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Clinical and cytopathological factors affecting the cellularity of urinary cell blocks and the implication for diagnosis and follow-up of urinary bladder urothelial carcinoma

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Objective: The methodology of cell blocks (CBs) has long been an integrated part of cytology. However, there are very few data on CBs derived from urine. Their main disadvantage is a lack of cellularity, which limits their broader clinical applicability. Factors affecting cellular adequacy in urine remain unclear. We assessed the impact of basic clinical and cytopathological factors on the adequacy of cellularity in urinary CBs.

Methods: Freshly voided urine was collected from 401 consecutive individuals. Of these, 167 patients were diagnosed with urothelial carcinoma. The remaining 234 patients had various benign urological conditions. Papanicolaou classes were determined and CBs produced. Cellular adequacy was assigned to each CB (acellular, hypocellular, moderate cellularity, high cellularity), and moderately and highly cellular CBs were considered as adequate. Several factors were analysed to find any correlation with the adequacy of the cellularity.

Results: In univariate analysis, seven factors significantly correlated with the adequacy of the CBs. In the multivariate model, positive sediment (OR = 3.7), female sex (OR = 2.7), positive urinary cytology (OR = 2.6) and positive leucocyturia (OR = 2.1) were independent predictors of adequate cellularity. Positive predictive value and negative predictive value of the model were 65.0% and 77.7%, respectively.

Conclusions: We determined four clinical and cytopathological factors which independently predict adequate cellularity in urinary CBs. Based on these results, several clinical situations have been proposed, in which the highest probability of adequate cellularity in urinary CBs can be achieved.

KEYWORDS
adequate cellularity, immunocytochemistry, urinary bladder, urinary cell block, urinary cytology, urothelial carcinoma

1 | INTRODUCTION

The urinary bladder is the most frequent site of origin of urothelial carcinoma (UC). It is the ninth most common cancer worldwide, the sixth most common cancer in the USA and represents the second most common genitourinary tumour.[1] It is a significant cause of morbidity and mortality worldwide, causing approximately 429 000 new cases resulting in 165 000 deaths annually.[2] Most of the patients are aged more than 65 years. Seventy percent of cases are diagnosed with non–muscle-invasive bladder cancer (NMIBC) with a favourable prognosis following transurethral resection (TURB) and intravesical chemotherapy or immunotherapy with Bacillus Calmette-Guérin.[3] Nevertheless, approximately 40% of these patients will progress to muscle-invasive disease during 5 years depending on histopathological features of the tumour.[4] Early diagnosis of NMIBC is therefore of utmost importance.

Current gold standard for the detection and surveillance of BC is a combination of cystoscopy and urinary cytology.[5] While cystoscopy remains a subjective and invasive tool that can cause discomfort and stress to the patient and can have potential side effects, urinary cytology is an easy, widely available noninvasive test with high specificity and high sensitivity in the detection of high grade UC (HG UC).[5] However, its low sensitivity in the detection of low grade UC (LG UC) and high inter- and intrapersonal variability represent a challenge.[6] Several systems for reporting of urinary cytology have been abandoned over time due to the absence of evidence-based data. In particular, abundance of indeterminate cytopathological diagnoses led to the decrease of its positive predictive value.[7] In recent years, an attempt to develop a standardised system has been observed.[8] However, there are still many controversies in exact definitions of cytopathological categories and general implementation of any guidelines to the clinical practice is missing.[9]

In recent decades, numerous molecular markers for the noninvasive detection of UC in urine samples have been studied, some of them with promising results.[10] Nevertheless, none of the investigated markers has been included into the clinical guidelines owing to low impact on clinical decision-making.[11] In general, an optimal marker should be more sensitive and specific, with easier, faster and cheaper methodology than the current standard.[12] A combination of cell morphology and immunochemistry—urinary immunocytochemistry—may have such a potential.[13]

There are two principal methods for the processing of urinary cytology samples: conventional cytospin method and modern liquid-based cytology (LBC). LBC was developed as an alternative to conventional methods to improve cell preservation. Comparing these two methods, conflicting results and opinions can be found with respect to the quality and quantity of the specimens.[14,15] Some authors still conclude that conventional methods remain more appropriate for the processing of urine samples and that modern form of LBC (eg, ThinPrep®), owing to its significantly higher cost, should be used essentially for cytology-based molecular studies.[16] Most of the published immunocytological studies used modern methods of LBC.[17-19]

In the current study, we implemented classical methods of urine cytospin and cell block (CB) production. CBs are cohesive cell pellets embedded in paraffin, processed the same way as tissue specimen by standard histological methods with haematoxylin-eosin staining. The main advantage of CBs compared to basic cytospin slides is a more efficient application of special stains including immunocytochemistry. However, there are several disadvantages of CBs, especially suboptimal cellularity, which was reported in up to 57% of fine needle aspiration cytologies.[20,21] Specifically in urinary CBs, the cellularity may be similarly low. As the adequate cellularity constitutes the critical factor for the interpretation of urinary cytology from CBs, we decided to correlate the cellularity of urine samples from patients with LG UC, HG UC and benign urological conditions with commonly available clinical and biochemical factors. On the basis of the results, we aimed to identify clinical scenarios in which the standard CBs technique may be capable of achieving sufficient cellularity and have the highest impact on the clinical decision-making.

2 | METHODS

2.1 | Characteristics of study population

In a period from June 2015 to August 2017, spontaneously voided urine was collected from 401 consecutive patients treated at the Department of Urology, Teaching Hospital Motol, 2nd Faculty of Medicine, Prague, Czech Republic. The study was approved by institutional review board and ethics committee of the same institution. Informed consent was signed by each subject. The majority of the patients had a common benign urological condition (benign group), the rest underwent surgery for LG or HG UC of the urinary bladder (cancer group). In all 401 individuals, white light cystoscopy and intravenous urography (or at least ultrasound of the kidneys) were performed. Patients with other malignancies and tumour of upper urinary tract were excluded. The benign group consisted of 234 individuals with urological conditions including stone disease, benign prostate hyperplasia, urethral stricture and papillary mucosal abnormalities of the urinary bladder with benign histology. Patients with UC underwent either radical cystectomy (40 patients; for muscle-invasive or high-risk NMIBC) or TURB for primary (93 patients) and recurrent (34 patients) UC of the urinary bladder.

Before surgery, all patients were asked to provide a fresh urine sample, avoiding the first-morning portion to minimise the rate of cytolysis. Patients with an indwelling permanent catheter were not included in the study. All urine samples were collected in special sterile containers with the minimal volume of approximately 50 mL and handled in the standard way to yield the best possible cell preservation.[22] A portion of 3 mL was analysed for urine culture, 10 mL was evaluated for the presence of erythrocytes (erythrocyturia), leucocytes (leucocyturia) and sediment (erythrocyturia or/and leucocyturia or/and other cells). The majority of the urine portion (at least 30 mL) was stored for the maximum of 2 hours in the fridge at 4°C and referred to the Department of Pathology and Molecular Medicine, Teaching Hospital Motol, 2nd Faculty of Medicine, Prague, Czech Republic, where the material was immediately processed. Cytospin (cytocentrifugation) and CB were prepared from each urine sample.

2.2 | Cytospin preparation

Urine was centrifuged for 10 minutes at 1000 rpm using a cell funnel (Cytospin 4; ThermoFisher Scientific, Waltham, Massachusetts, USA). The sediment on a slide, after short drying time, was stained with May-Grünwald and Giemsa-Romanowski. 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 centrifugation and the supernatant removed, blood plasma and thrombin were added to the sediment to form a cohesive pellet. The pellet was subsequently processed in the same way as tissue after fixation in 10% formalin.

Sections from the paraffin CB were stained by haematoxylin-eosin and Giemsa.

Cytospin and CB slides were independently evaluated by two pathologists trained in cytopathology (J.H. and P.S.). Urinary cytology was evaluated using classical Papanicolaou (PAP) terminology (classes 1-5) and semiquantitative cellularity (limited to epithelial cells) of CB was assessed in each sample. As previously reported, only classes PAP 4 and PAP 5 and samples suspicious for UC (PAP 3) were considered clinically positive.[6] Each CB slide comprised 10 high-power fields (HPFs) and an average cellularity was calculated. Cellularity (the quantity of epithelial cells) was defined by the consensus made between the cytopathologists as follows: acellular = no epithelial cells/HPF (magnification 400×), hypocellular = 1-5 epithelial cells/HPF, moderate cellularity = 6-20 epithelial cells/HPF and high cellularity = more than 20 epithelial cells/HPF (Figure 1). CBs with moderate and high cellularity were considered as *adequate*. Acellular and hypocellular CBs were considered as *inadequate*. All variables are summarised in Tables 1-3. All available clinical (age and sex), biochemical (sediment, erythrocyturia, leucocyturia) and cytopathological (presence of LG and HG UC, urinary cytology) parameters were tested for cellular adequacy in univariate analyses and factors with positive significance subsequently in multivariable analysis.

2.4 | Statistical analysis

The age was expressed as median and 95% confidence interval (CIs) of the medians. The two-sided Mann-Whitney test was used to compare the age between adequate and nonadequate cellularity groups as well as benign/cancer groups. Categorical parameters with 2×2 values were analysed using the Fisher's exact test. The chi-square test was used for comparison of categorical parameters with more than 2×2 values (tumour grade). The Fisher's exact test and the Bonferroni correction were performed for posthoc analysis in the tumour grade and cellularity comparison. The logistic regression was performed in the multivariate analysis. Only significant parameters in the logistic regression model were considered as the result of the multivariate analysis. A *P*-value less than 0.05 was considered statistically significant. Positive predictive values (PPV) and negative predictive values (NPV) were calculated for factors within univariate cellularity analysis as well as for logistic regression multivariate model. Odds ratio (OR) was used for the representation of the association between adequate and nonadequate cellularity groups in both univariate and multivariate analyses. Statistical analyses were performed in MedCalc Statistical Software version 17.9.6 (MedCalc Software bvba, Ostend, Belgium; http://www.medcalc.org; 2017).

TABLE 1 Characteristics of study population: age distribution

Parameter	Group	Sample size	Median	CI of median	*P*
Age	All	401	66	65-67	
	Cancer	167 41.6%	68	66-70	0.0002
	Benign	234 58.4%	64	62.5-66	

TABLE 2 Overview of clinical and cytopathological factors

Parameters	Sample size	Group/Result	Group size	Percentage (%)
Sex	401	Male	290	72.3
		Female	111	27.7
Cytology	393	Positive	110	28.0
		Negative	283	72.0
Erythrocyturia	391	Positive	190	48.6
		Negative	201	51.4
Leucocyturia	391	Positive	147	37.6
		Negative	244	62.4
Sediment	391	Positive	172	44.0
		Negative	219	56.0
Culture	389	Positive	72	18.5
		Negative	317	81.5
Cellularity	392	Adequate	152	38.8
		Inadequate	240	61.2
Group	401	Cancer	167	41.6
		Benign	234	58.4

TABLE 3 The cancer group in detail

Cancer group	Whole group size	Group size	Percentage (%)
Low grade	167	72	43.1
High grade		95	56.9

3 | RESULTS

Cytolysis occurred in seven urine samples (1.7%, three subjects from the benign group and four subjects from the cancer group). In the other two samples, only PAP class was assessed, and no CBs were available. An adequate cellularity was observed in 22 patients undergoing radical cystectomy (57.9%), where all patients presented with HG UC. Of the patients with primary tumours undergoing TURB, 38 had adequate cellularity in CBs (41.8%) and HG UC was found in 51.6% of these patients. Recurrent tumours, containing 26.5% of HG UC, yielded adequate cellularity of the CBs in only 36.4% of samples. In the benign group, adequate cellularity was found in 34.2%. Age did not correlate with adequate cellularity. Female sex, positive cytology, positive erythrocyturia, positive leucocyturia, positive culture, positive sediment and presence of HG UC significantly correlated with the cellularity of CBs. Individual PPVs, NPVs and tumour grade relations are listed in Tables 4-6, and Figure 2. Multivariate analysis demonstrated that female sex, positive cytology, positive leucocyturia and positive sediment were independent predictors of adequate cellularity of urinary CBs (Table 7). Odds values for individual factors are presented in Figure 3. PPV and NPV of the model were 65% and 77.7%, respectively.

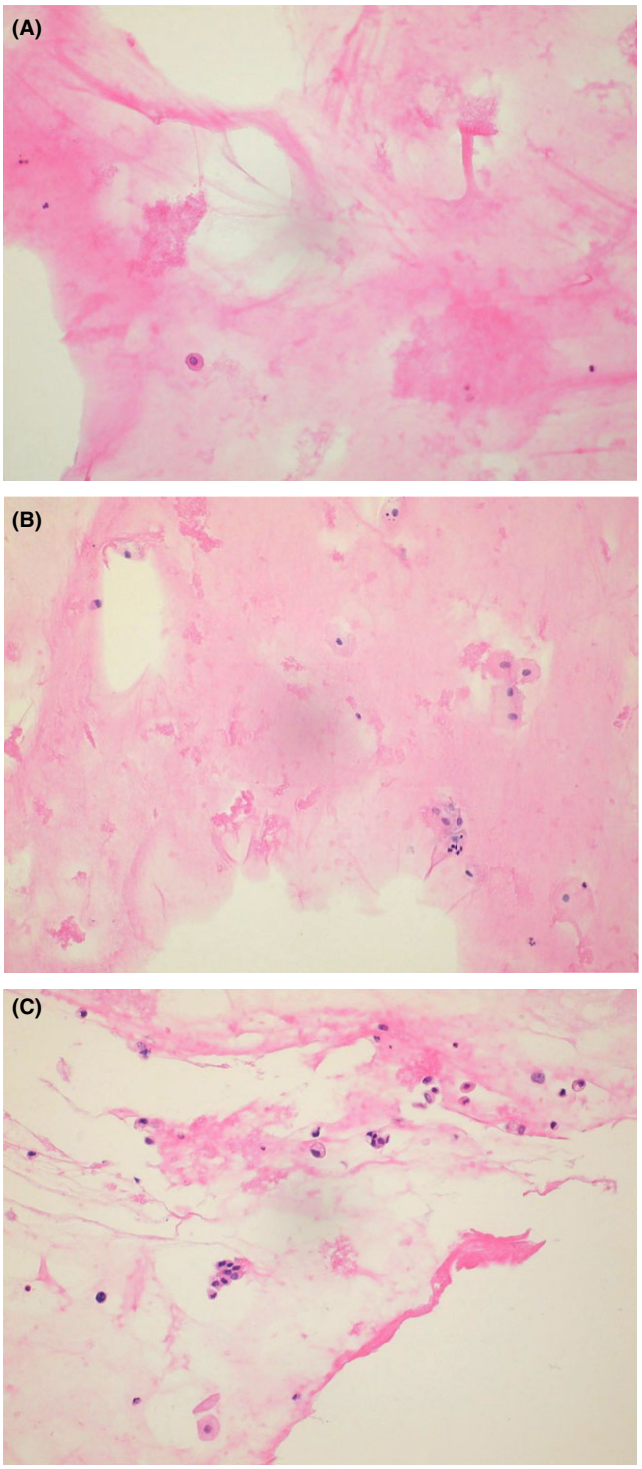

FIGURE 1 Illustrative images of the cell blocks with low (A), moderate (B) and high (C) cellularity (haematoxylin-eosin, 200×)

4 | DISCUSSION

Cystoscopy complemented with urinary cytology represents a widely used tool in diagnosis and follow-up of patients with UC. Apart from urinary cytology, there is no generally accepted urine marker.[23] Immunocytochemistry complements urinary cytology and may be promising in this regard. However, its use significantly depends on adequate cellularity of CBs. Most of the published studies used methods of modern LBC which yield good cellularity, although the results were not validated and translated into clinical practice.[19] The reasons might be a costly, technically and personally demanding methodology.[24]

By contrast, the technique of CBs is widely available at ordinary cytopathological laboratories and fulfils the criteria for a cheap, easy and fast methodology. CBs are commonly used for the evaluation of nongynaecological cytology specimens, but this technique is relatively uncommon in urinary cytology. Searching in the published literature, no relevant clinical or methodological information could be found. Adequate cellularity represents the main issue of CBs in the daily practice. There is no available evidence concerning nonmethodological factors which may affect the cellular adequacy of the CBs. Theoretically, decreased intercellular adherence due to neoplasia, inflammation or apoptosis may contribute to shedding of urothelial cells into urine.[25] However, it remains unclear whether these conditions would significantly affect the cellularity of urinary CBs. There are numerous methodological factors that can affect the cell adequacy in CBs, for example, the availability of rapid on-site evaluation, precise sample procurement, triage and sample loss while separating the supernatant from cells.[26]

In the present study, with the use of a simple and clearly described methodology, we tried to define the most common clinical and cytopathological factors that may affect cellular adequacy of urinary CB. We demonstrated significant impact of sex, cytopathological diagnosis, erythrocyturia, leucocyturia, urine sediment, culture and tumour grade on the adequacy of the urinary CB. When combining all these factors in a multivariate logistic regression analysis, female sex, positive urine cytology, positive urine leucocyturia and sediment predicted an adequate cellularity in urinary CBs. It is likely that the abundance of epithelial cells in the urinary CB among females results from the vulvovaginal contamination, although it may be difficult to distinguish squamous and urothelial cells by cytology and also some squamous cells may originate from the trigonal area of the urinary bladder. On the contrary, positive urine samples (PAP 3-5) tend to be more cellular because cancer cells are often shed in the urine due to deteriorated cell to cell adherence, which in turn leads to the adequate cellularity of the CBs. Positive leucocyturia and urine sediment refer to infection, inflammation or necrosis. These processes are responsible for the detachment of urothelial cells from intercellular connections.

There are certain limitations of our study. Age distribution between the benign group and the cancer group differed significantly (median of 68 and 64 years, respectively). UC of the urinary bladder is a disease of higher age. Bearing this in mind, the majority of consecutive patients with benign diagnoses aged over 50 years were accrued, and only 10% younger individuals were included. Despite that, the medians differed significantly, which in our opinion did not affect the results as age was not found to have a significant impact on the cellularity within the whole cohort (Table 4). Furthermore, in a separate multivariable analysis

TABLE 4 Age distribution with relation to cellularity

Parameter	Category	Sample size	Median	CI of median	*P*	Predictive values
Age	All	394	66	65-67		
	Adequate cellularity	152	67	65-68	0.21	
	Inadequate cellularity	240	66	64-68		

TABLE 5 Cellularity distributions in dependence on clinical and cytopathological factors

Parameter	Category	Sample size	Adequate cellularity	Inadequate cellularity	*P*	Predictive values
Sex	All	392	152	240	<0.0001	PPV = 55.0%
			38.8%	61.2%		
	Female	109	60	49		NPV = 67.5%
		27.8%	55.0%	45.0%		
	Male	283	92	191		OR = 2.54
		72.2%	32.5%	67.5%		
Cytology	All	391	152	239	0.0005	PPV = 52.7%
			38.9%	61.1%		
	Positive	110	58	52		NPV = 66.5%
		28.1%	52.7%	47.3%		
	Negative	281	94	187		OR = 2.22
		71.9%	33.5%	66.5%		
Erythrocyturia	All	382	148	234	<0.0001	PPV = 58.6%
			38.7%	61.3%		
	Positive	186	109	77		NPV = 80.1%
		48.7%	58.6%	41.4%		
	Negative	196	39	157		OR = 5.7
		51.3%	19.9%	80.1%		
Leucocyturia	All	382	148	234		PPV = 64.1%
			38.7%	61.3%		
	Positive	145	93	52	<0.0001	NPV = 76.8%
		38.0%	64.1%	35.9%		
	Negative	237	55	182		OR = 5.92
		62.0%	23.2%	76.8%		
Sediment	All	382	148	234	<0.0001	PPV = 57.2%
			38.7%	61.3%		
	Positive	215	123	92		NPV = 85.0%
		56.3%	57.2%	42.8%		
	Negative	167	25	142		OR = 7.59
		43.7%	15.0%	85.0%		
Culture	All	380	147	233	0.0004	PPV = 57.7%
			38.7%	61.3%		
	Positive	71	41	30		NPV = 65.7%
		18.7%	57.7%	42.3%		
	Negative	309	106	203		OR = 2.6
		81.3%	34.3%	65.7%		

which involved age and presence of UC (without other factors), age was not a significant predictor of adequate cellularity ($P = 0.45$).

Based on these results, we tried to outline several clinical situations, in which the chance to achieve the best cellular adequacy would be the most probable.

TABLE 6 Cellularity and tumour grade relation

Parameter	Category	Sample size	Adequate Cellularity	Inadequate Cellularity	*P*	Predictive values
Grade	All	392	152	240	0.059	
			38.9%	61.2%		
	LG	69	22	47		PPV = 53.8%
		17.6%	31.9%	68.1%		NPV = 73.2%
	HG	**93**	**50**	**43**	**0.003**	OR = 2.2
		23.7%	**53.8%**	**46.2%**		
	Benign	230	80	150		
		58.7%	34.8%	65.2%		

Values in bold are statistically significant ($P = 0.003$).

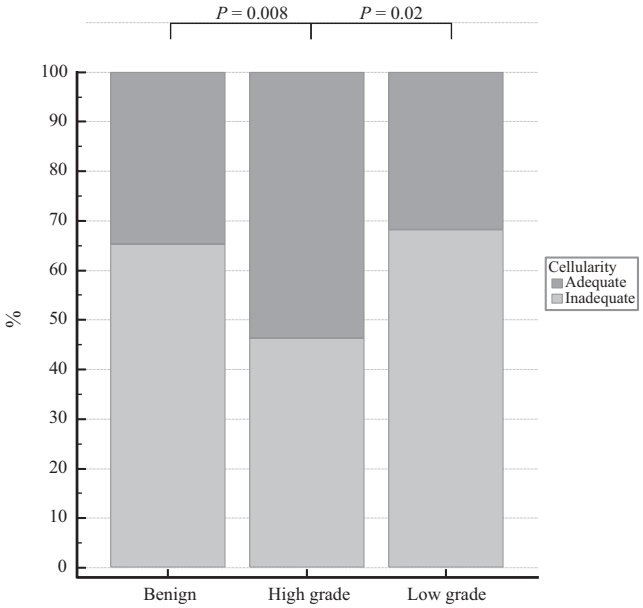

FIGURE 2 Pairwise comparison of tumour grade groups and benign group with respect to the cellularity of the cell blocks

4.1 | Screening

Early detection of UC of the urinary bladder obviously reduces cancer-specific mortality.[27] However, due to low tumour prevalence in general population, mass screening would not be cost-effective. Adoption of risk-adapted screening is still missing. Nevertheless, exposed patient populations (heavy smokers, professionally exposed etc.) with positive urine sediment or urinary cytology may potentially represent a patient subgroup that can be suitable for immunocytochemistry using urinary CBs.

4.2 | Follow-up of LG UC

There is a low risk of progression in these tumours.[28] Reliable urinary marker with high specificity may decrease the number of cystoscopies during surveillance. Cytology alone is inefficient in this setting.[6] Unfortunately, the majority of LG UC in our series recurred constantly as LG UC, which was followed by low cellularity of the CBs.

TABLE 7 Overview of logistic regression multivariate model parameters

Factor	*P*	Odds ratio	95% CI
Sex			
Female	0.0005	2.7	1.54-4.75
Cytology			
Positive	0.0007	2.6	1.49-4.52
Leucocyturia			
Positive	0.022	2.1	1.11-3.92
Sediment			
Positive	0.0002	3.7	1.88-7.32

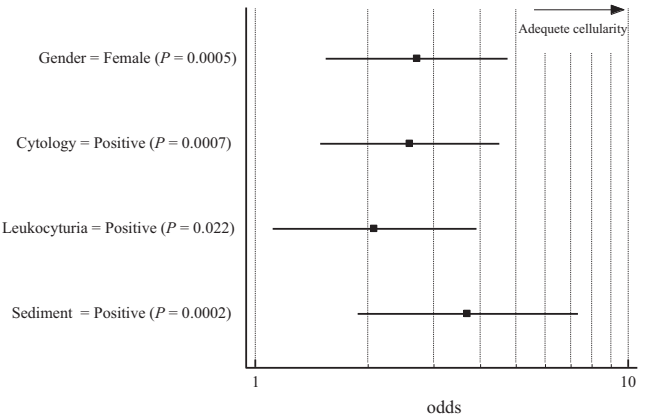

FIGURE 3 Forest plot for the adequate cellularity predictors based on logistic regression model

4.3 | Follow-up of HG UC

Sensitivity of urine markers is of utmost importance in this clinical scenario. Urinary cytology is very effective in this regard and complete diagnostic evaluation including endoscopy of the urinary bladder is therefore advocated in positive cases.[5] Nevertheless, immunocytochemistry may have a role as a prognostic marker of recurrence in this setting.

4.4 | Reflex testing

Immunocytochemistry may also help in evaluation of positive but indeterminate cytological results (PAP 3).

4.5 | Early reTURB

Early resection performed 2-6 weeks after TURB is recommended in all T1 tumours even with the presence of detrusor muscle in the specimen since T1 tumours belong to high-risk group following European Association of Urology risk group stratification.[23] However, using more complex scoring system and risk tables proposed by the European Organization for the Research and Treatment of Cancer, the risks of recurrence and progression differ when assessing other clinical and pathological factors, such as number of tumours, size, prior recurrence rate, concurrent carcinoma in situ and tumour grade.[28] Early repeated resection represents another invasive procedure that is costly and bears a risk of acute or late complications. Application of immunocytochemistry after resection of such tumours, when a significant number of different cells in sediment might be expected, could bring interesting diagnostic, prognostic or predictive information.

5 | CONCLUSIONS

In our study, we have determined four clinical and cytopathological factors (positive sediment, female sex, positive urinary cytology and positive leucocyturia), which significantly predicted adequate cellularity of the urinary CBs. To the best of our knowledge, this is the first attempt to demonstrate the diagnostic potential of this method and to determine the impact of common clinical and cytopathological factors on the adequacy of cellularity in urinary CBs. While low cellularity in CBs substantially limits their use, adequate cellularity may enhance their diagnostic value and enable the use of immunocytochemistry eventually. Thus, on the basis of our findings, we have proposed several clinical situations, in which the highest probability of adequate cellularity in urinary CB can be achieved.

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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—contributed with conceptualisation, resources 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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