vessel area. The association between Gleason grade and vessel regularity was weaker.

The HPFS prostatectomy cohort included almost one third whose tumors showed extraprostatic extension, nodal extension, or spread to the seminal vesicles or bladder on the basis of pathologic findings. Tumor vessel morphology and density were predictive of extraprostatic extension at time of prostatectomy. Pathologically advanced stage tumors exhibited greater microvessel density as well as smaller and less regularly shaped vessel lumen as compared with tumors that were localized.

Table 3. ORs and 95% CIs of Angiogenesis Markers to Predict Extraprostatic Disease: HPFS Prostatectomy Cohort, 1986 to 2000

Table 3. ORs and 95% CIs of Angiogenesis Markers to Predict Extraprostatic Disease After Prostatectomy, 1986 to 2006								
Biomarker and Quartile	What Is More Angiogenic?	Median of Quartile	Extraprostatic		OR*	95% CI	OR†	95% CI
			No.	%				
Microvessel density, vessels								
Q4 greater	Greater No.	115.8	56	39.4	2.5	1.5 to 4.2	1.7	1.0 to 3.0
Q3		75.3	49	34.3	2.0	1.2 to 3.4	1.6	0.9 to 2.9
Q2		58.8	34	24.1	1.2	0.7 to 2.1	0.9	0.5 to 1.7
Q1-fewer		41.4	30	21.3	REF		REF	
<i>P</i> , test for trend					.0002		.021	
Vessel area, μm^2								
Q1 smaller area	Smaller area	284	63	44.1	2.3	1.4 to 3.9	1.9	1.1 to 3.2
Q2		416	39	27.3	1.1	0.7 to 1.9	1.0	0.6 to 1.7
Q3		552	32	22.9	0.9	0.5 to 1.6	0.8	0.5 to 1.4
Q4-larger area		832	35	24.8	REF		REF	
<i>P</i> , test for trend					.0025		.038	
Vessel diameter, μm								
Q1 smaller diameter	Smaller diameter	19.7	63	44.1	2.5	1.5 to 4.2	2.0	1.2 to 3.5
Q2		23.0	34	23.9	1.0	0.6 to 1.8	0.9	0.5 to 1.5
Q3		26.1	39	27.7	1.2	0.7 to 2.1	1.0	0.6 to 1.8
Q4 larger diameter		31.4	33	23.4	REF		REF	
<i>P</i> , test for trend					.0017		.027	
Regularity of vessels, units								
Q4 less regular	Irregular shape	5.4	51	35.7	2.0	1.2 to 3.4	1.7	1.0 to 3.1
Q3		4.4	40	28.2	1.4	0.8 to 2.4	1.3	0.7 to 2.3
Q2		3.6	48	34.0	1.9	1.1 to 3.3	1.8	1.0 to 3.1
Q1 more regular		2.7	30	21.3	REF		REF	
<i>P</i> , test for trend					.036		.14	

Abbreviations: OR, odds ratio; HPFS, Health Professionals Follow-Up Study; REF, reference.

*Odds ratios adjusted for age at diagnosis.

†Odds ratios adjusted for age at diagnosis (continuous), Gleason grade (categorically, 2 to 6, 7, 8 to 10), and prostate-specific antigen at diagnosis (categorically, < 4, 4.1 to 10, > 10, not measured).

Vessel size and shape were most strongly associated with development of bone metastases or cancer death several years after diagnosis. Men whose tumors were infiltrated by vessels that were of smaller size and were more irregular in shape were at increased risk of developing lethal disease. These histopathologic angiogenesis measures were predictive beyond their correlations with Gleason grade, PSA levels, and stage, suggesting that biomarkers of prostate cancer angiogenesis at diagnosis can predict outcomes several years hence and may help to stratify a patient's risk of progression and perhaps suggest the need for additional therapy in the adjuvant setting.

Our data on vessel morphology and architecture further support the importance of angiogenesis in prostate cancer progression and the hypothesis that disease progression is marked by a rapid formation of neovasculature in tumors that are of smaller size and more poorly formed.^{14,15} The leaky and disordered function of these vessels has important implications for progression and prognosis.¹⁶ Because tumor angiogenesis predicts future outcomes, the biology of the prostate cancer, as reflected in tumor angiogenesis, seems to be determined early in its development. The overall number of vessels may not be as strong of an indication of neoangiogenesis, because microvessel density reflects both new and existing vasculature.

Our data suggest that microvessel morphology and architecture are linked to prostate cancer progression apart from age, Gleason grade, and PSA at diagnosis. The relation between microvessel density, vessel size, and shape as a predictor of extraprostatic disease suggests that angiogenesis biomarkers may be useful in guiding tumor staging

at diagnosis. Moreover, the finding that vessel size and irregularity were strong predictors of fatal disease many years in the future may have clinical utility and guide therapeutic choices, in combination with other tumor biomarkers or clinical information.

Several studies have shown that greater microvessel density is associated with worse tumor grade^{10,11} and more advanced stage.^{11,17} As a predictor of prognosis, greater microvessel density has been associated with an increased likelihood of biochemical failure after prostatectomy¹⁸⁻²⁰ and worse cancer-specific survival.^{11,21} However, other studies have found that microvessel density is not an independent predictor of prostate cancer outcomes after adjusting for Gleason grade.²²⁻²⁴ In our own data, microvessel density was a predictor of pathologic evidence of extraprostatic disease and high Gleason. On a univariate level, the data suggested a positive association with cancer death, albeit not significant, which may reflect the relatively small number of deaths in the cohort, with limited statistical power to detect modest effects. Given the strong correlation with Gleason grade, microvessel density did not independently predict lethal prostate cancer. In part, this may be due to the fact that microvessel density is reflecting both new and mature vasculature. Moreover, our findings may be attenuated, as they do not account for the amount of stromal tissue relative to tumor.

There are no published studies to date on the relation between morphologic aspects of vessels, such as size and shape, in relation to prostate cancer. Irregularly shaped vessels have been associated with

Table 4. HRs and 95% CIs of Angiogenesis Biomarkers to Predict Time to Lethal Prostate Cancer: HPFS Prostatectomy Cohort, 1986 to 2007

Biomarker and Quartile	What Is More Angiogenic?	No. of Deaths/ Metastases	HR*	95% CI	HR†	95% CI	HR‡	95% CI
Microvessel density								
Q4 greater	Greater No.	10	1.7	0.7 to 4.4	0.9	0.3 to 2.3	1.0	0.4 to 2.6
Q3		16	2.4	1.0 to 5.6	1.5	0.6 to 3.5	1.5	0.6 to 3.7
Q2		10	1.4	0.5 to 3.5	0.8	0.3 to 2.1	0.9	0.3 to 2.3
Q1-fewer		8	REF		REF		REF	
<i>P</i> , test for trend			.25		.94		.92	
Vessel area								
Q1 smaller area	Smaller area	23	6.6	2.0 to 21.9	4.3	1.3 to 14.7	4.0	1.2 to 13.3
Q2		13	3.5	1.0 to 12.4	3.0	0.9 to 10.8	3.2	0.9 to 11.4
Q3		5	1.6	0.4 to 6.7	1.3	0.3 to 5.6	1.5	0.4 to 6.4
Q4-larger area		3	REF		REF		REF	
<i>P</i> , test for trend			.0003		.0035		.0083	
Vessel diameter								
Q1 smaller diameter	Smaller diameter	22	6.0	1.8 to 20.0	3.9	1.1 to 13.2	3.6	1.1 to 12.1
Q2		11	2.9	0.8 to 10.3	2.1	0.6 to 7.7	2.3	0.6 to 8.2
Q3		8	2.6	0.7 to 9.7	1.9	0.5 to 7.4	2.1	0.6 to 8.0
Q4-larger diameter		3	REF		REF		REF	
<i>P</i> , test for trend			.0011		.013		.024	
Regularity of vessels								
Q4 less regular	Irregular shape	22	17.1	2.3 to 128	13.1	1.8 to 97.4	10.9	1.5 to 81.4
Q3		14	11.4	1.5 to 87.5	10.2	1.3 to 77.6	10.1	1.3 to 77.6
Q2		7	6.5	0.8 to 52.5	5.4	0.7 to 43.9	5.6	0.7 to 45.4
Q1 more regular		1	REF		REF		REF	
<i>P</i> , test for trend			.0001		.0007		.0038	

Abbreviations: HR, hazard ratio; HPFS, Health Professionals Follow-Up Study; REF, reference.

*Hazard ratios adjusted for age at diagnosis.

†Hazard ratios adjusted for age at diagnosis (continuous) and Gleason grade (categorically, 2 to 6, 7, 8 to 10).

‡Hazard ratios adjusted for age at diagnosis (continuous), Gleason grade (categorically, 2 to 6, 7, 8 to 10), and prostate-specific antigen at diagnosis (categorically, < 4, 4.1 to 10, > 10).

worse disease progression in pancreatic cancer,²⁵ Hodgkin's lymphoma,²⁶ laryngeal cancer,²⁷ and malignant melanoma.²⁸ Interestingly, smaller vessel diameter was associated with more advanced disease for bladder cancer,²⁹ whereas larger vessels were predictive of advanced melanoma.²⁸ Taken together with our results, these data suggest that the morphologic characteristics that reflect the pattern and maturity of the growing vascular network may be better indicators of neoangiogenesis, cancer aggressiveness, and metastatic potential.

Our study is one of the largest to evaluate biomarkers of angiogenesis and prostate cancer death. For validation of prognostic biomarkers, prostate cancer-specific death is a critical outcome. Although PSA recurrence is associated with an increased risk of prostate cancer death, most men with recurrences do not die of cancer,^{30,31} so studies based on intermediary measures may not provide the complete picture. The HPFS cohort is carefully monitored with prospective and complete follow-up, which is critical, because deaths can occur decades after diagnosis.^{1,2} The cohort has been carefully annotated with respect to important clinical characteristics. More than half of the participants were diagnosed in the PSA era, which allows an extrapolation of our findings to the recent clinical picture of prostate cancer. The cohort includes men in the United States, primarily white, and generally of a high socioeconomic status. Although these characteristics do not impact the study validity, they may influence the generalizability of the findings. Our study included prostatectomy specimens; evaluation of prostate biopsy specimens is an important next step. Concato et al²¹ directly assessed prostate cancer biopsies and

found a small positive association (hazard ratio, 1.8; 95% CI, 1.2 to 2.6) between microvessel density and total mortality, independent of clinical characteristics.

Experimental studies suggest that antiangiogenic therapies may normalize tumor vasculature and microenvironment, reduce vascular permeability, and restore the balance of pro- and antiangiogenic factors, at least transiently.¹⁵ Data from our study reflect that antiangiogenic therapy may represent an important drug target.

In conclusion, a quantitative assessment of microvessel architecture is informative in assessing the extent of prostate cancer neoangiogenesis. Microvessel size and shape may be valuable markers to distinguish cancers with indolent biology from those with an aggressive potential.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The author(s) indicated no potential conflicts of interest.

AUTHOR CONTRIBUTIONS

Conception and design: Lorelei A. Mucci, Edward Giovannucci, Steven K. Clinton

Financial support: Edward Giovannucci, Steven K. Clinton

Provision of study materials or patients: Lorelei A. Mucci, Steven K. Clinton

Collection and assembly of data: Lorelei A. Mucci, Anna Powolny, Edward Giovannucci, Zhiming Liao, Stacey A. Kenfield, Rulong Shen, Meir J. Stampfer, Steven K. Clinton

Data analysis and interpretation: Lorelei A. Mucci, Stacey A. Kenfield, Rulong Shen, Meir J. Stampfer, Steven K. Clinton

Manuscript writing: Lorelei A. Mucci, Anna Powolny, Steven K. Clinton

Final approval of manuscript: Lorelei A. Mucci, Anna Powolny, Edward Giovannucci, Zhiming Liao, Stacey A. Kenfield, Rulong Shen, Meir J. Stampfer, Steven K. Clinton

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