

Molecular markers and mortality in prostate cancer

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Accepted for publication 8 June 2007

Study Type – Prognosis (retrospective cohort study)
Level of Evidence 2b

OBJECTIVE

To evaluate prognosis in prostate cancer by assessing the independent effect of selected molecular factors (e.g. markers of cell-cycle regulation), in addition to the effect of traditional clinical factors (e.g. anatomical stage, histological grade), in predicting long-term mortality among men newly diagnosed with prostate cancer.

PATIENTS AND METHODS

In a community-based population of 64 545 USA veterans aged ≥ 50 years and receiving

ambulatory care during 1989–90 at nine Veterans Affairs (VA) medical centres in New England, 1274 had incident prostate cancer during 1991–95. We obtained the medical records and diagnostic tissue for these men, and then extracted demographic data and clinical information, and conducted immunohistochemical assays of molecular markers in biopsy tissue, as potential prognostic factors. In this interim analysis, data on 250 patients were analysed; the main outcome was overall mortality to 31 December 2003, providing 8–13 years of follow-up.

RESULTS

In 228 (91%) patients with available medical record and laboratory data, the median age was 72 years and the median prostate-

specific antigen level was 10.4 ng/mL. In adjusted (multivariate) analyses that included traditional prognostic factors, bcl-2 staining (hazard ratio 2.14, 95% confidence interval 1.27–3.58, $P=0.004$) and high microvessel density (1.76, 1.19–2.60; $P=0.005$) had an independent effect on the outcome.

CONCLUSIONS

Bcl-2 and microvessel density are independent predictors of subsequent death among men with prostate cancer and might have a clinical role in assisting in deciding on treatment.

KEYWORDS

prostatic neoplasms, prognosis, angiogenesis, bcl-2 proto-oncogene

INTRODUCTION

Published reports have examined various aspects of prognosis in prostate cancer. Patient-orientated research has focused on clinically relevant issues, e.g. predicting the pathological stage at surgery [1], assessing clinical or biochemical progression of disease after radical prostatectomy [2], examining the effect of preoperative PSA velocity on mortality after surgery [3] or radiation therapy [4], and describing long-term survival after conservatively treating localized prostate cancer [5]. Concurrently, laboratory-based and translational research has focused on molecular markers [6,7], but usually in studies that assess the correlation of such markers with tumour stage or grade, or as risk factors for resistance to androgen ablation or radiotherapy.

In a more comprehensive approach, a combination of clinical and laboratory-based factors would be evaluated, at the time of

diagnosis of prostate cancer (rather than in subgroups of patients who have received a particular therapy), to determine their effect on long-term survival. In this context, the objective of the present study was to determine whether factors related to angiogenesis and cell-cycle regulation, when assessed simultaneously with traditional clinical factors, provide independent predictive information about mortality among men with incident prostate cancer; in this report we describe an interim analysis from an ongoing study of prognosis in prostate cancer.

PATIENTS AND METHODS

The present sample was selected from a community-based population of 64 545 men receiving ambulatory care during 1989–90 at nine Veterans Affairs (VA) medical centres in New England, USA. Medical records and pathology registries identified 1274 men with incident prostate cancer during 1991–1995.

Candidate prognostic factors, assessed up to the time of initial treatment, include baseline demographic, clinical and molecular characteristics. These factors are being evaluated for their effect on overall mortality to 31 December 2003, providing a median (range) of 11 (8–13) years of follow-up. The primary analysis adjusted for treatment(s) received by patients; the secondary analyses included a treatment-stratified approach, and cause-specific (prostate cancer) death as the outcome.

This interim analysis includes over-sampling of mortality to provide adequate outcome events; 250 patients were identified based on their vital status as of 31 December 1999. To take advantage of additional follow-up, the current report considers mortality to 31 December 2003. Among the 250 patients, 228 (91%) had available medical records and biopsy tissue. Data were collected from three sources: first, a review of the medical records to obtain clinical data, based on a

standardized extraction form developed in a previous study [8]; second, paraffin-embedded blocks of tissue obtained at diagnosis (85% from needle biopsies) were retrieved and used to obtain data via immunohistochemical assays, with interpretation by one genitourinary pathologist unaware of the outcome; third, the vital status was determined using the VA Patient Treatment File, the VA Beneficiary Identifier Locator System [9], and the National Death Index [10] with cause-specific mortality based on a medical record review [8].

Potential prognostic factors included patients' age, race, and comorbidity (using the Charlson index [11]); the anatomical stage and histological (Gleason) grade of cancer; and PSA level. Using commercially available immunohistochemical antibodies, paraffin-embedded tissue from biopsy specimens was stained by a clinical (hospital) laboratory for β -3 integrin (implicated in tumour angiogenesis) [12], vascular endothelial growth factor (VEGF, angiogenic cytokine) [13], bcl-2 (apoptosis-related oncogene) [14], p53 (tumour-suppressor oncogene) [15], and microvessel density (MVD, a visual assessment of angiogenesis) [16]. The intensity of immunohistochemical staining in areas of carcinoma was rated on a 0, +, ++, +++ scale, except for MVD, which was counted as the number of vessels in a high magnification ($\times 400$) field, including representative 'hot spots', after antibody to Factor VIII-related antigen was used to highlight vessel endothelium. Primary treatment was coded as surgery (prostatectomy), radiotherapy (not adjuvant), hormone ablation, or watchful waiting/none.

Descriptive results were calculated as percentages or median values. Unadjusted and adjusted hazard ratios (HRs) for mortality and their 95% CIs were calculated using proportional-hazards analysis. Data were coded as 'unknown' when information on PSA level was lacking in medical records (43 men); irremediable technical problems occurred for bcl-2 in 38 samples, p53 in 40 and MVD in 48; or a single focus of cancer was 'too small to grade' histologically in five. The Institutional Review Board at each participating institution approved the protocol.

RESULTS

The baseline characteristics for the 228 men are shown in Table 1; the median age at entry

Characteristic	N (%)	TABLE 1 Characteristics of the 228 patients
Age, years		
50-59	3 (1)	
60-69	68 (30)	
70-79	134 (59)	
≥ 80	23 (10)	
Charlson Comorbidity:		
none (0)	40 (18)	
mild (1)	38 (17)	
moderate (2)	48 (21)	
severe (>2)	102 (45)	
Tumour differentiation (Gleason)		
well (2-4)	54 (24)	
moderate (5-7)	123 (54)	
poor (8-10)	46 (20)	
unknown	5 (2)	
Anatomical stage		
I	38 (17)	
II	156 (68)	
III	17 (7)	
IV	17 (7)	
PSA level, ng/mL		
≤ 4.0	24 (11)	
4.1-10.0	66 (29)	
10.1-20.0	44 (19)	
>20.0	51 (22)	
unknown (pre-diagnosis)	43 (19)	
Bcl-2		
negative	168 (74)	
positive	22 (10)	
unknown	38 (17)	
p53		
negative	141 (62)	
positive	47 (21)	
unknown	40 (18)	
MVD		
low (≤ 28)	92 (40)	
high (>28)	88 (39)	
unknown	48 (21)	
Mortality as of 31 December 2003	168/228 (74)	

into the cohort was 72 years; 6% of men were African-American; 66% of men had moderate or severe comorbidity; most pathology reports indicated moderate differentiation (Gleason scores of 5-7); and the median PSA value was 10.4 ng/mL. The primary treatment for prostate cancer included prostatectomy or radiation therapy in 102 (45%), hormonal ablation in 65 (29%), and watchful waiting or none in 61 (27%). With over-sampling of deaths, the cumulative (overall) mortality was 74% (168/228). Table 1 also shows the immunohistochemical results, with positive staining for bcl-2 in 10% (22) and p53 in 21% (47) of cancers; 'any' vs 'no' staining was used

in this interim analysis. For MVD, the median value (28 vessels per high-power field) was used as a threshold to designate 'high' vs 'low'. There were no cases of positive staining for CD-61, either in tumours or in vascular endothelium. In addition, despite staining of background (stromal) cells, there was no consistent or reproducible staining of malignant cells for VEGF, using different reagents from several vendors.

As shown in Table 2, unadjusted analyses confirmed the association of traditional prognostic factors (non-local anatomical stage; Gleason 8-10; PSA > 20 ng/mL) with

TABLE 2 Unadjusted and adjusted models of mortality in 228 patients

Candidate prognostic factor	HR (95% CI), P	
	unadjusted	adjusted*
Non-local tumour	1.58 (1.05–2.37), 0.03	1.66 (1.00–2.74), 0.05
Poor differentiation	2.62 (1.66–4.14), 0.001	1.17 (0.67–2.03), 0.59
PSA >20 ng/mL	1.83 (1.28–2.63), 0.001	1.21 (0.81–1.80), 0.35
Bcl-2 positive	1.75 (1.08–2.84), 0.02	2.14 (1.27–3.58), 0.004
p53 positive	1.32 (0.91–1.91), 0.15	1.31 (0.88–1.96), 0.18
High MVD	1.59 (1.14–2.23), 0.007	1.76 (1.19–2.60), 0.005
β-3 integrin†	–	–
VEGF‡	–	–

*Adjusted for age, race, comorbidity, and other baseline variables (including unknown values) listed in Table 1. †no staining for β-3 integrin using CD-61 antibody; ‡VEGF staining of background (stromal) cells only.

TABLE 3 Secondary analyses of Bcl-2 and MVD among selected groups of patients or with an alternative outcome

Group (assessing overall mortality)	Adjusted* HR (95% CI), P	
	bcl-2	MVD
All patients† (228)	2.14 (1.27–3.58), 0.004	1.76 (1.19–2.60), 0.005
PSA ≤ 20 ng/mL (134)	2.36 (1.09–5.11), 0.03	1.96 (1.13–3.39), 0.02
Stage I or II (194)	2.04 (1.08–3.87), 0.03	1.61 (1.04–2.49), 0.03
Needle biopsies (193)	2.20 (1.30–3.74), 0.004	1.90 (1.24–2.91), 0.003
Age < 70 years (71)	3.39 (1.02–11.23), 0.046	1.70 (0.76–3.76), 0.19
Outcome (among all patients)		
Disease progression or cause-specific death (228)	2.75 (1.14–6.33), 0.02	1.86 (0.81–1.80), 0.14

*Adjusted for age, race, comorbidity, and other baseline variables (including unknown values) listed in Table 1. †Results for bcl-2 and MVD as shown in Table 2. Numbers in parentheses are the numbers in each analysis.

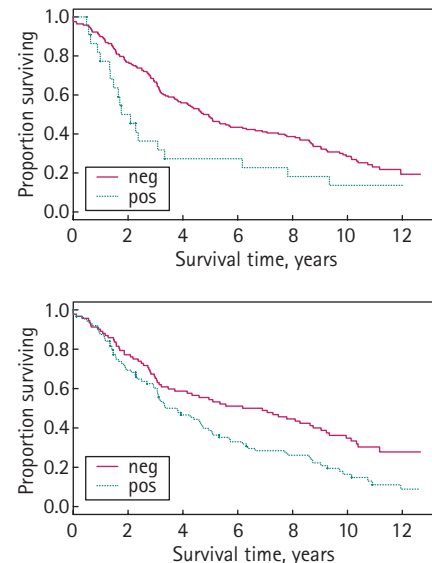
overall mortality. In addition, based on assays of biopsy specimens, bcl-2 (HR 1.75, 95% CI 1.08–2.84; $P = 0.02$) and high MVD (1.59, 1.14–2.23; $P = 0.007$) had statistically significant associations with the outcome; p53 did not (1.32, 0.91–1.91; $P = 0.15$). Figure 1 shows the probability of survival during the 8–13 years of follow-up, based on expression of bcl-2 and MVD.

Table 2 also shows the adjusted (multivariate) analysis of overall mortality among men with prostate cancer. Importantly, the associations of bcl-2 (HR 2.14, 1.27–3.58; $P = 0.004$) and high MVD (1.76, 1.19–2.60; $P = 0.005$) remained statistically significant in the context of the other prognostic factors. The prognostic effect of bcl-2 and MVD persisted

within patient strata defined by treatment category (data not shown). In the same multivariate model, treatment with surgery or radiotherapy, vs no therapy, was also associated with improved survival (data not shown). In addition, a bootstrap statistical procedure [17] confirmed the stability of the results by providing an internal validation (data not shown).

Several secondary analyses involved multivariate models that further examined the prognostic effect of bcl-2 and high MVD, as shown in Table 3. For example, among men with a PSA level of ≤20 ng/mL, the associations of both bcl-2 (HR 2.36, 95% CI 1.09–5.11, $P = 0.03$) and high MVD (1.96, 1.13–3.39, $P = 0.02$) with overall mortality

FIG. 1. The estimated probability of survival based on bcl-2 (top) and MVD (bottom).



were both statistically significant. Results were similar among men with stage I or II tumours, and when analysing data from men with prostate needle biopsies only. By contrast, among men aged <70 years, and in a model predicting disease progression or cause-specific death (among all men), bcl-2, but not high MVD, was independently associated with death.

DISCUSSION

When evaluating a 'panel' of molecular markers in an interim analysis of men with prostate cancer, we found that bcl-2 (an oncogene that inhibits apoptosis, or programmed cell death) and MVD (as a measure of angiogenesis) had statistically significant associations with mortality. The results for bcl-2 (based on analyses of archived biopsy tissue) indicate that this marker is involved in the balance between pro- and anti-apoptotic regulatory molecules in prostate cancer [7,14]. MVD might provide a direct and useful visual indication of the ability of a prostate tumour to grow and metastasize, although the results were less robust (compared to bcl-2) and determining vessels per high-power field is labour intensive [7,16].

The validity of our project is enhanced by fulfilling methodological criteria [18] for identifying true prognostic factors as

characteristics that are statistically significant and independent, as well as clinically relevant. We analysed data from a large, incident series of men spanning the entire spectrum of prostate cancer. In addition, we used clinical data that were generated at the time the men were diagnosed (e.g. original Gleason scores), and showed that immunohistochemical staining of tissue mainly from prostate needle biopsies (vs prostatectomy specimens) can provide useful prognostic information. Our start time [19] for assessing the prognosis (after a biopsy-based diagnosis of prostate cancer, but before a treatment decision is made) is the critical time for such information to be available [20], and the corresponding 'heterogeneous' patient population avoids selection bias associated with studies of patients after they receive therapy.

The limitations of the present study include its sample size, although the ongoing project will have extensive information on >1000 patients, with >10 years of follow-up, when completed. For example, the association of p53 and mortality (HR 1.31, 95% CI 0.88–1.96) might become statistically significant with a larger sample. Notably the present analysis answers the call for 'new studies of p53 status...on needle biopsy specimens, to achieve prognostic value for prospective treatment planning in prostate cancer.' [6]. Similarly, the full effect of MVD and bcl-2 (e.g. within strata defined by type of treatment) should be clarified in the larger study. An important negative finding is the lack of results for several factors described previously as relevant to prostate cancer; no specific, reproducible staining for VEGF or CD-61 (β -3 integrin) was obtained, due to limitations of immunohistochemical methods in paraffin-embedded needle-biopsy tissue, or other unidentified reasons.

In a research context, our findings are consistent with and extend similar studies of molecular markers, including evidence [21] linking survivin (another inhibitor of apoptosis) with progression of prostate cancer. In a clinical context, our study suggests that measuring bcl-2 and possibly MVD at the time of diagnosis, when men are counselled about treatment, can provide a more comprehensive assessment of the prognosis in prostate cancer than demographic and clinical factors alone. Perhaps as a direct measure of underlying mechanisms of cancer, molecular markers can help to explain tumour behaviour, even when

accounting for anatomical stage, histological grade, and the serum PSA level. Accordingly, bcl-2 and MVD have the potential to be useful clinically as prognostic markers, with HRs of a similar magnitude to those for Gleason score, PSA level and other factors.

Our ongoing study, when completed, will represent a rigorous and pragmatic ('effectiveness') evaluation of prognosis in prostate cancer. We used data that were obtained from, and apply to, the real world of men diagnosed with prostate cancer in a community setting. Although the diagnoses of prostate cancer were made in the 1990s, the follow-up period provided an opportunity to focus on the pertinent outcome of long-term mortality, rather than on surrogate outcomes (e.g. PSA failure). In addition, our observations on improved survival within groups defined by treatment are consistent with previous reports [22,23], but assessing the effect of therapy was not an objective of this research.

In conclusion, the present results confirm the independent effect of bcl-2 and perhaps MVD on survival among men with prostate cancer. The findings could improve the precision of estimates of the aggressiveness of prostate cancer at the time of diagnosis. This information can be useful to clinicians and patients when making treatment decisions, including the potential of recommending patients for clinical trial protocols evaluating new therapeutic agents.

ACKNOWLEDGEMENTS

The authors thank staff at the VA Clinical Epidemiology Research Center (K. Anderson, M. Aslan, D. Cavaliere, P. Crutchfield, N. Cummings, D. Orlando, M. Palmisano), VA Cooperative Studies Program Coordinating Center (P. Peduzzi), and Yale University School of Medicine (C. Bifulco, M. Helie) for assistance with this study; and Dr W. Kevin Kelly for reviewing a draft of the manuscript. This research was supported by grant funding from the Department of Veterans Affairs. No financial (or other) conflicts of interest exist in relation to this work. The Angiogenesis Foundation, a nonprofit organization, had no role in the funding, design, conduct, or analysis of the study.

CONFLICT OF INTEREST

None declared.

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Abbreviations: **VA**, Veterans Affairs; **VEGF**, vascular endothelial growth factor; **MVD**, microvessel density; **HR**, hazard ratio.