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Clinical Utility of Squamous and Transitional Nuclear Structure Alterations Induced by *Schistosoma haematobium* in Chronically Infected Adults with Bladder Damage Verified by Ultrasound in Ghana

Jean Naples, M.D., Ph.D., Sumit Isharwal, M.D., Clive J. Shiff, Ph.D., Kwabena M. Bosompem, Ph.D., and Robert W. Veltri, Ph.D.

OBJECTIVE: To evaluate the clinical utility of quantitative nuclear morphometry—i.e., alteration in nuclear size/shape, DNA content and chromatin structure—of intact cells obtained from the sediment of urine specimens collected from people living in an area highly endemic for *Schistosoma haematobium* in Ghana.

STUDY DESIGN: Digital images of Feulgen-DNA-stained squamous cell (SC) and transitional cell (TC) urothelial nuclei were captured using the AutoCyte imaging system, and nuclear morphometric descriptors (NMDs) were calculated. A total of 3,495 and 4,523 SC and TC nuclei from normal bladder ultrasound subjects ($n = 21$) and 3,465 and 3,064 SC and TC nuclei from severely abnormal bladder ultrasound subjects ($n = 20$) were captured.

RESULTS: Univariate logistic regression analyses of pooled SC and TC nuclei training sets showed that 27/40 NMDs and 24/40 NMDs were univariately significant for differentiating between SCs and TCs of subjects with normal and severely abnormal bladder ultrasound. Multivariate models constructed using NMDs with $\geq 50\%$ inclusion frequency yielded AUC-ROCs of 75.23% and 74.42% in the SC training and validation, and 69.90% and 66.70% for TC training and validation. Further, a squamous cell patient-specific model predicted severe bladder damage with an AUC-ROC of 86.90%, yielding the sensitivity, specificity and accuracy of 85.00%, 76.19% and 80.49%, respectively.

CONCLUSION: Quantitative nuclear structure alterations can be used to make a noninvasive assessment of

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cytologic changes observed in both SC and TC bladder epithelia due to *S haematobium* infection. (Anal Quant Cytol Histol 2009;31:143–152)

Keywords: bladder diseases, quantitative nuclear morphometry, *Schistosoma haematobium*, squamous cells, transitional cells.

As information that associates infectious disease agents (i.e., bacteria, viruses and some parasites) with the genesis of cancer collects,¹ society is realizing that these serious sequelae are preventable and that such prevention is dependent on early and accurate diagnosis. In the case of bladder cancer associated with urinary schistosomiasis the cells involved are almost always squamous cells (SCs). Definitive diagnosis is based on invasive cystoscopy, which is highly impracticable in the field situation for examining rural populations; thus, it is important to establish an alternative diagnostic procedure based on urine examination. In this study we utilized portable ultrasound (US) examination of the bladder as an indicator of severe, parasite-associated bladder damage and measured quantitative nuclear structure alteration characteristics of both SC and transitional cells (TCs) found in the urinary sediment.

The urinary parasite *Schistosoma haematobium* is associated with squamous cell carcinoma (SCC) of the bladder, and a causal relationship has been established conclusively in numerous studies, mainly from Egypt and other parts of Africa.^{1–5} The adult forms of this trematode parasite live in the vesicular veins. The fecund females move into the capillary plexus below the epithelium, where they deposit robust terminal spined eggs. These eggs, which are about 170 μm long, emit a highly immunogenic substance, the soluble egg antigen, which initiates inflammatory processes that enable the eggs to penetrate the epithelium and gain access to the urine, and they are passed to the exterior during micturition.^{4,6} If passed into fresh water, the eggs hatch to yield a free swimming miracidium that is able to infect aquatic snails of a particular species. This infection undergoes asexual multiplication and results in large numbers of an infective larval form, the cercariae, which finally emerge from the snail. These are also free swimming and will locate and penetrate the skin of any human who contacts the water where live cercariae are swimming. After penetration of the skin, cercariae metamorphose into juveniles and later into adults, which migrate to the

vesicular veins to complete the cycle. The parasite is long lived, most untreated infections will last for 8–10 years, but superinfection and reinfection can and does occur in endemic areas due to frequent contact with infested water through daily chores, play and agricultural labor. Urinary schistosomiasis is found in Africa and Western Asia, where it is estimated to infect some 100 million people.⁴ While the etiologic association with bladder cancer is known, there are no data available to determine the prevalence of associated bladder cancer in endemic communities. Unpublished hospital-based studies in urban Ghana have yielded evidence of cancer (both transitional cell carcinoma [TCC] and SCC), but formal epidemiologic studies using invasive or direct means of diagnosis are mostly absent for rural communities, and we are therefore unable to establish the public health impact of the infection.

Recently, the advent of the portable US apparatus has enabled noninvasive epidemiologic field studies to be undertaken to assess the prevalence of bladder damage in adults exposed to the infection. Several such studies have been carried out in different parts of Africa. Hodder et al,⁷ Brouwer et al⁸ and Shiff et al⁹ suggested that the effects of the chronic infection are far from benign. Now that the potential hazard has been demonstrated, the main problem facing investigators is to determine the extent to which severe internal damage to the bladder epithelium caused by the presence of the parasite and its eggs in the tissue will eventually become hyperplastic or neoplastic. Here, we report on the potential clinical utility of quantitative nuclear morphometry (QNM) determined from nuclear morphometric descriptors (NMDs) (i.e., measurements of alterations in nuclear size/shape, DNA content and chromatin structure) of intact cells obtained from the sediment of urine specimens collected from people living in a rural area highly endemic for *S haematobium* in Ghana.

Materials and Methods

Study Population

The goals of the study were to examine the effects of chronic urinary schistosomiasis, so the study population was defined as apparently healthy adults over the age of 18–19 years. The study was done in 3 villages in a rural area of southern Ghana near the capital, Accra. Village elders and the public at large were informed of the study and its consequences by meetings and discussion.¹⁰ On preannounced days, volunteers were recruited from the street, they com-

pleted an approved questionnaire, urine samples were provided and a US examination performed in the village clinic using a portable instrument. All examinations were carried out by one of us (J.N.); from the 3 villages a total of 378 individuals were examined.

Ethical Considerations

The study was approved by the institutional review board of the Noguchi Memorial Institute of the University of Ghana and by the institutional review board of the Johns Hopkins Bloomberg School of Public Health.

Parasitology

Urine specimens were prepared for parasitologic examination by centrifugation and filtration, followed by microscopic examination of the sediment for the presence of eggs. Part of the sediment was applied to charged microscope slides for fixation and preparation for Feulgen staining (see below). Serology to detect antischistosome antibodies was performed on dried blood specimens by enzyme-linked immunoassay.

US and Pathology

Examinations were done blind to the subject's infection status using an Aloka-SSD-500 portable US apparatus (Aloka, Tokyo, Japan) with a 3.5-MHz curvilinear probe. Classifications were made as per the World Health Association protocol (WHO 1991, 2000) approximately 1 hour after ingestion of a large volume of water to distend the bladder. The following abnormalities were noted: abnormal bladder shape, bladder wall irregularities or hyperplasia, discernable masses, presence of polyps, calcification and presence of hydronephrosis. Lesions were considered severely abnormal when any 4 of the above conditions were present or 3 with hydronephrosis. Lesions were considered negative when no specific lesions were observed.

Digital Measurement of Nuclear Morphometric Alterations

Feulgen DNA staining was performed on the preserved slide preparations per the manufacturer's instructions (TriPath Imaging Inc., Burlington, North Carolina, U.S.A.). Feulgen quantitatively stains DNA in cellular material by uncovering the free aldehyde groups in the DNA during the acid hydrolysis process, which then reacts with the Feulgen reagent to form a stable, colored compound

(blue) that absorbs light at 560 nm.^{11,12} Next, a minimum of ~125 intact, Feulgen-stained SC and TC nuclei from a subject's cytology slide were captured using an AutoCyte Pathology Workstation (TriPath Imaging) and the QUIC-DNA software (TriPath Imaging).¹³⁻¹⁷ A total of 3,495 and 4,523 SC and TC nuclei were captured from the normal bladder US subjects' (n = 21) cytology slides. Also, 3,465 and 3,064 SC and TC nuclei were captured from severely abnormal bladder US subjects' (n = 20) cytology slides. The AutoCyte QUIC-DNA software calculated 40 different NMDs for each nucleus captured,¹⁶ including nuclear size, shape, DNA content and chromatin texture features (at a step size of 1 pixel).

Statistical Analysis

All data were analyzed using Stata 10.0 statistical analysis software (Stata Corporation, College Station, Texas, U.S.A.). Cellular data were then randomly divided into 2 halves; 1 training set was used for model construction, and another validation set was used to validate the model. Univariate logistic regression analysis was performed, first on the training set to determine which independent variables were significant in the differentiation of cells of normal and abnormal US subjects. Next, using the NMDs as input variables that were significant by univariate analysis, 1,000 bootstrap samples of the training set were used to create multivariate logistic regression models applying backward stepwise selection at variable selection stringency of $p \leq 0.05$. The number of times each NMD variable was selected using the 1,000 bootstrap samples was determined, and only those variables that were selected in >50% of the bootstrap models were used for creating a final QNM multivariate model.¹⁸ Areas under the receiver operator characteristic (AUC-ROC) curves of the training and validation sets for the ability of such models to differentiate between urine cells of normal and severely abnormal bladder US subjects were calculated.

Results

Univariate logistic regression analyses of one-half of the pooled urine cytology SCs—i.e., SC training set—showed that perimeter, area, circular form factor, feret y, minimum feret, maximum feret, excess of gray values, skewness of gray values, standard deviation (SD) of gray values, maximum gray values, minimum gray values, intensity, minimum optical density (OD), maximum OD, median OD, SD of OD, skewness of OD, DNA ploidy, transmission,

Table I Inclusion Frequencies of Univariately Significant NMDs in Bootstrap Resampling with 1,000 Iterations of Squamous Cell Training Set

Morphometric measurement	Measurement type	Inclusion frequencies (%)
Perimeter	Size/shape	37.00
Area	Size/shape	100.00
Circular form factor	Size/shape	57.50
Feret y	Size/shape	82.20
Minimum feret	Size/shape	77.70
Maximum feret	Size/shape	20.60
Excess of gray values	DNA content	100.00
Skewness of gray values	DNA content	24.60
SD of gray values	DNA content	68.00
Maximum gray value	DNA content	42.30
Minimum gray value	DNA content	28.20
Intensity	DNA content	100.00
Minimum OD	DNA content	55.10
Maximum OD	DNA content	20.30
Median OD	DNA content	36.90
SD of OD	DNA content	74.10
Skewness of OD	DNA content	64.90
DNA ploidy	DNA content	99.80
Transmission	Texture	71.60
Sum variance-AC	Texture	33.70
Cluster shade	Texture	65.60
Diagonal moment-AC	Texture	17.00
Sum of homogeneity	Texture	18.40
Correlation	Markovian	44.50
Difference moment	Markovian	24.80
Inverse difference moment	Markovian	55.80
Information measure A	Markovian	50.20

sum variance-AC, cluster shade, diagonal moment-AC, sum of homogeneity, correlation, difference moment, inverse difference moment and information measure A were univariately significant NMDs for differentiating between SCs of normal and severely abnormal bladder US subjects.

Next, backward stepwise logistic regression analyses and a bootstrap resampling procedure em-

ploying 1,000 replications was performed on the SC training set. The goal was to identify the most important NMDs to compute a QNM for differentiating urine cytology of SCs from that of volunteers with normal vs. severely abnormal bladder US imaging results. Each variable in the replicated models was counted with a significance level of $p \leq 0.01$ for an NMD that entered the QNM model and $p \leq 0.05$ (variable selection cut-off) to remain in the final model. Table I lists the inclusion frequencies for all the univariately significant NMDs. Area, excess of gray value, intensity and DNA ploidy features were the most important to differentiate SCs of normal and severely abnormal bladder US subjects. Using selection criteria of at least 50% inclusion frequency, a QNM model was constructed for the SC training (Tables II and V), which yielded an AUC-ROC of 75.23%, and the same model in the SC validation set resulted in an AUC-ROC of 74.42% (Figure 1). Note that Figure 1A is the predictive probability results for the training and validation sets, while Figure 1B and C are the AUC-ROC curves for the SC training and validation sets.

Next, we analyzed the TC training set using univariate logistic regression and circular form factor, excess of gray values, skewness of gray values, SD of gray values, maximum gray values, minimum gray values, minimum OD, maximum OD, median OD, SD of OD, skewness of OD, DNA ploidy, transmission, sum average-AC, sum variance-AC, cluster shade, diagonal moment-AC, sum of homogeneity, correlation, difference moment, inverse difference moment, information measure A, maximal correlation coefficient and diagonal moment-M, which were significant NMDs for differentiating between the transitional cell of normal and severely abnormal bladder US subjects.

Following this, we applied a bootstrap resam-

Table II Squamous Cell Model

Morphometric measurement	Measurement type	β Coefficients	p Value
Area	Size/shape	-0.3375406	0.000
Feret y	Size/shape	-0.1337726	0.001
Minimum feret	Size/shape	0.394082	0.000
Excess of gray values	DNA content	-0.7292214	0.000
Intensity	DNA content	0.0001239	0.000
SD of OD	DNA content	7.689375	0.000
Skewness of OD	DNA content	0.3183693	0.000
DNA ploidy	DNA content	1.261996	0.000
Transmission	Texture	-8.741496	0.000
Cluster shade	Texture	-0.0072644	0.001
Model constant		8.334216	0.000

Table III Inclusion Frequencies of Univariately Significant NMDs in Bootstrap Resampling with 1,000 Iterations of Transitional Cell Training Set

Morphometric measurement	Measurement type	Inclusion frequencies (%)
Circular form factor	Size/shape	21.20
Excess of gray values	DNA content	37.10
Skewness of gray values	DNA content	75.20
SD of gray values	DNA content	45.10
Maximum gray value	DNA content	92.10
Minimum gray value	DNA content	45.90
Minimum OD	DNA content	33.30
Maximum OD	DNA content	41.60
Median OD	DNA content	28.80
SD of OD	DNA content	79.20
Skewness of OD	DNA content	40.10
DNA ploidy	DNA content	100.00
Transmission	Texture	78.50
Sum average-AC	Texture	44.60
Sum variance-AC	Texture	55.00
Cluster shade	Texture	24.50
Diagonal moment-AC	Texture	19.70
Sum of homogeneity	Texture	75.40
Correlation	Markovian	69.70
Difference moment	Markovian	54.80
Inverse difference moment	Markovian	17.00
Information measure A	Markovian	15.20
Maximal correlation coefficient	Markovian	89.40
Diagonal moment-M	Markovian	97.50

pling procedure employing 1,000 replications to perform backward stepwise logistic regression analyses on the TC training set. The goal was to identify the most important NMDs for differentiating TCs of normal and severely abnormal bladder US subjects. Each variable in the replicated models was counted with a significance level of $p \leq 0.01$ for an NMD that entered the model and $p \leq 0.05$ (variable selection cut-off) to remain in the model. Table III lists the inclusion frequencies for all the univari-

ately significant NMDs. DNA ploidy was the most important feature to differentiate TCs of normal and severely abnormal bladder US subjects. Using selection criteria of at least 50% inclusion frequency, a QNM model was constructed for the TC training set (Tables IV and VI), which yielded an AUC-ROC of 69.90%, and the same model in the TC validation set resulted an AUC-ROC of 66.40% (Figure 2). Note that Figure 2A is the predictive probability results for the training and validation sets, while Figure 2B and C are the AUC-ROC curves for the TC training and validation sets.

Further, we utilized the variance of NMDs, 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. Hence, 2 datasets were constructed, one using squamous nuclei and another using transitional nuclei. In squamous cell type patients' dataset, only 2 NMDs, skewness of gray values ($p = 0.007$, AUC-ROC: 81.67%) and maximum OD ($p = 0.024$, AUC-ROC: 70.24%) were univariately significant for predicting normal bladder vs. bladder damage caused by *Schistosoma* infection. A model was constructed using these 2 univariately significant NMDs that resulted an AUC-ROC of 86.90%. At a cutoff of 0.40 for the predictive probabilities, the sensitivity, specificity and accuracy of the model were 85.00%, 76.19% and 80.49%, respectively (Figure 3). In transitional cell type patients' dataset, no NMD was univariately significant at stringency of ≤ 0.05 , although skewness of gray values ($p = 0.069$), SD of gray values ($p = 0.075$) and SD of OD ($p = 0.062$) showed a trend toward statistical significance.

Discussion

Urinary schistosomiasis caused by *S haematobium* among adults primarily involved in farming and

Table IV Transitional Cell Model

Morphometric measurement	Measurement type	β Coefficients	p Value
Skewness of gray values	DNA content	0.9512663	0.000
Maximum gray values	DNA content	-0.0251895	0.000
SD of OD	DNA content	47.16716	0.000
DNA ploidy	DNA content	0.4946653	0.000
Transmission	Texture	12.06099	0.000
Sum of homogeneity	Texture	-10.22292	0.000
Correlation	Markovian	-1.336041	0.005
Maximum correlation coefficient	Markovian	0.5633883	0.000
Diagonal moment-M	Markovian	-0.8014619	0.000
Model constant		14.62134	0.000

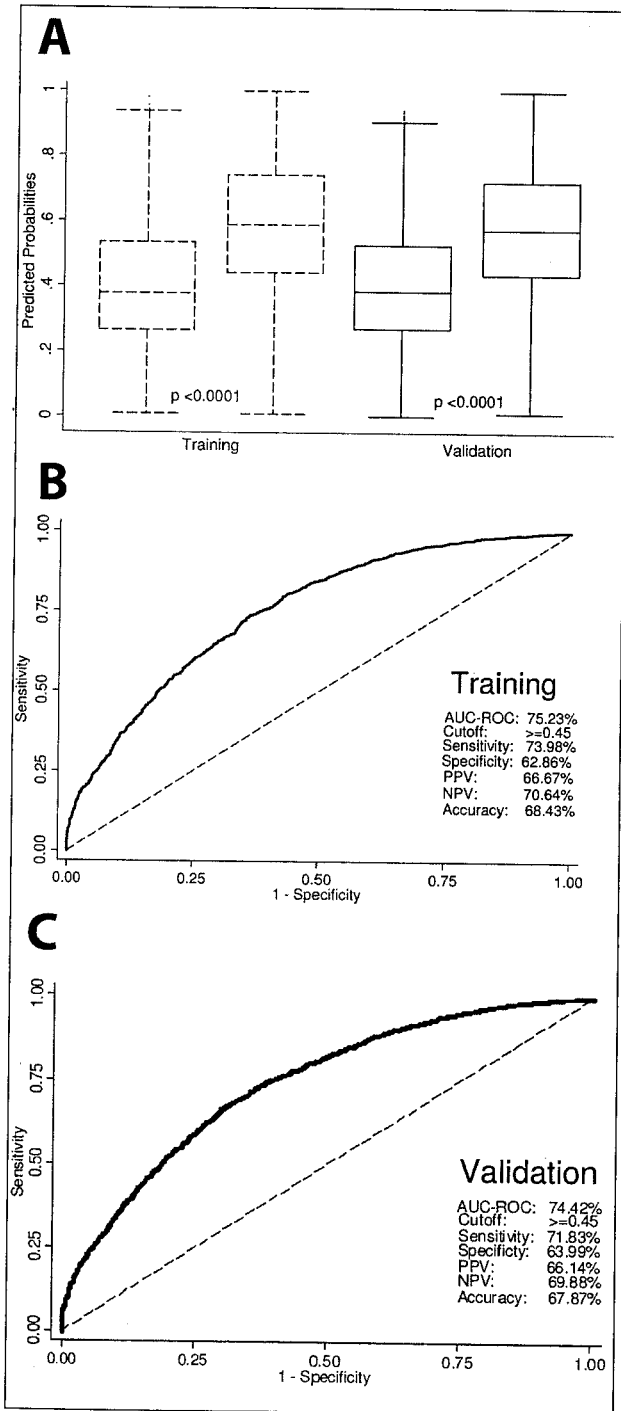


Figure 1 (A) Predictive probability results for the squamous cell training and validation sets. (B and C) Areas under the AUC-ROC curves for the squamous cell training and validation sets.

fishing occupations is seen as a chronic infection in endemic areas of Africa and frequently persists

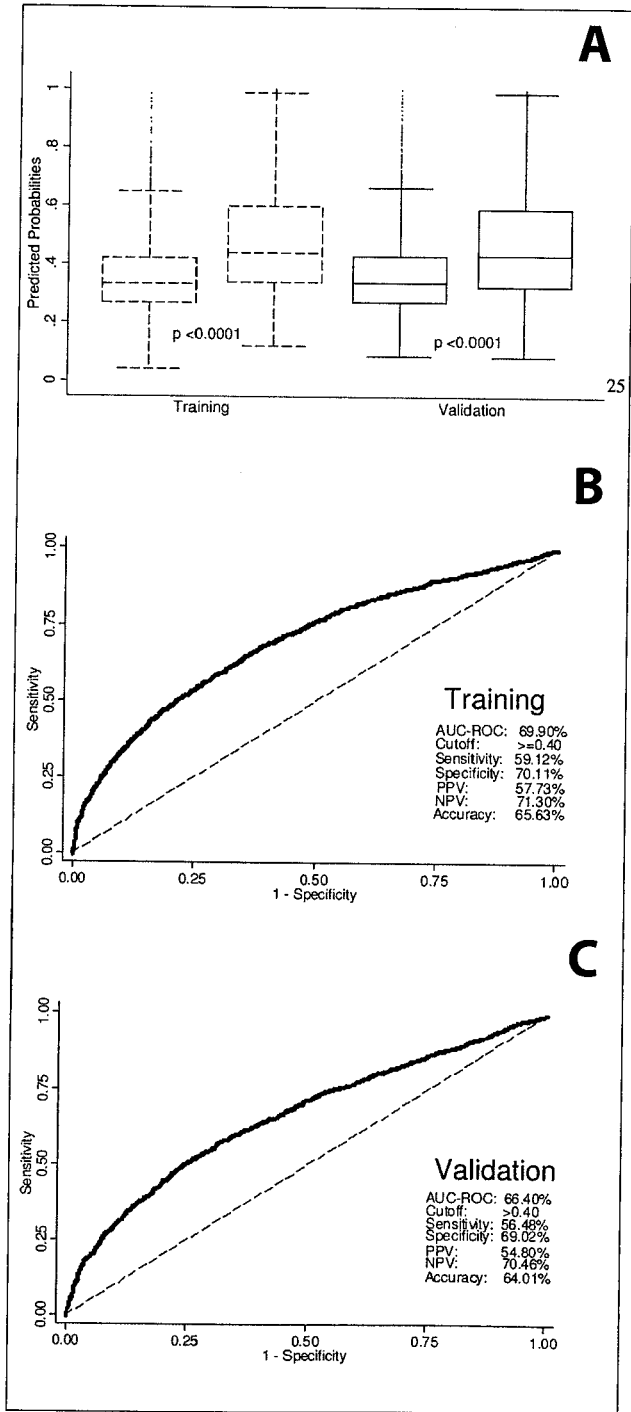


Figure 2 (A) Predictive probability results for the transitional cell training and validation sets. (B and C) AUC-ROC curves for the transitional cell training and validation sets.

through life, although it seems less prevalent as people age. Although it is considered a mildly de-

Table V Descriptive Characteristics (Mean \pm SD) of Univariately Significant NMDs Used to Construct Squamous Cell Training Model

NMDs	Squamous cell training set		p Value
	Normal ultrasound (N=1,715)	Abnormal ultrasound (N=1,722)	
Area	36.26 \pm 15.36	33.49 \pm 13.88	< 0.0001
Feret y	7.24 \pm 1.67	6.86 \pm 1.56	< 0.0001
Minimum feret	6.12 \pm 1.34	5.91 \pm 1.22	0.0001
Excess of gray value	0.018 \pm 0.930	-0.385 \pm 0.702	< 0.0001
Intensity	81,005.31 \pm 41,046.12	70,421.55 \pm 39,677.98	< 0.0001
SD of OD	0.079 \pm 0.033	0.123 \pm 0.090	< 0.0001
Skewness of OD	0.381 \pm 0.708	0.434 \pm 0.689	0.0157
DNA ploidy	1.87 \pm 0.621	2.07 \pm 0.45	< 0.0001
Transmission	0.648 \pm 0.102	0.586 \pm 0.109	< 0.0001
Cluster shade	747.16 \pm 20.24	750.39 \pm 28.89	0.0003

bilitating disease by some authorities and relegated to a low contributor to the disability-adjusted life year by the World Bank,¹⁰ extensive research, mainly in Egypt, has demonstrated its etiologic role in the onset of SCC of the bladder,¹⁹ and workers there have shown that in the past, bladder cancer was the most prevalent form of human cancer, with SCC occurring in >70% of these cases.²⁰ In Egypt there is an excellent rural health system that is able to detect and refer these cases. However, this is not the case in most of the African countries where urinary schistosomiasis is endemic, so there are few data currently available to assess the overall prevalence of severe bladder disease associated with the infection.

There is a dire need to improve and simplify the detection of bladder cancer among rural populations in the areas where *S haematobium* is endemic.²¹ Invasive cystoscopy is not routinely feasible in rural clinics in most of Africa, so it would help assess the

extent of the public health problem if improved diagnostics become available.

Several investigators^{13-17,22-29} have evaluated the diagnostic and prognostic value of quantitative nuclear structure alterations in prostate, renal and bladder tumors. In the current study, we assessed the potential clinical utility of QNM that measure nuclear features of size/shape, DNA content, chromatin texture and Markovian features of both SCs and TCs collected from bladder urine sediment from subjects with severe bladder damage or with normal bladders. All the volunteers were from 1 of 3 villages along the Densu River in southern Ghana. Several NMDs of nuclear size/shape, DNA content, chromatin texture and Markovian features were significant to differentiate SCs of subjects with severe bladder damage and normal bladders (Table I). Area, excess of gray value, intensity and DNA ploidy were the most important NMDs to differentiate SCs of subjects with severe bladder damage

Table VI Descriptive Characteristics (Mean \pm SD) of Univariately Significant NMDs Used to Construct Transitional Cell Training Model

NMDs	Transitional cell training set		p Value
	Normal ultrasound (N = 2,215)	Abnormal ultrasound (N = 1,529)	
Skewness of gray values	-0.311 \pm 0.549	-0.194 \pm 0.520	< 0.0001
Maximum gray values	243.13 \pm 12.58	244.45 \pm 12.83	< 0.0001
SD of OD	0.054 \pm 0.022	0.070 \pm 0.048	< 0.0001
DNA ploidy	1.99 \pm 0.48	2.15 \pm 0.56	< 0.0001
Transmission	0.740 \pm 0.087	0.709 \pm 0.108	< 0.0001
Sum of homogeneity	0.497 \pm 0.041	0.493 \pm 0.044	0.0001
Correlation	1.032 \pm 0.175	1.00 \pm 0.200	< 0.0001
Maximal correlation coefficient	0.582 \pm 0.348	0.558 \pm 0.360	0.092
Diagonal moment-M	18.89 \pm 0.86	18.83 \pm 0.85	0.029

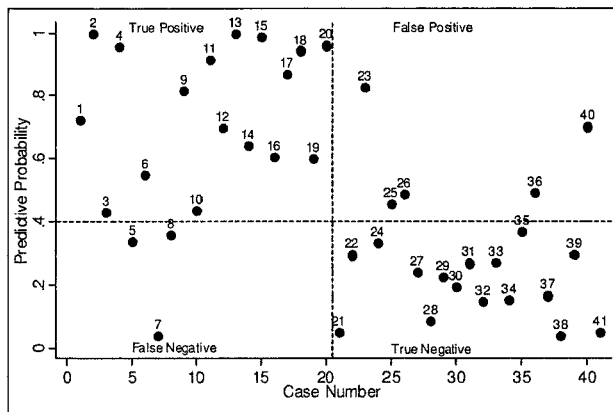


Figure 3 Predictive probability results for subjects with normal bladder vs. severely abnormal bladder caused by *Schistosoma* infection verified by ultrasound examination.

and normal bladders (Table I). However, to differentiate TCs of subjects, mainly nuclear DNA content, chromatin texture and Markovian features were significant (Table II). Further, only DNA ploidy was the most important NMD to differentiate TCs of subjects with severe bladder damage and normal bladder architecture (Table II). Multivariate QNM models constructed using NMDs with $\geq 50\%$ inclusion frequency yielded AUC-ROCs of 75.2% and 74.4% in the SC training and validation sets, and 69.9% and 66.7% for TC training and validation sets (Figures 1 and 2). Applying variance of squamous nuclei NMDs determined using the nuclei captured for each case, skewness of gray values ($p = 0.007$, AUC-ROC: 81.67%) and maximum OD ($p = 0.024$, AUC-ROC: 70.24%) were significant for predicting normal bladder vs. bladder damage in subjects caused by *Schistosoma* infection. A model constructed using these 2 univariately significant NMDs yielded an AUC-ROC of 86.90%, and at a cutoff of 0.40 for the predictive probabilities, the sensitivity, specificity and accuracy were 85.00%, 76.19% and 80.49%, respectively (Figure 3). The limitation of the study is small sample size for making patient-specific prediction for normal bladder vs. bladder damage caused by *Schistosoma* infection, and we are currently working to expand our study cohort to make accurate patient-specific prediction and to validate our results.

At the molecular level, studies have shown that elevated oxidative stress markers, DNA damage and DNA repair levels in *Schistosoma*-associated bladder cancer (SA-BC) occur more often as com-

pared to non-*Schistosoma* bladder cancer (NSA-BC).³⁰ Alterations in p16, p15 and p21WAF1/CIP1 expression levels also occur more frequently in SA-BC.^{31,32} Gutierrez et al³³ showed that SA-BC has more genes methylated than NSA-BC. Also, the extent of methylation in *S haematobium*-associated TCC (SA-TCC) was lower than in *S haematobium*-associated SCC (SA-SCC).³³ Further, the increased DNA copy number change (specifically gain and high-level amplification at 11q13 in SA-SCC and SA-TCC, and high-level amplification at 5p only in SA-SCC) is more often observed in SA-BC than in NSA-BC, which may be explained by the chromosomal instability mediated by either reactive oxygen species or urinary nitrosamines as a result of chronic inflammation and irritation in the urinary bladder by *S haematobium* infection.^{34,35} Additionally, *Schistosoma* infection has been reported to be directly involved in increased chromosomal breakage in the urothelial cells at the micronuclear level.^{36,37}

Clearly, both groups of cells (SC and TC) are affected by the presence of *S haematobium*, and they have different patterns of significant NMDs that predict abnormal US bladder changes. Notably, the effect of this infection results in significant changes in several nuclear size and shape features in the SCs rather than in TCs. The implication is that *S haematobium* infection and resultant inflammation has a predilection for SCs, causing squamous hyperplasia and perhaps ultimately carcinoma of the bladder. Since other investigators²³⁻²⁹ studying malignant changes in bladder epithelia have also shown the diagnostic and prognostic value of nuclear morphometry, understanding the molecular basis for such nuclear structure alterations is of utmost biologic and clinical importance. Importantly, one of the known transcriptional coactivators, p300 (histone acetyltransferase), has been shown to directly modulate nuclear size/shape in prostate cancer^{38,39} and should be studied in bladder cancer.

In conclusion, image analysis-based QNM can be added to the repertoire available to the cytopathologist in attempting to make a noninvasive assessment of changes observed in both SC and TC bladder epithelia due to *S haematobium* infection.

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References

- Parkin DM: The global health burden of infection-associated cancers in the year 2002. *Int J Cancer* 2006;118:3030-3044
- Jemal A, Siegel R, Ward E, Hao Y, Xu J, Murray T, Thun MJ: Cancer statistics, 2008. *CA Cancer J Clin* 2008;58:71-96
- Heyns CF, van der Merwe A: Bladder cancer in Africa. *Can J Urol* 2008;15:3899-3908
- Gryseels B, Polman K, Clerinx J, Kestens L: Human schistosomiasis. *Lancet* 2006;368:1106-1118
- Badawi AF, Mostafa MH, Probert A, O'Connor PJ: Role of schistosomiasis in human bladder cancer: Evidence of association, aetiological factors, and basic mechanisms of carcinogenesis. *Eur J Cancer Prev* 1995;4:45-59
- Yosry A: Schistosomiasis and neoplasia. *Contrib Microbiol* 2006;13:81-100
- Hodder SL, Mahmoud AA, Sorenson K, Weinert DM, Stein RL, Ouma JH, Koech D, King CH: Predisposition to urinary tract epithelial metaplasia in *Schistosoma haematobium* infection. *Am J Trop Med Hyg* 2000;63:133-138
- Brouwer KC, Ndhlovu PD, Wagatsuma Y, Munatsi A, Shiff CJ: Epidemiological assessment of *Schistosoma haematobium*-induced kidney and bladder pathology in rural Zimbabwe. *Acta Trop* 2003;85:339-347
- Shiff C, Veltri R, Naples J, Quartey J, Othare J, Anyan W, Marlow C, Wiredu E, Adjei A, Brakohiapa E, Bosompem K: Ultrasound verification of bladder damage is associated with known biomarkers of bladder cancer in adults chronically infected with *Schistosoma haematobium* in Ghana. *Trans R Soc Trop Med Hyg* 2006;100:847-854
- King CH, Dickman K, Tisch DJ: Reassessment of the cost of chronic helminth infection: A meta-analysis of disability-related outcomes in endemic schistosomiasis. *Lancet* 2005;365:1561-1569
- Gill JE, Jotz MM: Further observations on the chemistry of pararosaniline-Feulgen staining. *Histochemistry* 1976;46:147-160
- Schulte E, Wittekind D: Standardization of the Feulgen-Schiff technique: Staining characteristics of pure fuchsin dyes: A cytophotometric investigation. *Histochemistry* 1989;91:321-331
- Veltri RW, Partin AW, Miller MC: Quantitative nuclear grade (QNG): A new image analysis-based biomarker of clinically relevant nuclear structure alterations. *J Cell Biochem (suppl)* 2000;35:151-157
- Veltri RW, Khan MA, Miller MC, Epstein JI, Mangold LA, Walsh PC, Partin AW: Ability to predict metastasis based on pathology findings and alterations in nuclear structure of normal-appearing and cancer peripheral zone epithelium in the prostate. *Clin Cancer Res* 2004;10:3465-3473
- Veltri RW, Partin AW, Miller CM: Quantitative Nuclear Grade (QNG): The clinical applications of the quantitative measurement of nuclear structure using image analysis. *In* Cancer Chemoprevention. Edited by GJ Kelloff, ET Hawk, CC Sigman. Totowa, New Jersey, Humana Press, 2005, pp 97-108
- Veltri RW, Marlow C, Khan MA, Miller MC, Epstein JI, Partin AW: Significant variations in nuclear structure occur between and within Gleason grading patterns 3, 4, and 5 determined by digital image analysis. *Prostate* 2007;67:1202-1210
- Veltri RW, Miller MC, Isharwal S, Marlow C, Makarov DV, Partin AW: Prediction of prostate-specific antigen recurrence in men with long-term follow-up postprostatectomy using quantitative nuclear morphometry. *Cancer Epidemiol Biomarkers Prev* 2008;17:102-110
- Sauerbrei W: The use of resampling methods to simplify regression models in medical statistics. *Applied Stat* 1999;48:313-329
- Mostafa MH, Sheweita SA, O'Connor PJ: Relationship between schistosomiasis and bladder cancer. *Clin Microbiol Rev* 1999;12:97-111
- Lamm DL, Torti FM: Bladder cancer, 1996. *CA Cancer J Clin* 1996;46:93-112
- Farrow GM: Urine cytology in the detection of bladder cancer: A critical approach. *J Occup Med* 1990;32:817-821
- Carducci MA, Piantadosi S, Pound CR, Epstein JI, Simons JW, Marshall FF, Partin AW: Nuclear morphometry adds significant prognostic information to stage and grade for renal cell carcinoma. *Urology* 1999;53:44-49
- Kapur U, Antic T, Venkataraman G, Durazo-Arvizu R, Quek MM, Flanigan RC, Wojcik EM: Validation of World Health Organization/International Society of Urologic Pathology 2004 classification schema for bladder urothelial carcinomas using quantitative nuclear morphometry: Identification of predictive features using bootstrap method. *Urology* 2007;70:1028-1033
- Ramos D, Ruiz A, Morell L, Navarro S, Villamon R, Gil-Salom M, Llombart-Bosch A: Prognostic value of morphometry in low grade papillary urothelial bladder neoplasms. *Analyt Quant Cytol Histol* 2004;26:285-294
- Bol MG, Baak JP, Rep S, Marx WL, Kruse AJ, Bos SD, Kisman O, Voorhorst FJ: Prognostic value of proliferative activity and nuclear morphometry for progression in TaT1 urothelial cell carcinomas of the urinary bladder. *Urology* 2002;60:1124-1130
- Lipponen PK, Collan Y, Eskelinen MJ, Pesonen E, Sotarauta M: Morphometry in human transitional cell bladder cancer: Nuclear area and standard deviation of nuclear area: Relation to tumor grade (WHO) and prognosis. *Eur Urol* 1990;17:155-160
- Fukuzawa S, Hashimura T, Sasaki M, Yamabe H, Yoshida O: Nuclear morphometry for improved prediction of the prognosis of human bladder carcinoma. *Cancer* 1995;76:1790-1796
- van Velthoven R, Petein M, Oosterlinck W, De Wilde T, Matelaer J, Hardeman M, Kiss R, Decaestecker C: Identification by quantitative chromatin pattern analysis of patients at risk for recurrence of superficial transitional bladder carcinoma. *J Urol* 2000;164:2134-2137
- Wojcik EM, Miller MC, O'Dowd GJ, Veltri RW: Value of computer-assisted quantitative nuclear grading in differenti-

- ation of normal urothelial cells from low and high grade transitional cell carcinoma. *Anal Quant Cytol Histol* 1998;20:69-76
30. Salim EI, Morimura K, Menesi A, El-Lity M, Fukushima S, Wanibuchi H: Elevated oxidative stress and DNA damage and repair levels in urinary bladder carcinomas associated with schistosomiasis. *Int J Cancer* 2008;123:601-608
 31. Eissa S, Ali-Labib R, Khalifa A: Deletion of p16 and p15 genes in schistosomiasis-associated bladder cancer (SABC). *Clin Chim Acta* 2000;300:159-169
 32. Eissa S, Swelam M, Shaker Y, Abdel-Fattah M, Khalifa A: Expression of p21WAF1/CIP1 in bladder cancer: Relation to schistosomiasis. *IUBMB Life* 1999;48:115-119
 33. Gutierrez MI, Siraj AK, Khaled H, Koon N, El-Rifai W, Bhatia K: CpG island methylation in Schistosoma- and non-Schistosoma-associated bladder cancer. *Mod Pathol* 2004;17:1268-1274
 34. El-Rifai W, Kamel D, Larramendy ML, Shoman S, Gad Y, Baithun S, El-Awady M, Eissa S, Khaled H, Soloneski S, Sheaff M, Knuutila S: DNA copy number changes in Schistosoma-associated and non-Schistosoma-associated bladder cancer. *Am J Pathol* 2000;156:871-878
 35. Rosin MP, Anwar WA, Ward AJ: Inflammation, chromosomal instability, and cancer: The schistosomiasis model. *Cancer Res* 1994;54:1929s-1933s
 36. Rosin MP, Saad el Din Zaki S, Ward AJ, Anwar WA: Involvement of inflammatory reactions and elevated cell proliferation in the development of bladder cancer in schistosomiasis patients. *Mutat Res* 1994;305:283-292
 37. Rosin MP, Anwar W: Chromosomal damage in urothelial cells from Egyptians with chronic Schistosoma haematobium infections. *Int J Cancer* 1992;50:539-543
 38. Debes JD, Sebo TJ, Heemers HV, Kipp BR, Haugen DL, Lohse CM, Tindall DJ: p300 Modulates nuclear morphology in prostate cancer. *Cancer Res* 2005;65:708-712
 39. Isharwal S, Miller C, Marlow C, Makarov DV, Partin AW, Veltri RW: p300 (Histone Acetyltransferase) biomarker predicts prostate cancer biochemical recurrence and correlates with changes in epithelia nuclear size and shape. *Prostate* 2008;68:1097-1104