Minerva Urology and Nephrology 2025 April;77(2):149-51
DOI: 10.23736/S2724-6051.25.06372-4

MINERVA OPINION EDITORIAL

The current status of biomarkers for bladder cancer: progress and challenges

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Bladder cancer (BC) is one of the most common cancers worldwide with an estimate of 570,000 new cases and 210,000 deaths annually and an overall rate of 60% to 70% recurrence for non-muscle invasive bladder cancer patients.[1] Given the high recurrence rate, regular follow-up with diagnostic tests is essential for detecting recurrence as early as possible. Cytology and cystoscopy are commonly used in the clinical practice for bladder cancer diagnosis and monitoring. The former has high specificity but low sensitivity, especially for low-grade and stage tumors, the latter is invasive and cannot be used in screening programs.[2]

Several biomarkers have been studied over the years, potentially offering non-invasive diagnosis, prognostication, and treatment monitoring. In particular, urinary biomarkers have shown potential for their non-invasive nature, representing an alternative to cystoscopy for bladder cancer detection and disease monitoring. Among the most promising biomarkers UroVysion, a fluorescence in situ hybridization (FISH) test permits the detection of aneuploidies of chromosomes 3, 7, 17 and the loss of chromosome 9p21locus associated with BC.[2] UroVysion is an FDA approved test for monitoring high-risk patients, especially those with a history of recurrent or high-grade tumors. We previously demonstrated its potential for BC detection and monitoring also for symptomatic patients presenting irritative symptoms and micro/macrohematuria.[2]

However, it is not performed in all laboratories because the interpretation of the chromosomal abnormalities by microscope in the urine samples requires specialized personnel. Moreover, it has a relatively high cost and moderate sensitivity for low-grade tumors. NMP22, a protein marker that detects nuclear mitotic apparatus protein levels, has been approved by the FDA for detecting bladder cancer recurrence.[3] Unfortunately, its accuracy is still a matter of debate, especially in patients with hematuria or other urinary tract conditions. BTA stat and BTA TRAK

tests, which permit the detection of bladder cancer antigens in urine, have been FDA-approved for BC diagnosis and monitoring together with cystoscopy. However, they have not been adopted in clinical practice given their low sensitivity, especially for low-grade and stage tumors.[3]

Telomerase has been deeply investigated for BC early diagnosis and disease monitoring. Some authors evaluated its subunits hTERT, hTR and TP1, others telomerase activity.[2, 4] However, some biological and technical issues such as the presence of hTERT expression and telomerase activity also in normal cells (such as inflammatory cells) and the difficulty of having a good monoclonal antibody to detect hTERT expression discouraged its use in clinical practice.[4] We tried to combine the use of telomerase with cytology, TRAP, and FISH demonstrating the optimal balance between improved sensitivity and reduced specificity loss, particularly in non-bleeding patients, as well as those with low-grade and early-stage cancers.[2]

Other authors explored for research purposes and in clinical trials, the role of serum biomarkers such as FGFR3, TP53 and HRAS mutations for patient prognosis and BC treatment but with contradictory results.[5-7]

The clinical utility of some serum proinflammatory cytokines, such as IL-6 and IL-8, which promote tumor progression, are still under investigation.[8]

While traditional biomarkers provide valuable insights, the search for more accurate bladder cancer biomarkers easily detectable by liquid biopsy is ongoing. Liquid biopsy has the potential for detecting genetic mutations, monitoring the disease, and identifying minimal residual disease. One of the major issues is what to look for in liquid biopsy given that several biomarkers can be studied in it such as circulating tumor DNA (ctDNA), RNA, and exosomes.[9, 10] One promising area is the study of epigenetic biomarkers, such as DNA methylation, which have been shown to be able to distinguish between benign and malignant conditions.[11]

The Bladder EpiCheck test (Nucleix Ltd., Pekeris 3, Rehovot 7670203, Israel) is available for clinic use and permits to analyze 15 methylation-associated biomarkers by detecting specific based methylation profiles. The test is simple to be performed in urine samples processed by centrifugation. The DNA is extracted from the cell pellet using the EpiCheck DNA extraction kit and digested using a methylation-sensitive restriction enzyme. Bladder EpiCheck software analyzes the data obtained using a quantitative amplification real-time polymerase chain reaction (qPCR). EpiScore with values ranging from 0 to 100 allows to predict the outcome with a value ≥60 indicating a positive result [high risk for high grade urothelial carcinoma, HGUC] while a score <60 indicating a high probability of no bladder cancer or that the cancer is still in remission [negative or low risk for HGUC].[11] EpiScore of ≥90 indicates a diagnosis of HGUC. This test has a higher sensitivity than urine cytology during the follow-up of NMIBC patients but has a significantly lower specificity.[11]

Other authors demonstrated that Bladder EpiCheck can be used as a diagnostic tool in upper urinary tract tumors for its high sensitivity.[12]

Recently, Petrella *et al.* analyzed urinary metabolic profiles to predict NMIBC recurrence. Specific metabolites were found to be significantly related to cancer relapse. The study highlights the role of the severity of inflammation, immune suppression, and gut dysbiosis in predicting BC recurrence.[13]

However, liquid biopsy for bladder cancer is still in its early stages, and standardization of methods and validation of biomarkers are required before they can be translated into the clinical practice. The validation and standardization of biomarkers for routine clinical use are ongoing processes, with significant challenges arising from both biological and technical factors. Among the former, bladder cancer is a heterogeneous disease, and the presence or absence of biomarkers varies depending on the stage and grade of the disease. Among the latter, obstacles include the lack of specialized instruments, high costs, and poor reproducibility across different laboratories.

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Conflicts of interest
The authors certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

Authors' contributions
All authors read and approved the final version of the manuscript.

History
Manuscript accepted: March 6, 2025. - Manuscript received: January 22, 2025.

(*Cite this article as:* Leonardi R, Mantica G, Ambrosini F, Calarco A, Tufano A, Borges de Souza P, *et al*. The current status of biomarkers for bladder cancer: progress and challenges. Minerva Urol Nephrol 2025;77:149-51. DOI: 10.23736/S2724-6051.25.06372-4)