DOI: 10.5281/zenodo.4527239
UDC: 616.62-006.6-076

Diagnostic markers of urinary bladder tumors

[*]Ivan Vladanov, Alexei Plesacov, Artur Colta, Vitalii Ghicavii

Department of Urology and Surgical Nephrology
Nicolae Testemitanu State University of Medicine and Pharmacy, Chisinau, the Republic of Moldova

Authors' ORCID iDs, academic degrees and contributions are available at the end of the article

*Corresponding author: ivan.vladanov@gmail.com
Manuscript received January 04, 2021; revised manuscript February 08, 2021; published online February 22, 2021

Abstract

Background: The perfect method for laboratory diagnosis of bladder cancer should have high sensitivity and specificity, should be easily reproducible, inexpensive, be suitable for primary diagnosis, screening, and follow-up of patients, for timely detection of recurrence. In clinical practice, for bladder cancer diagnostics have been used the following markers: UBC, BTA, "ImmunoCyt", NMP22, "UroVision», and others. Each method has relative advantages and disadvantages. The study has demonstrated an influence on the test result of the histological structure and grade of the tumor, presence of hematuria, urolithiasis, chronic inflammatory malignancies, recent surgical procedures on the urinary tract. Apparently, the use of a palette of markers in connection with imaging techniques will increase the diagnostic capabilities, but it is still not clear which elements should be present in such palette.
Conclusions: At present, basic diagnostic methods for bladder cancer remain: USG, MRI, CT, and endoscopic methods. The laboratory methods that exist are not informative enough. Each marker has serious restrictions, but possibly the complex application will allow increasing the diagnostic value in the future, therefore it is necessary to develop new markers of bladder cancer or to study the results of the complex application of several known markers to increase the value of the laboratory diagnosis of primary bladder cancer and recurrent.
Key words: bladder tumors, tumor markers.

Cite this article
Vladanov I, Plesacov A, Colta A, Ghicavii V. Diagnostic markers of urinary bladder tumors. *Mold Med J.* 2021;64(1):71-75. doi: 10.5281/zenodo.4527239.

Introduction

Bladder cancer is an actual problem of modern oncourology, because of high rates of prevalence, recurrence, progression, and a major burden on the medical healthcare system and the economy. In 2015 incidence of bladder cancer ranked ninth and mortality thirteenth worldwide. It ranked the highest in high-sociodemographic index countries at position eighth for incidence and eleventh for cancer deaths [1]. Bladder cancer is divided into two main categories: non-muscle-invasive bladder cancer (NMIBC) which constitutes 75% and muscle-invasive bladder cancer (MIBC) which makes up 25% [2]. The perfect method of laboratory diagnosis of bladder cancer must be extremely sensitive and specific, easily reproducible, inexpensive, suitable for primary diagnosis, screening, and monitoring of patients, to detect recurrence on time [3].

Currently, the main diagnostic tool for the detection of bladder cancer remains cystoscopy, which is an effective but invasive method of diagnostics. Even after a flexible cystoscopy, we have pain during urination in 50% of cases, urinary frequency in 37%, visible hematuria at 19% of patients, and infection in 3% of cases [4]. Sensitivity and specificity of cystoscopy ranged from 62 to 84% and 43 to 98%, respectively, depending on the type, stage, and grading of the tumor, it has a low sensitivity for carcinoma in situ (CIS) [5]. Long-term observation thereby remains the keystone of long-term management, and cystoscopy for over 80 years remains the gold standard. However, cystoscopic access is economically expensive for the medical healthcare system and burdensome for patients, therefore, for decades, there has been a search for non-invasive urine biomarkers that can match or even improve the specificity and sensitivity of cystoscopy. However, current guidelines do not recommend the use of urinary biomarkers in the management of bladder cancer patients [6].

The use of urine-based biomarkers to detect bladder cancer seems to be an attractive alternative. Urinary biomarkers are in direct contact with the bladder and can come in a variety of forms, such as proteins, metabolites, deoxyribonucleic acid (DNA), different types of ribonucleic acid (RNA), and single nucleotide polymorphisms. The existence of variations in the expression of those molecules may be related to bladder cancer [7].

For the diagnosis of bladder tumors, cytological methods and different tumor markers are used. There are several classifications of tumor markers. According to the purpose of the research, markers are divided: which are used in the primary diagnosis and for the prognosis of recurrences, progression, and tumor metastasis [8]. Depending on the type

of material studied, the following markers are distinguished: urinary, serum, and tissue. The evaluation of markers in urine is of major clinical interest, because the given method is non-invasive, reduces the number of cystoscopes, and allows obtaining sufficient material for investigation [9].

However, in a lot of biomarker studies, we see displacement due to the severe and advanced cases of disease which probably rise apparent sensitivity (the percent of correctly identified cases) and inclusion of healthful volunteers which is probably to rise evident specificity (the percent of correctly identified controls), or the use of patients with big primary tumors when the aim is to discover slight recurrent tumors [10]. Another high pitfall in the measuring of urinary biomarkers for bladder cancer is haematuria: haematuria is a symptom and sign of bladder cancer but is not the biological reason for bladder cancer. Thereby, each protein represented in the blood can appear to act as a biomarker in case-control studies where haematuria is not matched, but will not be bladder cancer-specific [11].

Urinary cytology

Urine cytological investigation is the standard laboratory method for diagnosing bladder tumors with which other methods are compared. Cytology is used in clinical practice, it is a non-invasive method where voided or obtained with special instrument urine is examined for exfoliated cancer cells. We can mention as summary data that the diagnostics of cytology is not significant and constitutes in medium: specificity 40 – 44% and sensitivity 30 – 35%. There is a correlation between sensitivity and the degree of tumor differentiation: G1 – 13 – 16%; G2 – 31 – 36%; G3 – 70 – 84%, Tis – 92 – 94%, i.e. the more aggressive is the tumor, the greater is the possibility of detection, but to establish the diagnosis it is necessary to have a well-trained cytologist [12, 13].

Ajit D. et al. reported results of cytological investigations in 951 patients with bladder cancer, 1831 samples were performed. The histopathological examination of the bladder bioptate was performed as a control method. There were 173 false-negative and 6 false-positive results. The general specificity was 82% and the sensitivity – 96%. The main cause of false-negative results was related to high tumor differentiation, when the sensitivity of the method is lower. False-positive results can be explained by changes related to chronic inflammation of the urothelium [14]. Another example: Lokeshwar V. et al. studied 690 patients with the single episode of macrohematuria. All patients underwent urethrocystoscopy, ultrasound scan (USG), urine insemination, blood analysis, and urine cytology. Results: general sensitivity was – 40.2%, specificity – 98.7%, positive predictive value – 81.4%. The authors signed that with the help of the cytological examination it was not possible to highlight formations that would not be diagnosed with routine methods [15].

In 2016 the Paris Working Group published the standardized system for reporting by category the diagnosis of urinary cytology, which was validated in several retrospective studies [16, 17]. The Paris system includes the following groups [18]:

- Adequacy of urine specimens (Adequacy);
- Negative for high-grade urothelial carcinoma (Negative);
- Atypical urothelial cells (AUC);
- Suspicious for high-grade urothelial carcinoma (Suspicious);
- High-grade urothelial carcinoma (HGUC);
- Low-grade urothelial neoplasia (LGUN).

Tumor markers

There are several markers that are used in the diagnosis of bladder cancer (Table 1). In clinical practice, the following testing systems have received the most widespread: UBC cancer antigen, BTA, NMP-22, UroVision, ImmunoCyt, CYFRA 21-1, CK 20, and others.

Bladder cancer antigen (UBC) is a soluble fragment of cytokeratins 8 and 18 (intermediate microfilaments of epithelial cells). With the active proliferation and malignant cell transformation, cytokeratin expression increases [19]. The discriminant level is 32 μg/L. The sensitivity of the method is 60-78% for primary patients, the specificity can reach 95%. The correlation between the stage of the tumor

Table 1

Sensitivity and specificity of diagnostic tests for bladder cancer

Name of the test	Marker	Sensitivity %	Specificity %	Comments
Urinary cytology	Cytological examination of urine	40 - 44	30 - 35	Control method
UBC	Cytokeratin levels 8 and 18	54	97	Low sensitivity
BTA	Antigen, linked to urinary bladder cancer	50 - 80	50 - 75	Diagnostic significance decreases in the presence of urinary tract diseases
NMP-22	Nuclear matrix protein	50 - 90	70 - 85	Low sensitivity in invasive non-muscular tumors (50%) to invasive (90%), the high negative predictive value
ImmunoCyt	High molecular weight carcinoembryonic antigens and mucins	50 - 95	60 - 85	High sensitivity to well-differentiated tumors
UroVision	In situ fluorescence hybridization	70 - 100	66 - 93	Costly and time-consuming method
CYFRA 21.1	Cytokeratin levels 19	73	41	Low specificity
CK 20	Cytokeratin levels 20	85	76	
Survivin	Survivin levels	82	90	The costly and time-consuming analysis process

process and the proliferative activity of the tumor cells was observed. According to Todenhofer T. et al., who analyzed the results of the diagnosis of bladder cancer in 177 patients, at a discriminatory level of 12.3 ng/ml, the sensitivity of the method was 57.8% and the specificity – 66.7% [20]. For the bladder cancer antigen rapid test (UBC-rapid test), Ecke et al. in 2017 reported: sensitivity of 87% for detecting carcinoma in situ, 71% for high-grade non-muscle invasive bladder cancer, 60% for high-grade muscle invasive bladder cancer, and 30% for low-grade non-muscle invasive bladder cancer [21].

The bladder tumor antigen (BTA) is a single-chain protein, which is associated with human complement factor H (hCFHrg), with the property of a germ factor. BTA is determined in urine, discriminatory level 14 Un/ml. Leyh H. et al. studied 414 patients with invasive non-muscular tumors of the bladder. The sensitivity of the BTA test was 70%, specificity – 90%. A correlation was established between sensitivity and tumor degree of differentiation: an increase was marked in sensitivity from 17% in G1 to 64% in G2 and up to 92% in G3. The sensitivity of the method in recurrences was 67%. The sensitivity of the method also increases with increasing stage of the pathology: from 50% to 90%. For example, in stage Ta, the sensitivity of the BTA test was 53.8%, but in T1 – already 76% [22, 23].

The quantitative BTA (BTA TRAK®) test is performed in a specialized laboratory, whereas the qualitative BTA (BTA stat®) is a point-of-care test with an immediate result (Polymedco Inc., Cortlandt Manor, New York, USA). They have a sensitivity of 65% versus 64%, and a specificity of 74% versus 77%, respectively [24, 25]. However, the specificity of both of these tests is significantly decreased since false positives have been noted to occur due to the presence of human complement factor H-related protein in blood. Hematuria can be presented in different urological malignancies, such as urolithiasis, inflammation, recent use of instrumentation, other genitourinary malignancies, and intravesical Bacillus Calmette Guérin (BCG) therapy which causes local inflammation [24-27].

The European Association of Urology examines the diagnostic values of each of the proposed test systems. By combining the most preferred properties are considered: "ImmunoCyt", NMP-22, and "UroVision" [28].

Nuclear matrix protein 22 (NMP22) may be identified in urine as a biomarker of the death of the urothelial cells. This marker is often elevated in the urine of patients with bladder cancer and can thus be used in the finding of this disease. The NMP22®BladderChek® and NMP22®BC test kit is qualitative and quantitative enzyme immunoassay tests, respectively (originally Matritech Inc., Newton, MA, USA). The sensitivity of 69% and specificity of 77% quantitative NMP22 BC test kit is compared to a sensitivity of 58% and a specificity of 88% for the qualitative NMP22 test [25, 29]. However, are common false-positive results, because NMP22 is emitted from apoptotic cells which also occur in case of hematuria, infection, or inflammation [25, 30-32]. Its discriminatory level is 10 Un/ml. One of the benefits of the given test is a high negative predictive value. This marker is not widespread due to insufficient diagnostic value, but it is considered that its diagnostic role may be more significant when used in the palette of bladder cancer markers [33].

ImmunoCyt™/uCyt+™ is an immunocytochemical test that utilizes fluorescently marked antibodies that are guided against three antigens: two mucins which are specifically detected on malignant exfoliated urothelial cells and a glycosylated form of carcinoembryonic antigen [34]. This method has a high sensitivity in well-differentiated tumors and is less affected by concomitant inflammatory changes of the urinary tract, more preferably to be used in the primary diagnosis. Sensitivity is 50-95%, specificity – 60-85% [35]. The sensitivity of this test is higher than cytology, but the specificity is lower [36]. False positives are seen during infection or inflammation and there is poor sensitivity in T2 bladder cancers. Moreover, interobserver variability exists; trained cytopathologists are therefore necessary [37]. It is only approved for the surveillance of bladder cancer patients [38].

Widespread received the method for detecting chromosomal rearrangements using in situ fluorescence hybridization (FISH). FISH is a technique that uses fluorescently labeled deoxyribonucleic acid (DNA) probes to assess cells for genetic alterations [39]. Exfoliated urothelial cells are detected in voided urine, are hybridized on a slide. They are further examined for chromosomal aberrations which are found in bladder cancer: aneuploidy of chromosomes 3, 7, and 17, and a loss of locus 9p21 [38, 40]. In a meta-analysis, the specificity of the test was stated to be 83%, and the sensitivity to be 72% in the context of equivocal cytology [41]. Another recent meta-analysis of studies of UroVysion™ has calculated its sensitivity and specificity in detecting bladder cancer at 63% and 87%, respectively [39]. The lack of sensitivity for low-grade bladder cancers remains [42].

Jeong S. et al. analyzed the results of the CYFRA 21-1, NMP22, UBC and FDP tests in 250 patients. Of these, 54 were diagnosed with bladder cancer. The control group consisted of 196 patients with inflammatory diseases of the urinary tract, benign prostatic hyperplasia, hematuria of non-tumor etiology. The level of the studied markers was significantly higher in the study group than in the control group. The best results were observed with CYFRA 21-1 and NMP 22 [43].

Ludecke G. et al. investigated the influence of hematuria intensity on the level of UBC, NMP22, and BTA markers. As study material, they used freshly heparinized blood titrated in the urine of conventionally healthy people at different concentrations. The level of UBC and NMP22 did not increase at different intensities of macrohematuria. The BTA test showed the worst results: false-positive results were recorded in the presence of over 150 red blood cells in the visual field [44].

The combination of markers increases the diagnostic value compared to using each separate marker. Todenhöfer T. et al. studied the results of the application: urine cytological examination, FISH, ImmunoCyt, and NMP22. Diagnostic data from 808 primary care patients and 505 patients with non-invasive muscle bladder cancer recurrence

were analyzed. The complex application of these markers has demonstrated a high negative predictive value, which potentially makes it possible to use them as an additional control method between programmed cystoscopes [45].

Table 1 presents generalized data on sensitivity and specificity of different markers compared to cytological examination of urine.

If the main goal is to avoid unnecessary cystoscopies, rather than looking for markers with high sensitivity and specificity, the focus should be on identifying a marker with a very high negative predictive value. A test capable of predicting the absence of the tumor will be of great use in daily clinical practice [46]. Promising new urinary biomarkers, which evaluate several targets, have been tested in multicenter prospective studies with a very high negative predictive value [47, 48]. More studies are needed to obtain truthful information about diagnostic markers of bladder tumors for their implementation and use in daily practice.

Conclusions

It should be noted that currently the basic methods, routine for primary diagnosis and clarification of bladder cancer, remain: ultrasound scan (USG), magnetic resonance imaging (MRI), computed tomography (CT), and endoscopic methods (cystoscopy in white light, fluorescence, narrowband imaging, and others). The laboratory methods that exist are not informative enough, each method has relative advantages and disadvantages. Each marker has serious restrictions, but possibly the complex application will allow increasing the diagnostic value in the future. Apparently, the use of a palette of markers in connection with imaging techniques will increase the diagnostic capabilities, but it is still not clear which elements should be present in such palette.

Improving the diagnosis of bladder cancer is possible by combining the efforts of oncologists, urologists, morphologists, geneticists, and molecular biologists. Thus, the present study confirmed that it is necessary to develop new markers of bladder cancer or to study the results of the complex application of several known markers to increase the value of the laboratory diagnosis of primary bladder cancer and recurrent.

References

1. Fitzmaurice C, Allen C, Barber RM, Barregard L. Global, regional, and national cancer incidence, mortality, years of life lost, years lived with disability, and disability-adjusted life-years for 32 cancer groups, 1990-2015: a systematic analysis for the global burden of disease study. JAMA Oncol. 2017;3(4):524-548. doi: 10.1001/jamao ncol.2016.5688.
2. Woldu SL, Bagrodia A, Lotan Y. Guideline of guidelines: nonmuscle-invasive bladder cancer. BJU Int. 2017;119(3):371-80. doi: 10.1111/bju.13760.
3. Lokeshwar VB, Habuchi T, Grossman HB, Murphy WM, Hautmann SH, Hemstreet GP 3rd, Bono AV, Getzenberg RH, Goebell P, Schmitz-Dräger BJ, Schalken JA, Fradet Y, Marberger M, Messing E, Droller MJ. Bladder tumor markers beyond cytology: International Consensus Panel on bladder tumor markers. Urology. 2005;66(6 Suppl 1):35-63. doi: 10.1016/j.urology.2005.08.064.
4. Biardeau X, Lam O, Ba V, Campeau L, Corcoss J. Prospective evaluation of anxiety, pain, and embarrassment associated with cystoscopy and urodynamic testing in clinical practice. Can Urol Assoc J. 2017;11(3-4):104-110. doi: 10.5489/cuaj.4127.
5. Jocham D, Stepp H, Waidelichs R. Photodynamic diagnosis in urology: state-of-the-art. Eur Urol. 2008;53(6):1138-1148. doi: 10.1016/j.eururo.2007.11.048.
6. National Institute for Health and Care Excellence. Bladder cancer: diagnosis and management: NICE guideline. London: NICE; 2015 [cited 2020 Dec 12]. Available from: www.nice.org.uk/guidance/ng2/chapter/1-recommendations#diagnosing-and-staging-bladder-cancer-2
7. Leiblich A. Recent developments in the search for urinary biomarkers in bladder cancer. Curr Urol Rep. 2017;18(12):100. doi: 10.1007/s11934-017-0748-x.
8. Protzel C, Hakenberg OW. Molecular markers in the diagnostics and therapy of urothelial cancer. Urologe A. 2010;49(11):1415-1424. doi: 10.1007/s00120-010-2431-4.
9. Roos PH, Jakubowski N. Methods for the discovery of low-abundance biomarkers for urinary bladder cancer in biological fluids. Bioanalysis. 2010;2(2):295-309. doi: 10.4155/bio.09.174.
10. van Rhijn B, van der Poel H, van der Kwast T. Urine markers for bladder cancer surveillance: a systematic review. Eur Urol. 2005;47(6):736-48. doi: 10.1016/j.eururo.2005.03.014.
11. Bryan R, Wei W, Shimwell N, Collins S, Hussain S, Billingham L, et al. Assessment of high-throughput highresolution MALDI-TOF-MS of urinary peptides for the detection of muscle-invasive bladder cancer. Proteomics Clin Appl. 2011;5(9-10):493-503. doi: 10.1002/prca.201100011.
12. Riazantsev VE. Prognosticheskaia znachimost' obshcheklinicheskikh i molekuliarno-biologicheskikh markerov gruppy keratinov v rannei diagnostike raka mochevogo puzyria [Prognostic value of general clinical and molecular biological markers of the keratin group in the early diagnosis of bladder cancer] [dissertation]. Moscow; 2011. 156 p. Russian.
13. Yafi FA, Brimo F, Steinberg J, et al. Prospective analysis of sensitivity and specificity of urinary cytology and other urinary biomarkers for bladder cancer. Urol Oncol. 2015;33(2):66e25-31. doi: 10.1016/j.urolonc.2014.06.008.
14. Ajit D, Dighe S, Desai S. Has urine cytology a role to play in the era of fluorescence in situ hybridization? Acta Cytol. 2010;54(6):1118-1122. doi:10.1159/000325254.
15. Lokeshwar VB, Block NL. HA-HAase urine test. A sensitive and specific method for detecting bladder cancer and evaluating its grade. Urol Clin North Am. 2000;27(1):53-61. doi: 10.1016/s0094-0143(05)70234-2.
16. Cowan ML, Rosenthal DL, VandenBussche CJ. Improved risk stratification for patients with high-grade urothelial carcinoma following application of the Paris System for Reporting Urinary Cytology. Cancer Cytopathol. 2017;125(6):427-34. doi: 10.1002/cncy.21843.
17. Meilleroux J, Daniel G, Aziza J, et al. One year of experience using the Paris System for Reporting Urinary Cytology. Cancer Cytopathol. 2018;126(6):430-36. doi: 10.1002/cncy.21999.
18. Rosenthal DL, Wojcik EM, Kurtycz DF. The Paris System for Reporting Urinary Cytology. Switzerland: Springer; 2016. doi: 10.1007/978-3-319-22864-8.
19. Li T, Chen Z, Lin C. Value of urinary cytokeratins 8 and 18 as a diagnostic marker for transitional cell carcinoma. Chin J Urol. 2003;24:12.
20. Todenhöfer T, Hennenlotter J, Ritter R, Hoffmann U, Blutbacher P, Deja A, Hohneder A, Stenzl A, Schwentner C. Point-of-care testing for bladder cancer – the UBC Rapid test on the concile® Ω100 reader platform provides quantitative results. Eur Urol Suppl. 2013;12(1):e365. doi: 10.1016/S1569-9056(13)60850-7.
21. Ecke TH, Weiss S, Stephan C, Hallmann S, Barski D, Otto T, Gerulliss H. UBC ® Rapid Test for detection of carcinoma in situ for bladder cancer. Tumour Biol. 2017 May;39(5):1010428317701624. doi: 10.1177/1010428317701624.
22. Matveev BP. Klinicheskaia onkourologiia [Clinical oncourology]. Moscow; 2011. 934 p. ISBN 978-5-903018-23-9. Russian.
23. Leyh H, Hall R, Mazeman E, Blumenstein BA. Comparison of the Bard BTA test with voided urine and bladder wash cytology in the diagnosis and management of cancer of the bladder. Urology. 1997;50(1):49-53. doi: 10.1016/s0090-4295(97)00206-9.
24. Konety BR. Molecular markers in bladder cancer: a critical appraisal. Urol Oncol. 2006;24(4):326-337. doi: 10.1016/j.urolonc.2005.11.023.
25. Chou R, Gore JL, Buckley D, Fu R, Gustafson K, Griffin JC, Grusing S, Selphs S. Urinary biomarkers for diagnosis of bladder cancer: a systematic

review and meta-analysis. Ann Intern Med. 2015;163(12):922-931. doi: 10.7326/M15-0997.
26. Miyake M, Goodison S, Rizwani W, Ross S, Bart Grossman H, Rossers CJ. Urinary BTA: indicator of bladder cancer or of hematuria. World J Urol. 2012;30(6):869-873. doi: 10.1007/s00345-012-0935-9.
27. Guo A, Wang X, Gao L, Shi J, Sun C, Wans Z. Bladder tumour antigen (BTA stat) test compared to the urine cytology in the diagnosis of bladder cancer: a meta-analysis. Can Urol Assoc J. 2014;8(5-6):E347-E352. doi: 10.5489/cuaj.1668.
28. European Association of Urology. Non-muscle-invasive Bladder Cancer (TaT1 and CIS): EAU Guidelines. Arnhem: EAU; 2019 [cited 2020 Oct 23]. ISBN 978-94-92671-03-3. Available from: https://uroweb.org/guideline/non-muscle-invasive-bladder-cancer/
29. Grossman HB, Messing E, Soloway M, Tomera K, Katz G, Berger Y, Shens Y. Detection of bladder cancer using a point-of-care proteomic assay. JAMA. 2005;293(7):810-816. doi: 10.1001/jama.293.7.810.
30. Shariat SF, Marberger MJ, Lotan Y, Sanchez-Carbayo M, Zippe C, Ludecke G, Boman H, Sawczuk I, Friedrich MG, Casella R, et al. Variability in the performance of nuclear matrix protein 22 for the detection of bladder cancer. J Urol. 2006;176(3):919-926. doi: 10.1016/j.juro.2006.04.017.
31. Gaston KE, Pruthis RS. Value of urinary cytology in the diagnosis and management of urinary tract malignancies. Urology. 2004;63(6):1009-1016. doi: 10.1016/j.urology.2003.12.004.
32. Lotan Y, O'Sullivan P, Raman JD, Shariat SF, Kavalieris L, Frampton C, Guilford P, Luxmanan C, Suttie J, Crist H, et al. Clinical comparison of noninvasive urine tests for ruling out recurrent urothelial carcinoma. Urol Oncol. 2017;35(8):531.e15-531.e22. doi: 10.1016/j.urolonc.2017.03.008.
33. Xu K, Tam PC, Hou S, Wang X, Bai W. The role of nuclear matrix protein 22 combined with bladder tumor antigen stat test in surveillance of recurring bladder cancer. Chin Med J (Engl). 2002;115(11):1736-1738.
34. Greene KL, Berry A, Konetys BR. Diagnostic utility of the ImmunoCyt/uCyt+ Test in bladder cancer. Rev Urol. 2006;8(4):190-197.
35. Mowatt G, Zhu S, Kilonzo M, Boachie C, Fraser C, Griffiths TR, N'Dow J, Nabi G, Cook J, Vale L. Systematic review of the clinical effectiveness and cost-effectiveness of photodynamic diagnosis and urine biomarkers (FISH, ImmunoCyt, NMP22) and cytology for the detection and follow-up of bladder cancer. Health Technol Assess. 2010;14(4):1-331. doi: 10.3310/hta14040.
36. He H, Han C, Hao L, Zangs G. ImmunoCyt test compared to cytology in the diagnosis of bladder cancer: a meta-analysis. Oncol Lett. 2016;12(1):83-88. doi: 10.3892/ol.2016.4556.
37. Dhondt B, Van Deun J, Vermaerke S, de Marco A, Lumen N, De Wever O, Hendrixs A. Urinary extracellular vesicle biomarkers in urological cancers: From discovery towards clinical implementation. Int J Biochem Cell Biol. 2018;99:236-256. doi: 10.1016/j.biocel.2018.04.009.
38. Darwiche F, Parekh DJ, Gonzalgos ML. Biomarkers for non-muscle invasive bladder cancer: Current tests and future promise. Indian J Urol. 2015;31(4):273-282. doi: 10.4103/0970-1591.166448.
39. Halling KC, Kipps BR. Bladder cancer detection using FISH (UroVysion assay). Adv Anat Pathol. 2008;15(5):279-286. doi: 10.1097/PAP.0b013e3181832320.
40. Phillips JL, Richardsons IC. Aneuploidy in bladder cancers: the utility of fluorescent in situ hybridization in clinical practice. BJU Int. 2006;98(1):33-37. doi: 10.1111/j.1464-410X.2006.06189.
41. Hajdinjak T. UroVysion FISH test for detecting urothelial cancers: meta-analysis of diagnostic accuracy and comparison with urinary cytology testing. Urol Oncol. 2008;26(6):646-651. doi: 10.1016/j.urolonc.2007.06.002.
42. Dimashkieh H, Wolff DJ, Smith TM, Houser PM, Nietert PJ, Yangs J. Evaluation of urovysion and cytology for bladder cancer detection: a study of 1835 paired urine samples with clinical and histologic correlation. Cancer Cytopathol. 2013;121(10):591-597. doi: 10.1002/cncy.21327.
43. Jeong S, Park Y, Cho Y, Kim YR, Kim HS. Diagnostic values of urine CYFRA21-1, NMP22, UBC, and FDP for the detection of bladder cancer. Clin Chim Acta. 2012;414:93-100. doi: 10.1016/j.cca.2012.08.018.
44. Ludecke G, Pilatz A, Hauptmann A, Bschleipfer T, Weidner W. Comparative analysis of sensitivity to blood in the urine for urine-based point-of-care assays (UBC rapid, NMP22 BladderChek and BTA-stat) in primary diagnosis of bladder carcinoma. Interference of blood on the results of urine-based POC tests. Anticancer Res. 2012;32(5):2015-2018.
45. Todenhofer T, Hennenlotter J, Kuhs U, Mohrhardt S, Esser M, Aufderklamm S, Mundhenk J, Gakis G, Stenzl A, Schwentner C. Expedient combination of urine markers enhances their diagnostic performance in the detection of urothelial carcinoma. Eur Urol Suppl. 2013;12(1):364. doi: 10.1016/S1569-9056(13)60849-0.
46. Palou J, Brausi M, Catto JWF. Management of patients with normal cystoscopy but positive cytology or urine markers. Eur Urol Oncol. 2020;3(4):548-554. doi: 10.1016/j.euo.2019.06.017.
47. Kavalieris L, O'Sullivan P, Frampton C, et al. Performance characteristics of a multigene urine biomarker test for monitoring for recurrent urothelial carcinoma in a multicenter study. J Urol. 2017;197(6):1419-26. doi: 10.1016/j.juro.2016.12.010.
48. Ribal MJ, Mengual L, Lozano JJ, et al. Gene expression test for the noninvasive diagnosis of bladder cancer: A prospective, blinded, international and multicenter validation study. Eur J Cancer. 2016;54:131-138. doi: 10.1016/j.ejca.2015.11.003.

Authors' ORCID iDs and academic degrees
Ivan Vladanov, MD, PhD Applicant – https://orcid.org/0000-0002-9703-2775.
Alexei Plesacov, MD, PhD Applicant – https://orcid.org/0000-0002-0139-4772.
Artur Colta, MD, PhD Applicant – https://orcid.org/0000-0002-1291-1237.
Vitalii Ghicavii, MD, PhD, Assistant Professor – https://orcid.org/0000-0002-2130-9475.

Authors' contribution
IV, AP, AC designed the trial and drafted the first manuscript; VG interpreted the data and revised the manuscript critically. The authors revised and approved the final version of the manuscript.

Funding
This study was supported by *Nicolae Testemitanu* State University of Medicine and Pharmacy. The trial was the authors' initiative. The authors are independent and take responsibility for the integrity of the data and accuracy of the data analysis.

Ethics approval and consent to participate
No approval was required for this review study.

Conflict of Interests
The authors have no conflict of interests to declare.