Oncology

DNA Ploidy as Surrogate for Biopsy Gleason Score for Preoperative Organ Versus Nonorgan-confined Prostate Cancer Prediction

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OBJECTIVES Transformation of normal epithelium into cancer cells involves epigenetic and genetic changes and modifications in nuclear structure and tissue architecture. To evaluate nuclear morphometric alterations and clinicopathologic features for organ- vs nonorgan-confined prostate carcinoma (PCa) prediction.

METHODS Of 557 prospectively enrolled patients, 370 had complete information and sufficient tumor area for all evaluated parameters (281 organ-confined and 89 nonorgan-confined PCa cases). Digital images of Feulgen DNA-stained nuclei were captured from biopsies using the AutoCyte imaging system, and the nuclear morphometric alterations were calculated. Logistic regression analysis with bootstrap resampling was used to determine the factors important for differentiation of the 2 groups and to generate models for organ- vs nonorgan-confined PCa prediction.

RESULTS Several nuclear morphometric features were significantly altered and could differentiate organ- and nonorgan-confined disease. DNA ploidy was the most important factor among the significant nuclear morphometric features and was the second most important factor for organ- vs nonorgan-confined PCa prediction when considered with total prostate-specific antigen (PSA), complexed PSA, free/total PSA, biopsy Gleason score, and clinical stage. The combination of DNA ploidy with clinical stage, total PSA, and biopsy Gleason score showed an improvement of 1.5% in the area under the receiver operator characteristic curves compared with the combination of clinical stage, total PSA, and biopsy Gleason (73.97% vs 72.43%). The use of DNA ploidy in lieu of the biopsy Gleason score in each preoperative model evaluated resulted in equivalent or improved organ- vs nonorgan-confined PCa prediction.

CONCLUSIONS The results of our study have shown that DNA ploidy can serve as a surrogate biomarker that has the potential to replace biopsy Gleason scores for organ- vs nonorgan-confined PCa prediction. UROLOGY xx: xxx, xxxx. © 2009 Published by Elsevier Inc.

Prostate cancer (PCa) is the second leading cause of cancer death among men in the United States, with an anticipated 186 320 newly diagnosed cases and 28 660 deaths in 2008.[1] Most men with clinically localized PCa are treated with radical prostatectomy (RP), which provides excellent cancer control.[2] However, no consensus has been reached regarding the optimal management of locally advanced PCa.[3] The preoperative ability to assess the pathologic stage permits better counseling of patients, as well as more appropriate selection of therapy and consideration of novel clinical trials for those with more advanced disease.

Diamond et al.[4] were the first to use nuclear morphometric alterations (nuclear roundness factor) for the prediction of outcomes in patients with PCa with Stage B1 and B2 disease (Whitmore-Jewett staging). Subsequently, other investigators[5-8] have used nuclear morphometric alterations to predict the pathologic stage and prognosis for patients with PCa. Nuclear morphometric alterations measured by computer-assisted image analysis detect abnormal DNA content representing large-scale chromosomal alterations and reflect genetic instability in tumor cells.[9,10] The present study, using a single 5-μm section of biopsy tissue from a prospectively accrued patient cohort obtained at a single institution, assessed the ability of nuclear morphometric alterations and preoperative clinicopathologic parameters to predict for organ- vs nonorgan-confined PCa after RP in 370 men.

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Table 1. Patient demographics (n = 370)

Variable	Organ-confined PCa (n = 281)	Nonorgan-confined PCa (n = 89)	*P* Value
Age (y)			.028*
Mean	58.21 ± 6.03	59.75 ± 6.06	
Median	58.5	60.3	
tPSA (ng/mL)			<.0001*
Mean	5.95 ± 3.39	8.83 ± 5.47	
Median	5.32	7.49	
cPSA (ng/mL)			<.0001*
Mean	5.10 ± 3.03	7.70 ± 4.92	
Median	4.53	6.49	
fPSA (ng/mL)			.0009*
Mean	0.85 ± 0.57	1.13 ± 0.82	
Median	0.72	0.92	
f/tPSA ratio			.0432*
Mean	15.5% ± 7.1%	13.6% ± 5.8%	
Median	14.7%	13.1%	
Clinical stage (%)			.001†
T1c	210 (74.7)	48 (53.9)	
T2a	51 (18.2)	26 (29.2)	
T2b/c	20 (7.1)	15 (16.9)	
Biopsy Gleason score (%)			<.0001†
<7	247 (87.9)	57 (64.0)	
7	30 (10.7)	30 (33.7)	
>7	4 (1.4)	2 (2.3)	
Race (%)			1.000†
White	268 (95.4)	86 (96.6)	
Black	8 (2.9)	2 (2.3)	
Other	5 (1.7)	1 (1.1)	

tPSA = total prostate-specific antigen; cPSA = complexed PSA; fPSA = free PSA; f/tPSA = free/total PSA ratio.
* Wilcoxon's rank-sum test.
† Fisher's exact test.

MATERIAL AND METHODS

Patient Cohort

Of the 557 patients enrolled in a prospective PCa study from October 1998 to January 2000 scheduled for RP at the Johns Hopkins Hospital, 370 had complete information for all evaluated parameters, including serum samples for the evaluation of total prostate-specific antigen (tPSA), complexed PSA (cPSA), free PSA (fPSA), and free/total PSA (f/tPSA), a sufficient area of cancer on the biopsy section to determine the Gleason score on a hematoxylin-eosin-stained section and a sequential biopsy section stained with Feulgen from which a minimum of ~125 intact prostate cancer nuclei had been captured using a computer-assisted image analysis system. Patients were excluded from the cohort for the following reasons: microwave tissue overprocessing (which can degrade the ability to measure nuclear morphometric alterations; n = 39), preoperative hormonal therapy (n = 6), no biopsy material available (n = 45), no cancer observed on the recut biopsy material provided for the study (n = 56), no RP done (n = 9), PSA molecular forms missing from the evaluation (n = 22), and patients had declined to participate in the study (n = 10). All the men had undergone a minimum of a sextant biopsy that sampled tissue cores from the apex, mid, and base of the right and left lobes of the prostate gland, and none had received neoadjuvant hormonal therapy. All underwent RP at Johns Hopkins Hospital with routine PSA follow-up that consisted of assessment at 3 months postoperatively and annually thereafter. Pathologic interpretation of the Gleason score on the biopsy tissue was performed by pathologists in the Department of Pathology at Johns Hopkins Hospital. Table 1 summarizes the pretreatment information available for this patient cohort.

Digital Measurement of Nuclear Morphometric Alterations

Using ~5-μm tissue sections from the prostate biopsy cores, Feulgen DNA staining was performed according to the manufacturer's instructions (TriPath Imaging, Burlington, NC). Next, a minimum of ~125 intact, Feulgen-stained nuclei were captured from the cancer areas of each biopsy using AutoCyte Pathology Workstation (TriPath Imaging) and QUIC-DNA software.[5] The QUIC-DNA software calculated 40 different nuclear morphometric alterations for each nucleus captured (listed in the study by Veltri et al.[5]), including nuclear size, shape, DNA content, and chromatin texture features (at a step size of 1 pixel). We used the variance of the nuclear morphometric features, determined using the nuclei captured for each case, as our input variables, thereby reducing the complexity of the database to a single set of 40 variables for each case.[5]

To determine DNA ploidy, the image system was first calibrated using a control slide that contained Feulgen-stained rat hepatocytes. Using densitometry, rat hepatocytes representing diploid (2C) and tetraploid (4C) states were measured, and the QUIC-DNA software generated 2 reference points to define a calibration curve that was then used for the calculation of DNA ploidy. We used the variance of the DNA ploidy measurements in the nuclei captured from each case to generate a continuous DNA ploidy variance variable for each case included in our statistical analyses.

Statistical Analysis

All data were analyzed using Stata, version 10.0, statistical analysis software (Stata, College Station, TX). Univariate logistic regression analysis was performed first to determine the independent variables significant in the differentiation of organ- and nonorgan-confined PCa. Next, using the variables significant on univariate analysis, 500 bootstrap samples were used to create multivariate logistic regression models using backward stepwise selection at a stringency of $P \leq 0.05$ "bootstrap resampling." The number of times each variable was selected using the 500 bootstrap samples was determined, and only variables that were selected in >50% of the bootstrap models were selected for the final multivariate modeling. Areas under the receiver operator characteristic curves (AUC-ROC) for the ability of the logistic regression models to differentiate between organ- and nonorgan-confined PCa were calculated. Correlation of the nuclear morphometric alterations with the Gleason score, clinical stage, and all PSA derivatives were evaluated using Spearman's rank correlation coefficients.

RESULTS

The demographic, clinical, and pathologic information for organ- and nonorgan-confined PCa are listed in Table 1. Significant differences were found between the 2 groups for all clinical and pathologic variables assessed, with the exception of race.

Univariate logistic regression analyses of nuclear features showed that the area, perimeter, circular form factor, maximal Feret, minimal Feret, Feret X, Feret Y, DNA ploidy, transmission, and intensity were significantly altered and differentiated between the organ-confined and nonorgan-confined PCa groups (Table 2). Next, a bootstrap resampling procedure using 500 replications was used to perform backward stepwise logistic regression analyses. The goal was to identify the most important nuclear morphometric alterations for differentiating organ- and nonorgan-confined tumors. Each variable in the replicated models was counted with significance level of $P \leq 0.01$ for a variable that entered the model and $P \leq 0.05$ (variable selection cutoff) to remain in the model. The inclusion frequency for area, perimeter, circular form factor, maximal Feret, minimal Feret, Feret X, Feret Y, DNA ploidy, transmission, and intensity was 21.2%, 19.6%, 37.6%, 37.4%, 10.6%, 17.8%, 7.8%, 59.0%, 16.8%, and 22.6%, respectively. DNA ploidy had the greatest inclusion frequency of all the univariately significant nuclear morphometric features for differentiating organ- and nonorgan-confined PCa.

Table 2. Univariate logistic regression analysis—significant predictors of organ- vs nonorgan-confined prostate cancer

Variable	AUC-ROC (%)	*P* Value
Nuclear morphometric alteration		
DNA ploidy	62.91	.001
Circular form factor	59.57	.015
Maximal Feret	62.65	.001
Intensity	58.47	.05
Area	60.04	.014
Perimeter	61.71	.005
Feret X	59.25	.01
Transmission	56.61	.035
Minimal Feret	60.01	.02
Feret Y	60.48	.004
Clinicopathologic parameters		
tPSA	67.81	<.0001
cPSA	68.12	<.0001
fPSA	61.63	.001
f/tPSA	57.11	.027
Biopsy Gleason score	61.81	<.0001
Clinical stage	60.89	<.0001

AUC-ROC = area under receiver operator characteristic curve; other abbreviations as in Table 1.

All PSA molecular forms (ie, tPSA, cPSA, fPSA, and f/t PSA) were univariately significant for the differentiation of organ- and nonorgan-confined tumor (Table 2). The AUC-ROC of cPSA was not significantly greater than that of tPSA (68.12% vs 67.81%, respectively, $P = .48$) for differentiating between the 2 groups. The biopsy Gleason score (stratified as <7, 7, and >7) and clinical stage were also significant for differentiating organ- and nonorgan-confined tumors in the cohort (Table 2). No advantage was found to further stratifying the biopsy Gleason score 7 group into 3 + 4 (n = 38) and 4 + 3 (n = 22), because the proportion of nonorgan-confined tumors was equal in these 2 categories (50% in both; AUC-ROC 61.81% vs 61.81%; $P = 1.00$).

Next, we combined the most important nuclear morphometric measurement, DNA ploidy, with the univariately significant clinicopathologic parameters (ie, tPSA, fPSA, cPSA, f/tPSA, biopsy Gleason score, and clinical stage) in backward stepwise logistic regression analyses using 500 iterations of bootstrap resampling. Each variable in the bootstrapped model was counted with a significance level of $P \leq 0.01$ for a variable to enter the model and $P \leq 0.05$ to remain in the model. fPSA was removed from the model because of collinearity with the other PSA molecular forms and their derivatives. The inclusion frequency of clinical stage, biopsy Gleason score, DNA ploidy, tPSA, cPSA, and f/tPSA was 92.2%, 62.0%, 82.2%, 80.4%, 24.8%, and 16.0%, respectively. Clinical stage was the best and DNA ploidy the second best predictor of organ- and nonorgan-confined PCa.

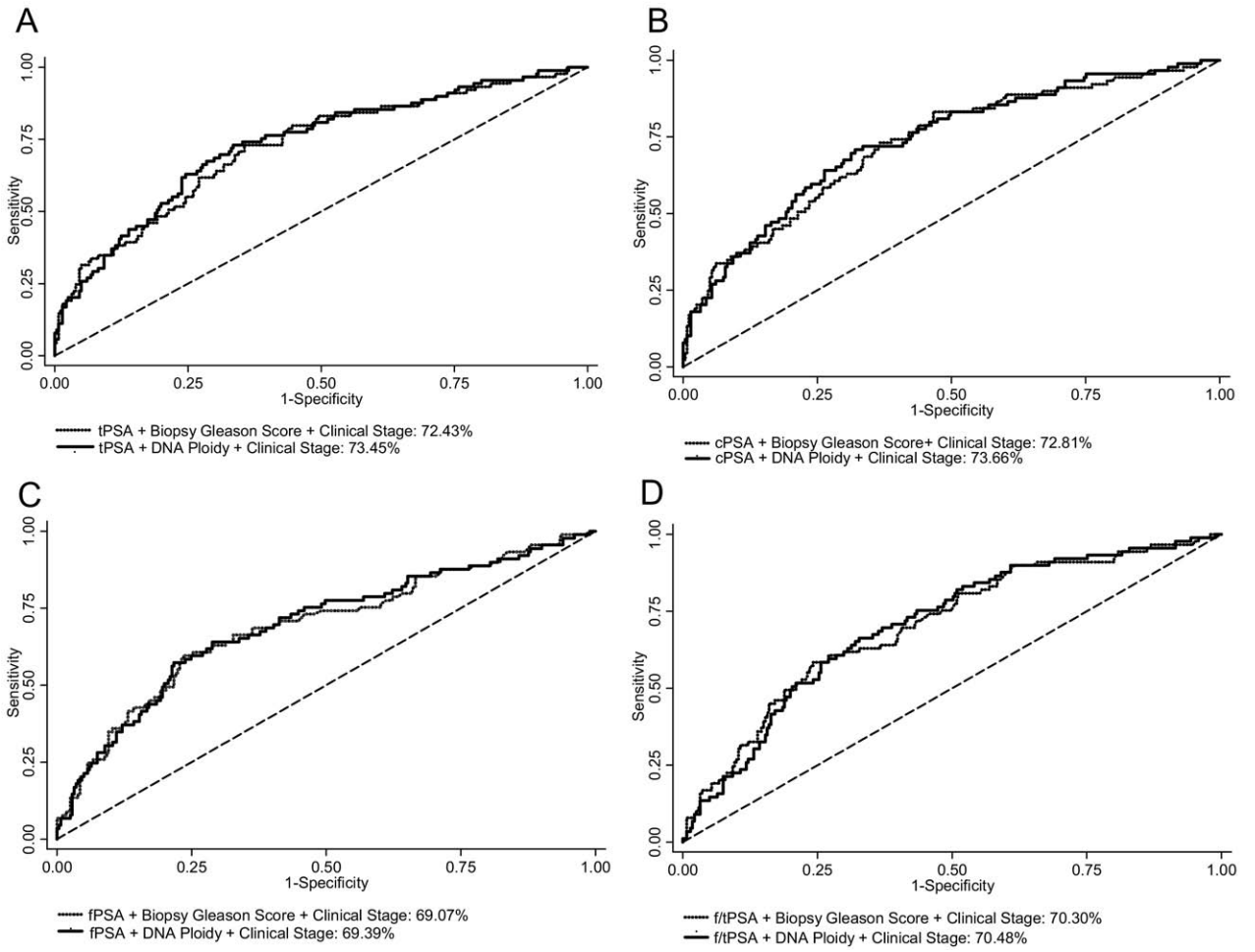

Figure 1. (A-D) Results of replacing biopsy Gleason score with DNA ploidy for prediction of preoperative organ- vs nonorgan-confined prostate cancer.

Among the PSA forms assessed, tPSA had the greatest inclusion frequency for differentiation of organ- and nonorgan-confined PCa.

The odds ratio, with the 95% confidence interval (CI) for clinical stage, biopsy Gleason score, DNA ploidy, tPSA, cPSA, fPSA, and f/tPSA was 1.90 (95% CI 1.36-2.67), 3.09 (95% CI 1.86-5.11), 12.95 (95% CI 3.02-55.53), 1.17 (95% CI 1.10-1.24), 1.19 (95% CI 1.11-1.28), 1.80 (95% CI 1.26-2.56), and 0.96 (95% CI 0.92-0.99), respectively. Using a cutoff criterion of ≥50% inclusion frequency, clinical stage, DNA ploidy, tPSA, and biopsy Gleason score were selected for a final multivariate logistic regression model, which resulted in an AUC-ROC of 74.0% and odds ratio of 1.83 (95% CI 1.27-2.34), 9.91 (95% CI 2.0-48.95), 1.15 (95% CI 1.08-1.22), and 1.87 (95% CI 1.08-3.24), respectively. Thus, the combination of DNA ploidy with clinical stage, tPSA, and biopsy Gleason score showed a modest improvement of 1.5% in the AUC-ROC compared with the multivariate model of clinical stage, tPSA, and biopsy Gleason (AUC-ROC 72.4%; odds ratio 1.83, 95% CI 1.28-2.63; odds ratio 1.15, 95% CI 1.08-1.22; and odds ratio 2.05, 95% CI 1.19-3.54, respectively).

DNA ploidy showed a significant correlation with biopsy Gleason score (rho = 0.211, $P < .0001$) and no correlation with clinical stage (rho = 0.066, $P = .206$), tPSA (rho = 0.057, $P = .276$), cPSA (rho = 0.061, $P = .242$), fPSA (rho = 0.028, $P = .588$), or f/tPSA (rho = −0.037, $P = .478$). To further explore the importance of DNA ploidy, we replaced the biopsy Gleason score with DNA ploidy for making preoperative organ- vs nonorgan-confined PCa predictions, and the AUC-ROC results showed equivalent or slightly improved prediction in each model (Fig. 1).

COMMENT

The identification of parameters predictive of the pathologic PCa stage has become a major focus in the area of PCa biology. The commonly used Partin tables[11] rely exclusively on clinicopathologic parameters (ie, tPSA, clinical stage, and biopsy Gleason score) to predict the pathologic stage. Numerous simple and complex parameters have been evaluated for the prediction of the pathologic stage, including tumor location, cancer volume on needle biopsy, the percentage of positive cores, the per-

centage of biopsy tissue with cancer, Gleason pattern 4/5, perineural invasion, vascular/lymphatic invasion, angiogenesis, DNA ploidy, reverse transcriptase-polymerase chain reaction detection of circulating PSA-positive cells, fluorescence in situ hybridization for chromosomal anomalies, and radiographic imaging methods.[12-15]

One of the limitations of the biopsy Gleason score can be the interobserver and intraobserver reproducibility; exact agreement has been reported in 43%-78% of cases, and agreement within ±1 score has been reported in 72%-87% of cases.[16-18] Gleason[19] noted that, on reexamination, exact duplication of Gleason scores occurred in only approximately 50% of cases and were within ±1 point in approximately 85% cases. Undergrading of PCa is the most common problem, occurring in ≤45% of cases, with overgrading of PCa occurring in ≤32% of cases.[16-18] However, Fine and Epstein[20] recently reported improvement in Gleason score reporting during the past decade, which can most likely be attributed to the increasing number of cores sampled from the prostate, comprehensive educational efforts by way of courses at meetings, on-line Web sites, and the medical literature.

Nuclear morphometric alterations can be accurately measured after capturing only ~125 Feulgen-stained nuclei from "an expert pathologist-selected" tumor area. Such an approach is objective and reproducible and thus reduces human error in making precise outcome predictions. The cost of measuring nuclear morphometric alterations using the AutoCyte imaging system was approximately $150.00, and a case took about 15 minutes to complete. In the present study, we showed that nuclear morphometric measurements of the area, perimeter, circular form factor, maximal Feret, minimal Feret, Feret X, Feret Y, DNA ploidy, transmission, and intensity were significantly altered and capable of differentiating between organ- and nonorgan-confined PCa (Table 2). DNA ploidy had the greatest inclusion frequency among all the univariately significant nuclear morphometric alterations for differentiating between organ- and nonorgan-confined PCa.

DNA ploidy was the second best predictor for differentiating between organ- and nonorgan-confined tumors when combined with tPSA, cPSA, f/tPSA, biopsy Gleason score, and clinical stage in a multivariate logistic regression model. The integration of DNA ploidy with the clinical stage, tPSA, and biopsy Gleason score model showed a modest improvement of 1.5% in the AUC-ROC. Replacing the biopsy Gleason score with DNA ploidy for the preoperative models showed equivalent or slightly improved organ- vs nonorgan-confined PCa prediction (Fig. 1). The use of tPSA was a better predictor for differentiating between organ- and nonorgan-confined tumors than was cPSA and f/tPSA in our cohort, and hence it should not be replaced by cPSA, as suggested by some investigators, for stage prediction.[21,22]

One limitation of our study was the lack of quantitative biopsy pathology information, which was not routinely determined, but that could have been used to compare their predictive ability with the nuclear morphometric alterations for differentiating between organ- and nonorgan-confined tumors.

Another limitation of our study was the lack of long-term follow-up information available for most of the patient cohort to assess the potential prognostic value of nuclear morphometric alterations detected in the needle core biopsy specimens. However, our group[5,6] has demonstrated the prognostic value of nuclear morphometric alterations detected in RP specimens for biochemical recurrence, distant metastasis, and death in men with PCa. Furthermore, our group[23] has shown that significant alterations occur in the nuclear structure between and within Gleason grading patterns 3, 4, and 5. Recently, Makarov et al.[24] used quantitative nuclear structure alterations to predict conversion to unfavorable biopsy pathologic features during surveillance, using the criteria of Carter et al.[25] for the selection of patients with PCa for expectant management. Because alterations in nuclear structure are so important, the identification of proteins that can modify nuclear chromatin organization, such as p300 expression, and others that have been shown to play an important role in PCa cell proliferation and PCa progression, is important.[26,27] Recently, our group demonstrated that valproic acid (histone deacetylase inhibitor) causes dose- and time-dependent changes in nuclear structure in PCa cells both in vitro and in vivo.[28] Clearly, the use of quantitative nuclear structure alterations and the molecular mechanisms that cause such changes provide a foundation for continued research in this area that could eventually change the treatment of patients with PCa.

CONCLUSION

The results of our study have shown that nuclear morphometric alterations provide an objective and reproducible quantitative measurement of nuclear structure and DNA ploidy features that can serve as a surrogate biomarker with the potential to replace biopsy Gleason scores for organ- vs nonorgan-confined PCa prediction.

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