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ORIGINAL ARTICLE

Diagnosis of urinary bladder urothelial carcinoma by immunocytology with p53, MCM5, MCM2 and Ki-67 antibodies using cell blocks derived from urine

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Abstract

Objective: Immunocytochemistry has attained a marginal role in urology so far. Combining the morphological and immunophenotypical changes of the urothelial cells retrieved from urine is a logical approach. The study aimed to analyse the diagnostic potential of immunocytological staining in the detection of high-grade and low-grade urothelial carcinoma.

Methods: Freshly voided urine was collected from 152 consecutive individuals, cytology classes were determined and cell blocks produced. A total of 77 patients were diagnosed with urothelial carcinoma and 75 patients had various benign urological conditions. Immunocytochemistry was performed using four antibodies: p53, MCM2, MCM5 and Ki-67. A diagnostic power to detect low grade and high-grade urothelial carcinoma was analysed for each antibody and their combinations with cytology.

Results: There were no significant differences between patients with low-grade tumours and control group. Antibodies p53 and Ki-67 slightly improved the sensitivity of urinary cytology while maintaining its specificity. The best negative predictive value was demonstrated in combinations of cytology and MCM5 (88.9%) and cytology, p53 and MCM5 (90.6%). In the diagnosis of high-grade tumours, all antibodies apart from MCM2 yielded better sensitivity and specificity than cytology alone (receiver operating characteristic curves: p53 = 0.853, MCM5 = 0.931, and Ki-67 = 0.895). Combined with cytology, the sensitivities went even higher for the cost of lower specificity. The best diagnostic performance was observed in the combination of MCM5 and Ki-67 (sensitivity = 96.2%; specificity = 80%).

Conclusions: Immunocytochemistry with p53, MCM5 and Ki-67 antibodies can improve the diagnostic power of urinary cytology in the detection and follow-up of urinary bladder urothelial carcinoma.

KEYWORDS

diagnosis, follow-up, immunocytochemistry, urinary bladder, urinary cell block, urinary cytology, urothelial carcinoma

1 | INTRODUCTION

Urothelial carcinoma (UC) is the most frequent histological type of urinary bladder cancer. In males, it is ranked the seventh most common cancer worldwide and that rank drops to the 11th when both sexes are taken into account. It represents a significant cause of morbidity and mortality worldwide, causing approximately 429 000 new cases resulting in 165 000 deaths annually.[1] Most of the patients are aged >65 years. Aggressive forms of cancer, with the ability of progression and dissemination, originate from muscle-invasive tumours. Seventy percent of the cases are diagnosed as non-muscle-invasive bladder cancer, which can be treated endoscopically with transurethral resection (TURB) followed by intravesical chemotherapy or Bacillus Calmette-Guérin immunotherapy.[2] Nevertheless, approximately 40% of these patients will progress to muscle-invasive disease during 5 years depending on pathological tumour features.[3] Early diagnosis of non-muscle invasive tumours, particularly those with unfavourable pathological features, is therefore of the utmost importance.

An ideal non-invasive marker of UC needs to be accurate and easy to perform using a cheap and fast methodology.[4] Over the last decades, numerous molecular markers have been studied, some of them with promising results. Nevertheless, none of the investigated markers has been included in the clinical guidelines owing to the low impact on the clinical decision-making.[5] Current gold standard in the detection and follow-up of UC is a combination of cystoscopy and urinary cytology (UCYT).[6] Cystoscopy is a subjective and invasive tool which can cause discomfort and complications to the patient. UCYT is a widely available urinary test with high accuracy in the detection of high-grade (HG) UC, which is used mostly as an adjunct method to cystoscopy.[6,7] However, its limitations lie in low sensitivity to detect low-grade (LG) UC and high intra- and interobserver variability among cytopathologists.[7,8]

Immunochemistry is an important adjunct of histopathology. Its combination with cytology, ie, immunocytochemistry (ICC; = immunocytology), has attained a marginal role in urology so far. A combination of cell morphology and immunophenotype is a logical approach. It has already become an established procedure in several benign diseases and malignancies other than UC.[9] There are several potential antibodies applicable for the diagnosis of UC. Immunocyt/uCyt is the only commercially available immunocytological assay; it is based on the detection of antigens by means of immunofluorescence. Its performance is good in reflex testing and as an adjunct to cytology in surveillance of patients with UC.[10,11] Compared to that, ICC is less demanding with respect to laboratory equipment and therefore it is more cost-effective. Based on a search in the literature, we have selected four antibodies and tested them as immunocytological stains with the use of cell blocks (CBs) derived from urine. Ki-67 is a nuclear protein that reflects cell proliferation. In urinary bladder cancer, expression of Ki-67 in the tissue correlated with poor cancer-specific survival and recurrence-free survival.[12] In urinary ICC, combined with cytology, Ki-67 and/or p53 increased the specificity without penalising the sensitivity.[13] MCM2 and MCM5 proteins are also essential for cell proliferation. There is evidence that ICC using MCM5 can improve the urinary detection of UC.[14] Immunoexpression of MCM2 in the tissue was an independent predictor of disease recurrence.[15] Tumour protein p53 is a tumour suppressor gene. Immunohistochemistry can indirectly detect its mutation in the majority of breast, lung, colon and urinary bladder neoplasms.[16] We designed a prospective single-institution study with the aim to investigate the potential of four selected antibodies to diagnose UC.

2 | METHODS

2.1 | Characteristics of the study population

In the period between June 2015 and August 2017, spontaneously voided urine was collected from 395 consecutive individuals treated at the Department of Urology, Teaching Hospital Motol, 2nd Faculty of Medicine, Prague, Czech Republic. The study was approved by the institutional review board and ethics committee of the same institution. Each subject signed an informed consent. A total of 152 samples (38.5%) provided a CB with adequate cellularity whereas those with inadequate cellularity (n = 243; 61.5%) were not included in the study (see Cell block preparation). Half of the patients enrolled into the study (n = 75) had a common benign urological condition (Control group), the rest (n = 77) underwent surgery for LG (n = 25) or HG (n = 52) UC of the urinary bladder (Patient group). Rates of adequate cellularity in particular groups were as follows: 75/230 (=32.6%) in Control group; 25/69 (=36.2%) in LG UC group and 52/96 (=54.2%) in HG UC group. To classify the tumour grade, WHO 2004 classification was used.[17] Patients with other malignancies and tumours of the upper urinary tract were not included. The control group consisted of individuals with urological conditions including stone disease, benign prostate hyperplasia, urethral stricture or papillary mucosal abnormalities of the urinary bladder with benign histology. Patients with an indwelling permanent catheter were not included in the study. In all individuals, white light cystoscopy and upper tract imaging (kidneys ultrasound or computed tomography urography if indicated) were performed. All subjects provided a fresh urine sample, avoiding the first-morning portion to minimise the risk of cytolysis. All urine samples were collected in special sterile containers with the minimal volume of approximately 30 ml and handled in the standard way to yield the best possible cell preservation.[18] The sample was stored for a maximum of 2 hours at 4°C and referred to the Department of Pathology and Molecular Medicine of the institution mentioned above, where the material was immediately processed. Cytospin (cytocentrifugation) and CB were prepared from each urine sample. Patients with UC underwent either radical cystectomy (for muscle-invasive or high-risk non-muscle-invasive bladder cancer) or TURB for primary and recurrent UC of the urinary bladder. Surgical specimens were processed and evaluated according to the standard pathology protocols and the final results were correlated with the previous cytological findings from urine samples.

2.2 | Cytospin preparation

Urine was centrifuged for 10 minutes at 1000 rpm using cell funnel (Cytospin 4; ThermoFisher Scientific). May-Grünwald and Giemsa-Romanowski stained the sediment on a slide after a short period of drying. Two cytospin slides were prepared from each urine sample.

2.3 | CB preparation

The plasma-thrombin method was used. Urine was centrifuged in tubes for 10 minutes at 3000 rpm. After the centrifugation has finished and the supernatant removed, blood plasma and thrombin were added to the sediment to form a cohesive pellet. The pellet was subsequently processed the same way as a tissue after fixation in 10% formalin. Sections from the paraffin CB were stained with haematoxylin-eosin and Giemsa.

Cell blocks and cytospin slides were independently evaluated by two pathologists trained in cytopathology (J.H. and P.S.). Urinary cytology was evaluated using the Paris System for Reporting Urinary Cytology 2016[8] and semiquantitative cellularity (limited to epithelial cells) of the CB was assessed in each sample. Epithelial cells were counted in 10 high power fields (HPFs, magnification 400×) in each CB slide and average cellularity was calculated. CBs with adequate cellularity (>5 cells per HPF, magnification 400×) were used for further immunocytochemical analysis. Cytological classes III (AUC = atypical urothelial cells), IV (suspicious for high grade urothelial carcinoma), V (HGUC) and VI (low grade urothelial neoplasia) were considered clinically positive whereas class II (negative for HGUC) was considered negative.

2.4 | Immunocytochemistry

Immunocytochemical staining was performed according to a standard protocol. Four-µm thick sections from selected CBs were deparaffinised, rehydrated and incubated in 3% peroxide solution for 10 minutes to block endogenous peroxidase activity. Antigen retrieval was performed by heating in pH 6.0 citrate buffer for 40 minutes. The sections were incubated overnight at 4°C with the following primary antibodies: Ki-67 (Dako, dilution 1:150), p53 (Dako, dilution 1:50), MCM-2 (Abcam, dilution 1:300), and MCM-5 (Abcam, dilution 1:100). The primary antibodies were detected by Mouse/Rabbit PolyDetector HRP with DAB (BioSB). Slides were counterstained with Harris's haematoxylin, dehydrated and mounted.

Immunocytochemical staining of Ki-67, p53, MCM2 and MCM5 antigens was evaluated in the epithelial cells (Figure 1). Nuclear positivity of medium and high intensity (ie, 2+ and 3+ on 0-3 scale) was considered as positive whereas no intensity (0) and mild intensity (1+) were considered as negative. Absolute numbers of positive cells within 10 consecutive high-power fields (at magnification 400×) were counted in all controls, LG tumours and HG tumours, and cut-off values were calculated for each antibody.

2.5 | Statistical analysis

The age was expressed as medians and 95% confidence intervals (CIs) of the medians. The Mann-Whitney test was used to compare the age between patients and controls. Categorical parameters with 2 × 2 values were compared using the Fisher's exact test (sex). Each marker was analysed by receiver operating characteristic analysis, separately for LG and HG UC, in order to determine Youden index (cut-off points).

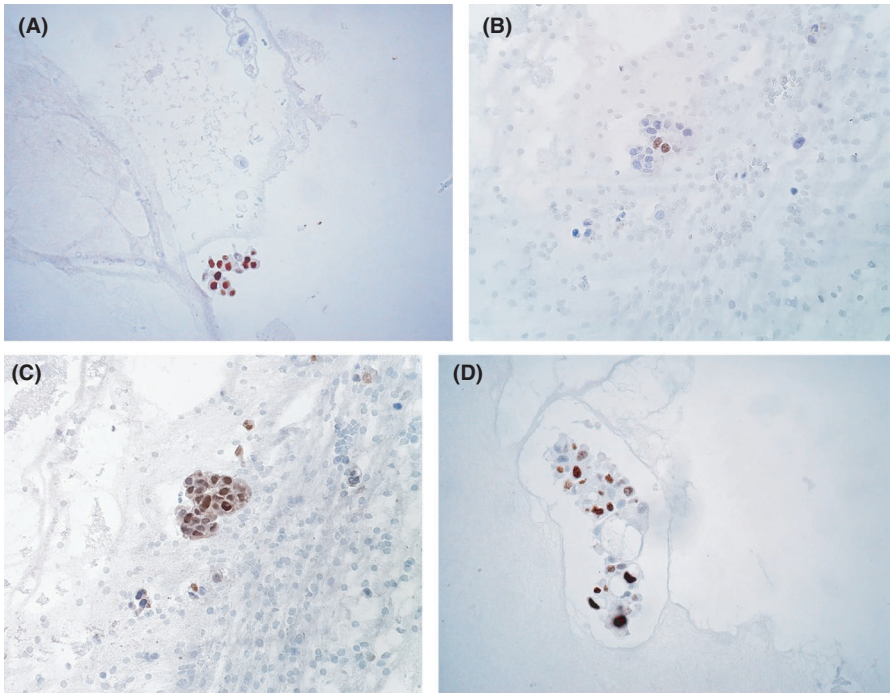

FIGURE 1 Illustrative images of immunocytochemical staining of high-grade urothelial carcinoma in cell block slides: p53 with 3+ intensity (A), MCM2 with 2+ intensity (B), MCM5 with 2+ and 3+ intensity (C), Ki-67 with 2+ and 3+ intensity (D) (magnification 400×)

TABLE 1 Characteristics of study population: age and sex

Parameter	Group	Sample size	Median	CI of median	*P*
Age	All	152	67	65-68.6	-
	Patient	77	68	66-70	.07
	Control	75	66	61-68	
Parameter		**Sample size**	**Patient**	**Control**	***P***
Sex	All	152	77 (50.7%)	75 (49.3%)	
	Female	56 (36.8%)	22 (28.6%)	34 (45.3%)	**.04**
	Male	96 (63.2%)	55 (71.4%)	41 (54.7%)	

The Youden index J was used for determination of the best combination of sensitivity and specificity and is defined as J = sensitivity + specificity - 1. Combinations of markers were created by a logical disjunction of particular markers used in the combination. In other words, only one positive marker was sufficient to give a positive result of the particular combination. For each marker and a combination, sensitivity and specificity were calculated and a comparison was performed by Fisher exact test for LG UC vs control and HG UC vs control groups, respectively. Moreover, negative predictive values (NPVs) were calculated for the follow-up of LG tumours. A *P*-value lower than 0.05 was considered as significant. Statistical analyses were performed in MedCalc Statistical Software version 17.9.6 (MedCalc Software bvba; http://www.medcalc.org; 2017).

3 | RESULTS

The median age of the Patient and Control groups was 68 and 66 years, respectively (*P* = .07). Female sex prevailed within the Control group (*P* = .04; Table 1). UCYT significantly discriminated Patients from Controls in both LG and HG tumours with the sensitivity of 40% and 69.2%, respectively. The specificity was 85.3%.

The average number (range) of positive cells in Control group was 0.7 (0-5); 0.2 (0-7); 0.4 (0-5) and 0.5 (0-6) with Ki-67, p53, MCM2 and MCM5, respectively.

The average number (range) of positive cells in LG UC group was 0.9 (0-5); 0.2 (0-3); 0.4 (0-5) and 2.3 (0-17) with Ki-67, p53, MCM2 and MCM5, respectively.

The average number (range) of positive cells in HG UC group was 8.4 (0-42); 12.7 (0-109); 2.8 (0-18) and 14.8 (0-104) with Ki-67, p53, MCM2 and MCM5, respectively.

Cut-off values to detect a disease with raw numbers of positive/negative results (including UCYT) are presented in Table 2. Immunochemistry using particular stains showed significant differences between the Control group and patients with HG UC. In the diagnosis of LG UC, only the combination of ICC and UCYT significantly discriminated Patient group and Control group except for the combination of UCYT and MCM2 (*P* = 0.55; Table 3, Figure 2).

3.1 | Low-grade tumours

The combination of UCYT with Ki-67 and p53 was able to keep a good specificity while improved the sensitivity of UCYT alone (Table 4). While the NPV of UCYT alone was 81% (Table 3), a combination of UCYT, MCM5 and p53 yielded a NPV of 90.6% (Table 4).

TABLE 2 Cut-off points (number of positive cells) for individual markers including urinary cytology (UCYT) at Control vs low-grade (LG)/high-grade (HG) urothelial carcinoma (Youden index) and raw numbers of positive/negative results

Marker	Result	Cut-off	LG	Control	Cut-off	HG	Control
UCYT	Positive	-	10	11	-	36	11
	Negative	-	15	64	-	16	64
p53	Positive	>0	3	5	>0	39	5
	Negative	=0	22	70	=0	13	70
MCM5	Positive	>0	12	23	>1	47	11
	Negative	=0	13	52	≤1	5	64
MCM2	Positive	=0[a]	21	58	>0	35	17
	Negative	>0	4	17	=0	17	58
Ki-67	Positive	>4	2	1	>1	44	13
	Negative	≤4	23	74	≤1	8	62

[a]There was no significant difference between Control group and LG tumours with MCM2 staining. Moreover, majority of LG tumours were negative using this stain (0 cells) thus cut-off value of 0 cells for positive result is irrelevant in this context.

3.2 | High-grade tumours

Apart from MCM2, all remaining antibodies had better sensitivity than UCYT while maintaining or even improving the specificity (Table 3). The sensitivities were 75%, 90.4%, 67.3% and 84.6% when applying p53, MCM5, MCM2 and Ki-67 antibodies, respectively. Individual receiver operating characteristic curves are depicted in Figure 3. All combinations with UCYT improved the sensitivity (76.9%-94.2%) of UCYT alone while maintaining an acceptable specificity (72%-80%; Table 3, Figure 4). The best results in multiple combinations with UCYT were obtained by MCM5 and p53 with the sensitivity and specificity of 96.2% and 73.3%, respectively. The best performance was observed when combining MCM5 and Ki-67 without UCYT with the sensitivity of 96.2% and the specificity of 80% (Table 5).

4 | DISCUSSION

Cystoscopy complemented with UCYT constitutes a gold standard in the diagnosis and follow-up of patients with UC. Besides UCYT, there is no generally accepted non-invasive urinary marker of UC that may potentially impact the clinical decision-making. The essential problem is a lack of standardisation of the methods, a lack of clinical validation and a lack of reproducibility on heterogeneous patient populations.[19] The specificity of UCYT has been historically high with a range of 83%-99% while its sensitivity depends on the grade of a tumour, reaching the median of 64% in grade 3 UC.[20] Reported sensitivity to detect LG UC drops as low as to 40%.[21] In the current study, the specificity of UCYT was lower than reported (85.3%) owing to the frequent occurrence of AUC and suspicious for high grade urothelial carcinoma classes among controls treated

TABLE 3 Sensitivity and specificity of urinary cytology (UCYT), single immunostains and their combinations[a]

	Low-grade UC vs Control				High-grade UC vs Control		
Marker(s)	Sensitivity (%)	Specificity (%)	NPV (%)	*P* value	Sensitivity (%)	Specificity (%)	*P* value
UCYT	40	85.3	81	.01	69.2	85.3	<.0001
p53	12	93.3	76.1	.4	75	93.3	<.0001
MCM5	48	69.3	80	.15	90.4	85.3	<.0001
MCM2	84	22.7	81	.58	67.3	77.3	<.0001
Ki-67	8	98.7	76.3	.15	84.6	82.7	<.0001
UCYT & p53	52	80	83.3	.004	86.5	80	<.0001
UCYT & MCM5	76	64	88.9	.0009	94.2	76	<.0001
UCYT & MCM2	88	18.7	82.4	.55	76.9	78.7	<.0001
UCYT & Ki-67	44	85.3	82.1	.004	92.3	72	<.0001

[a]Specificity differs between low-grade and high-grade tumours in those markers and combinations where cut-off points are different.
UC, urothelial carcinoma; NPV, negative predictive value

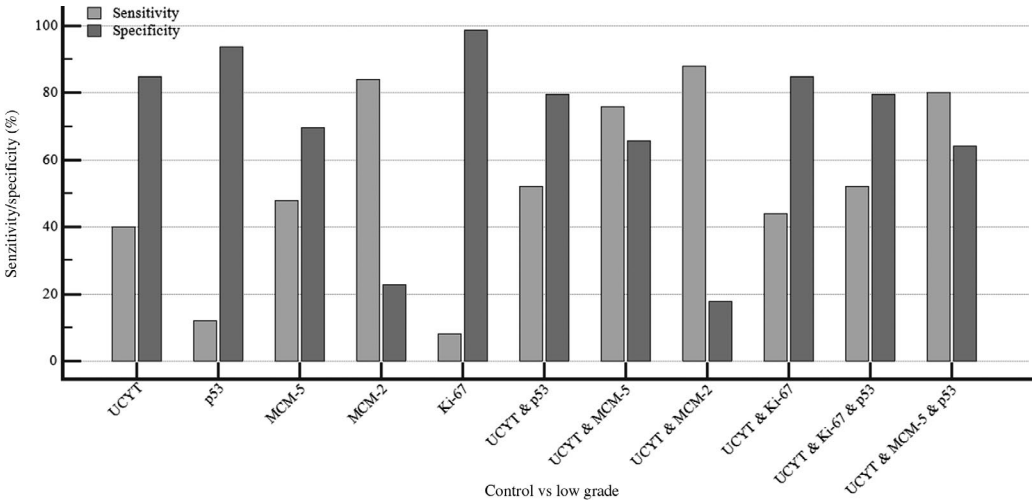

FIGURE 2 An overview of the sensitivities and specificities of immunostains to diagnose low-grade urothelial carcinoma (urinary cytology [UCYT] on the far left, continuing to the right with the individual antibodies and UCYT+antibody combinations)

TABLE 4 Multiple combinations at Control vs low-grade urothelial carcinoma (UC)

	Low-grade UC vs Control			
Marker(s)	Sensitivity (%)	Specificity (%)	NPV (%)	*P* value
UCYT & Ki-67 & p53	52	80	83.3	0.004
UCYT & MCM5 & p53	80	64	90.6	0.0002

NPV, negative predictive value

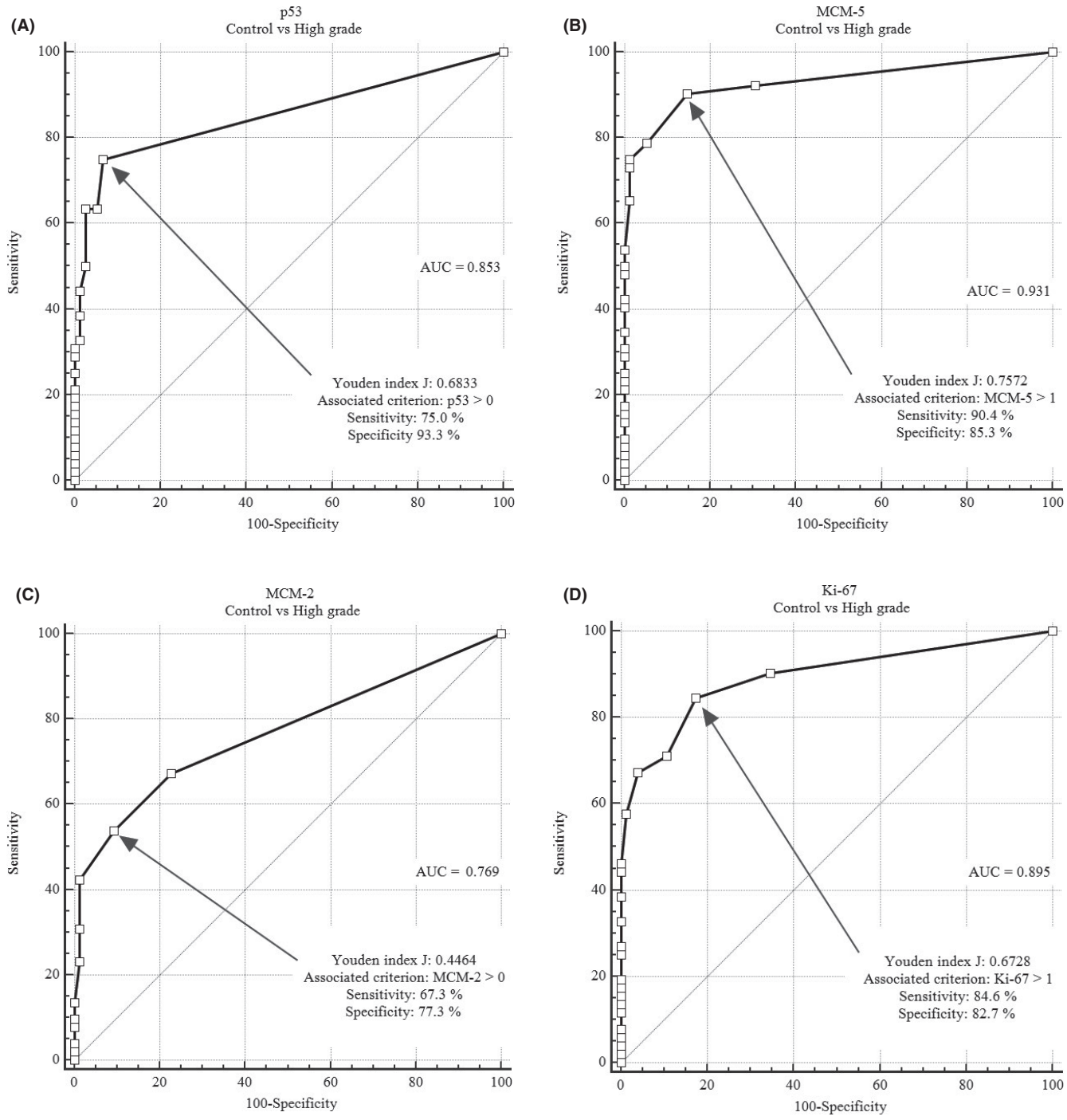

FIGURE 3 Receiver operating characteristic curves with the best sensitivity and specificity in a diagnosis of high-grade urothelial carcinoma using p53 (A), MCM5 (B), MCM2 (C) and Ki-67 (D)

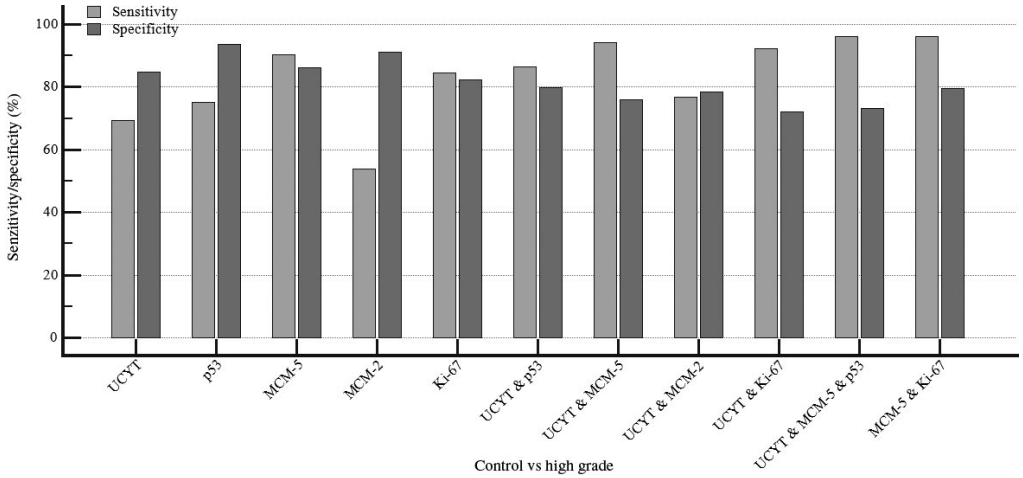

FIGURE 4 An overview of the sensitivities and specificities of immunostains to diagnose high-grade urothelial carcinoma (urinary cytology [UCYT] on the far left, continuing with the antibodies and UCYT+antibody combinations)

for stone disease with inserted ureteral catheters causing mucosal irritation.

Immunohistochemistry has been used as an adjunct to classic tissue histology for many years and its contribution to diagnosis, prognosis or prediction has been proven in many tumours. Many antibodies have been implemented as a standard adjunct to the histopathological analysis of urological specimens as well. A limited number of them have also been investigated as stains for ICC. Therefore, we designed a prospective study to investigate the value of four selected antibodies as an adjunct to traditional UCYT or a separate analysis using a methodology of urinary CBs.

Ki-67 is a nuclear protein that reflects cell proliferation. Antibody Ki-67 has been widely applied in histopathology.[22] It has been studied as a diagnostic and also as a prognostic marker in UC and other tumours, eg, head and neck squamous cell carcinoma linked to HPV infection,[23,24] including an immunocytological setting in UC.[13] Several authors reported that in bladder cancer, expression of Ki-67 in the tissue correlated with poor cancer-specific survival and recurrence-free survival.[12] Our results demonstrate the value of Ki-67 in ICC to detect UC. Although it slightly enhanced the diagnosis of LG UC (sensitivity of 44% when combined with UCYT), there was a significant improvement in the diagnosis of HG UC, especially in combination with MCM5 (sensitivity of 96.2%, specificity of 80%).

MCM2 and MCM5 (minichromosome maintenance complex) proteins of the pre-replication complex are also essential for cell proliferation. Anti MCM5 antibodies can improve the PAP smear test for precancerous lesions and carcinomas of the uterine cervix.[25] The combination of ICC with MCM5 and NMP22 improved the urinary detection of UC and identified 95% of clinically significant disease, ie, UC grade 3 or carcinoma in situ or UC stage ≥T1 with high specificity (72%, 95% CI = 69%-74%).[14] The levels of tissue MCM2 and MCM5 were significantly higher in HG ($P < .0001$), advanced-stage ($P = .001$), and non-papillary tumors ($P < .0001$).[26] As reported by Burger et al, during the follow-up of 71 patients with Ta/T1 UC, the grade and immunoexpression of MCM2 in the tissue were independent predictors of disease recurrence.[15] The efficacy of MCM2 in the diagnosis of both LG and HG UC has been shown to be inferior in our study. This result is not in contrast with Burger et al's study[15] where the markers were tested for the ability to predict recurrence and not to diagnose malignancy. MCM5 appeared to be the most effective in the diagnosis of both HG and LG UC regarding improving the sensitivity. However, the specificity in LG UC dropped to 64% when compared to UCYT alone (85.3%). By contraswt, its NPV was 88.9% (compared to 81% of UCYT alone) which is a solid figure for the follow-up of LG UC. In the diagnosis of HG UC, MCM5 worked well by outperforming the sensitivity of UCYT (90.4%), and when UCYT and MCM5 combined, the figure raised even to 94.2%. The combination with UCYT and p53 reached a sensitivity of 96.2%.

Tumour protein p53, also known as p53, is a group of protein isoforms encoded by homologous genes in various organisms, such as TP53 (humans) and Trp53 (mice). This homologue is crucial in multicellular organisms, where it prevents cancer formation thus functions as a tumour suppressor gene.[27] Mutations of p53 can be indirectly detected by immunohistochemistry in the majority of breast, lung, colon and bladder neoplasms.[16] Mutation of p53 was shown to be a predictive marker for the progression of urinary bladder UC.[28] Urinary

TABLE 5 Multiple combinations at Control vs high-grade urothelial carcinoma (UC)

	High grade UC vs Control		
Marker(s)	**Sensitivity (%)**	**Specificity (%)**	***P* value**
UCYT & MCM5 & p53	96.2	73.3	<0.0001
MCM5 & Ki-67	96.2	80	<0.0001

immunocytology for p53 and epidermal growth factor receptor was able to detect malignancy in 20 of 21 cytologically positive cases (out of 108 separate urine samples).[29] In the current study, p53 raised the sensitivity in the detection of LG UC from 40% to 52%, while maintaining satisfactory specificity. In HG UC, the sensitivity of UCYT was improved to 86.5% and even to 96.2% when combined with MCM5.

One of the main pitfalls of the ICC using CB can be its low efficacy in CBs with inadequate cellularity. In the current study, only 38.5% of urine samples provided CBs with moderate or high cellularity. In our previous research, the independent predictors of adequate cellularity were positive urine sediment, female sex, positive UCYT (PAP 3-5) and positive leucocyturia.[30] Due to these results, we proposed several clinical situations with the highest probabilities for an adequate CB cellularity.

So far, none of the investigated antibodies used for ICC has been validated on large clinical data sets and implemented into the standard cytopathological diagnostic algorithm. The reason for this maybe costly, technically demanding and non-standardised methodology. In this context, modern methods of liquid-based cytology were frequently used in the published studies. However, the methodology of CBs is widely available at ordinary cytopathological laboratories and, if standardised, it can fulfil the requirements to be a less expensive, easier and faster procedure.

To the best of our knowledge, the results from the current study represent the first evidence to demonstrate a clinical value of the ICC using p53, MCM5, MCM2 and Ki-67 antibodies in the detection of LG and HG UC in CBs. To summarise the overall results, none of the studied markers was able to increase the sensitivity of UCYT in the diagnosis of LG tumours significantly. However, missing an LG UC does not represent a serious clinical consequence in terms of the patient′s survival. Therefore, high NPV of immunocytology may add up a substantial benefit for the follow-up of LG UC by decreasing the number of an invasive procedure such as cystoscopy. The combination of UCYT, MCM5, and p53 gave the best result in this context (90.6%). Concerning HG UC, all of the studied markers increased the sensitivity of UCYT, reaching 96.2% by multiple combinations. Follow-up of HG tumours may therefore be a suitable clinical scenario for the use of these antibodies by ICC. Furthermore, since immunocytology detects changes in immunophenotype of urothelial cells which are closely linked to molecular changes, its role as a prognostic marker of recurrence of HG UC should be analysed and addressed by future studies. Reflex testing is another interesting clinical setting. Immunocytological evaluation of urine samples with indeterminate cytological results, ie, AUC class or PAP 3 in older PAP classification may be useful possible application. ICC may also affect an indication to early re-TURB after the resection of T1 HG UC. Early repeated resection means another invasive procedure in the urethra and urinary bladder in general anaesthesia, which is costly and bears the risk of acute or late complications. Evaluation of urine specimens by means of ICC shortly after resection of such tumours could bring interesting diagnostic, prognostic or predictive information.

There are certain limitations of our study. Age distribution between patients and controls differed, although not significantly (median of 68 and 66 years, respectively). UC is a disease of the higher age bracket. Bearing this in mind, a majority of consecutive patients with benign diseases aged over 50 years were accrued as controls. Furthermore, a higher prevalence of female individuals among controls also caused heterogeneity between the two groups. However, we were not able to affect the composition of both groups in this regard since there are objective differences in sex involvement between UC and benign urological diseases and patients were collected consecutively.

5 | CONCLUSION

We assessed the clinical value of ICC with p53, MCM2, MCM5 and Ki-67 antibodies separately and combined with UCYT to detect LG and HG UC. The highest NPV in the follow-up of LG UC was obtained by a combination of UCYT, MCM5 and p53. In the detection of HG UC, each antibody except MCM2 performed better than UCYT alone. When UCYT and an antibody were combined, each combination increased the sensitivity significantly while maintaining an acceptable specificity. The best diagnostic performance was achieved by a combination of UCYT with MCM5 and p53. A combination of MCM5 and Ki-67 without UCYT yielded even better specificity.

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CONFLICT OF INTEREST

The authors made no disclosures.

AUTHOR CONTRIBUTIONS

Antonín Brisuda, MD, has contributed with conceptualisation, resources, data curation, formal analysis, writing—original draft and project administration; Jaromír Háček, MD, has contributed with methodology, supervision, validation and writing—review and editing; Marcela Čechová, MD, has contributed with data curation, formal analysis, project administration and writing—review and editing; Petr Škapa, MD, PhD, has contributed with methodology, supervision, validation and writing—review and editing; Prof. Marek Babjuk, MD, PhD, has contributed with supervision and writing—review and editing.

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