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ORIGINAL RESEARCH

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Preliminary study on the diagnostic value of urine cell glucose metabolism detection for male urothelial carcinoma

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Abstract

This study assessed the potential of urine abnormal glycolytic metabolism detection versus urine cytology in diagnosing male urothelial carcinoma, using pathological results as the gold standard. Urine samples were collected from suspected urothelial carcinoma male patients at Zhejiang Cancer Hospital from September 2021 to February 2024. Both urine cell glycometabolism detection and urine cytology examination were performed on the same samples, with clinical data including tumor classification and grading gathered for statistical analysis. A total of 105 male patients were enrolled, with 83 (79.05%) diagnosed with urothelial carcinoma. Stratified analysis based on urine glycometabolism detection (high risk, low risk, no abnormalities) showed the sensitivity, specificity and Area Under Curve (AUC) values of "Glucose metabolism 1" (high risk) as 74.70%, 59.09% and 0.6689 (p = 0.0151), and "Glucose metabolism 2" (high/low risk) as 82.56%, 59.09% and 0.7082 (p = 0.0027). Urine cytology results showed the sensitivity, specificity, and AUC values of "Urine Exfoliative 1" (malignant tumor cells found) as 22.89%, 90.91% and 0.5690 (p = 0.3211), "Urine Exfoliative 2" (finding or suspecting malignant tumor cells) as 42.17%, 90.91% and 0.6654 (p = 0.0174), and "Urine Exfoliative 3" (finding/suspecting/not excluding malignant tumor cells) as 60.24%, 72.73% and 0.6648 (p = 0.0178). The combined diagnosis of "Glucose metabolism 2" and "Urine Exfoliative 4" (no tumor cells found) improved diagnostic efficiency, with sensitivity 62.65%, specificity 95.45% and AUC = 0.7905 ($p < 0.0001$). "Glucose metabolism 2" had a sensitivity of 86.21% for low-grade urothelial carcinoma, while "Urine Exfoliative 4" had a sensitivity of 58.62%. Compared to urine cytology, urine cell glycometabolism detection improved sensitivity for diagnosing urothelial carcinoma but had lower specificity. Combined diagnosis enhanced sensitivity and specificity, and glycometabolism detection showed superior sensitivity for low-grade urothelial carcinoma, serving as an efficient non-invasive diagnostic tool.

Keywords

Urine cell glycometabolism detection; Urine cytology examination; Urothelial carcinoma; Sensitivity; Specificity

1. Introduction

Urothelial Carcinoma (UC) is the second most prevalent malignant tumor of genitourinary system worldwide [1]. UC can occur in the upper (renal pelvis and ureter) or lower (bladder and urethra) urinary tracts [2]. Bladder Carcinoma (BLCA) accounts for 90–95% UC cases [3], which represent 6% of male and 2.1% of female cancer patients globally [4]. However, Upper Tract Urothelial Carcinoma (UTUC) including renal pelvis and ureteral cancer has lower incidence and accounts for ~5% of urothelial tumors [3]. Ureteral tumors are rare than renal pelvis tumors [4], however UTUC has increased in its incidence and mortality rates in recent years [5].

BLCA incidence has gender disparities with males being four times more likely to develop bladder cancer than females [6–8]. Previous studies [9] have attributed this difference to the higher smoking rates in males compared to females. However, the incidence rate of bladder cancer remains higher for males even in non-smoking populations [10]. *In vitro* and *in vivo* clinical studies have demonstrated differences in immune responses, hormones, hormone receptor expression, epigenetic and genetic changes between male and female bladder cancer patients, which may increase the susceptibility of bladder cancer in males [8].

Currently, UC diagnosis relies on cystoscopy, ureteroscopy-guided biopsy or postoperative pathology as the gold standard [11]. However, these invasive procedures have certain limitations which cause physiological discomfort to patients. The white light cystoscopy is less effective for detecting *in situ* UC. It is challenging after the prior intravesical treatments where

distinguishing inflammation from carcinoma can be difficult in cystoscopic examination. The international urological guidelines for UTUC recommend ureteroscopy [12, 13], however, the diagnostic value of ureteroscopy-guided biopsy samples is limited [14, 15]. The restricted access to more proximal portions of ureter and renal pelvis hinders adequate sampling. Even with the access to these regions, preoperative and intraoperative pathological diagnosis of UTUC is challenging with the obtained limited tissue [16], which leads to uncertainties in surgical strategies (*e.g.*, unilateral ureterectomy) [17].

UC at early stages has non-specific symptoms or asymptomatic hematuria, which demands conducive early screening and diagnostic methods to improve patient prognosis. Around 75–80% bladder cancer patients are initially diagnosed with Non Muscle Invasive Bladder Cancer (NMIBC), which is confined to mucosa with 5-year survival rate exceeding 85% [18]. The remaining cases progress to Muscle Invasive Bladder Cancer (MIBC). NMIBC requires transurethral resection of bladder tumors as the primary treatment. This procedure fragments the lesion for pathological examination, however it is controversial because of the risk of tumor cell implantation into healthy bladder mucosa which causes tumor recurrence [19]. The postoperative bladder instillation chemotherapy reduces recurrence rates [20], however 5-year and 10-year recurrence rates for bladder cancer are 65% and 81%, respectively [21, 22], with ~30% UTUC patients experiencing recurrence [23]. Lifelong follow-up examinations are required because of the high recurrence rates and prolonged survival of UC patients. A non-invasive diagnostic method would thus improve patient experience, streamline follow-up process, reduce examination time, and demonstrate market potential.

UC originates from urothelial mucosa. It is constantly exposed to urine. Highly abnormal tumor cells poorly adhere to tissues and thus shed into urine, which make their detection challenging. Urine cytology relies on the identification of shed tumor cells which has poor sensitivity and specificity, particularly for low-grade UC with sensitivity of 48–68% [4, 24], and also prone to false-negative results [25]. Novel urine biomarkers such as Nuclear Matrix Protein 22 (NMP22), Bladder Tumor Antigen (BTA), Fluorescence *in situ* Hybridization (UroVysion), and ImmunoCyt/uCyt+ have emerged in recent years [26], and received approvals from the US Food and Drug Administration (FDA) and the European Medicines Agency (EMA). Despite these biomarkers outperform cytology, they face challenges like high false-positive rates, sample storage, high cost, hindered clinical translation [5, 11] and limited large-scale validation studies [27]. The guidelines do not recommend routine usage of any urine biomarker in the initial UC diagnosis till date [28].

Tumor cells proliferation involves metabolic adaptations for promoting cell growth and division. Otto Warburg has first observed the abnormal energy metabolism of cancer cells [29]. It is characterized by a preference for glycolysis even in the oxygen presence, which leads to “aerobic glycolysis” or the Warburg effect [30]. This high glucose metabolism is clinically used in positron emission tomography (PET) for the cancer diagnosis. Innovative techniques based on the Warburg effect have emerged in recent years for identifying tumor cells in body fluids, such as the high-throughput screening of metabolically active tumor cells in pleural effusion [31, 32]. Compared to the cytological diagnosis, this new technique can detect rare malignant tumor cells in <1 mL effusion. It has thus the potential to detect rare circulating tumor cells in peripheral body fluids. However, its application to UC remains unclear or limited.

This study aims to analyze the glycolytic metabolism of shed tumor cells in urine. Pathological results are used as the gold standard and compared with shed cell cytology to validate its diagnostic value for male UC via the large-scale studies. Moreover, the feasibility of urine cell glycolytic metabolism testing for male UC diagnosis and its potential in follow-up and early screening are explored.

2. Methods

2.1 Research subjects

This study selected suspected urothelial carcinoma male patients at the Zhejiang Cancer Hospital from September 2021 to February 2024 as the research subjects.

2.1.1 Inclusion criteria

18–90 years age; clinical and routine examinations suggesting UC suspicion; patients undergone cystoscopy or surgery with pathological reports as the gold standard; preoperative concurrent urine glycolysis and shed cells’ cytology examinations; patients with good compliance.

2.1.2 Exclusion criteria

Age: <18 and >90 years; samples of incomplete case information; samples without pathological results or surgical/biopsy pathology indicating non-UC or unclear pathological results; improper sample collection or storage causing incomplete testing; patients with the history of systemic chemotherapy; patients having other malignant tumors.

2.2 Sample collection

2.2.1 Urine sample collection

For each suspected UC patient at the Zhejiang Cancer Hospital from September 2021 to February 2024, ~200 mL urine sample was collected and equally divided into two clean and labelled urine cups. Samples were delivered to the Pathology Department of Zhejiang Cancer Hospital within 2 hours. Urine cell glycolysis and shed cell cytology examinations were conducted.

2.2.2 Tissue sample collection

The enrolled male patients underwent standard diagnostic and therapeutic procedures including clinical evaluations, urinary tract ultrasonography (CTU), urinary system ultrasound, cystoscopy and other examinations for indicating the suspicion of UC. Surgical treatment or cystoscopic biopsy was conducted to obtain tissue samples for pathological diagnosis as the gold standard.

2.3 Grouping criteria

2.3.1 For urinary cell glycolysis testing

The cells with high-risk were selected from urine for the glycolysis examination via hexokinase 2 in the glycolytic pathway. Outcome was combined with inflammation markers, epithelial cells, and other biomarkers to exclude other interferences. The glycolysis results are presented as high risk, low risk and no abnormalities (Table 1).

TABLE 1. Results from urinary cell glycolysis testing and urinary shed cell cytology examination.

Urine cell glucose metabolism detection	Urine exfoliated cell examination
High risk	Finds malignant tumor cells
Low risk	Suspected tumor cells
No abnormality	Atypical urothelial cancer cells cannot exclude urothelial carcinoma
	Atypical cells have no clear meaning
	No tumor evidence

The urinary cell glycolysis testing was subdivided into two distinct evaluation groups for precise diagnostic indicators. In "Glucose metabolism 1" group, the positive result indicated high risk, while negative result reflected low risk and no abnormalities. In "Glucose metabolism 2" group, both the high risk and low risk results were classified as positive, which reflected the possibility of disease occurrence even at lower risk levels. Test result showing no abnormalities was considered negative. This grouping was aimed to provide personalized and segmented diagnostic information about the patients by interpreting risk levels. Furthermore, it assisted the clinicians for accurately assessing and managing patients' health conditions (Table 2).

TABLE 2. Grouping for urinary cell glycolysis testing.

Positive	Negative
Glucose metabolism 1	
High risk	Low risk
	No abnormality
Glucose metabolism 2	
High risk	No abnormality
Low risk	

2.3.2 For urinary exfoliated cell examination

The urinary exfoliated cells and postoperative tissue pathology were examined by the clinically experienced pathologists in the cytology laboratory of tumor diagnosis room at the Zhejiang Cancer Hospital. The urinary exfoliated cell examination results were classified as follows: identified malignant tumor cells, suspicious malignant tumor cells, non-excludable atypical UC cells, non-excludable atypical cells without clear significance and no tumor evidence (Table 2).

Four grouping strategies were employed for analyzing the urinary exfoliated cell examination to accurately identify and classify the patients' risk levels. In "Urine Exfoliation 1" group, the malignant tumor cells' presence was considered positive, while suspicious tumor cells, non-excludable atypical UC cells, non-excludable atypical cells without clear significance and no tumor evidence were considered negative. In "Urine Exfoliation 2" group, the presence of malignant and suspicious tumor cells were considered positive, while non-excludable atypical UC cells, non-excludable atypical cells without clear significance and no tumor evidence were considered negative. In "Urine Exfoliation 3" group, the presence of malignant and suspicious tumor cells, tumor cells and non-excludable atypical UC cells were considered positive, while non-excludable atypical cells without clear significance and no tumor evidence were considered negative. In "Urine Exfoliation 4" group, a negative result indicated no tumor cells' evidence, while the presence of malignant and suspicious tumor cells, tumor cells, non-excludable atypical UC cells and non-excludable atypical cells without clear significance were considered positive (Table 3).

TABLE 3. Grouping for urinary exfoliated cell examination.

Positive	Negative
Urine Exfoliation 1	
Finds malignant tumor cells	Suspected tumor cells
	Atypical urothelial cancer cells cannot exclude urothelial carcinoma
	Atypical cells have no clear meaning
	No tumor evidence
Urine Exfoliation 2	
Finds malignant tumor cells	Atypical urothelial cancer cells cannot exclude urothelial carcinoma
Suspected tumor cells	Atypical cells have no clear meaning
	No tumor evidence
Urine Exfoliation 3	
Finds malignant tumor cells	Atypical cells have no clear meaning
Suspected tumor cells	No tumor evidence
Atypical urothelial cancer cells cannot exclude urothelial carcinoma	
Urine Exfoliation 4	
Finds malignant tumor cells	No tumor evidence
Suspected tumor cells	
Atypical urothelial cancer cells cannot exclude urothelial carcinoma	
Atypical cells have no clear meaning	

2.4 Statistical analysis

The sensitivity (percent positive among malignant cases) and specificity (percent negative among normal cases) of urinary cell sugar metabolism detection and urinary exfoliated cell examination were separately calculated. Data were analyzed using statistical software SPSS version 25 (IBM, New York, NY, USA) and GraphPad Prism version 9.0 (Dotmatics, Boston, MA, USA). Initially, the descriptive statistical analysis outlined sample characteristics including age, gender distribution and other clinical parameters. Subsequently, the sensitivity, specificity and area under the Receiver Operating Characteristic (ROC) curve (AUC) were calculated. Statistical significance of results was determined at $p < 0.05$. Each diagnostic method was evaluated by calculating sensitivity, specificity and AUC values. Confidence intervals (95%) and *p*-values were computed to assess the statistical significance of results. Thereby, the relative strengths and limitations of urinary cell sugar metabolism detection and urinary exfoliated cell examination in clinical applications were evaluated. This methodology would provide quantitative basis for research, guide about future clinical practice and optimize the UC screening and diagnostic process.

3. Results

A total of 184 suspected urothelial carcinoma cases were identified from September 2021 to February 2024 at the Zhejiang Cancer Hospital, wherein 105 cases meeting the inclusion and exclusion criteria were analyzed. Based on the pathological reports, 83 cases (79.05%) were diagnosed with UC, where 4 (3.81%) were concurrent in bladder and upper urinary tract, 45 (42.86%) in bladder alone and 34 (32.38%) in upper urinary tract. In the remaining 22 patients, 15 cases (14.29%) were confirmed as benign bladder lesions, 5 (4.76%) had postoperative pathology showing renal tumors and 2 cases (1.90%) showed renal benign tumors on postoperative pathology (Fig. 1, Table 4).

3.1 Diagnostic efficiency of urinary cell sugar metabolism detection for UC

The sensitivity, specificity, and AUC values of "Glucose metabolism 1" using pathological results as the gold standard were 74.70% (95% Confidence Interva (CI): 64.40–82.81%), 59.09% (95% CI: 38.73–76.74%) and 0.6689 ($p = 0.0151$), and those for "Glucose metabolism 2" were 82.56% (95% CI: 73.20–89.14%), 59.09% (95% CI: 38.73–76.74%) and 0.7082 ($p = 0.0027$), respectively (Fig. 2A–C).

3.2 Diagnostic efficiency of urinary exfoliated cell examination for UC

The sensitivity, specificity, and AUC values of "Urine Exfoliation 1" using pathological results as the gold standard were 22.89% (95% CI: 15.17–33.01%), 90.91% (95% CI: 72.19–98.38%) and 0.5690 ($p = 0.3211$), those for "Urine Exfoliation 2" were 42.17% (95% CI: 32.12–52.91%), 90.91% (95% CI: 72.19–98.38%) and 0.6654 ($p = 0.0174$), those for "Urine Exfoliation 3" were 60.24% (95% CI: 49.48–70.09%), 72.73% (95% CI: 51.85–86.85%) and 0.6648 ($p = 0.0178$), and those for "Urine Exfoliation 4" were 73.49% (95% CI: 63.11–81.80%), 59.09% (95% CI: 38.73–76.74%) and 0.6629 ($p = 0.0192$), respectively (Fig. 2D–H).

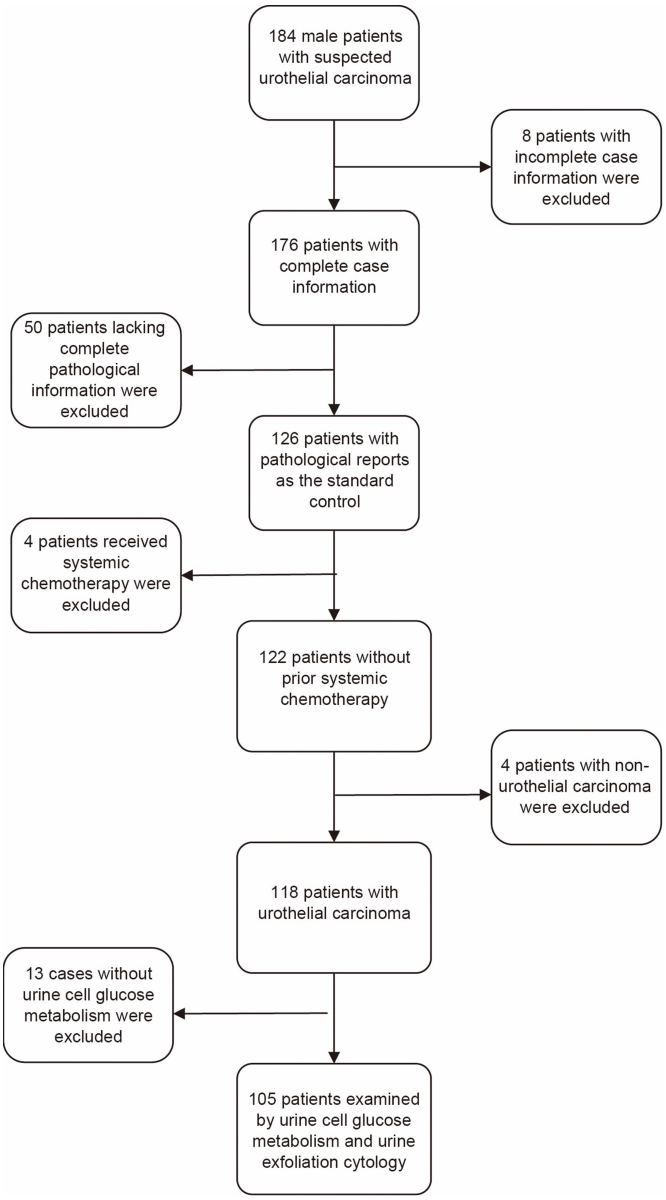

FIGURE 1. Flowchart of Patients' screening process.

3.3 Combined diagnosis by urinary cell sugar metabolism detection and urinary exfoliated cell examination for UC diagnostic efficiency

3.3.1 Grouping criteria for combined diagnosis

The combined diagnosis by urinary cell glycometabolism testing and urinary cell shedding examination was categorized as follows based on above results. "Glucose metabolism 1" was combined with "Urine Exfoliation 1" (combined A1/A2), "Urine Exfoliation 2" (combined B1/B2), "Urine Exfoliation 3" (combined C1/C2), and "Urine Exfoliation 4" (combined D1/D2). The positive criteria for combined A1/B1/C1/D1 required both glycometabolism and urinary shedding results

TABLE 4. Enrolled patients' information.

		Number of cases	Percent
Sex	Male	105	100%
Age (yr)			
	<45	5	4.8%
	≥45, <65	51	48.6%
	≥65, <85	46	43.8%
	≥85	3	2.9%
Pathology			
	Positive	83	79.0%
	Negative	22	21.0%
Carbohydrate metabolism			
	High risk	71	67.6%
	Low risk	6	5.7%
	No abnormality	28	26.7%
Urine exfoliating cells			
	Finds malignant tumor cells	21	20.0%
	Suspected tumor cells	16	15.2%
	Atypical urothelial cancer cells cannot exclude urothelial carcinoma	19	18.1%
	Atypical cells have no clear meaning	14	13.3%
	No tumor evidence	35	33.3%
Grade			
	Low	29	34.9%
	High	49	59.0%
	Unrated	5	6.0%

as positive for overall positive diagnosis. The negative criteria considered either test as negative for the overall negative diagnosis. For combined A2/B2/C2/D2, the positive diagnosis was made if either test resulted as positive, while both should be negative for the overall negative diagnosis. Similarly, "Glucose metabolism 2" was combined with "Urine Exfoliation 1, 2, 3 and 4" for the joint diagnosis (Tables 5,6).

3.3.2 Diagnostic efficacy of combined A for UC

The sensitivity, specificity, and AUC values for "Combined A1" were 20.48% (95% CI: 13.20–30.38%), 95.45% (95% CI: 78.20–99.77%), and 0.5797 ($p = 0.2519$), and those for "Combined A2" were 81.93% (95% CI: 72.30–88.73%), 54.55% (95% CI: 34.66–73.08%), and 0.6824 ($p = 0.0087$), respectively (Fig. 3A,B).

3.3.3 Diagnostic efficacy of combined B for UC

The sensitivity, specificity, and AUC values for "Combined B1" were 24.10% (95% CI: 16.17–34.31%), 95.45% (95% CI: 78.20–99.77%), and 0.5978 ($p = 0.1599$), and those for "Combined B2" were 81.93% (95% CI: 72.30–88.73%), 54.55% (95% CI: 34.66–73.08%), and 0.6824 ($p = 0.0087$), respectively (Fig. 3C,D).

3.3.4 Diagnostic efficacy of combined C for UC

The sensitivity, specificity, and AUC values for "Combined C1" were 24.10% (95% CI: 16.17–34.31%), 95.45% (95% CI: 78.20–99.77%), and 0.5978 ($p = 0.1599$), and those for "Combined C2" were 83.13% (95% CI: 73.66–89.68%), 54.55% (95% CI: 34.66–73.08%), and 0.6884 ($p = 0.0068$), respectively (Fig. 3E,F).

3.3.5 Diagnostic efficacy of combined D for UC

The sensitivity, specificity, and AUC values for "Combined D1" were 62.65% (95% CI: 51.90–72.28%), 86.36% (95% CI: 66.67–95.25%), and 0.7451 ($p = 0.0004$), and those for "Combined D2" were 85.54% (95% CI: 76.41–91.53%), 31.82% (95% CI: 16.36–52.86%), and 0.5868 ($p = 0.2120$), respectively (Fig. 3G,H).

3.3.6 Diagnostic efficacy of combined E for UC

The sensitivity, specificity, and AUC values for "Combined E1" were 21.69% (95% CI: 14.18–31.70%), 95.45% (95% CI: 78.20–99.77%), and 0.5857 ($p = 0.2179$), and those for "Combined E2" were 83.13% (95% CI: 73.66–89.68%), 54.55% (95% CI: 34.66–73.08%), and 0.6884 ($p = 0.0068$), respectively (Fig. 3I,J).

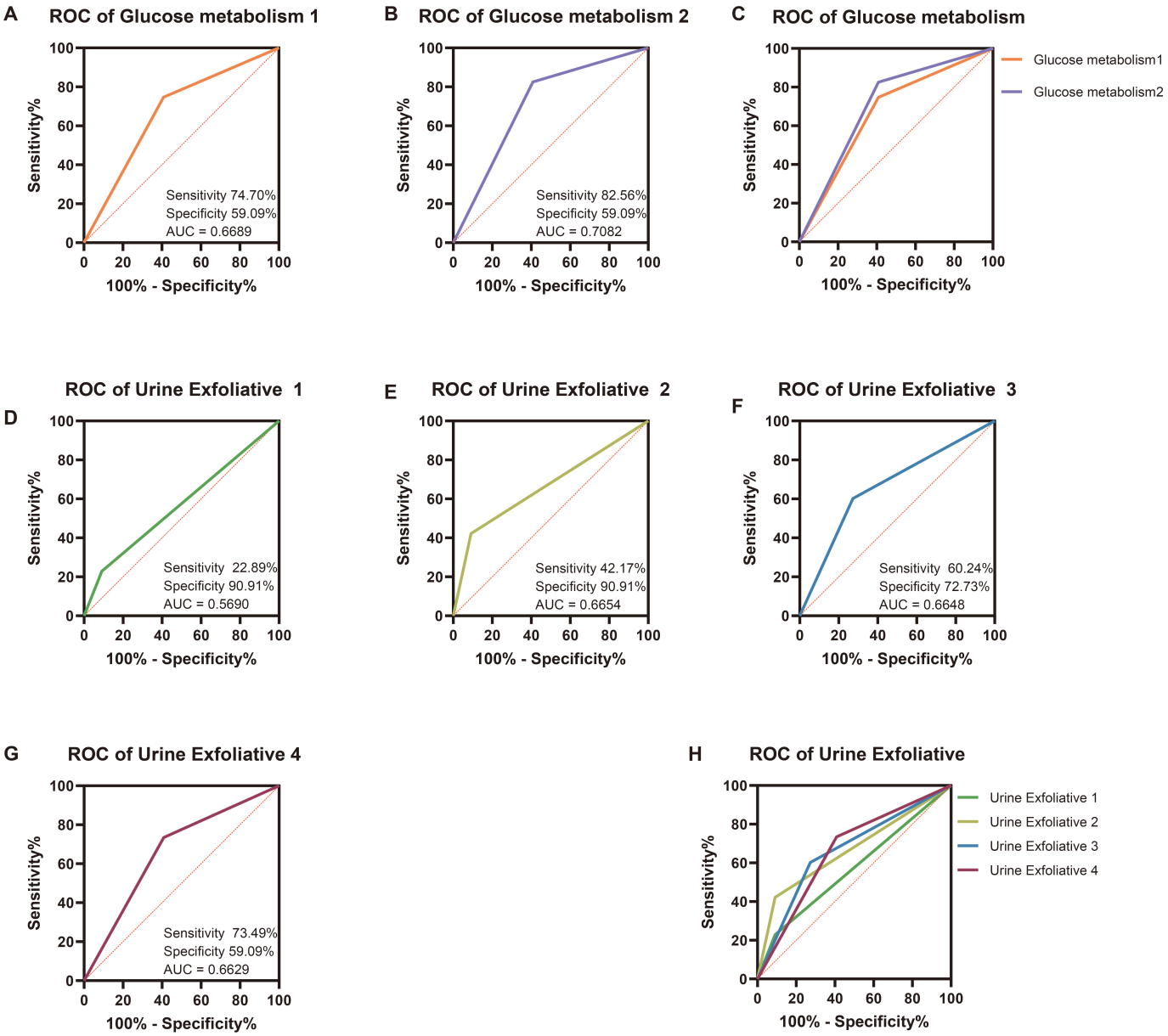

FIGURE 2. The diagnostic efficacy of glucose metabolism and urine exfoliation. (A) ROC curve of "Glucose metabolism 1"; (B) ROC curve of "Glucose metabolism 2"; (C) ROC curve of "Urine Exfoliation 1"; (D) ROC curve of "Urine Exfoliation 2"; (E) ROC curve of "Urine Exfoliation 3"; (F) ROC curve of "Urine Exfoliation 4"; (G) ROC curves of "Glucose metabolism 1" and "Glucose metabolism 2"; (H) ROC curves of "Urine Exfoliation 1–4". ROC: Receiver Operating Characteristic; AUC: Area Under Curve.

3.3.7 Diagnostic efficacy of combined F for UC

The sensitivity, specificity, and AUC values for "Combined F1" were 38.55% (95% CI: 28.81–49.31%), 95.45% (95% CI: 78.20–99.77%), and 0.6700 ($p = 0.0145$), and those for "Combined F2" were 84.34% (95% CI: 75.02–90.61%), 54.55% (95% CI: 34.66–73.08%), and 0.6944 ($p = 0.0052$), respectively (Fig. 3K,L).

3.3.8 Diagnostic efficacy of combined G for UC

The sensitivity, specificity, and AUC values for "Combined G1" were 55.42% (95% CI: 44.73–65.64%), 86.36% (95% CI: 66.67–95.25%), and 0.7089 ($p = 0.0027$), and those for "Combined G2" were 86.75% (95% CI: 77.81–92.44%), 50.00% (95% CI: 30.72–69.28%), and 0.6837 ($p = 0.0082$), respectively (Fig. 3M,N).

3.3.9 Diagnostic efficacy of combined H for UC

The sensitivity, specificity, and AUC values for "Combined H1" were 62.65% (95% CI: 51.90–72.28%), 95.45% (95% CI: 78.20–99.77%), and 0.7905 ($p < 0.0001$), and those for "Combined H2" were 87.95% (95% CI: 79.22–93.32%), 40.91% (95% CI: 23.26–61.27%), and 0.6443 ($p = 0.0380$) (Fig. 3O,P).

3.4 Sensitivity comparison between high-grade and low-grade UC

TABLE 5. Grouping criteria for combined diagnosis of urinary cell glycometabolism testing and urinary cell shedding examination.

Combined	Glycometabolic group	Urinary shedding group	Combined	Positive standard	Negative standard
A	1	1	A1	++	+−/−+/−−
			A2	++/+−/−+	−−
B	1	2	B1	++	+−/−+/−−
			B2	++/+−/−+	−−
C	1	3	C1	++	+−/−+/−−
			C2	++/+−/−+	−−
D	1	4	D1	++	+−/−+/−−
			D2	++	+−/−+/−−
E	2	1	E1	++	+−/−+/−−
			E2	++/+−/−+	−−
F	2	2	F1	++	+−/−+/−−
			F2	++/+−/−+	−−
G	2	3	G1	++	+−/−+/−−
			G2	++/+−/−+	−−
H	2	4	H1	++	+−/−+/−−
			H2	++	+−/−+/−−

TABLE 6. Diagnostic efficacy of combined diagnosis for urothelial carcinoma.

Combined	Combined	Sensitivity	Specificity	AUC value
A (G1 + U1)				
	A1	20.48%	95.45%	0.5797
	A2	81.93%	54.55%	0.6824
B (G1 + U2)				
	B1	24.10%	95.45%	0.5978
	B2	81.93%	54.55%	0.6824
C (G1 + U3)				
	C1	24.10%	95.45%	0.5978
	C2	83.13%	54.55%	0.6884
D (G1 + U4)				
	D1	62.65%	86.36%	0.7451
	D2	85.54%	31.82%	0.5868
E (G2 + U1)				
	E1	21.69%	95.45%	0.5857
	E2	83.13%	54.55%	0.6884
F (G2 + U2)				
	F1	38.55%	95.45%	0.6700
	F2	84.34%	54.55%	0.6944
G (G2 + U3)				
	G1	55.42%	86.36%	0.7089
	G2	86.75%	50.00%	0.6837
H (G2 + U4)				
	H1	62.65%	95.45%	0.7905
	H2	87.95%	40.91%	0.6443

G: Glucose metabolism; U: Urine Exfoliation; AUC: Area Under Curve.

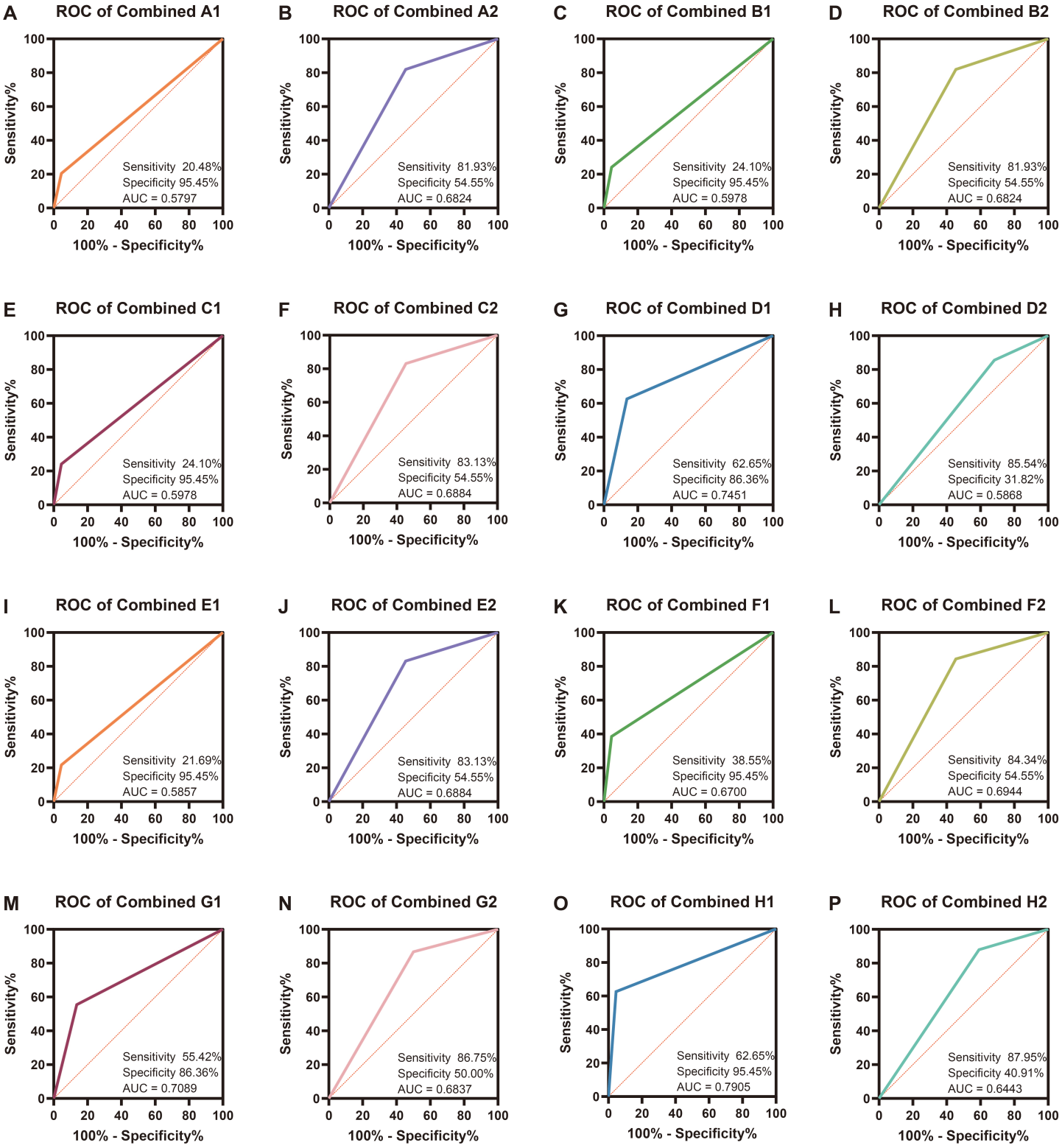

FIGURE 3. The diagnostic efficacy of combined diagnosis. (A) ROC curve of "Combined 1"; (B) ROC curve of "Combined A2"; (C) ROC curve of "Combined B2"; (D) ROC curve of "Combined B2"; (E) ROC curve of "Combined C1"; (F) ROC curve of "Combined C2"; (G) ROC curve of "Combined D1"; (H) ROC curve of "Combined D2"; (I) ROC curve of "Combined E1"; (J) ROC curve of "Combined E2"; (K) ROC curve of "Combined F1"; (L) ROC curve of "Combined F2"; (M) ROC curve of "Combined G1"; (N) ROC curve of "Combined G2"; (O) ROC curve of "Combined H1"; (P) ROC curve of "Combined H2". ROC: Receiver Operating Characteristic; AUC: Area Under Curve.

3.4.1 For urinary cell glycolysis testing and urine cytology

"Glucose metabolism 2" exhibited higher sensitivity than "Glucose metabolism 1" in low-grade and high-grade UC. The sensitivity of urine cytology was ranked as "Urine Exfoliation 4" > "Urine Exfoliation 3" > "Urine Exfoliation" > "Urine Exfoliation 1". The urine cytology sensitivity in diagnosing low-grade UC was lower than that of high-grade. For low-grade UC, the less sensitive "Glucose metabolism 1" (75.86%) in glycolysis group had advantage over the most sensitive "Urine Exfoliation 4" (58.62%) in urine cytology group. For high-grade UC, the sensitivity of "Urine Exfoliation 4" was higher than that of "Glucose metabolism 1" and "Glucose metabolism 2" (Fig. 4A,B).

3.4.2 For combined diagnostic methods

The sensitivity of Combined A2/B2/C2/D2/E2/F2/G2/H2 was higher than that of Combined A1/B1/C1/D1/E1/F1/G1/H1 for low-grade or high-grade UC. For low-grade UC, the sensitivity of Combined A1/B1/C1/D1/E1/F1/G1/H1 was lower compared to the higher-grade group, the sensitivity differences for different grades of UC were not significant in the Combined A2/B2/C2/D2/E2/F2/G2/H2 (Fig. 4C,D).

4. Discussion

Guidelines from the European Association of Urology (EAU) and the American Urological Association (AUA) recommend cystoscopy and urinary cytology as the primary diagnostic methods for urothelial carcinoma with the follow-up intervals of 3 months for first 2 years followed by every 6 months for next 3 years [33]. Some patients can experience anxiety or discomfort because of the invasive nature of cystoscopy, especially with the repeated examinations. Cost of these examinations adds to long-term economic burden on patients, which causes decreased compliance. There is an urgent need to develop non-invasive and cost-effective diagnostic method for improving accuracy and patient compliance.

Hexokinase (HK) catalyzes the glycolysis by phosphorylating glucose, wherein Hexokinase 2 (HK2) is minimally expressed or undetectable in most normal cells. In contrast, HK2 is highly expressed in cancers including epithelial and non-epithelial origin cancers [34–37]. HK2 as tumor marker can serve as the metabolic functional marker for rare circulating tumor cells in peripheral blood of non-small cell lung cancer patients, that surpasses epithelial markers [38, 39]. The metabolic analysis in this study is based on HK2 detection in urine, which is combined with inflammatory markers, epithelial cells, and other markers to exclude interferences in the detection of shed tumor cells in urine.

This study compares the sensitivity and specificity of urinary cell metabolic testing and urine shedding examination for 105 patients. It explores the diagnostic value of urinary cell glycolysis testing in UC. Results indicate higher sensitivity of "Glucose metabolism 2" subgroup (82.56%) under the same specificity. Increased vigilance is thus required to avoid missed diagnoses of patients classified as high- or low-risk based on glycolysis testing. Both "Glucose metabolism 1" and "Glucose metabolism 2" show higher sensitivity compared to all urine shedding examinations. Abnormal glycolysis being an important characteristic of cancer cells is studied as the potential biomarker [39, 40]. High sensitivity of such tests

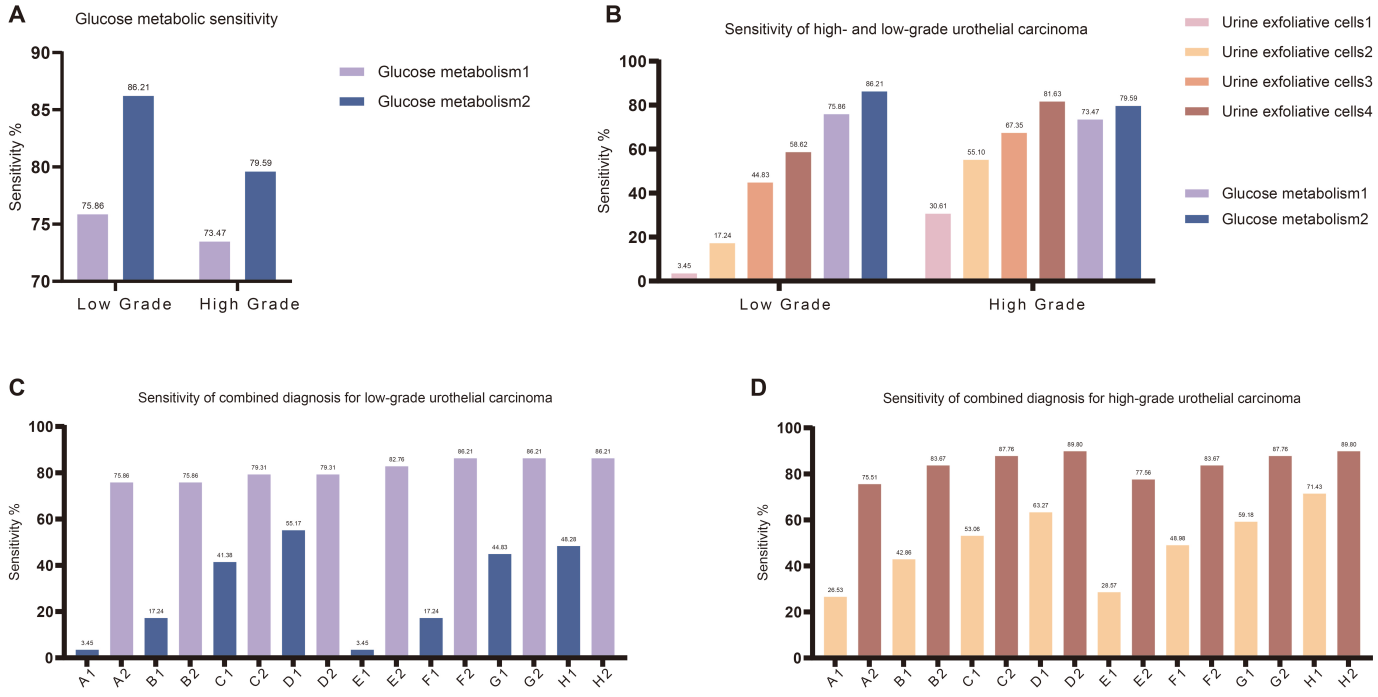

FIGURE 4. Comparison of sensitivity between high-grade and low-grade urothelial carcinoma. (A) Sensitivity of glucose metabolism test toward high-grade and low-grade urothelial carcinoma; (B) Sensitivity of glucose metabolism test and urine exfoliation toward high-grade and low-grade urothelial carcinoma; (C) Sensitivity of combined diagnosis for low-grade urothelial carcinoma; (D) Sensitivity of combined diagnosis for high-grade urothelial carcinoma.

may come from capturing of early changes in cancer cell metabolism, particularly in UC, having direct contact to urine.

However, the specificity of urinary cell metabolic testing in this study is lower than that of urine shedding cell examinations. This was different from previous study [12, 41] on a cohort of 384 individuals with bladder cancer and benign urologic and reproductive system diseases, where urinary cell metabolic testing demonstrated sensitivities and specificities of 90% and 88%, respectively. Benign control cases in that cohort were 60.68% (233/384), whereas in this study conducted at specialized tumor hospital, the proportion of confirmed UC cases was higher with only 20.95% (22/105) benign cases. This difference could contribute towards the lower specificity in this study compared to previous one.

Urinary shedding cytology as a medical detection method for cells in urine is useful for identifying abnormal cells in the urinary system (bladder, urethra, kidneys and ureters), which helps in diagnosing and monitoring UC and other diseases. This method relies on collecting urine samples and examining the cell characteristics under microscope. Therefore, it may require subjective interpretation because of the sample handling. It has advantages of non-invasiveness, simplicity and patient-friendliness. Studies have shown inadequate sensitivity of urinary shedding cytology despite its high specificity [42]. The sensitivity of urinary shedding cytology in a previous study was 38.57%, while the specificity reached 100% [43]. Herein, the sensitivity of four urinary shedding grouping criteria ranged from 22.89–73.49%, which highlighted its lower sensitivity. The highest sensitivity was seen in "Urine Exfoliation 4" positive diagnostic criteria which included finding malignant tumors, suspicious malignant tumors, not excluding malignant tumors, and atypical epithelial cells of indeterminate significance. Only the tumor cells absence was considered negative. Existing urinary shedding studies lack standardized criteria for benign and malignant results. Urologists based on the clinical experience consider results such as not excluding malignant tumors and atypical epithelial cells of indeterminate significance as benign lesions [44]. It is also reflected in this study's "Urine Exfoliation 2" grouping criteria for clinical diagnosis with sensitivity and specificity of 42.17% and 90.91%, respectively. Patients with excluding malignant tumors or atypical epithelial cells of indeterminate significance should thus be carefully evaluated via combined diagnostic methods or by integrating cytology with morphological analysis and cytokeratin-20 (CK-20) immunostaining to enhance the efficacy of urinary shedding cytology [45].

This study demonstrates the individual specificities of "Urine Exfoliation 4" and "Glucose metabolism 2" as 59.09%. However, the specificity of "Combined H1" increases to 95.45%. The individual sensitivities of "Urine Exfoliation 4" and "Glucose metabolism 2" are 73.49% and 82.56%, respectively, while that of "Combined H2" increases to 87.95%. Results from combined diagnosis indicate improved sensitivity and specificity. This integrated diagnostic has also been superior in diagnosing other cancer types [46]. The combined usage of urinary glycolysis testing and urinary shedding cytology provides comprehensive biological information and enhances the diagnostic accuracy through integrated analysis of this information.

The urinary tract UC tumor cells can be classified into low-grade and high-grade based on structural and cytological characteristics, according to the World Health Organization (WHO) classification of tumors of urinary system and male reproductive organs in 2022 [47]. High-grade urinary tract UC exhibits more invasiveness and poor prognosis compared to the low-grade UC [48]. This study reveals that the sensitivity of each urinary shedding group for diagnosing low-grade UC (3.45–58.62%) is lower than that for high-grade groups (30.61–81.63%). In contrast, glycolysis testing has high sensitivity for high-grade and low-grade UC (73.47–86.21%). This can be attributed to the lower nuclear heterogeneity and reduced nuclear division in low-grade UC cells [49, 50]. Urinary shedding cytology relies on capturing cells with heterogeneity and tumor characteristics [51]. The diagnostic potential of urinary shedding cytology is thus limited for low-grade UC patients [45], while urinary cell glycolysis testing has advantage regarding its high sensitivity.

Upper urinary tract UC originates from the malignant transformation of urothelial cells lining the renal collecting system or ureteral walls [52]. The standard surgery involves resection of kidney and ureter, including the bladder cuff excision [53, 54]. This has an impact on patient's renal function [55], which requires accurate preoperative diagnosis. The sensitivity of urinary shedding cell examination is from 31% to 60%, with sensitivity varying from 19% to 82%, and specificity from 86% to 100%. However, new biomarkers are unsuitable for clinical application because of low specificity and limited research data [56]. Urinary cell glycolysis testing and urinary shedding cytology should thus be combined with other examinations like urinary tract CTU for diagnosis.

This study provides a series of important findings, however there are certain limitations. Sample size is limited, and study scope is confined to the patients in specialized tumor hospitals, which results in insufficient controls from healthy populations. Future studies should thus expand the sample size and validate results in populations from multiple centers to enhance the universality and reliability of study findings. This study combines the urine glucose metabolism and urine cytology, however the diagnostic efficacy can still be improved. Combining imaging evaluations with other urinary biomarkers for diagnosis can further optimize the urinary cell glycolysis testing method.

5. Conclusions

Urinary cell glycolysis metabolism testing has the advantages of non-invasiveness, convenience, cost efficacy and higher sensitivity compared to urinary cell shedding examination. However, its specificity is lower compared to the urinary cell cytology. A high level of suspicion for male urothelial carcinoma is warranted to avoid missed diagnoses in male patients classified as high- or low-risk based on urinary cell sugar metabolism testing, as well as with urinary shedding results that do not exclude malignant tumor cells or show non-specific atypical cells. There is an increase in sensitivity, specificity and AUC value upon combining urinary cell sugar metabolism testing with urinary cell shedding examination for diagnosis, which improves the individual diagnosis efficiency. The urinary cell sugar metabolism testing depicts higher sensi-

tivity compared to urinary cell shedding examination in male patients with low-grade UC.

ABBREVIATIONS

AUC, Area Under Curve; UC, Urothelial Carcinoma; UTUC, Upper Tract Urothelial Carcinoma; BLCA, Bladder Carcinoma; NMIBC, Non Muscle Invasive Bladder Cancer; MIBC, Muscle Invasive Bladder Cancer; ROC, Receiver Operating Characteristic; CI, Confidence Interva; CK-20, cytokeratin-20.

AVAILABILITY OF DATA AND MATERIALS

The data presented in this study are available on reasonable request from the corresponding author.

AUTHOR CONTRIBUTIONS

SXZ—designed the research study. YYW—performed the research. ACY and ZGB—provided help and advice on the data analysis. YYW, HW and YPX—wrote the manuscript. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This study has been approved by ethics, with specific ethics approval numbers IRB-2023-1183 (IIT) and ethics approval committees (Medical Ethics Committee of Zhejiang Cancer Hospital). The patients provided informed consent and agreed to publication of the details of this research.

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CONFLICT OF INTEREST

The authors declare no conflict of interest. Yipeng Xu is serving as one of the Editorial Board members/Guest editors of this journal. We declare that Yipeng Xu had no involvement in the peer review of this article and has no access to information regarding its peer review. Full responsibility for the editorial process for this article was delegated to Biagio Barone.

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