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Molecular Markers and Death From Prostate Cancer

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Background: Current methods to assess the prognosis of prostate cancer at the time of diagnosis are limited.

Objective: To determine whether molecular markers of cell cycle regulation (bcl-2 and p53) and angiogenesis (β-3 integrin, vascular endothelial growth factor, and microvessel density) are associated with increased long-term risk for death among men with prostate cancer.

Design: Observational cohort study from 1991 to 2006.

Setting: The Veterans Affairs Healthcare System in New England.

Patients: Among 64 545 veterans at least 50 years of age, 1313 patients who had incident prostate cancer from 1991 to 1995 were identified. Clinical data were available for 1270 men and complete for 1172 men.

Measurements: Data were extracted from medical records, including patient age, race, and comorbid conditions, as well as tumor-related anatomical extent, histologic grade (Gleason score), prostate-specific antigen level, symptoms, and treatment. Immunohistochemical analyses of tissue obtained at diagnosis, which used antibodies against the selected markers, were also conducted. Proportional hazards analysis was used to evaluate the association of these factors with death from prostate cancer through 2006.

Results: At diagnosis, the median age was 72 years, the median prostate-specific antigen level was 10.0 μg/L, and most tumors were moderately differentiated. During an 11- to 16-year follow-up, 71.8% (842 of 1172) of men died, with 21.5% (181 of 842) of deaths attributable to prostate cancer. Among 1007 men with results for all pertinent markers and after adjustment for age and clinical characteristics, bcl-2 (adjusted hazard ratio [HR] for positive vs. negative staining, 1.61 [95% CI, 1.01 to 2.57]; $P = 0.045$), p53 (adjusted HR for positive vs. negative staining, 1.48 [CI, 1.06 to 2.08]; $P = 0.022$), and microvessel density (adjusted HR for highest vs. lowest quartile, 3.20 [CI, 1.77 to 5.78]; $P < 0.001$) were associated with death from prostate cancer.

Limitations: Results may be affected by residual confounding. Some patients were not included in complete case analyses because information was not available from clinical care records (7.5%) or tissue staining (12.6%).

Conclusion: Immunohistochemical evidence of bcl-2, p53, or high microvessel density in prostate cancer biopsy specimens at diagnosis is associated with an increased long-term risk for death from prostate cancer.

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The spectrum of severity in prostate cancer is highly variable, ranging from indolent to aggressive. Some men with prostate cancer have longevity similar to the general population, whereas others develop metastatic disease that can lead to death within months (1–3). Clinicians have limited ability to estimate survival in patients with newly diagnosed prostate cancer, and uncertainty therefore exists about optimal treatment decisions (4), especially for men with localized disease.

Current clinical strategies (5) for evaluating prognosis in prostate cancer at the time of diagnosis include determining anatomical extent, histologic grade (Gleason score), and serum levels of prostate-specific antigen (PSA). As a novel approach, molecular features—such as markers of cell cycle regulation and blood vessel formation—are potentially relevant prognostic factors. A recent review (6) reported that abnormal expression of various molecular markers is related to increasing stage and grade of prostate cancer but may or may not influence long-term health outcomes.

We sought to examine whether selected molecular factors are independently associated with death in men with prostate cancer. In particular, abnormal markers involved in apoptosis, tumor suppression, and angiogenesis may indicate poor prognosis. To evaluate this hypothesis, we identified a sample of men with prostate cancer; reviewed medical records to account for pertinent clinical characteristics of patients and their tumors; examined tissue obtained at the time of diagnosis for evidence of molecular markers; and measured long-term, cause-specific, and all-cause mortality.

Methods

Study Sample

The source sample included all 64 545 male veterans who were receiving ambulatory care linked to 9 Veterans Affairs (VA) medical centers in New England as of 1 January 1991. The institutional review board at each institution approved the research protocol with a waiver of informed consent. Pathology registries identified 1313 men

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Context

Whether molecular markers distinguish indolent from aggressive prostate cancer is unclear.

Contribution

This observational study of U.S. veterans found that markers of cell cycle regulation (bcl-2 and p53) and high microvessel density in biopsy specimens obtained at diagnosis were associated with increased risk for death from prostate cancer.

Caution

Some factors that might affect prognosis, such as family history, other molecular markers, and prostate-specific antigen velocity, were not assessed.

Implication

We need studies assessing whether molecular features that are associated with increased risk for death from prostate cancer are clinically useful in distinguishing patients who might and might not benefit from particular therapies.

—The Editors

with incident prostate cancer diagnosed from 1991 to 1995 (**Figure 1**). Medical records were unavailable (after at least 3 requests) for 16 (1.2%) men and were inadequate for 27 (2.1%) men (for example, missing entire sections). We collected data on candidate prognostic variables among the remaining 1270 men by using strategies for prognostic studies (7).

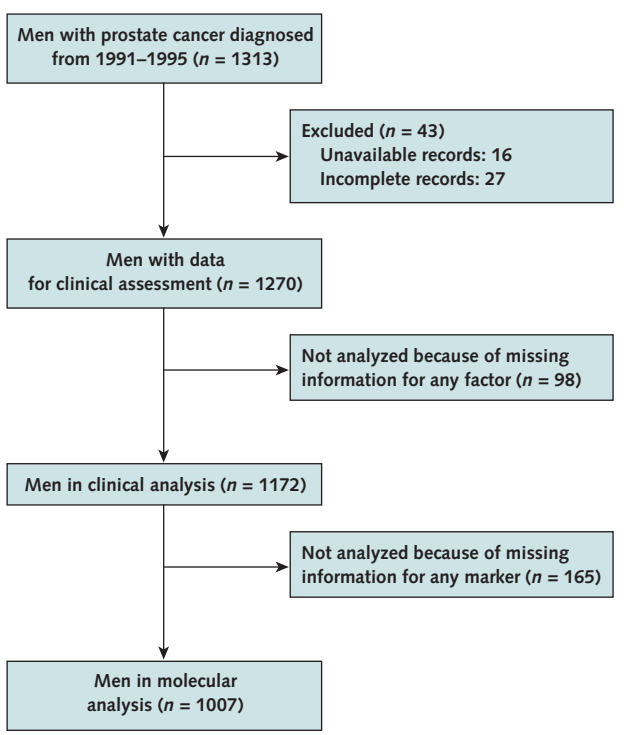
Data Collection

We analyzed 3 sources of data: paper and electronic medical records, immunohistochemical staining of prostatic tissue from which the initial diagnosis of cancer was made, and determination of vital status according to national databases. We first obtained clinical data before primary treatment (designated as "zero-time" [7]) through a comprehensive medical record review by using a standardized extraction form adapted from a previous study (8). We recorded each man's age (years), race (black or other), and comorbid condition (Charlson comorbidity index [9]). We recorded the anatomical extent (clinical stage) and histologic grade (Gleason score) of cancer on the basis of classification systems in use at the time. We also documented PSA levels and cancer-related symptoms (8). We always found certain factors, such as age, in the medical record. Other factors, such as at least 2 PSA tests before diagnosis (to calculate PSA velocity), were sometimes not available. Although our study was not designed to assess the effect of therapy, we coded initial treatment as surgery (prostatectomy), radiation therapy (nonadjuvant), hormone ablation, watchful waiting, or none.

We also requested diagnostic tissue blocks and slides for the men: 1149 (90.5%) from needle biopsies, 114 (9.0%) from transurethral resections of the prostate, 6 (0.5%) from prostatectomies for presumed benign disease, and 1 (0.1%) from a metastatic lesion. After confirming the presence of a tumor, our institutional pathology laboratory did immunohistochemical staining by using indirect immunoperoxidase methods with antibodies against selected factors and by blocking nonspecific staining (10). We evaluated the tissue for bcl-2 (11), an apoptosis-related molecule (dilution, 1:160; Dako, Carpinteria, California); p53 (12), a tumor-suppressor oncogene (dilution, 1:3000; Dako); β-3 integrin (13) (measured as CD-61), an adhesion molecule implicated in tumor invasion and angiogenesis (dilution, 1:40; Vector Laboratories, Burlingame, California); and soluble vascular endothelial growth factor (14), an angiogenic cytokine (dilution, 1:1; BioGenex, San Ramon, California). We recorded intensities of staining in areas of carcinoma on a scale from 0 to 3. In addition, we evaluated microvessel density (15) as a manifestation of tumor angiogenesis by using antibodies to factor VIII (dilution, 1:4000; Dako) in a more labor-intensive process of counting the number of antigen-stained blood vessel cross-sections seen on high-powered magnification (original magnification, $\times$400). A pathologist blinded to patient outcome did all of the readings.

We assessed the vital status of each patient by using the VA Patient Treatment File, the VA Beneficiary Identifier Locator System (16), and the National Death Index (17). Death from prostate cancer was determined while

Figure 1. **Study flow diagram.**



investigators were blinded to marker status through post-treatment medical record review and consensus decision (8). A censoring date of 31 December 2006 provided an 11- to 16-year range of potential follow-up after zero-time for each patient.

Statistical Analysis

On the basis of earlier work (8), we expected to identify as many as 1350 men with prostate cancer and observe an overall annual survival rate of 93%. For a type I error of 5%, 80% power, and at least an 8-year follow-up, we calculated the minimum detectable relative risks as 1.20, 1.17, 1.16, and 1.15 for a prevalence of molecular markers equal to 20%, 30%, 40%, and 50%, respectively. In addition, for as many as 13 variables (≤7 baseline + treatment + 5 markers) in a multivariable analysis, and based on an established criterion (18, 19) of at least 10 outcome events per independent variable, the anticipated number of deaths would exceed the suggested minimum threshold ($13 \times 10 = 130$).

Analyses of clinical data used a "complete case" approach for 1172 (among 1270) men with no missing information for any factor. We calculated descriptive results as percentages or median values and interquartile ranges. A category of "too small to grade" was used for molecular markers if only a single microscopic focus of cancer was found; molecular data were coded as "not available" if technical problems occurred. Molecular markers that did not yield any usable staining after 50 specimens were abandoned.

By using proportional hazards regression analysis, we first assessed "traditional" patient- and tumor-related characteristics for their association with death from prostate cancer. We subsequently used another multivariable proportional hazards model to evaluate the independent association of "novel" molecular markers with the same outcome, adjusting for the traditional prognostic factors.

We also examined death from any cause as an outcome variable, and we included treatment as an adjustment variable in a sensitivity analysis (using death from prostate cancer). In post hoc exploratory analyses, we assessed results on the basis of a validated system (20) for classifying low-, intermediate-, and high-risk groups according to combinations of clinical stage, Gleason score, and PSA level. We assessed key variables for interobserver variability in a 10% sample of medical records and intraobserver variability in a 10% sample of immunohistochemical stains. We did analyses by using SAS, version 9.1 (SAS, Cary, North Carolina).

Role of the Funding Source

The Office of Research and Development in the Veterans Health Administration funded the study. The funding source had no role in the study design, data collection, data analysis, data interpretation, or decision to submit the manuscript for publication.

Table 1. **Characteristics of Men With Prostate Cancer**

Characteristic	Men With Prostate Cancer (n = 1172)
Patient-related data	
Median age (IQR), *y*	72 (68–76)
Age, *n (%)*	
50–59 y	35 (3)
60–69 y	400 (34)
70–79 y	660 (56)
≥80 y	77 (7)
Race or ethnicity, *n (%)*	
Black	127 (11)
Other	1045 (89)
Comorbid conditions, *n (%)*	
0 (none)	317 (27)
1 (mild)	355 (30)
2 (moderate)	250 (21)
≥3 (severe)	250 (21)
Tumor-related data	
Anatomical stage, *n (%)**	
Localized (T1, T2)	1040 (89)
Regional (≥T3)	60 (5)
Metastatic (M1, M2)	72 (6)
Histologic differentiation	
Median Gleason score (IQR)	6 (5–7)
Gleason score, *n (%)*	
Good (2–4)	267 (23)
Moderate (5–7)	711 (61)
Poor (8–10)	194 (17)
Median baseline PSA level (IQR), *μg/L*	10.0 (5.7–21.0)
Baseline PSA level, *n (%)*	
0 to ≤4.0 μg/L (reference)	172 (15)
4.0 to ≤10.0 μg/L	413 (35)
10.0 to ≤20.0 μg/L	279 (24)
≥20.0 μg/L	308 (26)
Extent of disease, *n (%)*	
None	1024 (87)
Local only	86 (7)
Metastatic	34 (3)
Systemic	28 (2)
Immunohistochemical data, *n (%)*	
bcl-2	
Negative	837 (71)
Positive	67 (6)
Tumor too small	143 (12)
Not available	125 (11)
p53	
Negative	694 (59)
Positive	245 (21)
Tumor too small	142 (12)
Not available	91 (8)
Microvessel density	
0–19 vessels/hpf	197 (17)
20–28 vessels/hpf	226 (19)
29–39 vessels/hpf	250 (21)
≥40 vessels/hpf	235 (20)
Tumor too small	159 (14)
Not available	105 (9)
β-3 Integrin†	–
Vascular endothelial growth factor‡	–
Treatment and outcome data	
Initial treatment received, *n (%)*	
Prostatectomy	224 (19)
External beam or seed radiation	408 (35)
Hormonal therapy only	215 (18)
Watchful waiting or none	325 (28)
Death outcomes as of 31 December 2006	
Death from any cause, *n (%)*	842 (71.8)
Death from prostate cancer, *n/n (%)*	181/842 (21.5)
Median overall survival, *y*	7.7

hpf = high-power field; IQR = interquartile range; PSA = prostate-specific antigen.

* Anatomical stage based on applicable TNM classification system of the American Joint Committee on Cancer.

† No staining was observed for β-3 integrin using CD-61 antibody.

‡ Staining for vascular endothelial growth factor was observed in background (stromal) cells only.

Table 2. **Effect of Traditional Factors on Death From Prostate Cancer ($n = 1172$)**

Prognostic Factor	Adjusted Hazard Ratio (95% CI)*	*P* Value
Age (per year)	1.03 (1.01–1.06)	0.032
Race (nonwhite)	0.73 (0.45–1.18)	0.198
Comorbid conditions		
None	Reference	–
1	1.03 (0.69–1.56)	0.87
2	1.31 (0.85–2.03)	0.23
≥3	1.74 (1.14–2.67)	0.011
Anatomical stage		
Localized	Reference	–
Regional	2.14 (1.29–3.55)	0.003
Metastatic	6.52 (4.24–10.01)	<0.001
Differentiation (Gleason score)		
Good (2–4)	Reference	–
Moderate (5–7)	2.58 (1.40–4.74)	0.002
Poor (8–10)	3.98 (2.07–7.65)	<0.001
Prostate-specific antigen		
0–3.9 μg/L	Reference	–
4.0–9.9 μg/L	0.94 (0.45–1.96)	0.86
10.0–19.9 μg/L	1.81 (0.88–3.69)	0.106
≥20.0 μg/L	3.71 (1.87–7.37)	<0.001
Symptoms		
None	Reference	–
Local only	1.14 (0.68–1.93)	0.62
Metastatic	1.49 (0.85–2.60)	0.161
Systemic	1.68 (0.82–3.48)	0.158

* Results are adjusted for all other prognostic factors listed in this table.

RESULTS

Baseline Factors

Table 1 shows the characteristics of 1172 men with a prostate cancer diagnosis from 1991 to 1995. The median age at diagnosis was 72 years; 127 men (11%) were black, 672 (57%) had no or mild comorbid conditions, 1040 (89%) had clinically localized cancer, and 711 (61%) had moderately differentiated tumors (Gleason score, 5 to 7); and the median PSA level was 10.0 μg/L. Initial treatment included prostatectomy or radiation therapy in 632 (54%) men, hormonal ablation in 215 (18%) men, and watchful waiting or no treatment in 325 (28%) men. Among the latter group, 106 (33%) subsequently received therapy. The κ statistics for concordance (21) were 0.77 for comorbid conditions, 0.92 for histologic grade, and 0.80 for immunohistochemical readings.

Table 1 also shows results of the immunohistochemical analyses. Positive staining was found for bcl-2 in 67 (6%) and p53 in 245 (21%) specimens; coding of "any" or "no" staining was used in analyses of these factors because of low observed prevalence. Quartiles of vessels per high-powered field were used to code microvessel density. No positive staining in areas of carcinoma was seen for CD-61. Although positive background staining of prostatic stromal cells was seen for vascular endothelial growth factor, no reproducible staining of malignant cells was evident in biopsy or tissue from transurethral resection, despite use of reagents from several different vendors and several different antigen retrieval techniques (22).

Effect of Traditional Factors on Death From Prostate Cancer

After more than 15 years of follow-up, 71.8% (842 of 1172) patients died of any cause, with 21.5% (181 of 842) of deaths due to prostate cancer. The median overall survival was 7.7 years. As shown in **Table 2**, the effect of traditional patient- and tumor-level characteristics was confirmed in a multivariable model of death from prostate cancer, with increasing age, severe comorbid conditions, regional or metastatic prostate cancer, moderate or poorly differentiated tumor, and PSA level of at least 20 μg/L associated with shorter survival. Race and symptoms were not associated with death from prostate cancer and were therefore not included in subsequent analyses.

Effect of Molecular Markers on Death From Prostate Cancer

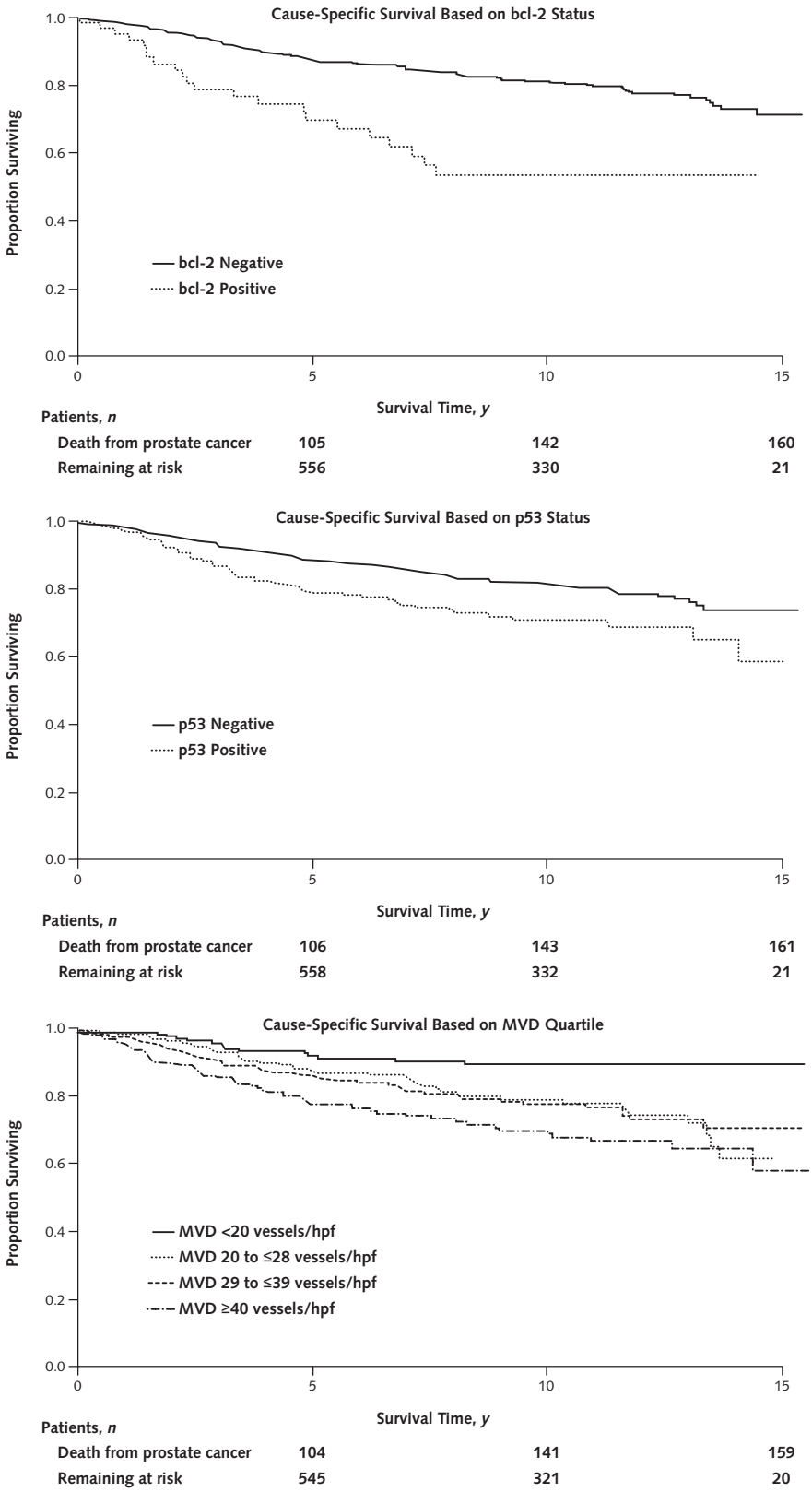
Because of missing data for the remaining markers (bcl-2, p53, and microvessel density), the sample size for subsequent multivariable analyses decreased to 1007 patients. Among men with complete information, 23.2% (169 of 728) of deaths were due to prostate cancer. **Figure 2** shows unadjusted cause-specific survival proportions, based on expression of bcl-2 and p53 and microvessel density, during the more than 15-year follow-up. **Table 3** shows the unadjusted and adjusted associations of molecular markers and death from prostate cancer. Positive (vs. negative) staining for bcl-2 (adjusted hazard ratio [HR], 1.61 [95% CI, 1.01 to 2.57]; $P = 0.045$) and p53 (adjusted HR, 1.48 [CI, 1.06 to 2.08]; $P = 0.022$) had statistically significant associations with death from prostate cancer. Similarly, the upper 3 quartiles of microvessel density were associated with increased death from prostate cancer (for example, adjusted HR for highest vs. lowest quartile, 3.20 [C.I. 1.77 to 5.78]; $P < 0.001$).

Secondary and Exploratory Analyses

Additional analyses further examined the primary results. For example, results were similar (data not shown) when death from any cause was used as the outcome variable. The effect of bcl-2, p53, or microvessel density on death from prostate cancer was also similar (data not shown) after adjustment for therapy, but because of concern about selection bias, we did not evaluate the effectiveness of specific treatments. In addition, having all 3 positive molecular markers (vs. all other categories) was associated with a greatly increased risk for death from prostate cancer (adjusted HR, 7.65 [CI, 2.34 to 25.0]; $P < 0.001$). Finally, results were similar (data not shown) when we did analyses only among men with prostate cancer diagnosed by needle biopsy.

Although the study was not powered to examine associations in selected groups of patients, **Table 4** shows post

Figure 2. **Estimated probability of cause-specific survival.**



Unadjusted results for bcl-2 ($P < 0.001$), p53 ($P = 0.002$), and MVD ($P < 0.001$) measured in quartiles of vessels per high-power microscopic field ($n = 1007$), excluding patients with tumors too small for staining (see text for details). hpf = high-power field; MVD = microvessel density.

Table 3. **Effect of Molecular Markers on Death From Prostate Cancer***

Candidate Prognostic Factor	Unadjusted Hazard Ratio (95% CI)	*P* Value	Adjusted Hazard Ratio (95% CI)*	*P* Value
bcl-2				
Negative	Reference	–	Reference	–
Positive	2.71 (1.73–4.25)	<0.001	1.61 (1.01–2.57)	0.045
Tumor too small	0.37 (0.19–0.72)	0.004	3.43 (0.50–23.8)	0.21
p53				
Negative	Reference	–	Reference	–
Positive	1.66 (1.20–2.30)	0.002	1.48 (1.06–2.08)	0.022
Tumor too small	0.35 (0.17–0.72)	0.004	0.42 (0.05–3.72)	0.44
Microvessel density				
<19 vessels/hpf	Reference	–	Reference	–
20–28 vessels/hpf	2.43 (1.35–4.37)	0.003	2.79 (1.51–5.16)	0.001
29–39 vessels/hpf	2.47 (1.37–4.42)	0.003	2.43 (1.32–4.47)	0.004
≥40 vessels/hpf	3.71 (2.10–6.55)	<0.001	3.20 (1.77–5.78)	<0.001
Tumor too small	0.78 (0.35–1.74)	0.54	1.36 (0.36–5.12)	0.65

hpf = high-power field.
* Sample size for this analysis was 1007 men. Hazard ratios are adjusted for statistically significant factors from **Table 2**.

hoc analyses that examined the effect of molecular markers in groups defined by clinical risk (20). Among men with low-risk clinical status and the greatest potential for cure, the magnitude of the HRs was increased for the association of positive (vs. negative) molecular markers and death from prostate cancer. A general pattern of increased hazard of death for positive markers was also found in the moderate- and high-risk groups.

Discussion

Molecular markers are not used routinely to assess men with a diagnosis of prostate cancer because their relevance to important health-related outcomes has been uncertain. Invoking a comparison with commonly used tests for women with a diagnosis of breast cancer (for example, estrogen and progesterone receptors and HER2 status), a recent report stated, "it is remarkable that comparable, generally accepted, clinically relevant markers are not available for prostate cancer" (23). This clinical situation exists despite more than 15 years of considerable research activity. An informal literature search (through November 2008) identified hundreds of articles each year reporting recently on bcl-2, p53, microvessel density, or other markers in prostate cancer. An overview of published studies indicates a sustained interest in molecular markers, involving both laboratory-based and clinic-based investigations. Some reports describe mechanisms at the genomic or molecular level, whereas others describe the clinical status of patients. Projects using tumor microarrays (24, 25) or assessing single nucleotide polymorphisms (26, 27) are increasingly common.

Among translational and clinical investigations that have focused on patient-level phenomena, 3 major methodological problems are apparent. First, rather than evaluating a longitudinal association with clinical outcomes (for example, death), many studies assessed only the cross-sectional relationship of molecular markers with tumor characteristics (for example, histology). Thus, a prognostic effect was not examined. Second, studies were often done among patients who had received a single type of therapy (attributable to having an available "case series"), rather than evaluating all patients at diagnosis. These reports are potentially biased because patient characteristics that influence therapy could be related to marker status and subsequent death. In addition, tissue obtained at prostatectomy is obviously not available at the time of diagnosis and decision about primary therapy. Accordingly, the appropriate strategy for assessing prognosis is to gather information after a biopsy is done but before a treatment is selected (7). Third, conflicting results on whether markers are associated with death include studies with small sample sizes and insufficient statistical power. Thus, some negative (null) reports have been described as unjustified claims of equivalence "after a failed test for superiority" (28).

These limitations of previous studies have been alluded to elsewhere, such as "most [studies of molecular markers in prostate cancer] do not link their data to clinical endpoints" and "many of these studies suffered from small patient groups" (23). In discussing a specific and representative marker, a review article identified "more than 100 studies reporting series of patients with prostate cancer evaluated for p53," and found that "this literature demonstrates increasing p53 expression with increasing grade and stage, with a prognostic effect that may or may not be independent of these two variables" (6). In this context, a recent report mentioned "one of the major scientific challenges will be the validation of several potential biomarkers in large enough and clinically well-characterized patient cohorts" (23). Similarly, a need was noted for research on " . . . molecular prognostic markers that could help to distinguish high-risk cases that culminate in metastatic spread and death from their indolent counterparts . . . " (29).

We did our research in a large sample, used data obtained at the time of diagnosis, evaluated patients who received various treatment modalities (including none), examined a panel of molecular markers, adjusted for pertinent factors, and assessed death as an end point. Therefore, our research can be viewed as advancing work done by previous investigations (6, 23, 29–31). In particular, long-term mortality from prostate cancer was affected by bcl-2, indicating inhibition of programmed cell death; p53, indicating loss of tumor suppression; and high microvessel density, reflecting angiogenesis.

Of importance, we studied patients in the "PSA era" and included long-term follow-up. We also used tissue obtained mainly from needle biopsies rather than from postprostatectomy specimens, thereby avoiding selection bias. The validity of our results is supported by methodological criteria (32) that help to identify true prognostic factors as statistically significant, independent, and clinically relevant. Validity is also supported by a lack of association between molecular markers and nonprostate cancer mortality (data not shown). The relatively low prevalence of positive staining precluded extensive analyses in subgroups of men, yet our results (**Table 4**) include a strong magnitude of associations, albeit not statistically significant, for men in a low-risk group for whom treatment decisions are difficult.

Study limitations include the potential for residual confounding due to unmeasured factors (for example, data on family history were infrequent and not used). We assessed archival data and specimens generated from clinical encounters, without a prospective research infrastructure, but this information represents "actual" health care and few medical records were missing. For complete case analyses, an individual factor in the medical record was sometimes lacking (7.5%), and data for molecular markers were sometimes not available (12.6%). Yet, when patients with incomplete information were included, each marker still had a statistically significant association with death from prostate cancer (data not shown). Other potential limitations include molecular factors not measured (33, 34), but we selected markers on the basis of evidence available when we planned our project.

Our participants included U.S. veterans, which influences the generalizability of results (for example, older men with comorbid conditions are over-represented) but does not affect the suitability of study design, breadth of data collected, or cogency of analysis. Additional characteristics of the study sample (for example, reported or calculated anatomical substage at diagnosis) are beyond the scope of the present work, as are nomograms intended for clinical use. We decided to balance the advantage of long-term follow-up with the disadvantage of reporting noncontemporary classifications of anatomical stage and histologic grade. For example, anatomical T1c tumors were diagnosed infrequently in the early 1990s, and the distribution of reported Gleason scores has subsequently shifted (35). Yet, despite such changes in reporting, the measurements used in the 1990s adequately assessed the status of prostate cancer.

The lack of specific and reproducible staining for 2 features of angiogenesis (β-3 integrin and vascular endothelial growth factor) is notable, although caution is warranted in interpreting our results when archived biopsy material was used. Among the factors that produced analyzable information (bcl-2, p53, and microvessel density), the association with death from prostate cancer is consistent with purported biological functions (36–43). Whether targeted therapy based on these mechanisms would improve patient outcomes, however, is uncertain (44–48).

In the current era of genomic medicine, evaluating genetic variants is considered cutting-edge research, yet such studies in prostate cancer (26, 27) have focused mainly on the risk for the disease. As mentioned in an editorial accompanying a recent genomic study, such work is therefore "of great mechanistic importance but of less clinical consequence, since the [specific haplotypes] do not distinguish between indolent and aggressive prostate cancer" (49). Another article mentioned " . . . how nice it would be if we could define, through molecular markers, those patients whose cancer will progress and benefit from treatment from those patients with indolent cancer . . . " (50). Our study represents incremental progress on this topic by assessing the severity of prostate cancer beyond

Table 4. **Exploratory Analysis of Molecular Markers and Death From Prostate Cancer in Selected Groups**

Molecular Marker*	Low-Risk Patients (n = 312)		Intermediate-Risk Patients (n = 303)		High-Risk Patients (n = 392)	
	Adjusted HR (95% CI)†	P Value	Adjusted HR (95% CI)†	P Value	Adjusted HR (95% CI)†	P Value
bcl-2	2.72 (0.29–25.4)	0.38	1.29 (0.29–5.73)	0.73	1.90 (1.16–3.11)	0.011
p53	4.82 (1.05–22.1)	0.043	2.04 (0.88–4.71)	0.098	1.33 (0.91–1.94)	0.141
Microvessel density	3.08 (0.58–16.3)	0.186	1.22 (0.53–2.80)	0.64	1.15 (0.80–1.66)	0.46

HR = hazard ratio.
* Results are shown for bcl-2 and p53 staining as positive vs. negative (reference); microvessel density was recoded as above vs. below (reference) the median value, to provide a single coefficient.
† Risk groups are based on clinical stage, Gleason score, and prostate-specific antigen level (as defined in reference 20). Hazard ratios are adjusted for age, comorbid conditions, and the other molecular factors.

anatomical stage, Gleason score, PSA level, and other characteristics currently used in medical practice.

Our results suggest that immunohistochemical staining of biopsy tissue for bcl-2, p53, and high microvessel density at the time of diagnosis of prostate cancer is associated with an increased long-term risk for death from this disease.

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