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TITLE: Combined Use of Tissue Morphology, Neural Network Analysis of Chromatin Texture & Clinical Variables to Predict Prostate Cancer Aggressiveness from Biopsy Material

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13. Abstract ***(Maximum 200 Words)*** *(abstract should contain no proprietary or confidential information)*

We proposed to combine standard prognostic methods (clinical stage, PSA, Gleason score, and biopsy information) with Neural Network analysis of chromatin texture and computer derived tissue morphology prospectively to predict pathologic stage. We also intended to retrospectively investigate in prostatectomy specimens using a similar combination of clinical, histologic, and computer derived characteristics to predict disease recurrence following surgery. This resulting technology and nuclear analysis would then be applied to a study group of men with long term follow up after surgery to develop and validate this technology in predicting recurrence following surgery. Lastly, we intended to use this methodology to develop and validate an accurate model for predicting time to metastatic progression/death after biochemical recurrence.

The scope of this project involved prospective enrollment of 500 men who were scheduled to undergo radical retropubic prostatectomy (year 01), development of an artificial neural network model (year 02), and prospective validation of this model (projected year 03). All models will be tested and developed for biopsy and prostatectomy material.

To date, we have completed prospective enrollment of 557 men. Tissue, serum and clinical pathologic information have been collected on all 557 cases (100%). Of these cases, 409 (83%) have retained enough cancer material on the archival biopsy specimens for image analysis. Image analysis is complete on all 409 cases evaluated. A predictive model of organ-confined prostate cancer was defined in January 2001. This model utilized a combination of unique biomarkers to address the contemporary challenge of patients in this cohort that presented with clinical stage T1c disease (69.4%). We are presently completing our evaluation of this technology on a cohort of 300 men (task 5) who have had biochemical recurrence following radical retropubic prostatectomy.

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FOREWORD

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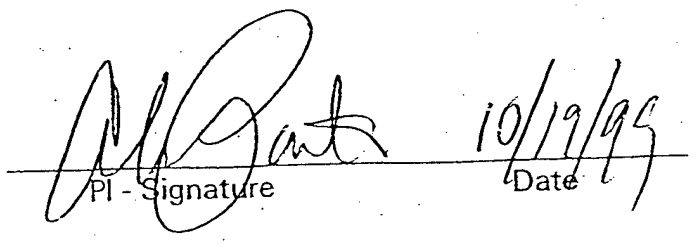

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Introduction:

Several specific objectives were outlined for our research proposal entitled *Combined use of Tissue Morphology, Neural Network Analysis of Chromatin Texture and Clinical variables to Predict Prostate Cancer Aggressiveness from biopsy Material.* We proposed to combine standard prognostic methods (clinical stage, PSA, Gleason score, and biopsy information) with Neural Network analysis of chromatin texture and computer derived tissue morphology prospectively to predict pathologic stage. We also intended to retrospectively investigate in prostatectomy specimens using a similar combination of clinical, histologic and computer derived characteristics to predict disease recurrence following surgery. This resulting technology and nuclear analysis would then be applied to study a group of men with long term follow-up after surgery to develop and validate this technology in predicting recurrence following surgery. Lastly, we intended to use this methodology to develop and validate an accurate model for predicting time to metastatic progression/death after biochemical recurrence. With these specific objectives outlined, a statement of work was submitted detailing the task and time line necessary to accomplish the goals of the proposal. Task one of our statement of work outlined the steps involved in the prospective enrollment of 500 men for prediction of pathologic stage model development. Completion of this objective was projected for 9 months following the initiation of this project. Below are the initial steps outlined in Task one, followed by an update of our progress to date.

Body: Specific Aims

A. Identification and prospective enrollment of consecutive radical prostatectomy cases performed at the Johns Hopkins Hospital.

557 patients have been enrolled with 409 successfully fulfilling all inclusion criteria. The exclusion of 148 patients was due to: canceled RRP, no response from original biopsy institution, no cancer present in remaining biopsy material.

B. Obtain tissue blocks for each case.

Tissue blocks have been obtained for all patients admitted into this research study.

C. Cut and prepare histologic sections.

Histologic sections have been obtained from all cases.

D. Measure nuclear features with the QNG model.

Image analysis has been completed on all 409 (100%) cases.

E. Enter all clinical, pathological, and quantitative nuclear data into the computer.

Clinical and pathological data for 409 patients has been collected and organized into a relational database.

F. Multivariate analysis to determine optimal prognosis prediction model.

DNA ploidy analysis and pathologic review has been completed on all 409 cases (100%). Model construction was completed in January 2001.

Task two of our approved statement of work details the steps necessary for prospective enrollment of 400 men for pathologic stage model validation. This portion of the project has a projected completion of 13 months following project initiation.

We have been unable to begin prospective enrollment for this objective due to the timing of the model completion and lack of continued funding.

Task three of the research proposal outlines the steps involved in predicting tumor aggressiveness from biopsy/prostatectomy specimens. This portion of the statement of work should be completed by month 14 of the study. Our progress to date is indicated below:

A. Obtain tissue blocks from 300 cases treated at Johns Hopkins with radical prostatectomy.

300 pathological specimens were identified and collected for analysis.

B. Cut histologic sections and prepare slides for QNG analysis.

We were not able to complete this portion of this objective. Please refer to the comments for task 4 for an explanation.

C. QNG determinations

Refer to task 3, section B comment.

D. Tissue morphology analysis.

Refer to task 3, section B comment.

F. Enter clinical data, pathological information, QNG results and tissue morphology into a database.

Clinical and pathological data for 300 patients has been collected and organized into a relational database.

G. Calculate model for prediction of post-operative progression from prostatectomy specimens.

Refer to task 3, section B comment

Task four involves validation analyses from prostatectomy specimens for prediction of tumor aggressiveness. Our initial statement of work projected completion of this portion of the project by month 30 (March 2001). We will not be able to complete this portion of the proposal before the end of the contractual period. The two previously listed tasks were highly time and manpower consuming.

Lastly, task five of this research study involves retrospective development of a model for prediction of development of metastasises/death following biochemical recurrence following

surgery. This task involves identification of 300 men who have exhibited biochemical or metastatic recurrence following surgery.

A. Obtain tissue blocks from 300 cases treated at Johns Hopkins with radical prostatectomy.

Tissue blocks for 304 cases have been collected. Of these cases, 277 (75%) have retained enough cancer material on the archival biopsy specimens for image analysis.

B. Cut histologic sections and prepare slides for QNG analysis.

Histologic sections have been obtained for all cases identified for this task.

C. QNG determinations

Feulgan staining has been completed on 232 (84%) cases. Pathologic review has begun on these cases. QNG analysis will proceed following pathologic review of the stained slides.

D. Tissue morphology analysis.

Tissue morphologic analysis is complete on 232 (84%). Analysis will continue accordingly, following Feulgan staining of remaining cases.

F. Enter clinical data, pathological information, QNG results and tissue morphology into a database.

Clinical and pathological data for 304 patients has been collected and organized into a relational database.

G. Determine the prognostic significance of combined variables to predict 3, 5 and 7 year likelihood of remaining metastases free by developing and validating a model for prediction.

This portion of task five will be begin following QNG and morphology analysis completion. We anticipate model completion by June 2001.

Research accomplishments:

Task one:

- Prospective enrollment of 557 patients.
- Biochemical profile (PSA, FPSA, Complex PSA) complete on 420 patients.
- Biopsy material obtained on 493 patients.
- Histology completed on 409 cases.
- Image analysis completed on 409 cases.

We evaluated the ability of using clinical and pathological data, computer assisted morphometric determinations, and neural network evaluation of chromatin texture, to predict pathologic stage as a surrogate for tumor aggressiveness from biopsy material. Approximately 69% of the

prospectively enrolled patients in task one were diagnosed with T1c disease. Because of the changing demographics of prostate cancer as represented by this clinical stage, PSA and Gleason scores, the model development utlilized the combination of existing and investigational biomarkers to address the new contemporary challenge.

Biomarkers assessed included: total PSA (tPSA), complexed PSA (cPSA), freePSA (fPSA), f/tPSA ratio, Quantitative nuclear grade (QNG), cPSA density, and biopsy gleason score. Logistic regression was used to determine the most accurate combination of variables for predicting OC disease. A cross-validation method of data analysis was performed.

Complete data were available for 254/386 (66%) men with T1c disease (average age, 58.8 +/- 6 years). A total of 49/254 (19%) had pathologically non-organ-confined disease. Univariate analysis of the pre-treatment variables showed that QNG, biopsy Gleason score, tPSA, calculated f/tPSA ratio, cPSA, and cPSA density were significant. Using backward stepwise logistic regression at a stringency of $p < 0.10$, only QNG, cPSA-density, and Gleason score remained in the model and yielded an area under the ROC curve of 81.6%. The sensitivity and specificity of the model at a cutoff of 0.14 was 75.5% and 73.2% respectively with a negative predictive value of 92.6%

We conclude, following this model construction, that this data demonstrates accurate pre-treatment prediction of organ confined disease in a contemporary series of men with T1c prostate cancer based upon only use of QNG, cPSA-density and biopsy Gleason score.

Task five:

- Image analysis and tissue morphology complete on 232/277 (84%).
- Clinical and pathological data for 277 (100%) patients has been collected.

Retrospective development of a model for the prediction of development of metastases/death following biochemical recurrence post surgery involves the following steps: requisition of reference slides from radical retropubic prostatectomy, identification of representative gleason pattern, PIN and normal tissue, collection of eight consecutive archival tissue sections from identified areas, tissue staining (Hematoxylin/ Eosin and Feulgan), and computer imaging of cancer, normal and PIN areas. The image processing involves complete analysis of sixty cells each of cancer, PIN and normal per 277 identified patients. Morphometric data is stored in a relational database and model construction awaits this completion.

Reportable outcomes:

- Manuscript: Steven R. Potter, M. Craig Miller, Leslie A. Mangold, Kerrie A. Jones, Jonathan I. Epstein, Robert W. Veltri, and Alan W. Partin. *Genetically Engineered Neural Networks for Predicting Prostate Cancer Progression after Radical Prostatectomy*. Urology, 54(5): 791-795, 1999.

- Poster presented at the American Urological Association Conference
  Prediction of Pathologic Stage in Clinical Stage T1c Prostate Cancer, Veltri, R.W., Miller, M.C., O'Dowd G.J., Mangold, L.A., Epstein, J.I., Partin, A.W., April 2000. (Attached)

- Poster to be presented at the American Urological Association Confernece, June 2001
  Improved Accuracy for Prediction of Organ-Confined Prostate Cancer (Pca) in a Contemporary Referral Series: The New Challenge, Veltri, R.W., Miller, M.C., Mangold, L.A., Epstein, J.I., Sokol, L.J., and Partin, A.W. (Attached)

Appendix A

AUA 2000 ABSTRACT #839

PREDICTION OF PATHOLOGICAL STAGE IN CLINICAL STAGE TIC PROSTATE CANCERS.
Robert W. Veltri, Michael C. Miller, Gerard J. O'Dowd, Oklahoma City, OK; Leslie A. Mangold, Jonathan I. Epstein, Alan W. Partin, Baltimore, MD.

INTRODUCTION AND OBJECTIVE: A new challenge for management of prostate cancer involves the ability to predict pathologic stage in patients with clinical stage T1c disease. We constructed a statistical model to predict the organ confinement status in these patients.
METHODS: A total of 101 patients with clinical stage T1c prostate cancer were prospectively evaluated. All patients underwent radical prostatectomy at the Johns Hopkins Hospital, and the pathological staging was performed by a single pathologist (JIE). Twenty-eight percent of these patients had non-organ confined disease. Feulgen stained, 5 micron sections from the positive biopsies of these patients were reviewed and the cancer areas were graded and marked (GJO). Approximately 125 cancer nuclei were captured from the highest Gleason score area of each case utilizing an AutoCyte Pathology Workstation with QUIC-DNA v120l software. The variance of 60 different nuclear size, shape, and chromatin texture features were calculated for each set of nuclei and used to determine a quantitative nuclear grade (QNG) for each case. The QNG, along with the patient age, highest Gleason grade (4/5), and pre-operative PSA were analyzed using logistic regression.
RESULTS: Using univariate logistic regression analysis, QNG provided the largest area under the curve (AUC 72%) compared to the other input variables, which ranged from an AUC = 58% - 63%. Applying backwards stepwise logistic regression at a stringency of $p < 0.05$ resulted in a model containing QNG, Gleason grade 4/5, and PSA with an AUC = 78% for the prediction of the disease organ confinement status. At a cutoff of 0.5, the accuracy of the model was 81%, with a positive predictive value of 74% and a negative predictive value of 83%.
CONCLUSIONS: Utilizing a new quantitative image analysis based variable, QNG, in combination with pre-operative biopsy and PSA data, we were able to more accurately predict post-operative stage in clinical stage T1c prostate cancer patients.

Source of funding: UroCor, Inc. and Department of Defense Grant #DAMD17-98-1-8468

INTRODUCTION

Prostate cancer (PCa) is the most common malignancy among men in the United States, affecting over 179,300 men and resulting in about 37,000 deaths in 1999 [1].

Approximately 30% of men who are treated for localized disease will recur, and a subset of these men will progress [2].

Prior to the commercial availability of the serum prostate specific antigen (PSA) test around 1987, the clinical staging of prostate cancer (PCa) utilized the digital rectal examination (DRE) and the transrectal ultrasound guided biopsy [2-4].

Most patients diagnosed early with organ-confined tumors are curable about 90-95% of the time with radical prostatectomy [5] or about 85-95% with radiation therapy [6].

There are a significant number (~60-70%) of patients with clinical stage T1c disease (PSA > 2.5 ng/ml and non-palpable disease) presenting at diagnosis that have advanced pathology (grade and stage) at radical prostatectomy [7-10].

Studies of various nuclear features, such as nuclear roundness and chromatin complexity, on PCa cells from radical prostatectomy sections demonstrated that nuclear morphometric descriptors (NMDs) from PCa epithelial cells are prognostic [10-11, 14].

Using computer-assisted image analysis, we applied a proprietary process to create a new pathological biomarker of genetic instability, termed Quantitative Nuclear Grade (QNG™) [10-11, 14-15] (*Figure 1*).

Using a new quantitative imaging system (*Figure 2*), we evaluated the use of the QNG™ variable in biopsy cases with Clinical Stage T1c to predict pathological stage.

MATERIALS AND METHODS

PATIENT SAMPLE

- From a total of 557 patients enrolled in a 2 ½ year prospective Prostate Cancer study funded by the Department of Defense (Grant # DAMD17-98-1-8468), we selected biopsies from a subset of men with clinical stage T1c disease where we had the following information (*Tables 1A & 1B*):
  - Age at the time of Biopsy
  - Pre-Operative PSA Level
  - Gleason Grades and Score of Biopsy
  - Feulgen Stained 5μ Tissue Section from Prostate Biopsy
  - Pathological Stage
- A total of 101 patients with clinical stage T1c underwent radical prostatectomy surgery at the Johns Hopkins Hospital, and pathological staging was performed by a single pathologist (JIE). Twenty-eight of these patients were determined to have non-organ confined disease (*Table 1A*).

QUANTATIVE NUCLEAR GRADE (QNG™) DETERMINATION:

- Feulgen stained, 5μ prostate biopsy tissue sections were reviewed and the cancer areas were graded and marked by a single pathologist (GJO).
- Approximately 125 cancer nuclei were captured from the highest Gleason score area of each case utilizing an AutoCyte Pathology Workstation with QUIC-DNA vl20l software (*Figures 1 & 2*).
- The variance of each of the 60 NMDs (i.e. different nuclear size, shape, DNA content, and chromatin texture features) were calculated for each case (*Figure 1*) [10, 11, 14, 15].
- Using univariate logistic regression analysis, the p-value and area under the receiver operator characteristics curve (ROC-AUC) for the variance of each NMD was determined (*Table 2*).
- Using backwards stepwise logistic regression at a stringency of $p < 0.20$, a multivariate model to calculate the QNG™ value was created, and it utilized 6 of the 17 univariately significant NMDs (*Table 2 & Figure 3*).

OC VS. NOC PREDICTIVE MODEL CONSTRUCTION:

- Univariate logistic regression analysis was used to determine the ability of the independent variables to predict the pathological stage (binary outcome of Organ confined [OC] vs. Non-organ confined [NOC]). (See *Table 3 & Figure 4*).
- Using the age, total PSA, presence of Gleason grade 4 and/or 5, the Gleason score, and the QNG™ value, a backwards stepwise logistic regression model was constructed with a stringency of $p < 0.05$. This multivariate model retained the total PSA, the presence of Gleason grade 4 and/or 5, and the QNG value to predict OC vs. NOC (*Table 3 & Figure 4*).

SUMMARY

- Clinical Stage T1c offers a new challenge for pre-treatment pathological staging and represents a very significant portion of prostate cancers being diagnosed today.
- Quantitative Nuclear Grade (QNG™) is an image-based morphometric measurement of genetic instability derived using a multivariately significant subset of 60 different NMDs that measure nuclear size, shape, DNA content, and chromatin organization features.
- QNG™, when combined with Gleason Grade 4/5 and total serum PSA information, predicted the pathological stage with an accuracy of 81% and a ROC-AUC of 78%.
- We plan to expand the training set to include additional biomarkers (i.e. molecular forms of PSA) and validate this clinical stage T1c pre-treatment staging algorithm.

CONCLUSIONS

- Quantitative image analysis offers a new and accurate tool to assess genetic instability cost effectively and reproducibly on both biopsy and radical prostatectomy material.
- In spite of the strong contribution of quantitative morphometry to predict the stage and progression, there remains a need to identify new and effective biomarkers that can aggregately make pre-treatment algorithms more accurate.
- Improved patient staging allows the urologist and patient to make more informed decisions for patient disease management from diagnosis through definitive treatment.

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Table 1A: Patient Sample Description (n=101 Clinical Stage T1c Prostate Cancers)

Pathologic Stage*	N	Mean Values (Median Values) tPSA (ng/ml)	Age at Biopsy	Biopsy Gleason	QNG Score
OC	73	6.1 (5.8)	57 (58)	6 (6)	0.23 (0.19)
NOC-CP	25	9.0 (6.5)	56 (56)	6 (6)	0.35 (0.28)
NOC-Mets	3	10.8 (10.6)	56 (57)	7 (7)	0.71 (0.72)

* OC = Organ Confined; NOC-CP = Non-Organ Confined due to Capsular Penetration Only; NOC-Mets = Non-Organ Confined due to Seminal Vesicle and/or Lymph Node Involvement

Table 1B:

Gleason Score	Biopsy	Radical
≤ 5	5 (5%)	6 (6%)
6	68 (67%)	79 (78%)
7	28 (28%)	14 (14%)
≥ 8	0 (0%)	2 (2%)

Figure 1: Method for QNG™ Determination

Analyze Specimen Using Image Analysis System, Generate a DNA Ploidy Histogram, and Save Nuclear Images for the Calculation of the Quantitative Nuclear Grade (QNG)

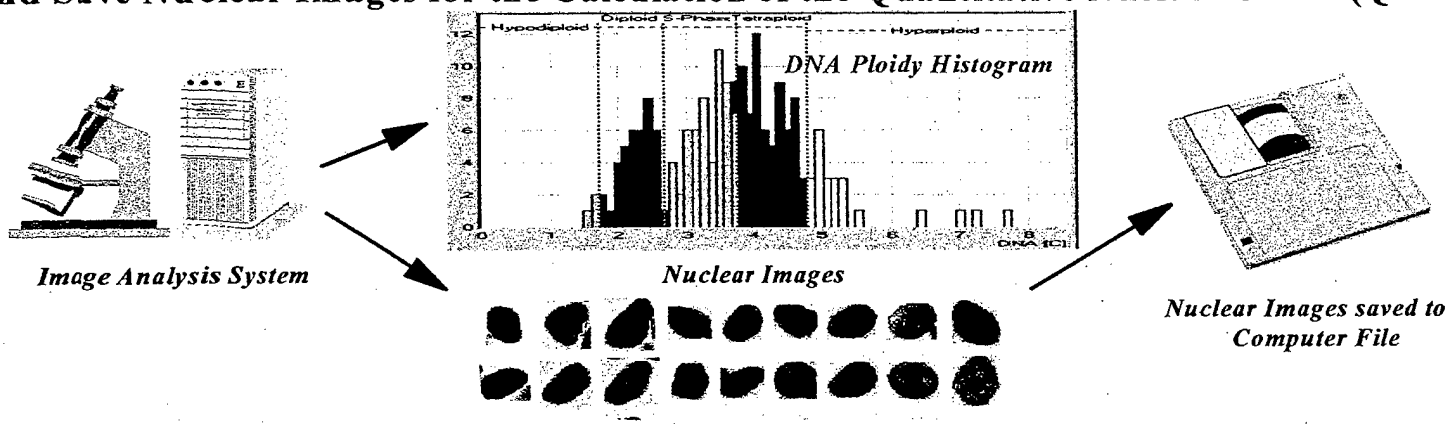
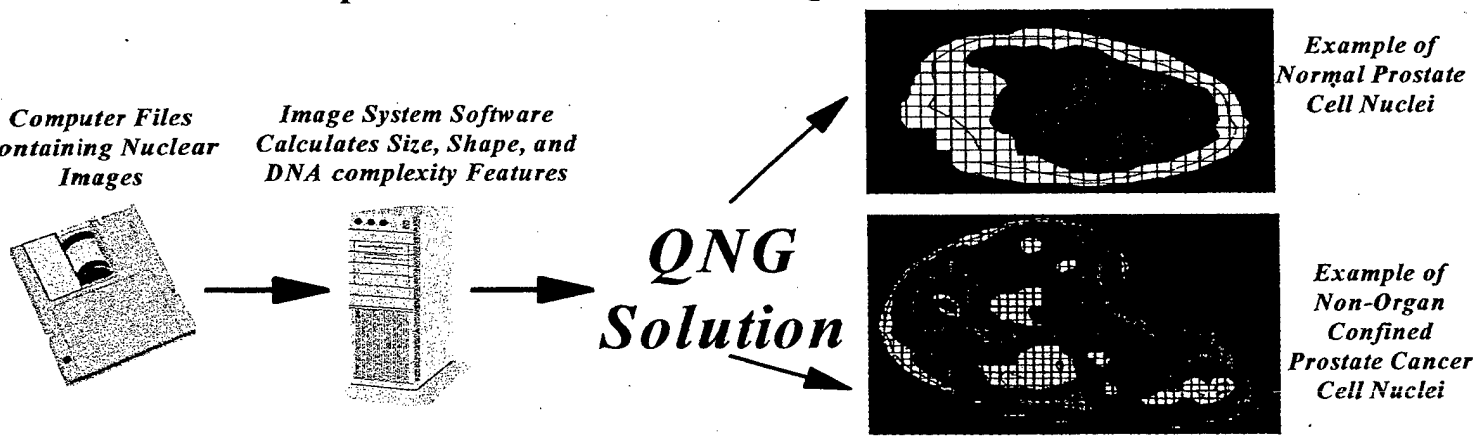


Image Analysis System

Nuclear Images

Nuclear Images saved to a Computer File

Calculate Size, Shape, and DNA complexity Features for each of the Nuclear Images saved in the Computer Files and Create the Quantitative Nuclear Grade Solution



Computer Files containing Nuclear Images

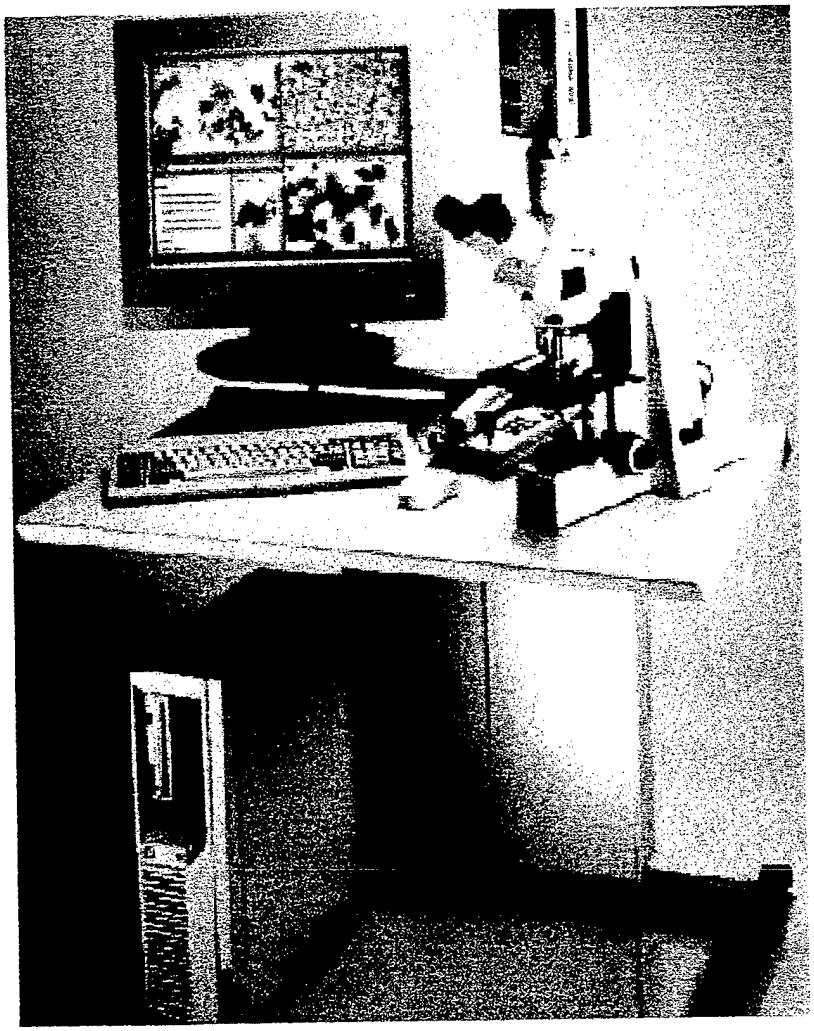
Image System Software Calculates Size, Shape, and DNA complexity Features

QNG Solution

Example of Normal Prostate Cell Nuclei

Example of Non-Organ Confined Prostate Cancer Cell Nuclei

Figure 2: AutoCyte™ Pathology Workstation (TriPath Imaging Inc., Burlington, NC)



- Zeiss Axioskop Microscope
- 3CCD Color Camera
- High Resolution (768x494)
- Square Pixels
- ~60 Nuclear Morphometric Descriptors
- User Friendly Software
- High Speed / High Capacity Computer System
- Commercially Available and not Cost Prohibitive

Table 2: Logistic Regression Analysis of NMDs

AutoCyte Morphometry Measurements		Univariate Analysis OC vs. NOC Prediction	
Variable	Variable Description	p-value	ROC-AUC
Var1	Cell Class	0.0615	63.31%
Var2	Perimeter	0.0205	67.27%
Var3	Area	0.0320	67.32%
Var4	Circular Form Factor	0.8890	56.36%
Var5	*Diameter Equivalent Circle**	*0.0171*	*68.98%*
Var6	Feret X	0.0707	62.33%
Var7	Feret Y	0.0148	68.93%
Var8	Minimum Feret	0.0413	66.78%
Var9	*Maximum Feret*	*0.0181*	*67.07%*
Var10	*Area Convex Hull*	*0.0327*	*67.12%*
Var11	*Perimeter Convex Hull*	*0.0187*	*68.00%*
Var12	Excess of Gray Values	0.4589	55.33%
Var13	Skewness of Gray Values	0.2702	55.68%
Var14	StdDev of Gray Values	0.2623	57.63%
Var15	Mean Gray Value	0.0361	62.57%
Var16	Median Gray Value	0.0405	61.59%
Var17	Maximum Gray Value	0.1345	57.34%
Var18	Minimum Gray Value	0.2503	59.44%
Var19	Intensity	0.0516	65.70%
Var20	*Integrated OD*	*0.0176*	*65.66%*
Var21	Minimum OD	0.1199	57.73%
Var22	Maximum OD	0.4021	48.53%
Var23	Median OD	0.1808	57.29%
Var24	Mean OD	0.1488	57.68%
Var25	StdDev OD	0.3431	53.96%
Var26	Skewness of OD	0.4047	52.35%
Var27	Excess of OD	0.5609	48.04%
Var28	DNA Ploidy	0.0465	67.07%
Var29	DNA Index	0.1294	67.86%
Var30	Transmission	0.0387	62.43%
Var31	Variance	0.9709	52.25%
Var32	Sum Mean	0.9392	49.85%
Var33	Sum Entropy-AC	0.2730	63.26%
Var34	Sum Variance-AC	0.1345	61.99%
Var35	Cluster Shade	0.0654	62.18%
Var36	Cluster Prominence	0.0517	62.43%
Var37	Diagonal Moment	0.0759	59.20%
Var38	Kappa	0.1015	58.17%
Var39	Sum of Homogeneity	0.0533	60.23%
Var40	Angular Second Moment	0.0159	65.90%
Var41	Contrast	0.2442	56.70%
Var42	Correlation	0.5103	54.31%
Var43	Difference Moment	0.2831	55.38%
Var44	Inverse Difference Moment	0.0694	59.78%
Var45	Sum Average	0.8554	50.20%
Var46	Sum Variance-M	0.4997	55.58%
Var47	Sum Entropy-M	0.2721	59.05%
Var48	Entropy	0.0470	61.59%
Var49	Difference Variance	0.3128	55.43%
Var50	Difference Entropy	0.1220	58.32%
Var51	Information Measure A	0.1433	57.68%
Var52	Information Measure B	0.3701	61.15%
Var53	Maximal Correlation Coefficient	0.6748	51.91%
Var54	*Coefficient of Variation*	*0.0299*	*62.33%*
Var55	Peak Transition Probability	0.2280	64.73%
Var56	Diagonal Variance	0.0159	65.90%
Var57	Diagonal Moment	0.1639	60.13%
Var58	Second Diagonal Moment	0.7463	53.13%
Var59	Product Moment	0.9392	49.85%

Areas shaded in gray indicate univariately significant NMDs.

Areas shaded in yellow indicate univariately significant NMDs that were retained in the multivariate QNG model.

T1c QNG Model Predictive Index (*Xb*) Formula (*Fig. 3*):

$$Xb = -6.037824 + (Var5)(24.53941) + (Var9)(4.157673) + (Var10)(-0.0388227) + (Var11)(-2.127776) + (Var20)(0.001242) + (Var54)(5605.381)$$

$$\text{QNG Value } (P_x) = e^{Xb} / (1 + e^{Xb})$$

Appendix B

IMPROVED ACCURACY FOR PREDICTION OF ORGAN-CONFINED PROSTATE CANCER (PCa) IN A CONTEMPORARY REFERRAL SERIES: THE NEW CHALLENGE Veltri RW, Miller, MC, Mangold LA, Epstein JI, Sokol LJ, and Partin, AW (Presentation by Dr. Partin)

INTRODUCTION: The choice of definitive therapy for men with localized PCa is often based upon ***their*** likelihood of having organ-confined (OC) disease. This decision is currently derived from limited pre-treatment clinical and laboratory information. Nomograms such as the "Partin Tables" offer clinically useful population statistics to guide this decision process, however, do not provide patient-specific results. The changing demographics of PCa in contemporary series (e.g. PSA, Gleason Score and Clinical Stage) are unable to accurately predict pathological stage patients at this critical decision step in disease management. This study utilizes a unique combination of existing and investigational biomarkers to address this contemporary challenge in patients with T1c disease.

METHODS: We prospectively enrolled 557 men between 10/98 and 01/00 scheduled for radical prostatectomy at a single institution and 386 (69%) were diagnosed with T1c disease. Exclusion criteria included neoadjuvant treatment or medications, which could effect serologic or histologic presentation of PCa. Pre-operative sera, biopsy histology slides, clinical demographic information, prostatectomy pathology and gland weight were obtained. Biomarkers assessed included: total PSA (tPSA), complexed PSA (cPSA), freePSA (fPSA), f/tPSA ratio, Quantitative nuclear grade (QNG), cPSA-density, and biopsy Gleason score. Logistic regression was used to determine the most accurate combination of variables for predicting OC disease. A cross-validation method of data analysis was performed.

RESULTS: Complete data were available for 254/386 (66%) men with T1c disease (average age, 58.8 +/- 6 years). A total of 49/254 (19%) had pathologically non-organ-confined disease. Univariate analysis of the pre-treatment variables showed that QNG, biopsy Gleason score, tPSA, calculated f/tPSA ratio, cPSA, and cPSA density were significant. Using backward stepwise logistic regression at a stringency of $p < 0.10$, only QNG, cPSA-density, and Gleason score remained in the model and yielded an area under the ROC curve of 81.6%. The sensitivity and specificity of the model at a cutoff of 0.14 was 75.5% and 73.2% respectively with a negative predictive value of 92.6%.

CONCLUSION: These data demonstrate accurate pre-treatment prediction of OC disease in a contemporary series of men with T1c PCa based upon only use of QNG, cPSA-density and biopsy Gleason score.

ELSEVIER

GENETICALLY ENGINEERED NEURAL NETWORKS FOR PREDICTING PROSTATE CANCER PROGRESSION AFTER RADICAL PROSTATECTOMY

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ABSTRACT

Objectives. To use pathologic, morphometric, DNA ploidy, and clinical data to develop and test a genetically engineered neural network (GENN) for the prediction of biochemical (prostate-specific antigen [PSA]) progression after radical prostatectomy in a select group of men with clinically localized prostate cancer.

Methods. Two hundred fourteen men who underwent anatomic radical retropubic prostatectomy for clinically localized prostate cancer were selected on the basis of adequate follow-up, pathologic criteria indicating an intermediate risk of progression, and availability of archival tissue. The median age was 58.9 years (range 40 to 87). Men with Gleason score 5 to 7 and clinical Stage T1b-T2c tumors were included. Follow-up was a median of 9.5 years. Three GENNs were developed using pathologic findings (Gleason score, extraprostatic extension, surgical margin status), age, quantitative nuclear grade (QNG), and DNA ploidy. These networks were developed using three randomly selected training (n = 136) and testing (n = 35) sets. Different variable subsets were compared for the ability to maximize prediction of progression. Both standard logistic regression and Cox regression analyses were used concurrently to calculate progression risk.

Results. Biochemical (PSA) progression occurred in 84 men (40%), with a median time to progression of 48 months (range 1 to 168). GENN models were trained using inputs consisting of (a) pathologic features and patient age; (b) QNG and DNA ploidy; and (c) all variables combined. These GENN models achieved an average accuracy of 74.4%, 63.1%, and 73.5%, respectively, for the prediction of progression in the training sets. In the testing sets, the three GENN models had an accuracy of 74.3%, 80.0%, and 78.1%, respectively.

Conclusions. The GENN models developed show promise in predicting progression in select groups of men after radical prostatectomy. Neural networks using QNG and DNA ploidy as input variables performed as well as networks using Gleason score and staging information. All GENN models were superior to logistic regression modeling and to Cox regression analysis in prediction of PSA progression. The development of models using improved input variables and imaging systems in larger, well-characterized patient groups with long-term follow-up is ongoing. UROLOGY **54:** 791–795, 1999. © 1999, Elsevier Science Inc.

Improvements in prostate cancer staging have dramatically increased the percentage of men presenting with clinically localized disease.[1,2] However, 30% to 40% of men undergoing radical retropubic prostatectomy (RRP) will have biochemical (prostate-specific antigen [PSA]) progression within 10 years.[1] Estimates of progression risk are based on tumor volume, surgical margin status, Gleason score, and pathologic stage.[2–5] Nuclear morphometry and DNA ploidy provide additional variables for use in predictive models.[4–7] Improvements in our ability to predict progression after definitive therapy are needed to help patients and physicians decide whether and when to initiate adjuvant therapy.

Statistical tools, such as logistic regression analysis, have routinely been used to analyze data and

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predict treatment outcomes.[2–6,8] However, the variability and complexity of the data may exceed the capacity of standard modeling methods. Artificial neural networks (ANNs) attempt to simulate human decision-making using adaptation and inference parameters.[9] ANNs can better define nonlinear patterns between predictor variables and previously unknown outcomes than linear statistical models.

Validation of an ANN requires separate training and testing phases. In the training phase, the ANN "learns" the relationships of input and outcome and assigns weights to the input variables. Once these weights are formalized, the ANN is considered "trained." The ANN must then be validated using a different data set. The term "genetic" in the phrase "genetically engineered neural network (GENN)" refers to a method of network development in which the network architecture is determined by the data presented to it.[10] The GENN develops the relationships between input variables and outcome, selects for the "fittest" solutions, and ultimately "evolves" an optimal network. Use of ANNs in urologic oncology has shown promise.[11–13]

Previously, we used logistic regression analysis to evaluate the ability of quantitative nuclear grade (QNG) and Gleason score to predict progression after RRP.[5] We determined that QNG and Gleason score stratified patients into low, moderate, and high-risk groups for prostate cancer progression. In follow-up to that retrospective study, we now compare the ability of GENNs and logistic regression modeling to predict progression in a subset of RRP patients in whom accurate prediction is especially difficult.

MATERIAL AND METHODS

Patients

A total of 214 men with prostatectomy Gleason score of 5 to 7 and clinical Stage T1b-T2c cancer were nonconsecutively selected from a cohort of more than 1800 RRP patients treated between 1982 and 1996 at one institution.[14] The selection of these men was based on adequate follow-up (at least 5 years for patients without progression), complete clinical data, and the availability of archival tissue. All men underwent anatomic RRP. Men with seminal vesicle invasion or lymph node involvement discovered at surgery were excluded because of the known high risk of progression. Men who underwent adjuvant or neoadjuvant hormonal or radiation therapy were also excluded, as the natural history of prostate cancer in these men could not be ascertained. Most were treated before the availability of preoperative PSA testing. These 214 men formed the training and testing groups for the development and analysis of the three GENN models and had a minimum follow-up among patients without progression of 5 years (range 5 to 16). All preoperative clinical, pathologic, and postoperative data were gathered prospectively and are summarized in Table I.

Men were followed up with serum PSA measurements at 3-month intervals for 1 year, at 6-month intervals for an additional year, and yearly thereafter (after PSA testing became available in 1987). An annual interview and digital rectal examination were performed. Biochemical recurrence was defined as a postoperative serum PSA greater than 0.2 ng/mL. No patient received radiation or hormonal therapy before biochemical disease recurrence.

TABLE I. *Summary of demographic and clinical data in 214 men presenting with clinically localized prostate cancer*

Average age (yr)	58.9 ± 6.4 (40–87)
Average follow-up time (yr)	7.8 ± 3.9 (1–16)
Average time to progression (yr)	4.5 ± 3.3 (1–14)
Average follow-up (nonprogression) (yr)	9.9 ± 2.7 (5–16)
Clinical stage (n)	
T1b–T1c	6 (3)
T2a	72 (33)
T2b	113 (53)
T2c	23 (11)
Prostatectomy Gleason scores (n)	
5	50 (23)
6	75 (35)
7	89 (42)

Numbers in parentheses for clinical stage and Gleason scores are percentages, all others are the range.
Data are presented as the average ± standard deviation, unless otherwise noted.

Image Data Acquisition

Representative sequential 5-μm-thick sections were cut from archival, formalin-fixed, paraffin-embedded tissue. Alternating sections were stained with hematoxylin-eosin and Feulgen reagents and areas of cancer marked.[15] Approximately 150 nuclei from each tumor were analyzed. Forty-one nuclear morphometric descriptors were measured for each image, including 11 DNA content, 22 markovian texture, and 8 nuclear shape features.[5]

Neural Network Analysis

All data were analyzed using NeuroGenetic Optimizer software, version 2.6 (BioComp Systems, Redmond, Wash), which builds predictive models using genetic algorithms. Input variables included prostatectomy pathologic findings (Gleason score and extraprostatic extension and surgical margin status), age, DNA ploidy, and QNG (the variance of 41 different nuclear morphometric descriptors). These variables were classified as nominal (extraprostatic extension and margin status), categorical (Gleason score and DNA ploidy), or continuous (age and nuclear morphometric descriptors).

Using pathologic findings and age (model 1), QNG and DNA ploidy (model 2), or a combination of all variables (model 3), we constructed three randomly selected training and testing sets balanced for the number of patients with (n = 84) and without (n = 87) progression in our cohort. The training sets consisted of 80% of the balanced sample; the testing sets used the remaining 20% of the balanced sample. The same three training and testing sets were used for network analysis and logistic regression modeling. To avoid network overfitting, each network was limited to a maximum of 200 training iterations.

Statistical Analysis

All data were analyzed with Stata version 5.0 statistical analysis software (Stata, College Station, Tex). Logistic regression

TABLE II. *Results of GENN models on randomly selected training and testing sets balanced for number of patients with and without progression*

	Model 1 (Pathology + Age)	Model 2 (NMDs + DNA Ploidy)	Model 3 (All Variables Combined)
Average for random training sets (n = 136)			
Sensitivity (%)	83.6 ± 0.0	53.7 ± 6.5	75.1 ± 2.3
Specificity (%)	65.5 ± 2.5	72.4 ± 7.4	71.8 ± 3.9
Accuracy (%)	74.4 ± 1.2	63.1 ± 6.3	73.5 ± 0.8
AUC (%)	79.4 ± 2.1	68.3 ± 5.8	79.6 ± 0.9
Average for random testing sets (n = 35)			
Sensitivity (%)	88.2 ± 5.9	74.5 ± 9.0	84.3 ± 9.0
Specificity (%)	61.1 ± 11.1	85.2 ± 3.2	72.2 ± 0.0
Accuracy (%)	74.3 ± 4.9	80.0 ± 2.9	78.1 ± 4.4
AUC (%)	71.3 ± 8.6	74.0 ± 4.0	73.5 ± 7.5

KEY: GENN = genetically engineered neural network; NMDs = nuclear morphometric descriptors; AUC = area under the curve.
Data presented as the average ± standard deviation.

analysis was used to evaluate the accuracy of the various GENNs. The outcome variable was biochemical progression. Receiver operating characteristic curves and the areas under the curves were calculated for each of the GENN models, as were sensitivity, specificity, and accuracy. Accuracy was defined as the overall percentage of cases that were correctly classified. Kaplan-Meier analysis was performed using the average results of model 3. The actuarial curve significance was determined using the log-rank test of equality and the Wilcoxon-Gehan test.

Logistic regression analysis was performed concurrently on the same three randomly selected training and testing sets using the same combinations of input variables. A multivariate significance stringency of $P < 0.25$ was used for backward stepwise logistic regression analysis. Again, receiver operating characteristic curves and the areas under the curves were calculated for each model, as were sensitivity, specificity, and accuracy. The Cox proportional hazards model was performed on the training and testing set output of model 3.

RESULTS

Of the 149 tumors (70%) with extraprostatic spread at pathologic staging, 66 (31%) also had positive margins. The remaining 65 tumors (30%) were organ confined. During a median follow-up of 9.5 years, 84 men (40%) developed biochemical progression within a median of 4 years (range 1 to 14). In the biochemical progression-free men (n = 130), 75% of the tumors had prostatectomy Gleason scores of 5 or 6; of the men with biochemical progression (n = 84), 67% had a prostatectomy Gleason score of 7.

The three GENN models achieved an average accuracy of 74.4%, 63.1%, and 73.5% for predicting progression in the training sets. The testing sets produced an average accuracy of 74.3%, 80.0%, and 78.1% (Table II). The use of QNG and DNA ploidy alone as input variables (model 2) had a lower sensitivity and higher specificity than the use of pathologic results and patient age (model 1). The training and testing sets were analyzed concurrently by logistic regression and Cox proportional hazards modeling (Table III). Logistic regression analysis maximized performance in the training sets, and the GENN models maximized performance in the testing sets. For the testing set, Cox analysis yielded a sensitivity of only 39%, a specificity of 67%, and an accuracy of 53% (Table III).

Kaplan-Meier analysis, performed on the average outputs of model 3 for the entire patient sample, allowed stratification of tumors into four biochemical recurrence risk groups (Fig. 1). The log-rank test of equality was used to calculate the significance levels for the differences between the risk groups (P value between groups I and II = 0.092; between groups II and III <0.0001; and between groups III and IV = 0.0113).

COMMENT

Although PSA testing has revolutionized the early detection of prostate cancer, PSA levels alone have a limited ability to predict progression. Prediction is especially problematic in men with clinically organ-confined cancer who, at surgery, have tumors with a Gleason score of 5 to 7 and negative seminal vesicles and lymph nodes.[14]

We developed and tested ANNs and compared them with the results of logistic regression analysis in a selected cohort of men at intermediate risk of cancer progression and with a lengthy follow-up. Our findings suggest that GENNs are useful in progression prediction and may aid in clinical deci-

TABLE III. ***Results of logistic regression models on randomly selected training and testing sets balanced for number of patients with and without progression***

	Model 1 (Pathology + Age)	Model 2 (NMDs + DNA Ploidy)	Model 3 (All Variables Combined)
Average for random training sets (n = 136)			
Sensitivity (%)	83.6 ± 0.0	74.1 ± 3.1	85.6 ± 2.3
Specificity (%)	65.5 ± 2.5	74.3 ± 4.8	86.4 ± 3.5
Accuracy (%)	74.4 ± 1.2	74.2 ± 3.9	86.0 ± 2.7
AUC (%)	79.8 ± 1.7	83.0 ± 1.4	93.7 ± 1.2
Average for random testing sets (n = 35)			
Sensitivity (%)	68.6 ± 3.4	56.9 ± 12.2	56.9 ± 9.0
Specificity (%)	64.8 ± 6.4	68.5 ± 6.4	59.3 ± 3.2
Accuracy (%)	66.7 ± 4.4	62.9 ± 7.6	58.1 ± 4.4
AUC (%)	68.0 ± 5.8	64.7 ± 7.3	64.7 ± 3.0

KEY: Abbreviations as in Table II.
Data presented as the average ± standard deviation.

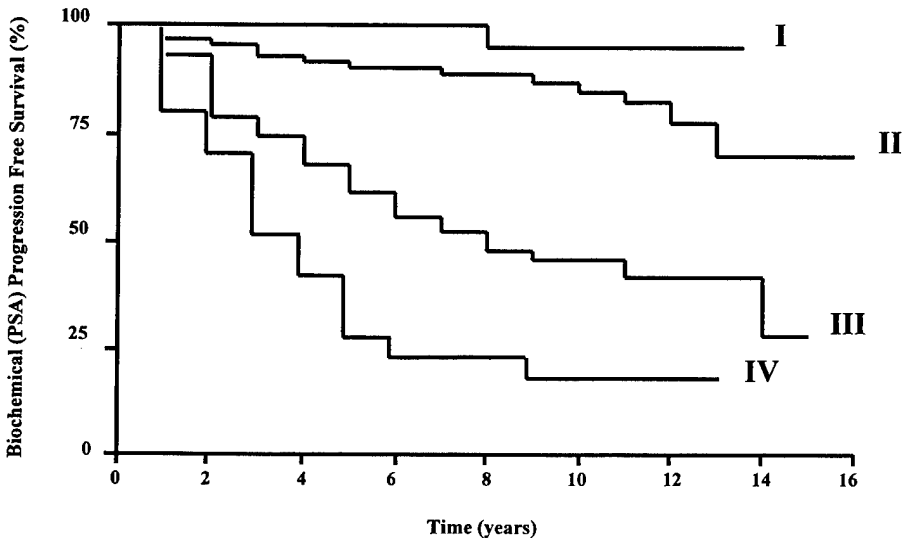

FIGURE 1. *Kaplan-Meier analysis of the average of the outputs for the entire patient sample (n = 214) using the trained model 3 GENN. The patients were separated into four distinct biochemical (PSA) progression risk groups. Group I, GENN less than 0.30 (n = 23;* P *= 0.0925); group II, GENN 0.30 or greater but less than 0.50 (n = 78;* P *<0.0001); group III, GENN 0.50 or greater but less than 0.70 (n = 92); and group IV, GENN 0.70 or greater (n = 21).* P *value between groups I and II = 0.092; between groups II and III <0.0001; and between groups III and IV = 0.0113.*

sion-making and the rational design of clinical trials. All GENN testing set models were superior to logistic regression modeling in predicting progression. Progression prediction using a Cox regression model was also inferior to ANN performance. Development of three different GENN models allowed comparison of different input variables.

The use of ANNs in predicting outcome after surgery shows promise, but some limitations are apparent. Currently, a pathologist and imaging technician are required to select cancer nuclei for QNG determination. The utility of QNG (models 2 and 3) was reduced by the limitations of the nuclear imaging system used. Analysis with current state-of-the-art systems is ongoing and will likely improve the contribution of QNG in these models.

Because of limitations on patient numbers necessitated by our desire for a lengthy follow-up and intermediate progression risk, we did not construct a separate set of previously unstudied patients to serve as a validation cohort. This does not invalidate the comparison of GENN and logistic regression analysis results. Because the testing set patients were not used to adjust the input weights

in our networks, the testing set results are useful in assessing these networks as tools for predicting progression. The collection of a validation patient cohort is underway.

The absence of PSA values as input variables, necessary because the length of follow-up achieved meant that most men had undergone RRP before the PSA era, was potentially limiting. However, new input variables, such as PSA or other serologic, immunohistochemical, or molecular markers, can be incorporated into GENNs with relative ease and are likely to increase the predictive value. Few of these men had Stage T1c lesions, and the development of predictive models using a more representative percentage of nonpalpable cancers is ongoing.

CONCLUSIONS

The application of ANNs in progression prediction shows promise in men at intermediate risk of progression in whom prediction has historically been most inaccurate. GENN creation is a logical step in the development of progression modeling. Networks were developed with high sensitivity and specificity for the prediction of prostate cancer progression in a group of men with long-term prospective follow-up after RRP. Advances in nuclear imaging systems and input variable selection promise further improvements. The development of these improved models in larger, well-characterized patient groups with long-term follow-up is ongoing. Further development of GENNs will provide improved prognostication after radical prostatectomy, allowing early and appropriate evaluation of investigational adjuvant therapies.

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