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Quantitative Nuclear Morphometry, Markovian Texture Descriptors, and DNA Content Captured on a CAS-200 Image Analysis System, Combined With PCNA and HER-2/*neu* Immunohistochemistry for Prediction of Prostate Cancer Progression

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Abstract One hundred and twenty-four localized prostate cancer patients operated on at Johns Hopkins Hospital (JHH) since 1975 were identified. The sample was optimized for evaluation of prostate cancer progression. Based upon accurate clinical histories, these radical prostatectomy patients included 50 progressors and 74 non-progressors using appearance of serum PSA as an indication of recurrence (mean follow-up = 8.6 ± 1.8 years, range 7–15 years). All patients included in the study had no involvement of their seminal vesicles or lymph nodes at the time of prostatectomy. Average time to progression was 3.6 ± 2 years, range of 1–8 years. Using paraffin-embedded specimens, several five micron sections were cut and placed on Probe-On™ slides; one slide was H&E-stained and the other was Feulgen-stained. The H&E and Feulgen-stained slides were screened and "dotted" by pathologists at JHH and CytoDynostics, Inc. A CAS-200 Image analysis system (Cell Image Systems, Elmhurst, IL) equipped with a Cell Measurement Program version 1.2β, was used to capture the Feulgen-stained images and to perform the calculations. From the "dotted" areas, 150 cancer cells were selected for measurement of DNA content and 27 nuclear morphometric shape and size factors, including 21 Markovian chromatin texture variables. Additional sections were used for immunochemistry staining with an alkaline phosphatase streptavidin-biotin complex stain to detect and quantitate cancer cells binding monoclonal antibodies directed against proliferating cell nuclear antigen (PCNA) and HER-2/*neu* antigen. All data were entered into a statistical program (STATA™) for further analysis and univariate and multivariate statistical analysis was performed using logistic regression and its stepwise variant. The biomarkers of greatest utility to detect progressors when analyzed univariately included post-operative Gleason score ($p = <0.0001$), HER-2/*neu* antigenicity ($p = 0.0147$), CAS-200 DNA ploidy ($p = 0.008$), and twelve Markovian nuclear texture and shape features ($p = <0.0001$), whereas PCNA ($p = 0.160$) failed. The optimal set of nuclear morphometry progression tumor features were selected using backward stepwise logistic regression estimate analysis which drops variables due to collinearity. Although post-operative Gleason score is a strong univariate predictor of progression, DNA ploidy and HER-2/*neu* contributed significantly to further stratification of higher risk groups within the low

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Gleason score subpopulation. The best Markovian features combined with post-operative Gleason score generated sensitivity = 90%, specificity = 96%, positive predictive value = 94%, negative predictive value = 93% and the area under the receiver operator curve was 0.975. © 1994 Wiley-Liss, Inc.

Key words: Chromatin texture, DNA ploidy, nuclear morphometry, prostate cancer progression, tumor markers

Prostate cancer is diagnosed in 100/100,000 white males and in 70.1/100,000 black males in the United States. It is the second leading cause of male cancer deaths and the most commonly diagnosed cancer in men in the United States, representing 21% of all newly diagnosed cancers. In 1993, an estimated 165,000 men in the United States will be diagnosed with clinically apparent prostate cancer and 35,000 will succumb to the disease [1–3]. The age-specific increase in incidence achieves a maximum of 1000/100,000 in men >75 years of age [3]. The lifetime risk of developing clinical prostate cancer in the U.S. is 8.7% for white and 9.4% for black Americans with a lifetime risk of dying being 2.6% and 4.3% respectively [4]. The risk of developing prostate cancer has risen 42.6% since 1975 as compared to an increase of only 26% in the risk of developing lung cancer for that same time period. Approximately 65% of prostate cancers are clinically localized at the time of diagnosis and potentially curable with standard surgical techniques [1], yet only 50% of men are found to have disease confined to the prostate at the time of surgery [5,6]. Pack and Spitz [3], reviewing the epidemiology of prostate cancer, indicated several definable risk factors such as age, race, dietary fat consumption, vasectomy, and familial aggregation with at least a two-fold increased risk for first generation relatives of men with prostate cancer (rare autosomal dominant inheritance). These casual correlations, though impressive, can not yet explain the complex etiology, biologic heterogeneity, and rapidly increasing incidence of this disease and await further investigations of genetic, epigenetic and environmental factors.

The mortality rate for prostate cancer has been steadily increasing over the past 40 years and will continue to do so as our population ages [7]. This clinically evident disease represents only the tip of the iceberg in that nearly 30 percent of all men over age 50 harbor a silent microscopic form of latent prostate cancer [4,8,9]. Current early detection methods [4] are increasing the numbers of this latent form of cancer identified, which now represents more than 11 million cases within the male population in the United States. Growth rate studies indicate that these tumors appear to grow very slowly and the great majority should remain clinically silent.

Recent advancements in transrectal ultrasonography and the development of a serum-based assay prostate specific antigen (PSA) for early detection, premalignant neoplasias, and prostate cancer diagnosis has increased at an alarming rate [4]. Many of these newly diagnosed neoplasias could represent the non-aggressive, potentially latent form of the disease that may never have become clinically evident if followed without therapy [10]. Unfortunately, no accurate and specific methods presently exist to distinguish the more potentially aggressive form of prostate cancer from the latent form of the disease; thus most patients diagnosed are presently treated as though they had the aggressive form of the disease. We are presently limited to estimates and significance of tumor volume [11–13], pre- and post-operative histological grading of cancer [14–19] and high-grade intraepithelial neoplasia [20,21,13], clinical and pathological stage [12,13,22–24], DNA ploidy [25] and serum PSA [4,26–29] to predict biological aggressiveness of prostate cancer, and these techniques have only marginal predictive value.

It is well accepted that the epigenetic and genetic transformation of a normal prostatic epithelial cell to a cancer cell with progression to a metastatic phenotype requires multiple steps [30]. The development of methods to accurately quantify these changes in order to better predict tumor aggressiveness has been the subject of extensive experimental work in prostate cancer [31,32].

Diamond and associates [33,34] were the first to employ a simple nuclear shape factor (nuclear roundness) to describe the shape of cancerous

nuclei for patients with stage B1 and B2 (Whitmore-Jewett staging) prostate cancer and accurately predict the outcome for these patients. Since then, several investigators have used this method to predict prognosis for patients with various stages of prostate cancer [12,19,35–43]. More recently, Partin *et al.* [19] used a multivariate analysis of the variance of nuclear roundness, clinical stage, Gleason score, and patient age to predict disease-free survival among a group of 100 post-operative patients with localized prostate cancer. The use of chromatin texture feature data extracted from either H&E-stained [44,45] or Feulgen-stained sections [46–48] correlate well with classification of malignant cells. However, the sensitivity of Markovian texture measurements is complicated by the level of pixel grey level resolution capabilities of the image system. The CAS-100 image analysis cell measurement software has been developed to measure 22 Markovian texture features at a single pixel step size of resolution [48]. Dawson *et al.* employed the CAS-100 hardware and software to identify 10 features which discriminated chromatin patterns in breast cancer images captured by the CAS-100 [47]. Christen *et al.* [45] has applied another method, using linear discriminant statistical model analyses of shape, size, and texture features on H&E-stained prostate nuclei to obtain a high efficiency (93%) correct classification of normal and abnormal cells.

There has been little progress in terms of new effective therapy with regard to prostate cancer and at present, only pathologically localized prostate cancer is considered potentially curable [49]. It has been estimated that only approximately 40–50% of new cases of prostate cancer will represent disease confined to the organ [4,7]. Within this population of potentially curable patients, the relative risk of progression during the next 10–15 years approaches 25% even after definitive surgery [27,41,50–54]. These observations mandate new and better methods for determining the biological potential for progression of treatable prostate cancers using serum, core biopsies, or radical specimens in order to: 1) improve evaluation of patients who have tumors that need treatment, 2) determine the prognosis of patients with prostate cancer pathologically localized to the gland after surgery so that adjuvant treatment of those patients with a high probability of disease progression might begin earlier in the natural course of the disease, and 3) provide more objective means to select patients for chemoprevention trials using dietary modifications, retinoids, and hormonal manipulation (*i.e.*, 5-alpha reductase inhibitors) [21,55]. This paper describes a well-defined localized prostate cancer patient sample followed for recurrence based on PSA elevation, local recurrence, metastasis, or prostate cancer-specific death. The work describes the use of Feulgen-stained sections to characterize DNA ploidy in combination with immunohistochemistry for tumor markers, nuclear morphometry, and texture features which can correctly identify patients at risk for tumor progression.

MATERIALS AND METHODS

Collection and Processing of CAS-200 Cell Measurement Program/DNA Data

The morphometry data from radical prostatectomy specimens are captured using the Cell Measurement Program (CMP) version 1.2β software from a CAS-200 Image Analysis System, the same system currently used by CytoDiagnostics, Inc. (Oklahoma City, OK). The CAS-200 system at 40X magnification has an image field size of 256 X 256 pixels with a grey level resolution of 229 levels and each pixel area is 0.19 μm^2. For this study, at least 150 cells were captured and measured from each 5 µm Feulgen-stained section using CMP v1.2β software in the QDA Morphology mode. This analysis mode allows the measurement of the DNA content as well as 27 different size, shape, and texture descriptors for each cell [46–48]. The Markovian features utilized a single pixel step size and an 8 X 8 grey level transition field matrix constructed from individual normalized cell images [48]. The DNA histogram and cell images are saved to a list-mode file (*.ILM), while the 27 morphometric measurements were saved to a CMP vector file (*.VEC) and then converted to a Lotus 1-2-3 file (*.WK1) using the CMP Exporting Utility (a feature of the CMP v1.2β software). The Lotus 1-2-3 file (*.WK1) is then transferred to a 486 PC equipped with Windows v3.1 and Excel v4.0 for Windows, and converted to an Excel v4.0 file (*.XLS). A separate *.XLS file containing the 27 morphometric measurements, as well as the means, standard deviations, variances, minima,

and maxima for every cell captured from a particular case is created. The DNA information contained in the *.ILM is extracted using a Cortex program written by Michael P. Bacus (MIS supervisor) to a comma delimited text file. This text file is converted to an Excel v4.0 file, and the DNA information (*i.e.*, pg DNA) for each cell is added to the *.XLS file for every case. Each *.XLS file contained the information listed below for every cell captured from all cells for each case (Table I).

IMMUNOCHEMICAL STAINING

HER-2/*neu* (c-*erb*B-2) monoclonal antibody (Ab-3, OP-15) was provided by Oncogene Sciences, Inc. (Uniondale, NY) as a gift. PCNA (PC-10 clone, M-879) monoclonal antibody was purchased from DAKO (Carpenteria, CA). The SuperSensitive MultiLink™ kit (BioGenex Inc., San Ramon, CA), which employs the streptavidin-biotin complex (ABC) alkaline phosphatase labeling method, was used for monoclonal antibody detection. All staining was performed with the MicroProbe™ manual staining system (Fisher Scientific, Pittsburgh, PA) which utilizes capillary action vertical staining principles developed by Dr. David Brigati [56]. Incubation temperature for monoclonal antibody was 4°C overnight and remaining staining methods followed the recommended procedures of the BioGenex MultiLink™ kit.

THE PATIENT SAMPLE

A group of 124 patients with localized prostate cancer patients (74 non-progressor and 50 progressors) were used in the study. The patient samples were optimized for evaluation of tumor progression and were collected by Drs. Alan Partin and Jonathan Epstein at the Johns Hopkins Hospital (JHH). The patients have clinically and pathologically localized prostate cancer and were followed for evidence of progression based on a significant change in their PSA levels, local recurrence, or evidence of metastasis following radical prostatectomy. None of the patients had seminal vesicle or lymph node invasion. A partial description of the patient sample is shown in Table II.

STATISTICAL ANALYSIS

Statistical analyses used multiple independent parameters (morphometric and DNA content) as independent variables to ascertain the optimal multivariate parameter combinations that yield the greatest sensitivity and specificity values for the projected progression model. Analyses proceeded from an array of univariate

TABLE I. Description of Nuclear Features Captured by CAS-200 Image System

1.	(OBSD) Object Sum OD	15.	(TXK001) Difference Entropy
2.	(OBSZ) Object Size	16.	(TXL001) Information Measure A
3.	(OBSH) Object Shape	17.	(TXM001) Information Measure B
4.	Picograms of DNA	18.	(TXN001) Maximal Correlation Coefficient
5.	(TXA001) Angular Second Moment	19.	(TXO001) Coefficient of Variation
6.	(TXB001) Contrast	20.	(TXP001) Peak Transition Probability
7.	(TXC001) Correlation	21.	(TXQ001) Diagonal Variance
8.	(TXD001) Difference Moment	22.	(TXR001) Diagonal Moment
9.	(TXE001) Inverse Difference Moment	23.	(TXS001) Second Diagonal Moment
10.	(TXF001) Sum Average	24.	(TXT001) Product Moment
11.	(TXG001) Sum Variance	25.	(TXU001) Triangular Symmetry
12.	(TXH001) Sum Entropy	26.	(TXV001) Blobness
13.	(TXI001) Entropy	27.	(TXW) Standard Deviation
14.	(TXJ001) Difference Variance	28.	Cell Classification (1 = Hypodiploid, 2 = Diploid, 3 = S-Phase, 5 = Tetraploid, 6 = Hyperploid)

TABLE II. Patient Sample Description

Average Age:	59.6 ± 6.4 years [40–87 years]
Non-Progressors:	74 (60%)
Progressors:	50 (40%)
Average Time to Prog:	3.62 ± 2.1 years
Time to Assess:	6.6 ± 3.1 years [1–15 years]
Local Recurrence:	11 (9%)
Distant Mets:	5 (4%)
Non-Organ Confined:	95 (77%)
Organ Confined:	29 (23%)

TABLE III. Univariate Analysis using Logistic Regression (STATA™) as Predictor of Progression: [N = 124]

Independent Variable	p Value
Post-operative Gleason Score	$p \leq 0.00001$
17/28 CAS-200 Nuclear Descriptors	$p \leq 0.05$
CAS-200 DNA Ploidy	$p = 0.008$
HER-2/*neu* Antigenicity	$p = 0.0147$
PCNA [Clone PC-10]	$p = 0.160$**

**** Not statistically significant**

analyses to combinations of (weighted) multivariate parameters. Statistical significance consisted of values ($p = \leq 0.05$). The binary dependent variable in the analysis consisted of a relevant clinical outcome (*e.g.*, progression, organ-confinement, rise in PSA, death, *etc.*) Both univariate and multivariate non-parametric logisitic analyses was performed using the STATA™ statistical software package (Stata Corporation, Release 3.1, College Station, TX). Multivariate analysis was employed to select and weigh relevant clinical parameters through a backwards stepwise logistic regression analysis process. The software program generated receiver operator characteristic (ROC) curves for continuous variables with either default cutoffs of $p = 0.5$ or investigator selected cutoffs, resulting in optimized percentages for sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV).

RESULTS

Table III clearly illustrates the statistical correlation of the independent variables assessed to the outcome progression using the STATA™ software program. When the 28 morphometric parameters were analyzed, a total of 17 were demonstrated to be significantly correlated to this same endpoint. DNA ploidy and the biomarker HER-2/*neu*, but not PCNA (PC-10) were also correlated to progression when analyzed univariately by logistic regression.

Next, we analyzed the above univariately significant variables using the STATA™ software program termed stepwise variant of logistic regression [swlogis], where all the significant variables are analyzed together and those which are determined to be collinear in the model are dropped. Table IV summarizes the model results attained using this method of analysis compared

TABLE IV. Multivariate Analysis Using Stepwise Variant of Logistic Regression (STATA™) as Predictor of Progression in Radical Prostatectomy Specimens: [N = 124]

COMPLETE MODEL:

- Post-op Gleason
- HER-2/*neu* FDN Antigenicity
- CAS-200 DNA Ploidy
- 12/17 Best CAS-200 Nuclear Descriptors

MODEL STATISTICAL POWER
Stepwise Variant of Logistic Regression (STATA™)

	Post-op Gleason	Post-op Gleason and Nuclear Descriptors	Complete Model
Overall Model p Value	$p \le 0.0001$	$p \le 0.0001$	$p \le 0.0001$
Area Under the ROC	0.826	0.94	0.967
Sensitivity	78%	88%	90%
Positive Predictive Value	74%	87.76%	93.75%
Specificity	81%	91.89%	95.95%
Negative Predictive Value	85%	90.67%	93.42%

TABLE V. Stratification of Progressors Among Well to Moderately Differentiated Prostate Cancers Using DNA Ploidy Cytometry

Group	DNA	Gleason	Progressors (N=50)	Non-Progressors (N=74)	Total (N=124)
0	Diploid	< 7	3 (7.5%)	37 (92.5%)	40
1	Diploid	≥ 7	16 (72.7%)	6 (27.3)	22
2	Non-diploid	< 7	8 (25.0%)	24 (75%)	32
3	Non-diploid	≥ 7	23 (76.7%)	7 (23.3%)	30

to post-operative Gleason scores obtained by the JHH Pathology department at radical prostatectomy. A notable increase in the model's power was achieved using the nuclear descriptors alone and in combination with the additional biomarkers. The [swlogis] software also includes the logistic receiver operator curve to determine model sensitivity, specificity, NPV and PPV (Table IV). Figure 1 demonstrates the ROC curve for the optimized and complete model; Table V summarizes the actual number of progressors and non-progressors correctly identified with this model.

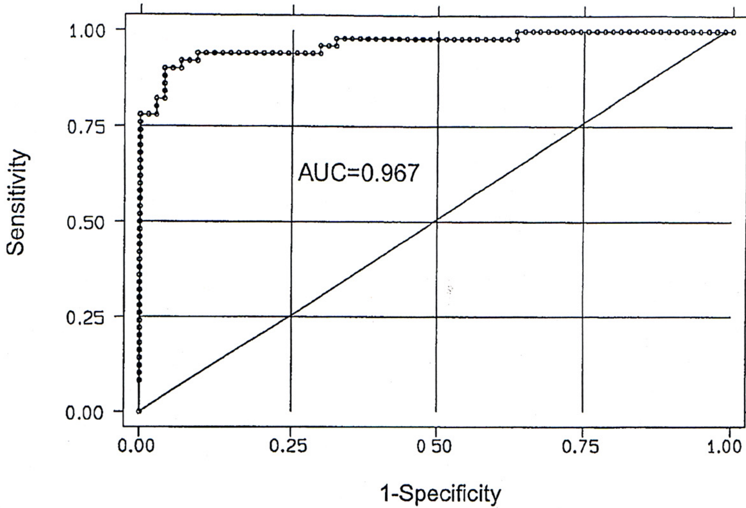
Besides contributing to the overall model predictive value, another advantage for DNA ploidy analysis achieved with our patient sample was the ability of non-diploid DNA content to select for a subpopulation of higher risk individuals with low to moderate Gleason scores who progress (Table V). Likewise, the HER-2/*neu* biomarker FDN (focal, diffuse, and none output results) selected a group of higher risk patients who progress in spite of a low to moderate Gleason score (Table VI).

DISCUSSION

This study describes the utility of nuclear morphometry and selected biomarkers to identify prostate cancer patients at risk for progres-

FIGURE 1

Identification of Progressors using CAS-200 Nuclear Descriptors, Post-operative Gleason Score, and Biomarkers



Sensitivity = 90%, specificity = 96%, negative predictive value = 93%, positive predictive value = 94%

TABLE VI. Stratification of Progressors Among Well to Moderately Differentiated Prostate Cancers Using HER-2/*neu* Antigenic Expression

Group	DNA	Gleason	Progressors	Non-Progressors	Total
0	HER-2/*neu* –	< 7	1 (5.3%)	18 (94.7%)	19
1	HER-2/*neu* –	≥ 7	5 (55.6%)	4 (44.4%)	9
2	HER-2/*neu* +	< 7	10 (19.2%)	42 (80.8%)	52
3	HER-2/*neu* +	≥ 7	34 (77.3%)	10 (22.7%)	44

sion (recurrence). The patient sample is clinically defined as localized stage A or B, and pathologically 95 cases were definitively classified as stage C1 without seminal vesicle or lymph node involvement. The mean follow-up period was 6.6 years (range 1–5 years) with a mean time to recurrence of 3.6 years based upon demonstration of serum PSA and/or clinical recurrence. Our laboratory, in collaboration with Johns Hopkins University Departments of Urology and Pathology, applied the v1.2β software and the CAS-200 image analysis system (Cell Image Systems, Inc., Elmhurst, IL) to measure chromatin texture of five μm sections of formalin-fixed, paraffin-embedded radical prostatectomy specimens stained using the Feulgen method [47]. Chromatin texture has often been measured using computer-assisted image analysis using a variety of software programs configured with necessary image hardware [44–48]. The methods used to address chromatin texture features have included mathematical interpolation of transitional field probability distributions of the grey levels of pixels, texels, and grains within a defined nuclear area [47,48]. The Markovian method of texture analysis was introduced by Pressman in 1976 [46]. This method was adapted to the CAS-100 and successfully used to assess texture by Bacus and Grace [48], as well as Dawson *et al.* [47]. When nuclear descriptors including shape, size, texture, DNA ploidy, and biomarkers were assessed univariately using nonparametric logistic regression, all were significantly correlated to progression except for PCNA. When these variables were assessed multivariately using non-parametric variant of the stepwise logistic regression method, significant improvements over Gleason scores were obtained with respect to identification of the progressors. Previous studies on archival prostate material by Partin *et al.* [12,18,19] used nuclear morphometry (nuclear roundness variance) in a similar patient sample to successfully identify prostate cancer progressors. Epstein [23] has clearly characterized pathological variables such as capsular penetration, seminal vesicle involvement, positive lymph nodes, and surgical margins as predictors of progression. Others investigators [11–18,22,24] similarly demonstrated that grade, positive seminal vesicles, or lymph nodes were such strong predictors of progression that their influence in evaluation of progression should always be considered for their significant contribution.

In summary, this study confirms that, in spite of artifacts of fixation, specific patterns of chromatin texture and biomarkers can be quantified reliably and configured into a statistical algorithm with a very high predictive value as determined by ROCs. Also, DNA ploidy and HER-2/*neu* can identify a subpopulation of well-to-moderately differentiated adenocarcinomas of the prostate at significant risk for progression. The contribution of DNA ploidy performed by flow cytometry for predicting progression is well documented [25]. Myers *et al.* [57] has recently described the expression of HER-2/*neu* and its detection in prostate cancer as well as intraepithelial neoplasia, and suggests that such expression may correlate to prostate cancer progression [57]. The algorithm next will be run on matched sextant core biopsies and radical surgical specimens to assess correlation coefficients, followed by application of the algorithm to make predictions with biopsies.

REFERENCES

1. Boring CC, Squires TS, Tsong T: Cancer Statistics. CA, Cancer J Clin 41:19–36, 1991.
2. American Cancer Society, Inc: Cancer Facts and Figures. New York: 1993.
3. Pack R, Spitz MA: Epidemiology of prostate cancer, with emphasis on familial clusters. The Cancer Bulletin 45:384–388, 1993.
4. Pisters LI, Babaian RJ: Status of early prostate cancer detection. The Cancer Bull 45:389–396, 1993.
5. Catalona WJ, Bigg SW: Nerve-sparing radical prostatectomy: Evaluation of results after 250 patients. J Urol 143:538–543, 1990.
6. Partin AW, Carter HB, Chan DW, Epstein JI, Oesterling JE, Rock RC, Weber JP, Walsh PC: Prostate specific antigen in the staging of localized prostate cancer: Influence of tumor differentiation, tumor volume and benign hyperplasia. J Urol 143:747–752, 1990.
7. Carter HB, Coffey DS: Prostate cancer: The magnitude of the problem in the United States. In: Coffey DS, Resnick MI, Dorr FA, Karr JP (eds): "A Multidisciplinary Analysis of Controversies in the Management of Prostate Cancer." New York: Plenum Publishing Corp, 1988, pp 1–7.
8. Franks LM: Latent carcinoma of the prostate. J Pathol Bacteriol 68:603–616, 1954.
9. Scardino PT: Early detection of prostate cancer. Urol Clin North Am 16:635–655, 1989.
10. Austenfeld MS: Treatment of stage A1 prostate cancer: The case for observation. Semin Urol XI:58–63,

1993.
11. Bostwick DG, Graham SD, Napalkov P, Abrahamsson P-A, di Sant'Agnese PA, Algaba F, Hoisaeter PA, Lee F, Littrup P, Mostofi FK, Denis L, Schroeder F, Murphy GP: Staging of early prostate cancer: A proposed tumor volume-based prognostic index. Urology 41:403–411, 1993.
12. Partin AW, Epstein JI, Cho KR, Gittelsohn AM, Walsh PC: Morphometric measurement of tumor volume and percent of gland involvement as predictors of pathologic stage in clinical stage B prostate cancer. J Urol 141:341, 1989.
13. McNeal JE, Kindrachuk RA, Freiha FS, Bostwick DG, Redwine EA, Stamey TA: Patterns of progression in prostate cancer. Lancet 1:60–63, 1986.
14. Gleason DF, Mellinger GT, The Veterans Administrative Cooperative Urological Research Group: Prediction of prognosis for prostatic adenocarcinoma by combined histological grading and clinical staging. J Urol 111:58–64, 1974.
15. Gleason DF: Histologic grading of prostatic carcinoma. In: Bostwick DG (ed): "Pathology of the Prostate." New York: Churchill-Livingstone, 1990, pp 83–90.
16. Mostofi FK: Grading of prostatic carcinoma. Cancer Chemother Rep 59:111–117, 1975.
17. Murphy GP, Gaeta JF, Pickren J, Wajsman Z: Current status of classification and staging of prostate cancer. Cancer 45:1889–1895, 1980.
18. Partin AW, Walsh AC, Pitcock RV, Mohler JL, Epstein JI, Coffey DS: A comparison of nuclear morphometry and Gleason grade as a predictor of prognosis in stage A2 prostate cancer: A critical analysis. J Urol 142:1254–1258, 1989.
19. Partin AW, Steinberg GD, Pitcock RV, Wu L, Piantadosi S, Coffey DS, Epstein JI: Use of nuclear morphometry, Gleason histologic scoring, clinical stage and age to predict disease free survival among patients with prostate cancer. Cancer 70:161–168, 1992.
20. Bostwick DG, Amin MB, Dundore P, Marsh W, Schultz DS: Architectural patterns of high grade prostatic intraepithelial neoplasia. Hum Path 24:298–310, 1993.
21. Boone CW, Kelloff GJ: Intraepithelial neoplasia, surrogate endpoint biomarkers, and cancer chemoprevention. J Cell Biochem 17F (Suppl):37–48, 1993.
22. Cantrell BB, deKlerk DP, Eggleston JC, Boitnott JK, Walsh PC: Pathological factors that influence prognosis in stage A prostatic cancer: The influence of extent versus grade. J Urol 125:516–520, 1981.
23. Epstein JI, Pizov G, Walsh PC: Correlation of pathologic findings with progression after radical retropubic prostatectomy. Cancer 71:3582–3593, 1993.
24. Freiha FS: Selection criteria for radical prostatectomy based on morphometric studies. NIH Consensus Development Conference. Management of Clinically Localized Prostate Cancer, Program and Abstracts, pp 73, June 15–17, 1987.
25. Shankey VT, Kallioniemi O-P, Koslowski JM, Leiber ML, Mayall BH, Miller G, Smith GJ: Consensus review of the clinical utility of DNA content cytometry in prostate cancer. Cytometry 14:497–500, 1993.
26. Partin AW, Yoo J, Carter HB, Pearson JD, Chan DW, Epstein JI, Walsh PC: The use of prostate specific antigen, clinical stage and Gleason score to predict pathologic stage in men with clinically localized prostate cancer. J Urol 150:105–110, 1993.
27. Partin AW, Pound CR, Clemens JQ, Epstein JI, Walsh PC: Prostate specific antigen after anatomic radical prostatectomy: The Johns Hopkins Experience after ten years. Urol Clin North Am 20:713–725, 1993.
28. Oesterling JE: Prostate specific antigen: A critical assessment of the most useful tumor marker for adenocarcinoma of the prostate. J Urol 145:907–923, 1991.
29. Oesterling JE: PSA leads the way for detecting and following prostate cancer. Contemp Urol 5:60–81, 1993.
30. Nowell PC: Mechanisms of tumor progression. Cancer Res 46:2203–2207, 1986.
31. Isaacs JT: Prostate cancer cell and molecular mechanisms in diagnosis and treatment. Cancer Surv 11:1–287, 1991.
32. Bostwick DG, Montironi R, Nagle R, Pretlow T, Miller G, Wheeler T, Epstein JI, Sakr W: Current and proposed biologic markers in prostate cancer. J Cell Biochem 16H (Suppl):65–67, 1992.
33. Diamond DA, Berry SJ, Umbricht C, Jewett HJ, Coffey DS: Computerized image analysis of nuclear shape as a prognostic factor for prostatic cancer. Prostate 3:321–332, 1982.
34. Diamond DA, Berry DS, Jewett HJ, Eggleston JC, Coffey DS: A new method to assess metastatic potential of human prostate cancer: Relative nuclear roundness. J Urol 128:729–734, 1982.
35. Mohler JL, Partin AW, Lohr DW, Coffey DS: Nuclear roundness factor measurement for assessment of prognosis of patients with prostatic carcinoma. I. Testing of a digitization system. J Urol 139:1080–1084, 1988.
36. Mohler JL, Partin AW, Epstein JI, Lohr DW, Coffey DS: Nuclear roundness factor measurement for assessment of prognosis of patients with prostatic carcinoma. II. Standardization of methodology for histologic sections. J Urol 139:1085–1090, 1988.
37. Partin AW: The development of a system for the quantitative analysis of tumor cell motility: Application to prostate cancer. Doctoral Thesis, The Johns Hopkins University, 1988.
38. Miller GJ, Shikes JL: Nuclear roundness as a predictor of response to hormonal therapy of patients with stage D2 prostatic carcinoma. In Karr JP, Coffey DS, Gardner W (eds): "Prognostic Cytometry and Cytopathology of Prostate Cancer." New York: Elsevier Science Publishing Co., Inc., 1988, pp 349–354.
39. Narayan P, Michael M, Jajodia P, Stein R, Gonzalez J, Ljung B, Chu K, Mayall B: Automated image analysis—a new technique to predict metastatic potential of prostate carcinomas? J Urol 141 (Suppl):

183, 1989 (Abstract #56).

40. Clark TD, Askin FB, Bagnell CF: Nuclear roundness factor: A quantitative approach to grading in prostatic carcinoma, reliability of needle biopsy tissue, and the effect of tumor stage on usefulness. Prostate 10:199–206, 1987.
41. Paulson DF, Stone AR, Walther PJ, Tucker JA, Cox EB: Radical prostatectomy: Anatomical predictors of success or failure. J Urol 136:1041–1043, 1986.
42. Eichenberger T, Mihatsch MJ, Oberholzer M, Gschwind R, Rutishauser G: Are nuclear shape factors good predictors of the disease course in patients with carcinoma of the prostate. Prog Clin Biol Res 243A:533–537, 1987.
43. Epstein JI, Berry SJ, Eggleston JC: Nuclear roundness factor: A predictor of prognosis in untreated stage A2 prostate cancer. Cancer 54:1666–1671, 1984.
44. Bibbo M, Kim DH, di Lareto C, Galera-Davidson H, Thompson D, Dytch HE: Architectural morphometric and photometric features and their relationship to the main subjective clues of grading prostate cancer. Anal Quant Cytol Histol 12:85–90, 1990.
45. Christen R, Xiao J, Minimo C, Gibbons G, Fitzpatrick BT, Galera-Davidson H, Bartels PH, Bibbo M: Chromatin texture features in hematoxylin and eosin-stained prostate tissue. Anal Quant Cytol Histol 15: 383–388, 1993.
46. Pressman NJ: Markovian analysis of cervical images. J Histochem Cytochem 24:138–144, 1976.
47. Dawson AE, Cibas ES, Bacus JW, Weinberg DS: Chromatin texture measurement by Markovian analysis. Anal Quant Cytol Histol 15:227–235, 1993.
48. Bacus JW, Grace LJ: Optical microscope system for standardized cell measurements and analyses. Applied Optics 26:3280–3293, 1987.
49. Bahnson RR: Treatment of stage A1 prostate cancer: The case for treatment. Semin Urol XI:54–57, 1993.
50. Robey EL, Schellhammer PF: Local failure after definitive therapy for prostatic cancer. J Urol 137:613–619, 1987.
51. Gibbons RP, Correa RJ Jr, Brannen GE, Mason JT: Total prostatectomy for localized prostatic cancer. J Urol 131:73–76, 1984.
52. Walsh PC, Jewett HJ: Radical surgery for prostatic cancer. Cancer 45:1906–1911, 1980.
53. Middleton RG, Smith JA Jr, Metzer RB, Hamilton PE: Patient survival and local recurrence rate following radical prostatectomy for prostatic carcinoma. J Urol 136:422–424, 1986.
54. Myers RP, Fleming TR: Course of localized adenocarcinoma of the prostate treated by radical prostatectomy. Prostate 4:461–472, 1983.
55. Thompson IM, Brawley O, Kramer B: Chemoprevention in carcinoma of the prostate. Oncology 7 (Suppl):49–53, 1993.
56. Reed JA, Manahan LJ, Park CS, Brigati DJ: Complete one-hour immunochemistry based on capillary action. Biotechniques 13:434–443, 1992.
57. Myers RB, Srivastava S, Oeschlager DK, Grizzle WE: Expression of p^{erbB-3} and $p185^{erbB-2}$ in prostatic intraepithelial neoplasia and prostatic adenocarcinoma. J Natl Cancer Inst (in press).