Original paper

Clinical value of digital image analysis in the diagnosis of urinary bladder cancer, particularly in aggressive tumors: a preliminary report

Tomasz Borkowski[1], Anna monika Dulewicz[2], Andrzej Borkowski[1], Dariusz Piętka[2], Piotr Radziszewski[1]

[1]Department of Urology, Medical University of Warsaw, Warsaw, Poland
[2]Nałęcz Institute of Biocybernetics and Biomedical Engineering PAS, Warsaw, Poland

The aim of the project was to evaluate the clinical value of a computer analysis of cytological specimen images obtained from urine and bladder washing samples.
Three sample types (voided urine, catheterized urine and bladder washing) from 59 patients with primary or recurrent tumor were analyzed. All patients underwent cystoscopy and biopsy or resection. The histological results were compared with the results of the image analyzing computer system of collected urine samples. The consistency between the computer diagnosis and the clinical or histological diagnosis both in the presence and absence of cancer was as follows: 77% for voided urine samples, 72.5% for catheterized urine samples and 78% for bladder washing samples. The specificity of the method at the standard pathology level was 71%, and the sensitivity was 83%. The positive and negative predictive values (PPV and NPV) were 87.5% and 63% respectively. The sensitivity for G3 or CIS or T2 or T3 tumors reached nearly 100%.
Computer analysis of urine provided correct diagnoses in cancer and control patients with the sensitivity of 83% and specificity of 71% and gave excellent results in aggressive tumors such as T2, T3, G3 and in CIS.

Key words: urothelial carcinoma, cytology, digital image analysis.

Introduction

Bladder cancer is one of the most common malignant tumors of the genito-urinary organs, and it ranks 7th in incidence among all cancers in men and 17th in women. In Europe, the age-standardized incidence rate is 27/100,000 for men and 6/100,000 for women, whereas the age-standardized mortality rate is 8 and 3 per 100,000 for men and women, respectively; 75% of bladder cancers are limited to the epithelium and stroma and constitute superficial bladder cancers (non-muscle-invasive bladder cancers – NMIBC). Because of the limited progression potential, patients with NMIBC have a low cancer mortality rate (CSM; cancer-specific mortality).

Although the CSM in patients with cancers of the infiltrating muscle membrane (muscle-invasive bladder cancers – MIBC) is higher, the higher number of patients with NMIBC and their prolonged survival with high recurrence (RR, recurrence rate) causes the morbidity (prevalence) of bladder cancer to be the highest of all "urologic" cancers [1].

Cystoscopy and histological assessment following biopsy of small or flat suspicious lesions is the only accurate method for diagnosing bladder tumors before they grow sufficiently large to be visible as tumors on

ultrasonography or as filling defects in imaging studies using contrast medium (intravenous pyelogram – IVP and computed tomography). For patients with CIS (carcinoma *in situ*), cystoscopy is the best option for the diagnosis. Although cystoscopy can frequently be performed on an outpatient basis using a flexible cystoscope, given the high incidence and prevalence of bladder cancer, the burden on urological departments and national health systems is very high.

Even when performed using flexible instruments, cystoscopy remains an invasive test. Therefore, there have been numerous attempts to limit the number of performed cystoscopies, e.g., by screening groups of patients with elevated risk factors (smokers and patients who work in some industries) or by using control tests (monitoring) of patients after NMIBC resections.

Numerous urinary molecular marker tests has been proposed: UroVysion, microsatellite analysis, ImmunoCyt, nuclear matrix protein 22, BTA stat, BTA TRAK, and cytokeratins. In general, these approaches have a higher sensitivity but a lower specificity than the cytological urine test, and they show higher sensitivity for tumors with low-grade histological differentiation (high-grade tumors). The sensitivity and specificity depend on the clinical context; usually, the sensitivity is higher for detection of the primary tumor than for monitoring of recurrence. Some false-positive results can be verified by a subsequent recurrence and are undetectable during the first endoscopy (especially for UroVysion and microsatellite analysis) [2, 3]. There is a general consensus that these methods cannot replace cystoscopy for screening and diagnostic tests in patients with macroscopic hematuria. None of these techniques can replace cytology and cystoscopy to monitor recurrence in patients with high-risk NMIBC because monitoring of NMIBC with the above-mentioned techniques in low- and intermediate-risk patients still does not detect half of the tumors that are detected by cystoscopy [1, 4, 5, 6].

Cytological urine and bladder washing tests have high sensitivity for high-grade tumors and low sensitivity for low-grade tumors. The sensitivity in CIS cases is 28-100% [7]. Cytological tests are often negative in cases involving low-grade tumors. Cytological interpretation is user-dependent, and the specificity with extensive experience increases to 90% [5].

In various clinical situations, a combination of cytological tests with cystoscopy is the best option to limit the rate of diagnostic errors. However, cytological tests by experienced cytopathologists are not available everywhere, making these tests difficult and expensive.

Therefore, in this paper, we present the initial results for a computer method for early detection of bladder cancer using a non-invasive measurement technique based on material collected from urine and bladder washes.

Further studies will determine whether our method is reliable enough to reduce the number of required cystoscopies and classic cytological examination.

Material and methods

In this study we tested the ability of digital image processing and analysis to diagnose the presence of bladder cancer based on an analysis of urine and bladder washing samples.

After signing informed consent, urine from 87 subjects was collected. As not all preparations obtained from the collected samples were suitable for the final evaluation and computer analysis, samples from only 59 patients, including 45 men and 14 women (at the age 47-87 years, average 70.9, SD 10.79) were used for the analysis.

The tested group in this study consisted of patients with a clinical diagnosis of primary bladder tumor that was not histologically confirmed and patients with remission after a previous transurethral resection and cancer patients.

Among those 59 patients, 17 had no cancer (3 in which a clinical diagnosis of primary bladder tumor was not confirmed and 14 with remission after previous transurethral resection). The 42 remaining patients with histologically diagnosed cancer were classified as follows: 10 G1 (9 Ta, 1pTx), 28 G2 (16 pTa, 9 pT1, 3 pT2), 3 G3 (2 pT2, 1 pT3a), and 1 CIS. Histological classification was performed according to the WHO 1973 grading system of bladder urothelial carcinomas, which was formally in force until 1998.

Eighteen samples of voided urine from healthy volunteers were additionally tested as a training set and were not taken into account in the summary of results.

Urine samples were collected from voided and catheterized urine from all patients. The samples were collected from the second or subsequent voiding, then the patients were catheterized, and the samples from catheterized urine were collected.

Similarly, we tried to take bladder wash samples from all patients, but this was not always possible.

All 50 ml samples were prefixed after collection for 24 hours. The prefix solution doubled the sample volume and was composed of 100% ethanol, PEG 300 (Merck, Poland) and distilled water. Then, the mixtures were centrifuged at 2000 g for 10 minutes. The supernatant was removed, and 500 μl of prefix solution was added to rinse the pellets. Then, 200 μl of the mixture was placed in a Shandon Cytospin 4 system (Thermo Electron Corporation, United Kingdom) and centrifuged for 6 minutes at 1000 g to separate and deposit a monolayer of cells on microscopic slides. Then, the slides were dried for approximately 5 minutes at room temperature and finally

fixed using the Bohm fixation method [8], followed by purification by repeated immersion in 100%, 96%, 70%, 50% ethanol solutions and distilled water. Staining was performed with the Feulgen method [9] using 5 N HCL for one hour at room temperature followed by treatment with Schiff's reagent (Merck, Poland).

The stained preparations were then scanned and analyzed using our image processing computer system consisting of an IBM/PC computer and a digital Coolscope microscope (Nikon, Japan). The scanned images of the preparations were transmitted to the computer system. The size of the recorded images was 480 × 640 pixels (8 bits). In most cases, a single captured image contained between a few and a few hundred nuclei. The objective magnification was 20 : 1.

Image analysis was performed using a digital image processing system (CytoAnalyzer) designed by the authors as a software package equipped with specialized procedures [10, 11, 12] to scan microscope slides and perform urinary and washing sediment analysis [10, 11]. The cytospin technique was used to obtain the concentration of sediment on a small area of the microscopic slides, which significantly shortened the time required for scanning microscopic preparation.

Description of the system

The tested system, which in addition to the computer contains the digital microscope, had at the time of the measurements a fixed set of scanning parameters (size of the magnification, lens aperture and brightness of the image).

The CytoAnalyzer application implemented in the system is equipped with a procedure for standardization of the brightness of the image that runs prior to analysis of each measuring scanned frame image.

The procedures of image processing and analysis were developed and described in detail in our previous works [10, 11, 12, 13]. Now we would like only to mention the main ideas of our approach.

In the first stage, the CytoAnalyzer software performs preliminary image preprocessing comprising image background correction and normalization, which are essential procedures for correct comparison of objects derived from different images.

The method of image **background correction** in our software was created based on a two-dimensional correction matrix and made out of 10 images of an "empty field" derived from different places of the analyzed specimen.

The next step of image processing, **normalization of the images analyzed**, was achieved in such a way that that the most frequent pixel light intensity in the background gained saturation (maximum light intensity). This was achieved by multiplying all the pixels in the image by a coefficient calculated for each image based on a histogram of light intensity distribution.

The next processing stage, **extraction of objects**, was also based on the light distribution histogram of the image. The minimum of the histogram of light intensity level was taken as a threshold value and used for performing the thresholding operation.

The subsequent step consisted of **measurement of parameters** of the defined features describing objects in analyzed images as unequivocally as possible.

At the beginning the system was built and tested on the basis of the material from bladder washings supplied in the form of cytospins from the Netherlands (LCP laboratory). Then the first clinical test was performed from bladder washings of Polish patients.

When selecting the features and developing a method of analysis we focused on well-known processes occurring in cell nuclei in the neoplastic process, such as:

- an increased number of large cell nuclei in images of cancer patients (Fig. 1),

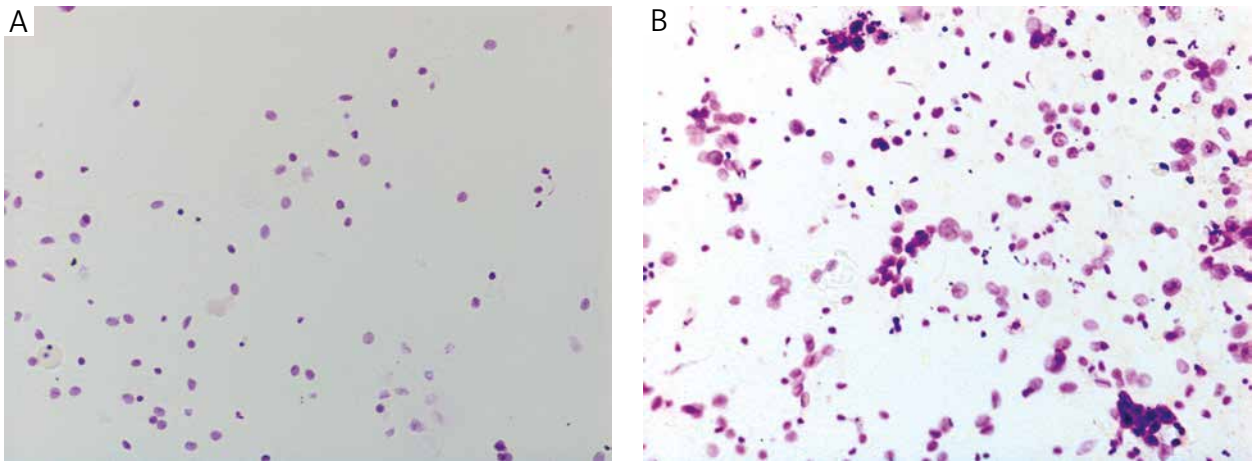

Fig. 1. The typical example of normal nuclei (A), and the typical example of nuclei of a cancer patient (B)

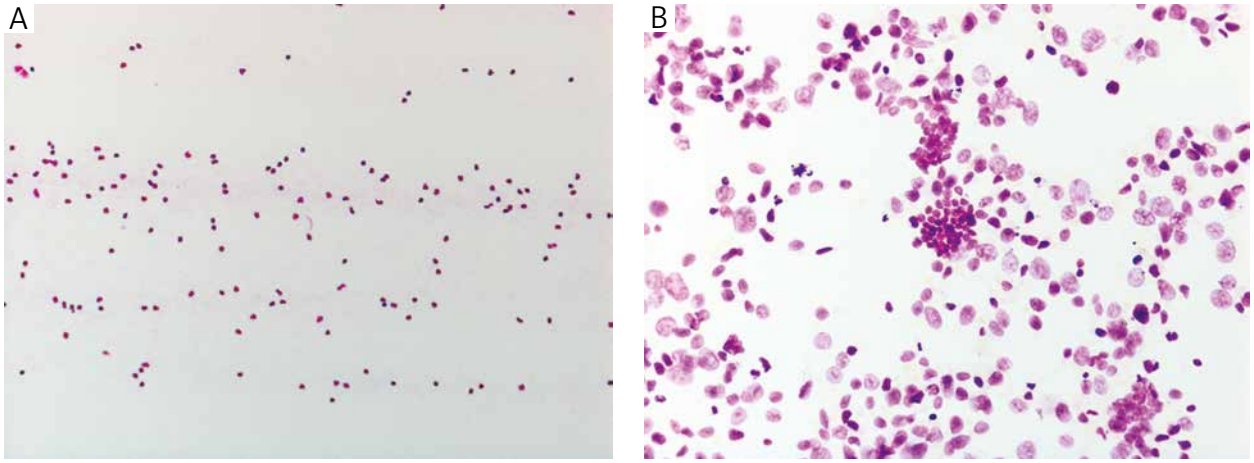

Fig. 2. The increased exfoliation of epithelial cells in the image of a cancer patient (B) than in the image of a healthy patient (A)

- increased exfoliation of cancerous epithelial cells relative to normal cells (Fig. 2),
- relatively large difference in the size and structure of nuclei clusters in the images of preparations from cancer patients (Fig. 3).

All chosen features were defined on the basis of a histogram of object size distribution in a population of cell nuclei. In total 7 features were defined. Five of them were defined on the basis of object size distribution in defined ranges of pixel size. The sixth was a measure of the ratio of the number of nuclei clusters to the total number of individual nuclei and granulocytes in the preparation. The seventh feature was the percentage of granulocytes in relation to the number of all objects.

On the basis of the defined features a multistage classification algorithm (in the shape of a decision tree) was designed and implemented in CytoAnalyzer application.

Results

In total, the sensitivity of the tested method based on all three types of samples was 83% (35/42), and the specificity of the method was 71% (12/17).

Sensitivity and specificity of the test was dependent on the type of sample to be analyzed:

- the **specificity** of the voided urine analysis was 75%,
- the **sensitivity** of the voided urine analysis was 75%,
- the **specificity** of the catheterized urine analysis was 61%,
- the **sensitivity** of the catheterized urine analysis was 78%,
- the **specificity** of the bladder washing analysis was 57%,
- the **sensitivity** of the bladder washing analysis was 89%,

Computer analysis provided correct diagnoses in cancer and non-cancer patients in 77% of the prepa-

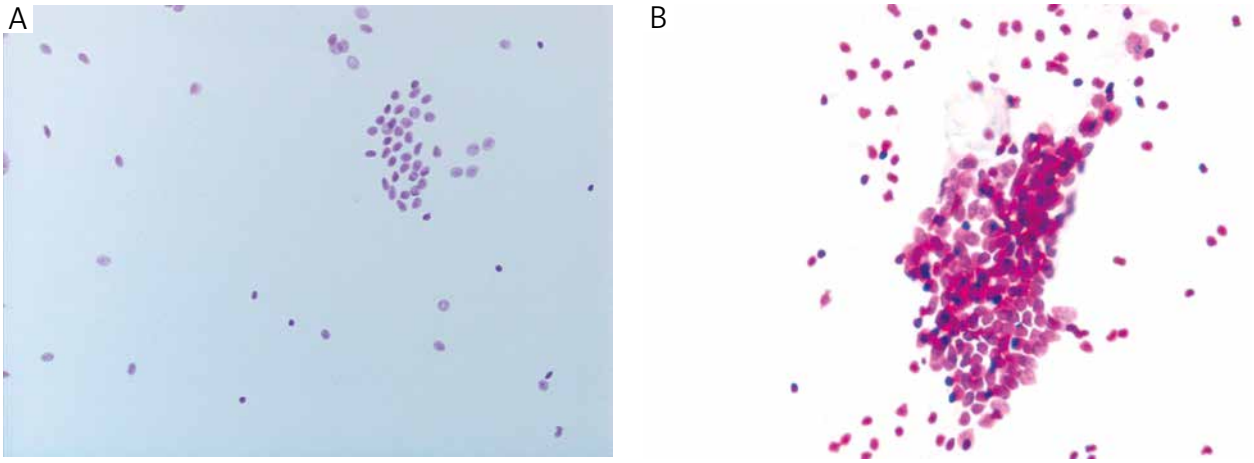

Fig. 3. The typical example of a cluster of normal cell nuclei (A), and the typical example of a cluster of cancer nuclei (B)

Table I. Comparison of the results of histological assessment with the results of the computer analysis based on all types of samples

Patient group	Histological diagnosis	Computer diagnosis	Number of incompatible diagnoses
Patients with cancer	42	35	7
Patients without cancer	17	12	5

Table II. Accordance of histological and computer diagnoses on the basis of the type of samples for all grades of cancer histological malignancy

	Voided urine samples	Catheterized urine samples	Bladder wash samples	All three types of samples
Patients with a G1 grade tumor	67%	60%	75%	70%
Patients with a G2 grade tumor	75%	78%	90%	86%
Patients with a G3 grade tumor	100%	100%	100%	100%
Patients with a CIS tumor	–	100%	100%	100%

Table III. Accordance of histological and computer diagnoses on the basis of three type of samples for non-cancer patients

Patient group	Voided urine samples	Catheterized urine samples	Bladder wash samples	Of all three types of samples
Without primary bladder tumor	50%	100%	100%	67%
Without recurrent bladder tumors	80%	54%	50%	64%

Table IV. Relationship between stage of tumor and correct computer diagnosis in both primary and recurrent tumors from different types of preparation

Type of preparation	Type of tumor	Detection of all grades of tumor malignancy in respective stages				
		Ta (n)	T1 (n)	T2 (n)	T3 (n)	CIS (n)
Voided urine	primary	67% (3)	75% (4)	100% (1)	100% (1)	–
	recurrent	75% (12)	75% (4)	100% (3)	–	–
Catheterized urine	primary	0% (2)	75% (4)	100% (1)	100% (1)	–
	recurrent	77% (13)	100% (3)	100% (3)	–	100% (1)
Bladder washing	primary	80% (5)	100% (4)	–	100% (1)	–
	recurrent	90% (10)	100% (4)	67% (3)*	–	100% (1)

rations from voided urine, 72.5% of the preparations from catheterized urine and 78% of the preparations from bladder washes. Table I shows a comparison of the results of histological assessment with the results of the computer analysis, based on all types of samples.

The accordance of computer analysis and histopathologically confirmed diagnosis of bladder tumors varied depending on histological grading. It was higher in G3 and CIS tumors and lower in G1 and G2 lesions (Table II).

In 10 patients **with G1 tumors** (9 pTa, 1 pTx), 7 were diagnosed with cancer: 4 grade G1 and 3 G2. In the 3 remaining cases, cancer was not diagnosed.

One patient had a primary tumor, 9 had recurrent tumors. The primary tumor case (Ta) was not recognized either in voided urine or in bladder wash preparations.

In total, 28 patients **with G2** (16 pTa, 9 pT1, 3 pT2) **tumors** were examined: 10 of them had primary tumors and 18 had recurrent tumors. In the

pTa and pT1 stages, the **voided urine** computer analysis was accurate in **80%** (4/5 analyzed) of primary tumors and in 67% of recurrent tumors (8/12 analyzed). The computer analysis was correct in 100% (3/3) of pT2 tumors. With **catheterized urine**, the computer analysis was correct in 60% (3/5) of primary tumors and 82% (9/11) of recurrent tumors in stages pTa and pT1 and in 100% (2/2) of stage pT2 tumors. In the pTa and pT1 stages, the computer analysis of **bladder washes** was correct for all analyzed primary tumors and in recurrent tumors in 90% of pTa (7/8 analyzed) and 91% of pT1 (10/11 analyzed).

In all **G3** (3 patients) and **CIS** (1 case) cases, all preparations of both primary and recurrent tumors were correctly diagnosed by the computer analysis. In the bladder washing analysis, these tumors were recognized as G3.

The **positive predictive value and negative predictive value of the computer analysis were 87.5% and 63% respectively. The sensitivity for aggressive tumors G3 and CIS and T2 and T3 was very high, approaching almost 100%.** In the pT2 stage, 1 of 4 tumors was not recognized correctly because of extensive inflammation.

Computer analysis of urine samples produced 5 false positive results (5/17). One in three patients had unconfirmed primary bladder tumor and there were 4 cases of recurrent bladder tumor.

Detailed comparative results for patients **without** primary or recurrent bladder tumors are presented in Table III.

The relationship between **the stage of the tumor** and the correct computer diagnosis in both primary and recurrent tumors from different types of preparations is shown in Table IV. Best compatibility occurred for T2, T3 and CIS cases both for primary and recurrent tumors regardless of the type of preparation.

A 100% correct diagnosis rate was achieved for samples from the voided urine of healthy volunteers.

Discussion

In the study we analyzed the clinical value of a computer-aided diagnostic system for bladder cancer. There have been many studies on this topic [14, 15, 16, 17, 18, 19, 20, 21, 22, 23], which in general focused on the identification and analysis of individual cancer cells. The problem, however, proved to be the precise identification of all of the cells in the preparation. Our approach was not to classify individual nuclei into specific types but rather to classify each entire specimen into diagnostic categories to prevent erroneous classification resulting from faulty diagnoses of individual components (nuclei).

Classification into various grades of malignancy was based on our multilevel algorithm and performed using all components contained in the preparation. The preparations were classified into the particular malignancy grades used in histological evaluations. Our approach of classifying the entire preparation made it possible to avoid erroneous classification of the preparations resulting from faulty diagnoses of individual components (nuclei). In addition, similar results were obtained in the diagnosis of high-grade cases in previous studies [14, 15, 24].

Papanicolaou staining is commonly used for the evaluation of cancer cells [14, 19, 25, 26]. We used the Feulgen method of staining to avoid problems with double extraction of cytoplasm and nuclei from the image. As in our previous works [10, 11, 12, 13] focused on the development of image analysis and carried out on ready preparations (prepared in the Netherlands), also this time it was assumed that the information contained in the nuclei of the cells was sufficient to detect cancer. The nuclei in the analyzed preparations were subjected to multi-level classification. At every level of the decision tree, another feature of the cell nuclei size distribution histogram was analyzed. The analyzed characteristics were quantified not in absolute terms but rather in relative terms or as a percentage of the fixed ranges in the histogram or in relation to all components (or clusters of nuclei) in the preparation. Similar to previous studies, no attempt was made to identify abnormal or cancerous cell nuclei.

Our main goal was to detect cancer and, in the next step, to determine the grade of histological malignancy.

However, taking into account that images of preparations obtained from samples of voided urine, catheterized urine and bladder washes differ in terms of the observed number of nuclei and background, in which urinary crystals and traces of mucus may be present or absent, the classification algorithms for each of these sample types required adaptation. Hence, different grading procedures were developed for voided and catheterized urine and bladder washes.

The correct diagnosis in patients with necrosis and inflammation of the bladder but with an unconfirmed clinical diagnosis of primary bladder tumors was obtained from all types of samples (voided and catheterized urine and bladder washes). In the case of inflammation with purulent infiltration, the correct diagnosis was obtained only from samples of voided and catheterized urine. In the case of hemorrhagic cystitis, the correct diagnosis was obtained only from a sample of catheterized urine. In summary, a correct diagnosis rate of 67% was achieved based on the analysis of all three sample types.

For computer analysis of patients with no recurrence after previous resection of a bladder tumor, the

bladder wall appearance in these patients is no longer similar to that in a healthy person. Consequently, it would be necessary to test a larger number of cases and appropriately modify the classifying algorithm. It is possible that in these patients, the computer cytological examination would be more meaningful than histological examination, which is exclusively limited to the biopsy of only noticed suspicious bladder wall fragments obtained during cystoscopy, while the computer examination is based on the analysis of exfoliated nuclei of the entire bladder. However, the validity of this approach can only be confirmed by prolonged observation of treated patients, e.g., by observing recurrence, which was not possible in this study.

The most accurate diagnosis of patients was based on the simultaneous use of the three types of samples: voided, catheterized urine and bladder washes.

70% compliance in diagnosis was achieved for the G1 cases. All cases of recurrent tumors, except two, were diagnosed as cancerous, and in 1 case of a primary tumor, the computer analysis was incorrect for both voided urine and bladder washes.

For G2 grade a compliance rate of 86% was achieved. At least two preparations were examined from each sample. However, not all preparations were suitable for analysis because they were technically poor or did not contain nuclei; these preparations were omitted.

The most reliable test for this group of cancer patients was that based on preparations obtained from bladder washes (90% correct diagnosis rate), whereas the number of correct diagnoses from urine was lower. From the voided and catheterized urine, correct diagnosis rates of 75% and 78% were achieved, respectively. A substantial correlation between the stage of the tumor and the correct diagnosis rate using the computer technique was not observed. Ta and T1 cases of this grade of malignancy were detected based on a urine test with correlations at the level of 73%, whereas with the bladder washes, Ta and T1 cases were correctly detected with an accordance of 89%. The cases in stage T2 were recognized correctly in all tests, except for one case from a bladder washing sample. The best result, 100% cancer detection, was achieved for stages T2 and T3 as well as for CIS cases on the basis of urine and wash samples.

In addition, a 100% correct diagnosis rate was achieved for samples from the voided urine of healthy volunteers. It is worth mentioning that sensitivity of the visual cytology is around 60%.

Obtaining good quality of urine samples was the biggest challenge in this study. Most of the technically poor preparations were obtained from samples of voided urine, whereas most of the bladder wash samples were correct. The sampling, fixation, transportation and specimen preparation methods were very important for the quality of the preparation. We are concerned that imprecise patient compliance with the sampling rules will always constitute a problem. Therefore, the sampling rules must be fully developed, simplified and standardized to enable the application of these tests in routine clinical trials and screening, given their particular value as non-invasive, low-cost and feasible methods that can be performed with any frequency during therapy.

The lack of full consistency between the computer analysis results and the histological diagnosis of malignancy grades may have resulted from the inadequate quality of the preparations or samples. However, it is also possible that our analysis, which was based on a few thousand nuclei, may have actually been more reliable than a histological analysis based on an analysis of only locally collected samples of tissue. In this case, the lack of consistency of our results with the standard method could actually indicate greater sensitivity of our technique.

As mentioned above, appropriate sampling for testing and careful handling of the preparations in accordance with the developed procedure are important for obtaining the correct computer diagnosis. The lack of a sufficient number of exfoliated epithelial cells in the sample and the presence of impurities (urate crystals and mucus) impede the computer analysis or make it unreliable or inaccurate. Therefore, in the future, for absolutely reliable diagnosis, several preparations obtained from patient samples should be examined.

Given that the images obtained from samples of bladder washes and voided and catheterized urine differed in terms of the observed number of nuclei and the presence or absence of urate crystals and mucus, the classification algorithms for each of these groups had to be adjusted accordingly. These developed algorithms that were adapted to the specifics of the analyzed images have been implemented in the computer system. Moreover, it should be emphasized that the process of both scanning slides and image analysis is fully automated and requires no user intervention We are aware that recognition of the developed system as a routine diagnostic test will require clinical trials on a much larger sample of clinical cases and healthy controls.

In future, the accuracy of the system could be improved by utilization of a larger sample of cases such as patients after treatment (chemotherapy and radiotherapy), non-malignant conditions of the urinary tract (nephrolithiasis) or primary tumors in a learning process.

The next task that requires further research is the qualitative optimization of the urine sample collection and preparation process, which could finally establish the standardized method as more sensitive and specific than classic cytology – a method that is

fast (the process of computer scanning and the analysis of a single slide is relatively fast as it takes 17 minutes in total), inexpensive enough to be widely available, and on top of that an application which does not require an expert skilled operator.

It should be stated that the proposed method is not meant to replace classic morphological assessment but rather to be an additional useful tool.

Conclusions

Computer analysis of urine provided correct diagnoses in cancer and control patients with the sensitivity of 83% and specificity of 71% and gave excellent results in aggressive tumors such as T2, T3, G3 and in CIS.

The authors declare no conflict of interest.

Scientific work financed by research project funds allocated for science in the years 2009-2013 (N NCN403 197937).

References

1. Babjuk M, Burger M, Zigeuner R, et al. EAU Guidelines on Non-Muscle-invasive Urothelial Carcinoma of the Bladder: Update 2013. Eur Urol 2013; 64: 639-653.
2. Bergman J, Reznichek RC, Rajfer J. Surveillence of patients with bladder carcinoma using fluorescent in-situ hybrydization on bladder washings. BJU Int 2008; 101: 26-29.
3. De Bekker-Grob EW, van der Aa MN, Zwarthoff EC, et al. Non-muscle invasive bladder cancer surveillance for which cystoscopy is partly replaced by microsatellite analysis of urine: a cost-effective alternative? BJU Int 2009; 104: 41-47.
4. Glas AS, Roos D, Deutekom M, et al. Tumour markers in the diagnosis of primary bladder cancer. A systematic review. J Urol 2003; 169: 1975-1982.
5. Lokeshwar VB, Habuchi T, Grossman HB, et al. Bladder tumor markers beyond cytology: international consensus panel on bladder tumor markers. Urology 2005; 66 (6 suppl 1): 35-63.
6. Lotan Y, Roehrborn CG. Sensitivity and specificity of commonly available bladder tumor markers versus cytology: results of a comprehensive literature review and meta-analysis. Urology 2003; 61: 109-118.
7. Têtu B. Diagnosis of urothelial carcinoma from urine. Mod Pathol 2009; 22 Suppl 2: 53-59.
8. Bohm N, Sandritter W. DNA in human tumours: a cytophotometric study. In: Current Topics in Pathology, Vol. 60, Grundmann E, Kirsten WH (eds.). Springer-Verlag, Berlin 1975; 151-219.
9. The Feulgen Procedure for DNA. Available at: http://homepages.gac.edu/~cellab/chpts/chpt2/ex2-5.html, last access 03.04.2016.
10. Dulewicz A, Pietka D, Jaszczak P, et al. Value of Digital Image Analysis in Research and Diagnosis of Urine Bladder Cancer. In: Computer Recognition Systems 2. Advances in Soft Computing. 45. Kurzynski M, Puchala E, Wozniak M, Zolnierek A (eds.). Springer, Berlin Heidelberg 2007; 613-620.
11. Dulewicz A, Piętka D, Jaszczak P. A Study on Diagnostic Potential of a Computer-Assisted System for Identification of Neoplastic Urothelial Nuclei from the Bladder. In: Information Technologies in Biomedicine. Advances in Soft Computing. 47. Pietka E, Kawa J (eds.). Springer, Berlin Heidelberg; 2008; 403-417.
12. Dulewicz A, Piętka D, Jaszczak P. Digital image analysis in research and diagnosis of urinary bladder cancer. In: Bladder Cancer: Etymology, Diagnosis and Treatments. Nilsson WE (ed.). Nova Science Biomedical Books, New York 2010; 211-228.
13. Dulewicz A, Piętka D, Jaszczak P, et al. Computer identification of neoplastic urothelial nuclei from the bladder. Anal Quant Cytol Histol 2001; 23: 321-329.
14. Koss LG, Sherman AB, Adams SE. The use of hierarchic classification in the image analysis of a complex cell population. Experience with the sediment of voided urine. Anal Quant Cytol 1983; 5: 159-166.
15. Koss LG, Deith D, Ramanthan R, Sherman AB. Diagnostic value of cytology of voided urine. Acta Cytologica 1985; 29: 810-816.
16. Sherman A, Koss LG, Adams S, et al. Bladder cancer diagnosis by image analysis of cells in voided urine using a small computer. Anal Quant Cytol 1981; 3: 239-249.
17. Sowter C, Sowter G, Slavin G, et al. Morphometry of bladder carcinoma: definition of a new variable. Anal Cell Pathol 1990; 2: 205-213.
18. Tanaka N, Ueno T, Ikeda H, et al. CYBEST Model 4. Automated cytologic screening system for uterine cancer utilizing image analysis processing. Anal Quant Cytol Histol 1987; 9: 449-454.
19. de Voogt H, Rathert P, Beyer-Boon M. Urinary Cytology and its Relationship to Histology of the Urinary Tract. Urinary Cytology: Springer, Berlin Heidelberg 1977; 15-41.
20. Wied G, Koss LG, Reagan J. Compendium on Diagnostic Cytology. Wied G, Keebler CM, Koss LG, Reagan JW (eds). 5th ed. International Academy of Cytology, Chicago 1983; 419-423.
21. Wiener HG, Vooijs GP, van't Hof-Grootenboer AE. Accuracy of urinary cytology in the diagnosis of primary and recurrent bladder cancer. Acta Cytol 1992; 37: 163-169.
22. Wong EK, Liang E, Lin E, et al. A selective mapping algorithm for computer analysis of voided urine cell images. Anal Quant Cytol Histol 1989; 11: 203-210.
23. Zieliński, KW, Strzelecki M. Computer assisted analysis of biomedical images. Introduction to the morphometry and quantitative pathology. Polish Scientific Publishers PWN, Warsaw 2002; 101-153.
24. Konety BR, Getzenberg RH. Urine based markers of urological malignancy. Journal of Urology 2001; 165: 600-611.
25. Boon ME, Drijver JS. Routine cytological staining techniques: Theorethical background and Practice. Macmillan Education Ltd., London 1986.
26. Jarkrans T. Algorithms for cell image analysis in cytology and pathology. Acta Universitatis Upsaliensis, Upsala 1996; 6-26.

Address for correspondence

Tomasz Borkowski
Lindleya 4
02-005 Warsaw, Poland
e-mail: tomasz.borkowski@wum.edu.pl