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Using Nuclear Morphometry to Predict the Need for Treatment Among Men With Low Grade, Low Stage Prostate Cancer Enrolled in a Program of Expectant Management With Curative Intent

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Abstract

PURPOSE—We assessed the use of quantitative clinical and pathologic information to predict which patients would eventually require treatment for prostate cancer (CaP) in an expectant management (EM) cohort.

EXPERIMENTAL DESIGN—We identified 75 men having prostate cancer with favorable initial biopsy characteristics; 30 developed an unfavorable biopsy (Gleason grade >6, >2 cores with cancer, >50% of a core with cancer, or a palpable nodule) requiring treatment and 45 maintained favorable biopsies throughout a median follow-up of 2.7 years. Demographic, clinical data and quantitative tissue histomorphometry determined by digital image analysis were analyzed.

RESULTS—Logistic regression (LR) modeling generated a quantitative nuclear grade (QNG) signature based on the enrollment biopsy for differentiation of Favorable and Unfavorable groups using a variable LR selection criteria of $P_z < 0.05$. The QNG signature utilized 12 nuclear morphometric descriptors (NMDs) and had an area under the receiver operator characteristic curve (ROC-AUC) of 87% with a sensitivity of 82%, specificity of 70% and accuracy of 75%. A multivariable LR model combining QNG signature with clinical and pathological variables yielded an AUC-ROC of 88% and a sensitivity of 81%, specificity of 78% and accuracy of 79%. A LR model using prostate volume, PSA density, and number of pre-diagnosis biopsies resulted in an AUC-ROC of 68% and a sensitivity of 85%, specificity of 37% and accuracy of 56%.

CONCLUSIONS—QNG using EM prostate biopsies improves the predictive accuracy of LR models based on traditional clinicopathologic variables in determining which patients will ultimately develop an unfavorable biopsy. Our QNG-based model must be rigorously, prospectively validated prior to use in the clinical arena.

Keywords

prostate cancer; expectant management; quantitative nuclear grade; prostate biopsy; low grade and low stage cancer

INTRODUCTION

Prostate cancer (CaP) is the most common malignancy among men in the US with approximately 230,000 new cases and approximately 30,000 deaths reported in 2006 [1]. With widespread screening, men are diagnosed with CaP at a younger age, exhibit lower stage disease and smaller tumors, and have lower serum PSA, Gleason score 6 or 7, and nonpalpable disease (75%) [1–14]. While a great number of men are still diagnosed with advanced disease and approximately 25% of all CaP patients detected will recur [2,4,12], approximately 25% of men undergoing PSA testing in referral and screened populations will have well differentiated (Gleason score <7), small volume (<0.5 cc) tumors known to demonstrate a long natural history [8,9,15]. The Epstein criteria, PSA density <0.15 ng/ml/g without adverse biopsy pathology (Gleason score <7, <3 cores involved with cancer, and <50% cancer involving any single core), correctly predict small volume, low grade tumors in 73% of men undergoing radical prostatectomy [8]. Carter et al. prospectively confirmed this finding in an expectant management (EM) cohort of whom 69% maintained favorable characteristics and never needed CaP treatment [16–18]. We sought to determine whether we could identify at enrollment into EM, based on serum PSA biomarkers, demographic information and quantitative nuclear grading (QNG) using image analysis of biopsy tissue, men who would never require treatment for prostate cancer.

MATERIALS AND METHODS

Prostate Tissue Biopsies

As of 1/06, 320 men (median age 65 years) were enrolled in a program of EM with curative intent for CaP (median follow-up 2.7 years). Enrollment criteria are PSA density 0.15 ng/ml/cc, clinical stage T1c, and cancer on a 12 core biopsy demonstrating <3 cores, Gleason score 6, and <50% of any core. Patients are monitored by semiannual PSA tests and annual 12 core surveillance needle biopsies. Development of a palpable nodule, Gleason score >6, 3 cores involved with cancer or cancer involving 50% of any single core results in a recommendation for treatment [19]. One hundred fourteen men had at least two biopsies on separate dates demonstrating CaP and 75 of these men had tissue from both biopsies available for study. The first biopsy, B₀, is the initial diagnostic biopsy or the 12 core confirmatory biopsy obtained at JHH. B_L is either the unfavorable biopsy generating the recommendation for treatment or the most recent favorable biopsy demonstrating cancer for those still undergoing EM surveillance. Variables examined included demographic information, serum biomarkers (total PSA and free PSA), and tissue biomarkers (nuclear morphometry based on Feulgen DNA stain). We selected for analysis available prostate biopsy blocks from any given case demonstrating the highest Gleason grade or those with the greatest percent core positive for cancer. A dedicated genitourinary pathologist (JIE) determined representative regions for analysis on a given slide. Four patients were excluded, as many patients in EM have small areas of cancer and some slides did not demonstrate cancer on deeper sectioning into the paraffin block.

Feulgen DNA Stain and Nuclear Morphometry Measurements

Feulgen reagent specifically and quantitatively stains DNA by uncovering the free aldehyde groups during the acid hydrolysis process, reacting with Feulgen reagent to form a stable compound absorbing at 560 nm [20,21]. Feulgen staining was performed per the manufacturer's instructions (TriPath Imaging, Inc., Burlington, NC). QNG determination involved capturing images of approximately 200 intact Feulgen-stained nuclei for each patient using the AutoCyte Pathology Workstation (APW) (TriPath, Inc.) with QUIC-DNA software [22]. Forty nuclear morphometric descriptors (NMDs), including nuclear size, shape, DNA content and chromatin texture features, were determined from each nucleus.

For each patient, the NMD variance was calculated then evaluated using backwards-stepwise logistic regression (LR) to predict Favorable versus Unfavorable future biopsy status [22]. The resulting model, consisting only of NMD variances significant on multivariable LR, is termed a QNG solution.

Statistical Analysis

All data were analyzed with Stata™ v9.1 statistical analysis software (Stata Corporation, College Station, TX). Nonparametric, Wilcoxon ranksum testing and chi-squared analysis were utilized to test for differences in clinical parameters. The tissue morphometry, based upon image analysis collection of nuclear morphometric descriptors (NMDs), was used to derive quantitative nuclear grade (QNG) computational solutions using backwards-stepwise logistic regression (LR) with a variable inclusion exclusion criteria of $P_z \leq 0.05$ [22].

RESULTS

Patient Characteristics of Men Undergoing EM for PCa at B₀ (Entry)

Of the 75 patients satisfying EM enrollment criteria with at least two biopsies demonstrating CaP available for analysis at JHH, 30 developed unfavorable pathology on a subsequent biopsy, generating a recommendation for treatment (Unfavorable Group), and 45 have maintained favorable pathology on surveillance biopsies (Favorable Group). Median age of the Favorable and Unfavorable groups was 65.0 and 66.5 years, respectively, and median years of enrollment were 2001 and 2002 (Table I). At enrollment, there were no significant differences between groups with respect to year of B₀, age, prebiopsy tPSA, prebiopsy %fPSA, prostate volume, maximum B₀ core fraction, and number of involved B₀ cores. The groups were effectively controlled for clinical stage, PSA density, and Gleason grade by the enrollment criteria.

Patient Characteristics of Men Undergoing EM of PCa at B_L (Monitoring)

Of the 30 patients in the Unfavorable Group, 8 had a higher Gleason grade at B_L (Table II). As B₀ may represent either the initial diagnostic biopsy or the first positive biopsy obtained at JHH, we examined and found no difference in either the number of total biopsies prior to B₀ or in the number of positive biopsies prior to B₀ between Favorable and Unfavorable groups. Median (range) time of follow-up for the Unfavorable and Favorable groups was 2.3 years (0.5–5.6) and 3.6 years (0.8–8.5) respectively, $P = 0.009$ (Table II). Median (range) maximum core fraction of B_L between the Favorable and Unfavorable groups were 10% (1–40%) and 30% (1–90%), $P = 0.0011$, and the number of cores of B_L involved with cancer were 1 (1–2) and 3 (1–5), $P < 0.0001$. Patients in the Unfavorable group had a significantly higher B_L Gleason grade as compared to the Favorable, $P = 0.003$.

Calculation of a QNG Solution to Predict B_L

The QNG solution ($N = 71$) used variances of 12 NMDs, 3 relating to nuclear size and shape, 3 to the measurement of DNA content within the nucleus, 3 to texture, and 3 based on Markovian analysis (Table Ia). This solution predicts future biopsy pathology with an AUC-ROC of 87% (Fig. 1b). At a threshold of 0.35, the QNG solution demonstrates a sensitivity of 82%, a specificity of 70%, and an accuracy of 75% in predicting the future biopsy status of a patient meeting inclusion criteria for EM. In Figure 1c a scatter plot of the predictive probabilities for the QNG LR solution illustrates the diagnostic performance based upon the patient-specific responses at the cutoff probability of 0.35.

A Comparison of QNG Versus Clinicopathological Information

Univariate and multivariable LR modeling was performed using clinicopathologic data (Table I) (N = 75). Serum free PSA fraction (N = 60) <20% or 20% predicted Unfavorable biopsy, $P < 0.07$ (data not shown). Using a variable selection criteria of $P_z < 0.50$, a multivariable, backwards stepwise LR model was determined combining 3 statistically significant clinicopathologic variables (prebiopsy PSA, biopsies prior to B₀ and maximum B₀ core fraction). This produced an AUC-ROC of 68% (Fig. 2b).

QNG Combined With Clinicopathological Parameters

Combining the QNG signature model with the clinicopathologic model from the previous example changed the AUC-ROC from 87% (Fig. 2a) to 88% (Fig. 2c). The summary of the diagnostic performance for combining the two models is shown above the ROC curves, demonstrating a slight improvement in specificity and accuracy (Fig. 2).

DISCUSSION

Approximately 30% of men at autopsy harbor CaP, but only 16% are diagnosed with CaP and 3% ultimately die of it [23]. To minimize the morbidity of treating clinically insignificant CaP, our group has an institutionally approved expectant management program with curative intent for selected men with low stage, low grade CaP [24]. Approximately 30% of the men in this group will require treatment for CaP because of changes in the pathologic characteristics of subsequent biopsies. We sought to determine, using clinical and pathological information, whether we could predict at the time of enrollment, which men would ultimately require treatment for CaP. In our experience, receiver operator characteristic (ROC) curves with AUCs in the 60–65% range (Fig. 2b) indicate models with little predictive power. Using the paradigm developed by Kattan [25] and Kattan and Katz [26] for evaluation of new cancer biomarkers, we found the addition of clinicopathologic data only marginally improved the predictive power of the model based on QNG signature alone.

One previous study examined criteria predicting pathologic progression in an EM cohort [27]. A model based on PSA velocity, %fPSA >20%, and prostate volume determined which patients fail at subsequent biopsy. Applying %fPSA >20% approached significance ($P = 0.07$) in our EM study. The current study examines only men already qualifying for EM, while Khan et al. included men enrolled in EM as well as newly evaluated men with T1c disease, some already excluded from EM based on initial biopsy. Requiring all patients to have two biopsies demonstrating cancer excludes patients with the smallest tumors, only demonstrating cancer on a single biopsy. Our work represents the first attempt of its kind to determine the outcome of patients with low stage, low grade CaP satisfying the Epstein criteria for small volume disease based on data from tissue biomarkers available at enrollment.

Researchers have hypothesized that CaP behaves heterogeneously and potentially aggressively due to numerous genetic or epigenetic alterations potentially identifiable long before they become clinically significant [28–30]. The molecular basis of alterations in nuclear structure that determine clinical risk for disease recurrence and progression to distant metastasis appear to be an important factor in these events. Early work on the importance of the nuclear matrix demonstrated that aberrant gene expression in cancer cells leads to alterations in nuclear structure identified early in the malignant cell's life cycle [31–33]. One approach to determine these molecular changes uses digital image analysis to quantify changes and variations in the shape of a CaP cell's nucleus [34–36]. More recently, Debes et al. [37] studied the role of a transcriptional regulatory gene, P300 (histone

acetyltransferase, HAT) in altering nuclear size and shape architecture in CaP. The authors demonstrate that the transformation of LNCaP cell lines with P300 gene and variations in expression of P300 among CaP cells collected from patients can produce alternations in nuclear size and shape [37]. Seligson et al. [38] have shown that molecularly modified histones H3 (acetylated) and H4 (methylated) correlate with PSA-recurrence; however, the correlation with nuclear structure alterations has not been made. Further investigation has demonstrated that certain reproducible alterations of morphometric nuclear features were identified as a cell underwent malignant transformation and progression to metastasis, and these could be used clinically as biomarkers [22,39,40].

Quantitative nuclear grade (QNG) is an image analysis-based biomarker proven effective in predicting clinical outcomes from Feulgen DNA stained prostate tissue. Early work on radical prostatectomy specimens showed that QNG could predict biochemical progression with an AUC-ROC of 93.5% when using LR modeling [41] and accuracies as high as 80.0% when using a genetically engineered neural network [42]. Further work demonstrated that QNG solutions determined from prostatectomy specimens could also predict the downstream endpoint of metastasis both from whole mount prostatectomy samples [43] and from a tissue microarray [44]. Significant work has also correlated QNG solutions obtained from CaP biopsies with pathologic stage, both retrospectively [45] with AUC-ROC of 86% and prospectively with AUC-ROCs of 81% and 82% for QNG alone and 82.4% with QNG combined with Gleason score and cPSA-density, respectively [11].

Our biopsy expectant management study clearly illustrates the potential value of QNG applied to biopsy specimens; however, it does have several important limitations. It is retrospective, based on a small sample and uses many variables, risking model over-fitting. Our definition of unfavorable pathology is arbitrary. Selection bias exists when examining only patients with two cancer biopsies; excluded are those with the most benign and the most aggressive disease. Since the follow-up for the entire cohort is relatively short, verification bias is a limitation as well; those currently classified in the Favorable group may eventually fall into the Unfavorable group.

Whether detecting global changes in a gland under-sampled by needle biopsy or predicting future aggressive morphologic and molecular transformation within temporarily quiescent cells, analysis of initial prostate biopsies by QNG, compared to clinicopathologic data alone, improves prediction of patients developing subsequent unfavorable biopsies. Such analysis may prevent unnecessary treatment of men with indolent CaP. Continued improvements in our model may be possible with addition of new biomarkers [28–30]. While this QNG model provides insight into the natural history of low volume CaP, it must be rigorously and prospectively validated prior to use in a clinical arena.

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Fig. 1. Development of nuclear morphometric model. **a:** Significant variables for QNG. **b:** ROC for QNG solution. **c:** Plot of predictive probabilities.

	QNG	Clinicopathology	QNG + Clinicopathology
ROC-AUC	87%	68%	88%
Sensitivity	82%	85%	81%
Specificity	70%	37%	78%
Accuracy	75%	56%	79%

Fig. 2.
Comparison and Combination of nuclear morphometric model with clinicopathologic model.

TABLE I

Characteristics of Men Undergoing Expectant Management of Prostate Cancer at Study Entry

Characteristic	Favorable group	Unfavorable group	P-value	Statistical test
No. of patients	45	30		
Median biopsy year	2001 (1997–2004)	2002 (1996–2004)	0.49	Rank sum
Median age (range)	65 (57–76)	66.5 (53–75)	0.58	Rank sum
Pre-biopsy PSA level (ng/ml) median	4.8 (1–15)	5.35 (1.2–10.4)	0.15	Rank sum
% free PSA level median	17 (8.7–37.3)	16 (7.1–24.9)	0.54	Rank sum
Prostate volume	55 (18.2–109)	50 (30–211)	0.35	Rank sum
Number total biopsies including B ₀	1 (0–3)	1 (0–2)	0.32	
Number positive biopsies including B ₀	1 (0–2)	1 (0–2)	0.44	
Maximum cancer core fraction	1 (1–40)	1 (1–40)	0.94	
Number cores involved with cancer			0.17	Chi squared
1 core	35 (78%)	19 (63%)		
2 cores	10 (22%)	11 (37%)		

TABLE IICharacteristics of Men Undergoing Expectant Management of Prostate Cancer at B_L

Characteristic	Favorable group	Unfavorable group	<i>P</i> -value	Statistical test
No. of patients	45	30		
Follow up, median (range)	3.6 (0.8–8.5)	2.3 (0.5–5.6)	0.009	Rank sum
Number total biopsies at B _L	5 (2–8)	3 (2–7)	0.0003	Rank sum
Number positive biopsies at B _L	3 (2–6)	3 (2–5)	0.1	Rank sum
Maximum cancer core fraction at B _L	10 (1–40)	30 (1–90)	0.0011	Rank sum
No. cores involved with cancer at B _L	1 (1–2)	3 (1–5)	<0.0001	Rank sum
Gleason score at B _L			0.003	Chi squared
5	1 (2%)	0 (0%)		
6	44 (98%)	22 (73%)		
7	0 (0%)	7 (23%)		
8	0 (0%)	1 (3%)		