Prostate cancer aggressiveness and age: Impact of p53, BCL-2 and microvessel density

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ABSTRACT

Older men are more likely to have advanced prostate cancer at time of their diagnosis, but whether prostate tumors are inherently (biologically) more aggressive with advancing age is uncertain. To address this gap in knowledge, we analyzed data from veterans (n=971) diagnosed with prostate cancer during 1991–1995. Factors included age, detection of prostate cancer by screening, prostate-specific antigen (PSA) level, anatomic stage, and Gleason score. Information on molecular markers obtained from immunohistochemical staining of prostate tissue, included B cell lymphoma-2 (bcl-2), p53, and microvessel density (MVD), each having a previously documented association with disease progression and increased risk of prostate cancer death. We first examined the bivariate association of demographic, clinical, and molecular factors with age, and found evidence that race, screening status, Gleason score, PSA, bcl-2, p53, and MVD varied across categories of age in this study population. After further characterizing the association between age and Gleason score, we used logistic regression to examine the association between age and molecular markers—accounting for race, screening status, PSA, and Gleason score. Comparing men older than 80 years to those younger than 70 years, adjusted ORs and 95% CIs were 1.89 (0.73 to 4.92), 1.91 (1.05 to 3.46), and 2.00 (1.06 to 3.78), for positive bcl-2, p53, and MVD markers, respectively; no statistically significant associations were found for men 70–79 years old, compared with men younger than 70 years. These novel findings suggest that very elderly men often present with biologically aggressive prostate cancer; the results also have potential implications for therapeutic decision-making.

INTRODUCTION

Increasing age is a risk factor for being diagnosed with prostate cancer, and compared with younger men, older men tend to be diagnosed with more advanced disease[1 2] and are more likely to die.[3 4] Less screening, accompanied by later disease detection,[5] is a potential reason for more advanced disease at diagnosis among older men. An additional or alternative explanation is that the tumors of the elderly may be inherently more aggressive.[2] Based on published studies,[6 7] several molecular markers are known to be associated with prostate-cancer mortality across the entire age spectrum. If such markers are more prevalent in older than in younger men—after accounting for clinically relevant factors, including histology of the tumor—then such evidence would suggest that prostate cancer has increased aggressiveness in the older group, perhaps warranting modified treatment approaches.

This report focuses on the antiapoptotic protein B cell lymphoma-2 (bcl-2), tumor suppressor protein p53, and microvessel density (MVD) as molecular markers. The protein product of the proto-oncogene *BCL-2*, when overexpressed, permits tumor cell survival and proliferation by inhibiting programmed cell death.[8 9] *TP53* is the most frequently mutated gene across a wide range of human cancer types, with the majority of mutations somatically acquired.[10–14] This gene may lose tumor-suppression function, and it may acquire gain-of-function mutations that promote tumorigenesis and contribute to malignant progression.[13 14] MVD, reflecting tumor angiogenesis, has been associated with pathological features and poor prostate cancer outcomes in some, but not all, studies.[15]

Few studies of prostate cancer have examined the pattern of age differences in the occurrence of these molecular markers, and published reports[9 16–21] have often involved relatively small sample sizes, unadjusted analyses (ie, ignoring covariates), and inconsistent findings. To address this gap in knowledge, we examined potential age differences in molecular markers bcl-2, p53, and MVD, in a relatively large sample of veterans. We hypothesized that older (vs younger) men would be more likely to have tumors with positive bcl-2 or p53 staining, and greater MVD, both before and after adjusting for relevant demographic and clinicopathologic features in multivariable analyses.

MATERIALS AND METHODS

The study population, and methods used to assemble the database, have been described previously.[6] Briefly, among 64,545 male veterans receiving ambulatory care at nine Veterans Health Administration (VHA) medical centers in the Northeast as of 1 January 1991, pathology records identified 1331 men

diagnosed with prostate cancer during 1991–1995. Of these patients, 1172 (88.1%) had complete medical record data, and 971 (82.8%) had available data on specimens for at least one of the three markers under study.

Immunohistochemical (IHC) staining of prostatic tissue for bcl-2, p53, and MVD was obtained at initial diagnosis by needle biopsy (90.4%), transurethral prostate resection (9.1%), or prostatectomy for presumed benign disease (0.5%). Our institutional pathology laboratory conducted the staining, and a pathologist, blind to patient outcomes, reported the results. The laboratory used indirect immunoperoxidase methods, with antibodies (Dako, Carpinteria, California, USA) against selected factors and blocking of non-specific staining, with dilutions of 1:160 for bcl-2, and 1:3000 for p53. The intensity of staining in areas of carcinoma was scored on a 0–3 scale. The laboratory used antibodies (Dako) to factor VIII at a dilution of 1:4000 to assess MVD, and counted the number of stained blood vessels under 400 × magnification. For this research (and as in previous reports), we coded bcl-2 and p53 staining as yes versus no; and MVD as 0–28 versus 29+ vessels/high-power field, based on the median value observed.

Demographic and clinical data, including whether prostate cancer was detected by a screening test, were obtained from a comprehensive medical record review, using a standardized extraction form.[6] Recognizing that the median age of our population was approximately 70 years, and that results of regression analyses can be easier to interpret for intervals of age than for 1 year age increments, we divided age into categories of less than 70 years, 70–79 years, and 80 years or older for further analyses. Specific features of disease at time of diagnosis and initial treatment, or the decision not to treat, included baseline prostate-specific antigen (PSA) level, histologic grade (Gleason score), and clinical (anatomic) stage. We also report on race, first-degree prostate cancer family history, and comorbid conditions, as well as initial treatment and mortality for descriptive purposes.

We first examined the association of demographic and clinical characteristics with age, using χ^2 or Fisher's exact tests. We then conducted bivariate analyses to evaluate whether older men were more likely than younger men to exhibit positive molecular marker staining, including greater MVD. We subsequently used logistic regression to focus on the relationship between age and Gleason scores, in: a) an unadjusted model, and b) a model adjusted for factors that were statistically significant in bivariate analyses or clinically relevant. Finally, to examine the independent impact of the molecular markers, we generated logistic models focusing on the association between age and 'positive' markers, both unadjusted and adjusted for cogent factors, including Gleason score. We did not impute missing data. All analyses were done with SAS software, V.9.4 (SAS System for Windows, 2002–2012 SAS Institute, Cary, North Carolina, USA).

RESULTS

Table 1 shows baseline characteristics for 971 men with data on molecular markers; men who were excluded (n=201) did not differ in terms of age, race, family history of prostate cancer, comorbidity, cancer stage, or mortality (data not shown). African-Americans (vs other races) tended to be younger, but self-reported prostate cancer family history was not associated with age at diagnosis. Among clinical and pathological factors, more severe comorbidity and higher anatomic stage were somewhat more common, although not statistically significant, among the very elderly. Most (34/60; 57%) of the oldest men were not screen detected, a finding with borderline statistical significance (p=0.065), and Gleason score (p=0.049) and baseline PSA level (p=0.009) were higher (worse) in the oldest group.

Tumors of the oldest men were more likely to be positive for molecular markers, with statistically significant results for p53 (p=0.004) and MVD (p=0.016), but not for bcl-2 (p=0.072). For example, 44.3% (27/61) of men 80 years old or older were positive for p53, compared with 24.4% (88/361) of men younger than 70 years. Treatment with prostatectomy or radiation therapy, as potentially curative modalities, was less common among the oldest men. Prostate-cancer and all-cause mortality increased with advancing age; among men at least 80 years old, 30.2% (19/63) and 95.2% (60/63) had these outcomes, respectively.

The association between age and Gleason score is further characterized in table 2, with older age conferring increased risk of poor histology. Among men 80 years old or older, compared with men younger than 70 years, ORs and 95% CIs for poor histology were 2.21 (1.30 to 3.76) and 1.58 (0.90 to 2.76), respectively, in analyses that were unadjusted and adjusted for race, screening status, and PSA. These results suggest that age is associated with more aggressive prostate cancer, per routine clinical considerations.

Representing our main objective, table 3 shows that the association between age and molecular markers was quantitatively impactful and statistically significant in unadjusted analyses. In addition, even when accounting for impactful covariates, including the Gleason score, these associations were largely maintained. Specifically, when comparing men 80 years old or older to men younger than 70 years , fully adjusted associations of age and molecular markers for bcl-2, p53, and MVD were 1.89 (0.73 to 4.92), 1.91 (1.05 to 3.46), and 2.00 (1.06 to 3.78), respectively.

DISCUSSION

We found evidence of biologically more aggressive disease in men ≥80 years old than in men younger than 70 years, based on a) evidence consistent with previously reported associations of age and Gleason score,[22] and b) novel results showing twofold and statistically significant increases in odds of positive p53 staining and higher MVD, after adjusting for race, screening status, PSA level, and Gleason score. The strength of association for bcl-2 staining was similar for men aged 80 years and older, but the result was not statistically significant in adjusted analyses.

Most of the studies of p53[9 16–21] or bcl-2 staining[9 20 21] in prostate cancer that we reviewed did not emphasize age differences in molecular markers, and few of these studies explicitly reported on men aged 80 years and older. In addition, sample sizes in these reports were relatively small (usually <200 patients). Of note, some of the studies examined only radical prostatectomy samples, which tend to exclude older men, thereby precluding a direct comparison with our results, and some studies conducted only bivariate analyses. Importantly, the age-marker associations we

Table 1 Characteristics of patients in the analytic sample (n=971), stratified by age

	Age:								
	<70 years (n=373)		70–79 years (n=535)		80+ years (n=63)		P values	Total* (n=971)	
	n	(%)	n	(%)	n	(%)		n	(%)
Patient characteristics									
Race							<0.001		
Black	66	(17.7)	44	(8.2)	4	(6.3)		114	(11.7)
All other	307	(82.3)	491	(91.8)	59	(93.7)		857	(88.3)
Positive family history							0.685		
No	347	(93.0)	504	(94.2)	60	(95.2)		911	(93.8)
Yes	26	(7.0)	31	(5.8)	3	(4.8)		60	(6.2)
Charlson Comorbidity Index							0.210		
0	103	(27.6)	139	(26.0)	16	(25.4)		258	(26.6)
1	118	(31.6)	166	(31.0)	12	(19.0)		296	(30.5)
2	79	(21.2)	106	(19.8)	20	(31.7)		205	(21.1)
3+	73	(19.6)	124	(23.2)	15	(23.8)		212	(21.8)
Tumor characteristics									
Screen-detected cancer							0.065		
No	163	(48.8)	275	(56.9)	34	(56.7)		472	(53.8)
Yes	171	(51.2)	208	(43.1)	26	(43.3)		405	(46.2)
Baseline PSA (µg/l)							0.009		
0 to <4.0	65	(17.4)	65	(12.1)	3	(4.8)		133	(13.7)
4.0 to <10.0	125	(33.5)	179	(33.5)	17	(27.0)		321	(33.1)
10.0 to <20.0	85	(22.8)	136	(25.4)	14	(22.2)		235	(24.2)
≥20.0	98	(26.3)	155	(29.0)	29	(46.0)		282	(29.0)
Histology (Gleason score)									
Good (2–4)	81	(21.7)	107	(20.0)	7	(11.1)	0.049	195	(20.1)
Moderate (5–7)	226	(60.6)	333	(62.2)	36	(57.1)		595	(61.3)
Poor (8–10)	66	(17.7)	95	(17.8)	20	(31.7)		181	(18.6)
Anatomic stage							0.317		
Local	329	(88.2)	463	(86.5)	53	(84.1)		845	(87.0)
Regional	23	(6.2)	34	(6.4)	2	(3.2)		59	(6.1)
Metastatic	21	(5.6)	38	(7.1)	8	(12.7)		67	(6.9)
Molecular marker status									
Bcl-2							0.072		
Negative	330	(94.3)	458	(92.2)	49	(86.0)		837	(92.6)
Positive	20	(5.7)	39	(7.8)	8	(14.0)		67	(7.4)
p53							0.004		
Negative	273	(75.6)	387	(74.9)	34	(55.7)		694	(73.9)
Positive	88	(24.4)	130	(25.1)	27	(44.3)		245	(26.1)
Microvessel density							0.016		
<29 vessels/HPF	170	(49.0)	236	(47.0)	17	(28.8)		423	(46.6)
≥29 vessels/HPF	177	(51.0)	266	(53.0)	42	(71.2)		485	(53.4)
Treatment and outcomes									
Initial treatment							<0.001		
Watchful waiting or none	61	(16.4)	172	(32.1)	24	(38.1)		257	(26.5)
External beam/seed XRT	128	(34.3)	204	(38.1)	13	(20.6)		345	(35.5)
Androgen deprivation only	52	(13.9)	116	(21.7)	26	(41.3)		194	(20.0)
Prostatectomy	132	(35.4)	43	(8.0)	0	(0.0)		175	(18.0)
Cancer-specific mortality							0.011		
No	318	(85.3)	439	(82.1)	44	(69.8)		801	(82.5)
Yes	55	(14.7)	96	(17.9)	19	(30.2)		170	(17.5)
All-cause mortality							<0.001		
No	148	(39.7)	112	(20.9)	3	(4.8)		263	(27.1)
Yes	225	(60.3)	423	(79.1)	60	(95.2)		708	(72.9)

*Totals may differ due to missing values for some factors.
P values are for comparisons across age groups.
HPF, high-power field; PSA, prostate-specific antigen; XRT, radiotherapy.

Table 2 Association of age and 'poor' rating for prostate cancer histology (based on Gleason score 8–10); n≤971

	'Poor' histology (as "outcome")	
	Unadjusted	Adjusted*
Age	OR (95% CI)	OR (95% CI)
<70 years	(ref)	(ref)
70–79 years	1.10 (0.81 to 1.38)	0.95 (0.72 to 1.27)
≥80 years	2.21 (1.30 to 3.76)	1.58 (0.90 to 2.76)

*ORs adjusted for race, screen-detected cancer, and PSA.
PSA, prostate-specific antigen; ref, reference group.

detected were based on diagnostic (pretreatment) biopsy samples from almost 1000 men, and we used multivariable adjustment to account for cogent factors.

Our findings are consistent with an emerging literature that evaluates age differences for gene sequencing in prostate cancer. Recent studies have found dysregulated genes associated with DNA repair and androgen signaling in men over age 70 years,[23] and also substantial differences in genes associated with progression to metastatic disease between older and younger groups.[22] In addition, although published results are not entirely consistent, investigators are finding age differences for molecular markers in other tumor sites, including overexpression of *TP53* in older (versus younger) individuals in meningioma, gastric cancer, and endometrial cancers.[24–26] Given that most mutations in *TP53* are acquired somatically and that such mutations increase with age, the increased risk of cancer with ageing[14 27] can be explained, at least in part, on this basis.

In clinical practice, elderly men are less likely than younger men to be offered curative treatment with radiotherapy or prostatectomy.[28 29] Recent findings, however, highlight a decrease in mortality among older men with high-risk tumors who received curative versus conservative treatments[30 31] and stress the importance of considering comorbidities, rather than age alone, in treatment decisions.[32 33] Our results, if confirmed, could provide support for more aggressive therapy in selected older men, and point to the need for further study of age-related molecular markers to aid in identifying appropriate treatment candidates. The results are also relevant to guidelines that address screening for prostate cancer, in that even with recent updates—such as by the US Preventive Services Task Force in 2018[34]—recommendations have consistently advised against screening for elderly men (eg, 70 years and older).

From a methodological perspective, manual evaluation of stained tissues precludes evaluation of certain features available with automated techniques, yet such technology was validated based on manual techniques as a gold standard,[35–37] and a manual approach was customary at the time of the original project and is still used commonly today. In terms of the markers themselves, how best to measure MVD is controversial,[15 38–40] as is the question of whether increased MVD has prognostic implications.[15 41–43] Regarding the latter point, prior research[6] by our group can be viewed as supportive of an MVD-outcome association. A parallel issue for p53 involves how to optimize staining and its characterization, as described for gastric[44] and ovarian[45] cancers. Such reports, however, suggest that a modest change in the precision of measurements wouldn't threaten the validity of our categorical (yes-no) coding.[46]

In terms of our study itself, IHC data on 201 men were missing due to inadequate tumor tissue or technical problems, although baseline characteristics for these men were consistent with the analytic sample. In addition, statistical power for analyses with lower marker staining prevalence and smaller age group sample sizes was reduced, resulting in wider CIs for some point estimates. A similar scenario occurred for the adjusted analysis of age and Gleason score, but the magnitude of association and overall consistency of results suggests robust relationships for corresponding associations. Also, the data were collected in the 1990s, when anatomic stage tended to be higher and Gleason scores tended to be lower, but this issue should not threaten the internal validity of our findings. Finally, study participants were US veterans, which may impact generalizability.

Strengths of our study include the relatively large sample size (n≈1,000), 'rich' clinical data, and a complete spectrum of prostate cancer severity. In addition, follow-up was robust, with the most relevant outcome (death) occurring in a majority of the study population. Our analyses also used clinically pertinent cut points for age that had previously been understudied, and the results accounted for differences in clinicopathologic disease features that could potentially confound the relationships of interest. Lastly, previous work had demonstrated an association of molecular markers with biochemical recurrence[7 9 47] and survival,[6] extending the relevance of our results to an important health outcome.

Our findings suggest that continued exploration of age differences in molecular markers of prostate cancer has the potential to enrich our understanding of the biology of this disease, contribute to identifying factors associated with aggressive tumors (and possibly worse outcomes) in older men, and potentially improve clinical decision making.

Table 3 Association of age and 'positive' molecular marker staining; n≤971

	bcl-2 positivity		p53 positivity		MVD positivity	
	Unadjusted (n=904)	Adjusted* (n=817)	Unadjusted (n=939)	Adjusted* (n=847)	Unadjusted (n=908)	Adjusted* (n=820)
Age	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)
<70 years	(ref)	(ref)	(ref)	(ref)	(ref)	(ref)
70–79 years	1.41 (0.81 to 2.46)	1.17 (0.63 to 2.17)	1.04 (0.76 to1.42)	0.94 (0.67 to 1.31)	1.08 (0.82 to 1.42)	1.01 (0.75 to 1.36)
≥80 years	2.69 (1.13 to 6.45)	1.89 (0.73 to 4.92)	2.46 (1.41 to 4.31)	1.91 (1.05 to 3.46)	2.37 (1.30 to 4.33)	2.00 (1.06 to3.78)

*ORs adjusted for race, screen-detected cancer, PSA, and Gleason score.
MVD, microvessel density (see text for description of other markers); PSA, prostate-specific antigen; ref, reference group.

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Ethics approval VA Connecticut. Institutional review boards at the nine Veterans Affairs (VA) medical centers contributing data approved the original research protocol and waived informed consent. This analysis was approved by the Veterans Administration (VA) Healthcare System Human Studies Subcommittee and was conducted in accordance with the World Medical Association's Declaration of Helsinki.

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