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ORIGINAL ARTICLE

A Model Based on Automated Urinalysis Parameters for Urothelial Carcinoma Risk Stratification in Suspected Patients

Chunyun Ren [1, 2], Wenjian Qian [3]

[1] *Department of Laboratory Medicine, The First Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou City, China*
[2] *Key Laboratory of Clinical In Vitro Diagnostic Techniques of Zhejiang Province, Hangzhou City, China*
[3] *Department of Laboratory Medicine, Yi Wu Hospital of Traditional Chinese Medicine, Yi Wu City, China*

SUMMARY

Background: **The aim of this study was to develop and validate a risk stratification model for the screening of patients with suspected urothelial carcinoma (UC).**
Methods: **We enrolled 671 consecutive patients with suspected UC and generated a risk stratification model based on urinary parameters by using an automated urinalysis analyzer (Sysmex UN-9000). All patients received urine cytology examination from January 1, 2019, to October 31, 2022.**
Results: **Out of the 671 patients, 191 (28.5%) were ultimately diagnosed with UC. The four features associated with the presence of malignancy on multivariable analysis can be summarized by using the mnemonic UC-PAAS: UC, protein vs. creatinine ratio (P/C), age, atypical cells (Atyp.C), and small round epithelial cell (SRC). Major criteria include Atyp.C ≥ 0.1/μL (2 points) and age ≥ 65 years (2 points); minor criteria include SRC ≥ 2.7/μL (1 point) and abnormal P/C results (1 point). The model evidenced good discrimination (area under the curve = 0.802, 95% confidence interval [0.756, 0.848]) in the training group. A UC-PAAS cutoff of more than 4 points identified a high-risk population, of whom 37 of 59 (62.7%) had UC; the negative predictive value was 0.867. The validation group yielded similar findings.**
Conclusions: **We present a urinalysis-based screening model, the UC-PAAS, that may serve as an accessory clinical tool for the evaluation of patients with suspected UC, because the model identifies patients at higher risk who require closer follow-up than others or additional examinations.**
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Correspondence:
Chunyun Ren
Department of Laboratory Medicine
The First Affiliated Hospital
Zhejiang University School of Medicine
79 Qingchun Rd.
Hangzhou City, 310003
China
Phone/Fax: + 86 57187236383
Email: 1506110@zju.edu.cn

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KEYWORDS

automated urinalysis, Sysmex UN-9000, urothelial carcinoma, Atyp.C parameter

INTRODUCTION

Urinalysis is commonly used to detect and manage a wide range of disorders, including genitourinary tract and other diseases [1-3]. Bladder cancer, which is the principal urinary tract malignancy, is the 10th most common cancer worldwide, with nearly 91,900 new diagnoses and 43,000 deaths in China in 2022 [4,5]. Although various diagnosis and treatment methods have been applied in clinical research or practice, the 5-year survival rate has not significantly improved since 1985, and annual incidences have been increasing [6]. Given

the steadily rising costs of healthcare, bladder cancer has become a global problem [7]. Over the past decades, many reports have shown that early detection and management of cancer reduces cancer-specific mortality, and high-level cancer screening also improves survival [8,9]. The use of automated routine urinalysis for bladder cancer screening may reduce morbidity and mortality in patients with asymptomatic microscopic hematuria [10-13]. However, it is difficult to identify high-risk patients who require screening. The establishment and validation of a urine-based risk stratification model would be useful.

Dipstick urinalysis coupled with conventional sediment examination or automated urinalysis is both widely available and cost-effective. The UN-9000 is a fully automated analyzer that features a dipstick urinalysis module (UF-3500) and a flow cytometric urine particle analyzer (UF-5000). Previous studies have reported that albuminuria, hematuria, or abnormal urine particle parameters raise a strong suspicion of neoplastic proliferation, most probably UC [10,11,14]. These tests are simple and fast, and thus suitable for screening. We first compared changes in urinary chemistry and particle parameters in cases with suspected UC. Our objective was to evaluate the performance of automated urinalysis parameters in the screening context and to develop and validate a novel screening model for patients with suspected UC.

MATERIALS AND METHODS

Study design

Ethical approval

The study protocol was approved by the Research Ethics Committee of the First Affiliated Hospital, School of Medicine, Zhejiang University (approval no. 2018-1069). Informed consent was obtained from most patients before enrollment; the others were informed of their right to opt out via telephone, but no one chose to do so. All procedures followed the Chinese guidelines for informed consent.

Reference standard

The gold reference standard for UC status is a subsequent histological diagnosis, if any, obtained from the medical records. In the absence of histological diagnosis, given the high specificity of abnormal urinary tract cytology (UTCy) results in terms of malignancy, a suspicious or confirmed malignancy serves as a reference standard when defining the presence of UC. Patients with negative initial cytology but concurrent suspicious or confirmed malignancies on follow-up are also considered to have UC. Cases are considered true-negative if at least one subsequent negative follow-up result (i.e. UTCy or biopsy) is recorded.

Clinical specimens

Suspected patients were decided by urologists, and if patients declined ancillary tests, specimens would not be sent to our clinical laboratory. Otherwise, all urine specimens from eligible patients were collected from January 1, 2019, to October 31, 2022. Suspected patients were selected from specific at-risk groups (e.g. hematuria, abnormal findings of abdomen ultrasound or CT scan). To avoid UN-9000 misdetections, diluted specimens, aggregates, or specimens containing visible particles that could not be resuspended were excluded. We also excluded patients who underwent organ transplantation, patients with a history of previous UC, or patients with concurrent malignant tumors other than UC or distant metastases. We prospectively collected preoperative urine specimens from patients undergoing UTCy, cystoscopy, transurethral resection of a bladder tumor, or radical cystectomy, all of whom finally obtained a histological or UTCy diagnosis. In total, 770 consecutive patients were enrolled. Voided and catheterized urine samples were collected in sterile containers. Each sample was divided into two sterile containers: one for urinary cytopathology and the other was sent to the laboratory medicine department for UN-9000 analysis. We excluded 99 patients based on the exclusion criteria. Age, gender, and the pathological diagnosis were obtained from the electronic medical records.

UN-9000 analysis

The UN-9000 analyzer (Sysmex Corporation, Kobe, Japan) features two components: the UC-3500 and UF-5000 modules. These rapidly evaluate the dipstick parameters and urine particle status of unstained, unfixed, and uncentrifuged specimens, and the results of dipstick chemistry and urine particle parameters are obtained simultaneously. The UC-3500 yields semiquantitative data on the urobilinogen (URO), glucose (Glu), protein (Pro), blood (BLD), nitrite (NIT), bilirubin (BIL), ketone (KET), leukocyte esterase (LEU), creatinine (CRE), albumin (ALB), pH, protein/creatinine ratio (P/C), and albumin/creatinine ratio (A/C) parameters and quantitative data on the specific gravity (SG). The UF-5000 module yields data on white blood cell (WBC), red blood cell (RBC), bacteria (BACT), and urothelial parameters, including atypical cells (Atyp.C), squamous epithelial cells (Squa.EC), non-squamous epithelial cells (Non.SEC), transitional epithelial cells (Tran.EC), small round epithelial cells (SRC), and epithelium cells (EC). Commercial control materials (UC and UF controls) were, according to the manufacturer's instructions, used as daily internal quality control, and all parameters were in control. All UN-9000 analyses were completed within 2 hours after urine specimen collection and were performed by expert staff. Urine specimens were defined as dilute, when A/C or P/C resulted in "dilute".

Urinary cytopathology and follow-up histological diagnoses associated with UTCy

One of two board-certified cytopathologists read and diagnosed all UTCy slides. The two professionals consulted regularly with specialized cytopathologists and proposed the following four diagnostic categories: 1) benign/negative; 2) with atypical urothelial cells; 3) suspicious of malignancy; and 4) confirmed malignancy [15].

Further tests ordered by the urologists were based on the UTCy results, patient preferences, and practice patterns. Follow-up histological diagnoses were obtained from our electronic health record system in the following six months. Patients were lost to follow-up if later tests were performed outside our hospital.

Statistical analysis

Continuous variables are presented as medians (with in terquartile ranges, IQRs) and semiquantitative data as frequencies (with percentages). Continuous variables were compared by using the *t*-test or Mann-Whitney U test, and categorical variables were compared by using the chi-squared (χ^2) or Fisher's exact test, as appropriate. The association between age and the risk of UC was analyzed by using generalized linear models with logit links. Univariate and multivariate analyses were used to identify predictors of UC. Variables exhibiting two-sided p-values < 0.1 on univariate analysis were included in the forward, stepwise, and multivariable logistic regression model. The score of the final model was obtained by rounding the coefficient of the logit model. Predicted and observed risks were calculated for each score. Areas under the receiver operating characteristic curves (AUCs), sensitivities, specificities, positive predictive values (PPVs), and negative predictive values (NPVs) were calculated by using cross tabulation, and the final risk groups were chosen based on the NPV and the false-negative (FN) rate. The AUCs were used to assess the powers of various scores in terms of diagnosis. Internal validation was performed by using a bootstrap procedure with 500 bootstrapped samples. Youden's index was used to determine the optimal cutoff values predictive of clinical outcomes. Microsoft Excel 2016 (Redmond, WA, USA) and SPSS, version 25.0, software (IBM Corp., Armonk, NY, USA) for Windows were used for statistical analyses. All tests were two-sided, and a p-value < 0.05 was considered statistically significant.

RESULTS

Patient demographics

In total, 671 of 770 patients were included until October 31, 2022. Out of the 99 patients who were excluded, two had undergone organ transplantation, eight had non-urothelial tumors, 24 had diluted specimens, and 65 had a history of UC (Figure 1). Gross hematuria occurred in 43.5% (292/671) of the patients, followed by microscopic hematuria in 26.2% (176/671) and voiding symptoms in 19.8% (133/671). Other 175 patients without hematuria or voiding symptoms had a history of hematuria or abnormal findings on imaging. The median age of the study population was 63 (54 - 72) years; 62.1% (417/671) were men and 37.9% (254/671) were women. From January 1, 2019, to March 31, 2021, patients were set as the training group and from April 1, 2021, to October 31, 2022, patients were set as the validation group. Table 1 lists their characteristics.

On cytological analysis, 146 were diagnosed with atypical urothelial cells, 30 with suspected malignancies, 69 with confirmed malignancies, and the remaining 426 as negative. Out of the 30 patients with suspected malignancies, 19 were confirmed to have high-grade urothelial carcinoma (HGUC) and 5 to have low-grade urothelial carcinoma (LGUC) on follow-up histology; the remaining 6 were lost to follow-up.

Out of the 671 patients, 237 received histological diagnoses and 434 were diagnosed via UTCy (81 with atypical urothelial cells, 6 with a suspected malignancy, 5 with confirmed malignancies, and the remaining 342 as negative). In total, 179 histological diagnoses were classified as HGUC (78.8%, 141/179) or LGUC (21.2%, 38/179), and out of the remaining cases, 57 were diagnosed as benign and one as a carcinoma *in situ*, based on the World Health Organization/International Society of Urological Pathology criteria [16]. Out of the 179 patients with abnormal histological diagnoses, 59 were diagnosed as bladder cancer and 120 were diagnosed as upper tract urothelial carcinoma. Furthermore, a total of 145 individuals underwent radical nephroureterectomy, 78 individuals underwent transurethral resection of bladder tumor, and the remaining 14 individuals underwent radical cystectomy. Finally, we diagnosed 191 patients (28.5%, 95% CI [25.1, 31.9]) with UC. Figure 1 presents a study flow chart.

Factors associated with UC

Figure 2a presents the association between age and UC incidence; the incidence increased with age. When we stratified age by quartile, the incidences (from the first to the fourth quartiles) were 9.9%, 21.0%, 34.8%, and 50.0% in the training group and 15.7%, 22.2%, 39.4%, and 30.1% in the validation group (Figure 2b). There was no statistically significant difference in the incidence of UC between the two groups, except for the 4th age quartile. Based on Youden's index, the optimal age cutoff was 65 years; the incidence was higher (40.0%, 124/310) in older patients. Therefore, those aged ≥ 65 years were stratified as high risk.

Table 2 lists the parameters affecting the UC incidence as revealed by univariate and multivariable analyses. UC patients exhibited higher Atyp.C (0.1/μL vs. 0.0/μL) and SRC (3.3/μL vs. 1.4/μL) values and a higher rate of A/C (76.3% vs. 51.9%), PRO (78.0% vs. 52.3%), and P/C (77.1% vs. 50.9%) abnormalities. Similar findings were evident in the validation group (Table S1). Three significant factors associated with the UC incidence

Table 1. Patient characteristics.

Characteristic (n = 671)		Training group *	Validation group *	p-value
No.		403	268	
Age, median (IQR), years		64 (55 - 72)	62 (54 - 70)	0.262
Gender				
Male		252 (62.5%)	165 (61.6%)	0.801
Female		151 (37.5%)	103 (38.4%)	
Dipstick parameters **				
BLD	normal	118 (29.3%)	76 (28.4%)	0.796
	abnormal	285 (70.7%)	192 (71.6%)	
LEU	normal	288 (71.5%)	196 (73.1%)	0.636
	abnormal	115 (28.5%)	72 (26.9%)	
PRO	normal	162 (40.2%)	133 (49.6%)	0.016
	abnormal	241 (59.8%)	135 (50.4%)	
BIL	normal	400 (99.3%)	266 (99.2%)	0.998
	abnormal	3 (0.7%)	2 (0.8%)	
GLU	normal	358 (88.8%)	243 (90.7%)	0.446
	abnormal	45 (11.2%)	25 (9.3%)	
KET	normal	396 (98.3%)	264 (98.5%)	0.807
	abnormal	7 (1.7%)	4 (1.5%)	
NIT	normal	379 (94.0%)	262 (97.8%)	0.023
	abnormal	24 (6.0%)	6 (2.2%)	
URO	normal	393 (97.5%)	260 (97.0%)	0.692
	abnormal	10 (2.5%)	8 (3.0%)	
SG		1.014 (1.011 - 1.019)	1.014 (1.011 - 1.019)	0.925
ALB (mg/L; RR 0-10)		30 (10 - 150)	30 (10-80)	0.105
CR (g/L)		0.5 (0.5 - 1.0)	0.5 (0.5 - 1.0)	0.608
pH (RR 4.5-8.0)		6.0 (5.5 - 6.5)	6.0 (5.5 - 7.0)	0.925
A/C ratio	normal	165 (40.9%)	135 (50.4%)	0.016
	abnormal	238 (59.1%)	133 (49.6%)	
P/C ratio	normal	167 (41.4%)	144 (53.7%)	0.002
	abnormal	236 (58.6%)	124 (46.3%)	
Urine particle analysis (/μL)				
Atyp.C		0.0 (0.0 - 0.1)	0.0 (0.0 - 0.0)	0.064
Squa.EC		1.0 (0.3 - 3.0)	1.1 (0.3 - 3.1)	0.794
Non.SEC		1.1 (0.1 - 4.3)	1.7 (0.5 - 4.5)	0.011
Tran.EC		0.1 (0.0 - 0.4)	0.1 (0.0 - 0.4)	0.956
SRC		1.9 (0.5 - 5.0)	1.7 (0.7 - 4.4)	0.863
EC (RR 0 - 9.6)		4.0 (1.7 - 9.5)	3.8 (1.4 - 10.2)	0.981
RBC (RR 0 - 22.7)		79.2 (9.4 - 1,577.4)	80.4 (8.2 - 990.6)	0.413
WBC (RR 0 - 16.9)		24.5 (5.9 - 103.3)	23.9 (5.5 - 67.6)	0.227
BACT (RR 0 - 130.7)		14.9 (3.4 - 69.9)	11.5 (2.2 - 55.7)	0.187

*** Unless otherwise indicated, data are expressed as number (percentage) of patients. ** Qualitative test: negative result was defined as normal; positive result was defined as abnormal.**
IQR - interquartile range, RR - reference range, BLD – blood, LEU - leukocyte esterase, PRO - protein, BIL - bilirubin, GLU - glucose, KET - ketone, NIT - nitrite, URO - urobilinogen, SG - specific gravity, ALB - albumin, CR - creatinine, A/C - albumin vs. creatinine ratio, P/C - protein vs. creatinine ratio, Atyp.C - atypical cell, Squa.EC - squamous epithelium cell, Non.SEC - non-squamous epithelium cell, Tran.EC - transitional epithelium, SRC - small round epithelial cell, EC - epithelium cell, RBC - red blood cell, WBC - white blood cell, BACT - bacteria.

Table 2. Univariate and multivariate analyses of factors associated with the UC incidence in the training group.

Parameter	Non-UC	UC	Univariate		Multivariate	
	n = 285	n = 118	OR (95% CI)	p-value	OR (95% CI)	p-value
Age (years)	61 (52 - 69)	70 (63 - 77)	1.06 (1.04 - 1.08)	< 0.001	1.05 (1.03 - 1.07)	< 0.001
Atyp.C	0.0 (0.0 - 0.0)	0.1 (0.0 - 0.4)	1.33 (1.04 - 1.69)	0.021	1.15 (0.97 - 1.37)	0.116
SRC	1.4 (0.4 - 3.5)	3.3 (1.5 - 8.4)	1.06 (1.03 - 1.10)	< 0.001	1.04 (1.00 - 1.07)	0.024
A/C (abnormal)	148 (51.9%)	90 (76.3%)	2.98 (1.83, 4.83)	< 0.001		
PRO (abnormal)	149 (52.3%)	92 (78.0%)	3.23 (1.97, 5.29)	< 0.001		
P/C (abnormal)	145 (50.9%)	91 (77.1%)	3.25 (2.00, 5.30)	< 0.001	2.48 (1.46 - 4.20)	< 0.001

Atyp.C - atypical cell, SRC - small round epithelial cell, A/C - albumin vs. creatinine ratio, PRO - protein, P/C - protein vs. creatinine ratio.

Table 3. Multivariate analyses of UC indicators in the training group.

Parameter	OR (95% CI)	β Coefficient	p-value	Point score
Age (years)				
< 65	1	1		
≥ 65	3.83 (2.31 - 6.36)	1.34	< 0.001	2
Atyp.C (/µL)				
< 0.1	1	1		
≥ 0.1	4.00 (2.37 - 6.75)	1.39	< 0.001	2
SRC (/µL)				
< 2.7	1	1		
≥ 2.7	2.04 (1.23 - 3.40)	0.71	0.006	1
P/C				
Normal	1	1		
Abnormal	1.82 (1.03 - 3.20)	0.60	0.038	1

Atyp.C - atypical cell, SRC - small round epithelial cell, P/C - protein vs. creatinine ratio.

Table 4. Performance of the risk stratification algorithm in terms of UC diagnosis in the training and validation groups.

Group	AUC (95% CI)	Sensitivity	Specificity	Positive PV	Negative PV
Training group	0.802 (0.756-0.848)	0.695	0.825	0.621	0.867
Validation group	0.728 (0.660-0.797)	0.658	0.682	0.436	0.842

AUC - area under the receiver operating characteristic curve, PV - predictive values.

were revealed by multivariate analysis. Based on the previous study and significant performance in suspected UC patients, Atyp.C was still included. This can be summarized by using the mnemonic acronym UC-PAAS (UC, P/C ratio, age, atypical cells and small round epithelial). None of the A/C or PRO parameters was significant in this context (Table 2).

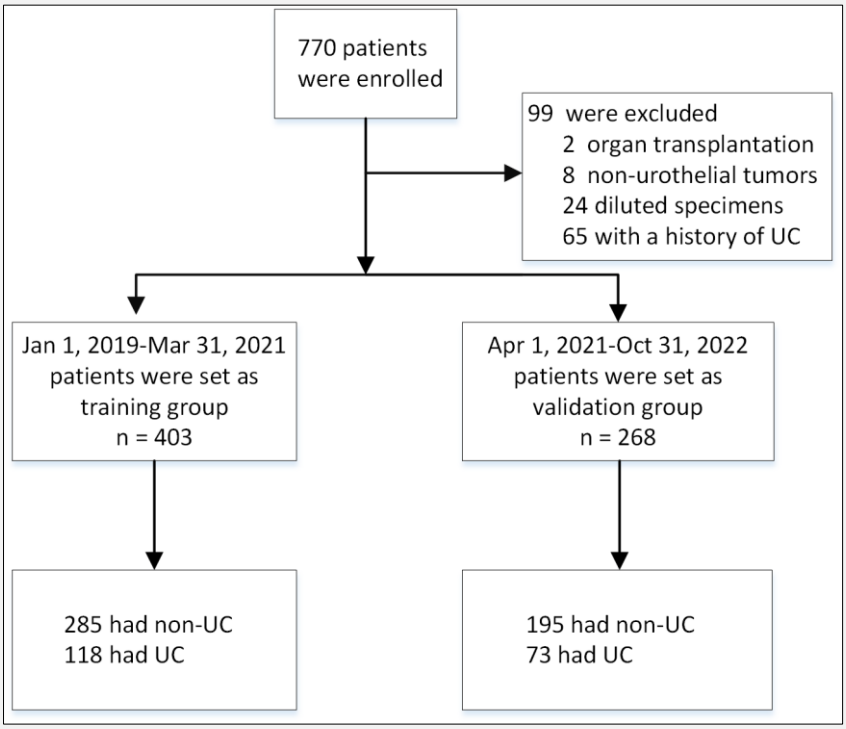

Figure 1. A flow chart of the study showing patient demographics.

UC - urothelial carcinoma.

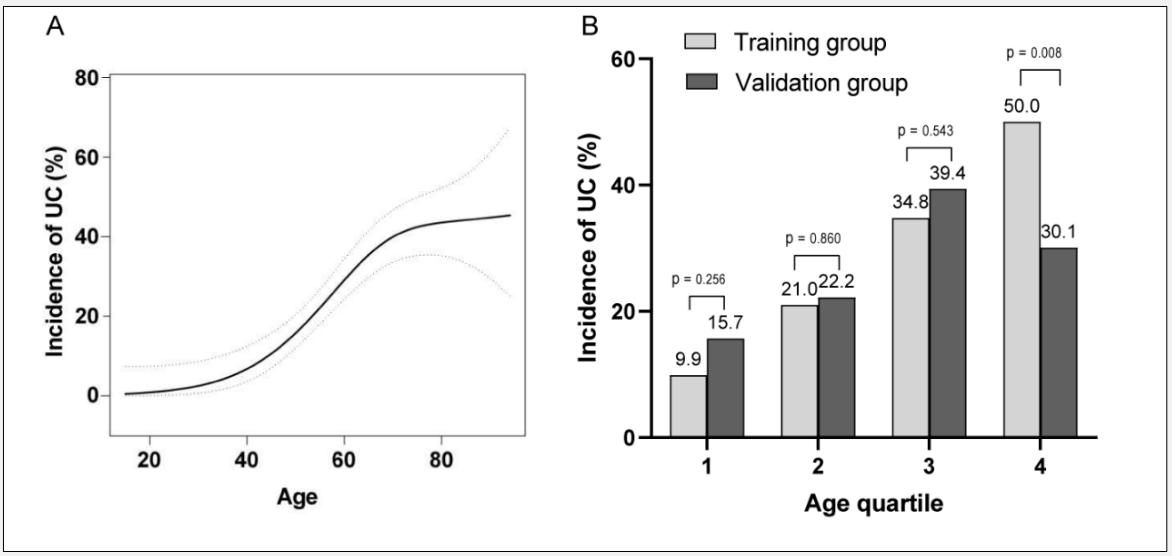

Figure 2. The Age and incidence of urothelial carcinoma (UC).

A - The incidence increased with increasing age. B - Incidences by the age quartiles in the training and validation groups.

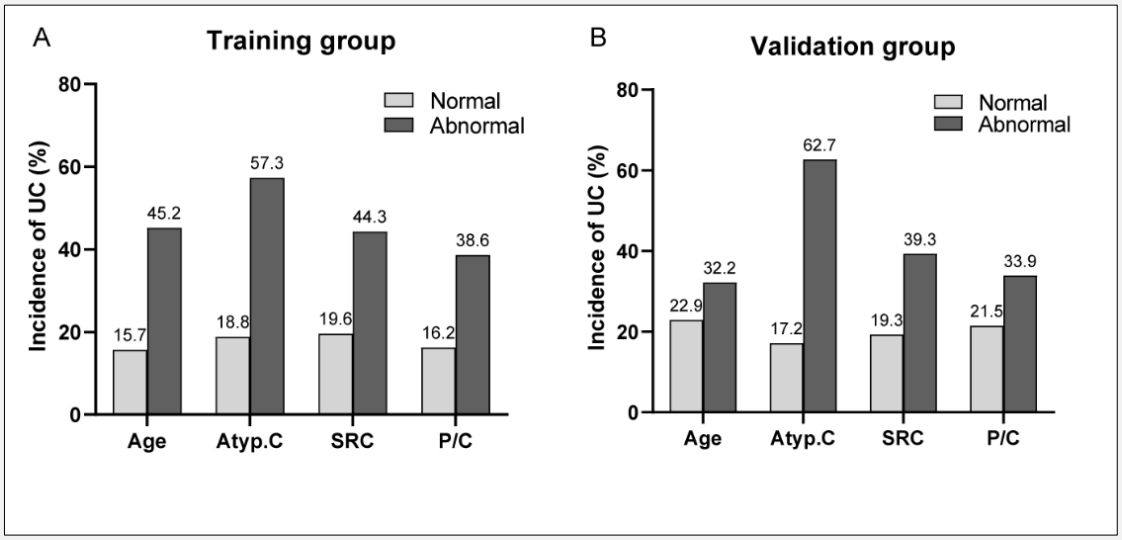

Figure 3. UC incidences after risk stratification of the training and validation groups.
A - Training group. B - Validation group.
Atyp.C - atypical cell, SRC - small round epithelial cell, P/C - protein vs. creatinine ratio.

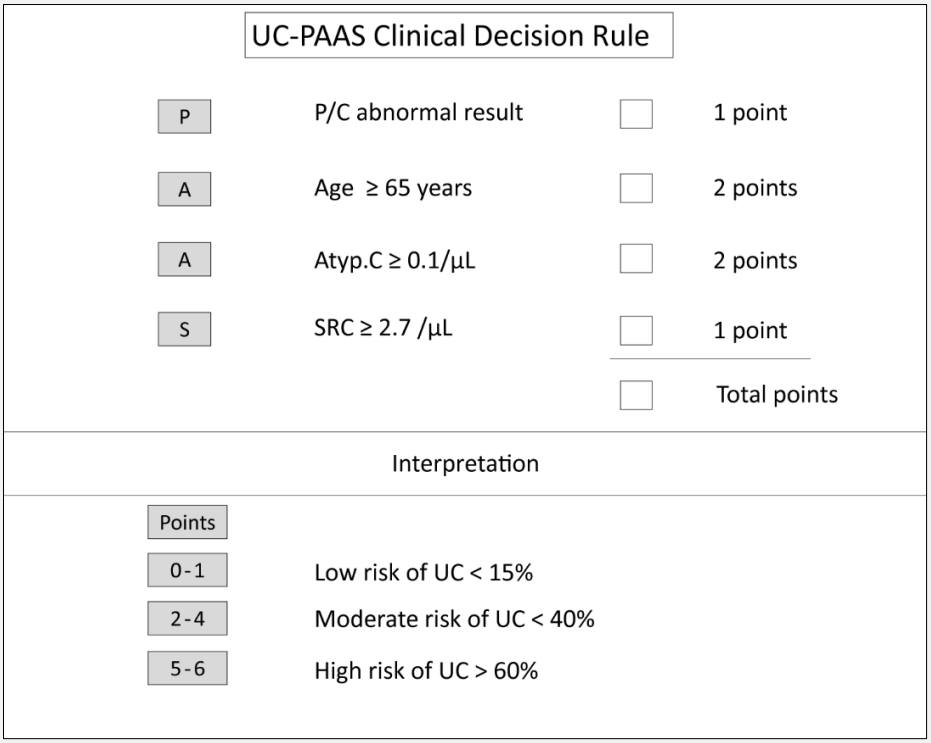

Figure 4. The UC-PAAS decision rule for UC risk stratification.

P/C - protein vs. creatinine ratio, Atyp.C - atypical cell, SRC - small round epithelial cell, UC - urothelial carcinoma.

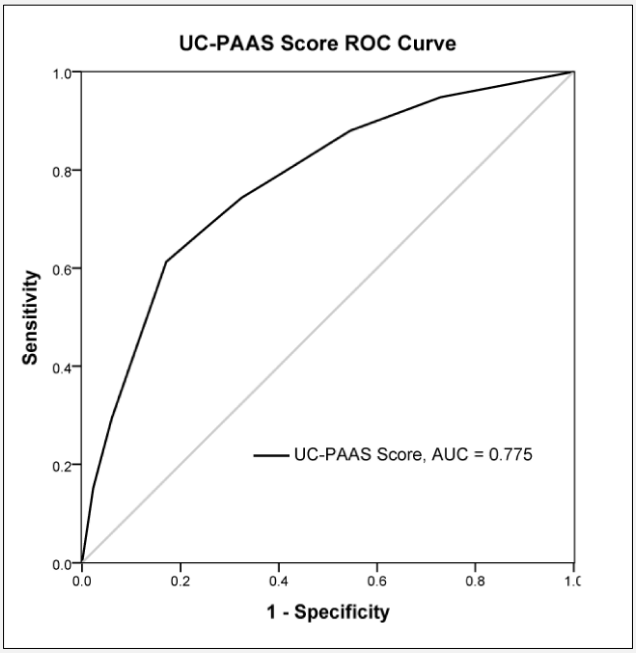

Figure 5. The ROC curve of UC-PAAS.

AUC - area under the receiver operating characteristic curve.

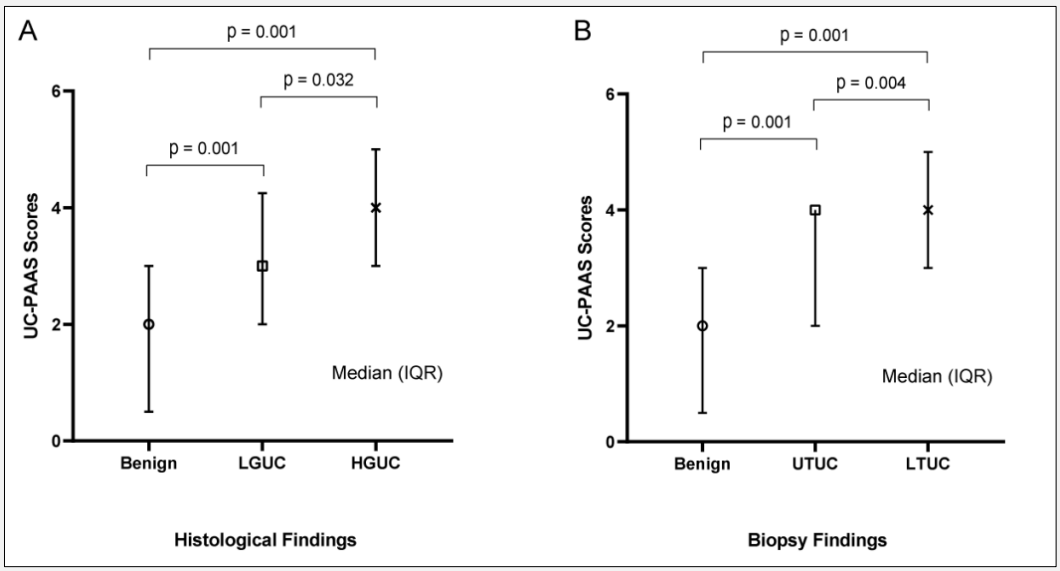

Figure 6. Distributions of scores by the follow-up histology.

A - Benign, LGUC, and HGUC groups. B - Benign, UTUC, and LTUC groups.
LGUC - low-grade urothelial carcinoma, HGUC - high-grade urothelial carcinoma, UTUC - upper-tract urothelial carcinoma, LTUC - lower-tract urothelial carcinoma.

The performance of UC-PAAS in patients with suspected UC

The AUCs for Atyp.C and SRC in the UC training group were 0.690 and 0.683, respectively. In the validation group, the AUCs were 0.708 and 0.691 (Figure S1). Based on Youden's index, the optimal cutoff values for Atyp.C and SRC were 0.1/μL and 2.7/μL, respectively.

The UC incidence was significantly higher than that of non-UC carcinoma in patients with Atyp.C ≥ 0.1/μL (57.3% vs. 18.8%) and SRC ≥ 2.7/μL (44.3% vs. 19.6%) and in those with abnormal P/C results (38.6% vs. 16.2%) in the training group, and also in the validation group, where the figures were 62.7% vs. 17.2%, 39.3% vs. 19.3%, and 33.9% vs. 21.5% for the Atyp.C, SRC, and P/C subgroups, respectively (Figure 3).

Based on multivariate logistic regression analysis, major criteria included Atyp.C ≥ 0.1/μL (2 points) and age ≥ 65 years (2 points). Minor criteria included SRC ≥ 2.7/μL (1 point) and abnormal P/C results (1 point) (Table 3). The model afforded good discrimination (AUC = 0.802 (95% CI, [0.756, 0.848]) in the training group, and internal verification yielded an AUC = 0.800 (95% CI, [0.754, 0.845]). The trend was consistent in the validation group, for which the AUC was 0.728 (95% CI [0.660, 0.797]) (Table 4).

Finally, we defined three risk groups: low risk (0 - 1 points), with a UC risk of 8.1%; moderate risk (2 - 4 points) (UC risk 35.2%); and high risk (5 - 6 points) (UC risk 62.7%). In the validation group, the incidences were 11.8% (0 - 1 points), 28.9% (2 - 4 points), and 73.1% (5 - 6 points), respectively (Figure 4). A cutoff value of more than 5 points served to identify high-risk patients in the training group; 59 patients were at higher UC risk and 62.7% (37/59) had UC. In addition, the UC-PAAS score system yielded an AUC = 0.775 (95% CI, [0.736, 0.814]) for UC risk stratification (Figure 5). The sensitivity was 0.880 and NPV was 0.905 in patients with moderate to high risk scores.

Histological diagnosis and the UC-PAAS score

UC-PAAS scores differed significantly among the benign, LGUC, and HGUC groups, and the trend was strongly associated with the urothelial grade, especially in the HGUC group. A high score indicated a high grade of UC; the median (IQR) scores were 3 (2 - 4) vs. 4 (3 - 5) (LGUC vs. HGUC, p = 0.032, Mann-Whitney U-test) (Figure 6a). Notably, the UC-PAAS score was significantly higher in the lower-tract UC group (LTUC) than in the upper-tract UC group (UTUC); the median (IQR) scores were 4 (3 - 5) vs. 4 (2 - 4) (p = 0.004, Mann-Whitney U-test) (Figure 6b).

DISCUSSION

Given the lack of cancer-specific symptoms in patients with urothelial malignancies, detection and follow-up of UC remains challenging. Although cystoscopy is usually accepted as the gold standard for detection of LTUC (mainly bladder cancer), ureteroscopy is the only standard method for UTUC diagnosis. These methods are invasive and must be performed in hospital. Screening requires noninvasive and low-cost methods, such as urine-based techniques. In this study, we assessed whether automated urinalysis parameters could identify patients at risk of or with UC. Early detection and treatment reduces cancer-specific mortality [17,18]. As the molecular alterations associated with urothelial malignancy became better known, many markers have been assessed in terms of screening, diagnosis, and surveillance. However, given the few strong markers and the low prevalence of UC, the use of molecular markers for mass screening is not cost-effective. Automated urinalysis has become widely employed in recent years [19-21]. We used automated urinalysis parameters to develop a clinical decision-making model that stratified populations into those with suspected and certain malignancies. The method can be used in primary or community institutions, especially in low-resource settings that lack advanced equipment. The UN-9000 evaluates unstained and uncentrifuged urine samples and yields dipstick and particle parameters rapidly. No previous study has evaluated the utility of a model based on these parameters in terms of detecting suspected UC.

The SRC, A/C, PRO, and P/C parameters were significantly higher in UC patients of both the training and validation groups. We previously showed that the Atyp.C level could indicate suspected UC, improving malignancy screening [22]. Proteinuria or Albuminuria were also an indicator for UCs. Elevated levels of other parameters reflect chronic irritation of the urinary tract epithelium, which may increase cancer risk.

To identify high-risk patients, we used: an Atyp.C ≥ 0.1/μL (2 points), age ≥ 65 years (2 points), a SRC ≥ 2.7/μL (1 point), and abnormal P/C results (1 point). We defined three risk groups: low risk (0 - 1 points), moderate risk (2 - 4 points), and high risk (5 - 6 points). A UC-PAAS cutoff score of 5 points equated to NPVs of 0.867 and 0.842 in the training and validation groups, respectively. Any time the score exceeded half of the total, this group should be categorized as moderate to high risk. The sensitivity at this point was 0.613 and the specificity was 0.829.

We developed the clinical decision-making UC-PAAS model to risk-stratify suspected UC patients in clinical practice. Model predictability might be conserved, because we used urinary cytopathology as the reference standard when histological data were lacking. Bernd et al. [9] reported that the sensitivity of urinary cytology ranged from 34 - 55%; therefore, patients with negative cytology but UC-PAAS scores higher than 5 points may be underestimated. Importantly, the UC-PAAS score was significantly higher in the HGUC than in the LGUC and benign groups. Thus, high-risk patients with UC-PAAS scores ≥ 5 require close surveillance, not only cytology. We also found that UC-PAAS performance was superior for the LTUC group, compared with the UTUC group. Thus, our model may optimally

detect bladder cancer compared to other UCs; this should be further investigated. However, we encountered only one carcinoma *in situ*, so we failed to draw a definitive conclusion.

Our study had several limitations. The single-center study design and the small sample size restricted the numbers of included factors. External validation with a large prospective patient cohort is required. We encountered only one carcinoma *in situ*, so the model performance requires further investigation in this context. Also, although automated urinary analysis is widely available, standard procedures for specimen selection and processing prior to urinary tract tumor screening are lacking.

In recent years, many studies have sought to use molecular alterations to manage urinary tract lesions, but few such approaches have been approved by the US Food and Drug Administration (FDA) [9]. Also, cytology and immunocytology data depend greatly on the experience and skills of pathologists [23]. The UC-PAAS decision-making rules use parameters obtained from automated urinary testing. First morning midstream urine and testing completed within two hours can help them to improve screening efficiency. Such rules can be applied in primary or community medical institutions and may help urologists to better manage suspected UC patients by their risk stratification, thereby reducing mortality, discomfort, and cost.

CONCLUSION

This study developed a simple, rapid, and cost-effective screening model (UC-PAAS) that could stratify the urothelial malignancy risk and especially identifies high-risk patients. The model may be useful in most clinical practice to screen out patients who require close follow-up or additional examinations.

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The authors declare that they have no competing interests.

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