New tumor markers in prostate cancer – an update from the literature

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ABSTRACT

In recent years, urinary biomarkers appeared as a promising alternative to prostate-specific antigen (PSA) for the detection of prostate cancer (PCa). These predominantly non-invasive biomarkers provide enhanced specificity regarding both the presence and biological aggressiveness of Pca. The urinary biomarkers like PCA3, ERG (TMPRSS2-ERG), and SPDEF and their corresponding diagnostic platforms represent a substantial advancement in the early detection and risk stratification of prostate cancer, facilitating a more personalized, accurate, and less invasive diagnostic approach. Meanwhile, blood-based biomarkers remained the most utilized, PSA remaining in the top of this scale, even if there are new scores like PHI, 4K or molecules like PCA3 or MCM, all presenting promising outcomes but having different limitations in detection, follow-up, development costs of applicability in clinical practice. In this context, artificial intelligence could represent a helpful solution into oncologic research holding substantial promise for improving patient outcomes and advancing precision medicine in Pca. In conclusions, there are some limitations as, the high cost, the regulatory approval processes, the patient-related factors, which continue to limit the widespread implementation in clinical practice of the new markers, but the new tools like AI could make these things happening faster and more efficient.

Key words: prostate cancer, urinary biomarkers, blood markers, AI.

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Introduction

Prostatic neoplasms, encompassing both prostate adenocarcinoma and benign prostatic hyperplasia (BPH), constitute a substantial public health burden. Epidemiological data indicates that the lifetime probability of men developing histologically confirmed BPH reaches approximately 70% among individuals aged 61–70 years [1], whereas prostate cancer (PCa) has been established as the most prevalent malignancy in men over the age of 66 years [2]. Despite extensive investigation, the precise etiopathogenic mechanisms underlying both conditions remain incompletely understood.

The complex crosstalk between inflammatory processes, immune responses, and the tumor microenvironment (TME) in prostatic tumors suggests that interleukin-6 (IL-6) plays a pivotal role in modulating tumor progression. Emerging evidence supports the concept that a bidirectional interaction between malignant cells and their surrounding microenvironment promotes tumor proliferation while simultaneously impairing immune-mediated tumor elimination [3]. Furthermore, the TME undergoes profound phenotypic and functional remodeling. Fibroblasts differentiate into cancer-associated fibroblasts, macrophages facilitate enhanced cytokine production, and myeloid-derived suppressor cells migrate from the bone marrow to the tumor niche, where they contribute to stromal reprogramming. Concurrently, adipocytes secrete tumor-promoting factors that facilitate invasion and metastatic potential. Additionally, tumor-infiltrating immune cells play a critical role in establishing immune tolerance and enabling tumor immune evasion.

Collectively, the tumor microenvironment functions as a biological shield, protecting malignant cells from both immunological surveillance and the cytotoxic effects of antineoplastic therapies [3–5]. The dynamic polarization of macrophages further delineates the balance between pro-inflammatory (M1) and anti-inflammatory (M2) phenotypes, thereby shaping the inflammatory landscape of the tumor [6]. Moreover, the convergence of inflammatory stimuli, microbiome alterations, androgen/androgen receptor signaling status, hypoxic conditions, and genomic instability facilitates neoplastic transformation through mechanisms largely mediated by oxidative stress [7–9].

Systemic cytokines, including IL-6, TNF-α, IL-10, and TGF-β, along with chemokines such as CCL2, CCL4, and CXCL10, contribute to intercellular communication pathways that orchestrate inflammatory regulation within the tumor milieu [3,8,10]. IL-6, in particular, is critically involved in the initiation, propagation, and resolution phases of the inflammatory response. Its principal cellular sources include macrophages, B and T lymphocytes, fibroblasts, endothelial cells, and neoplastic cells. Within the tumor microenvironment, IL-6 exerts both pro-inflammatory and anti-inflammatory effects predominantly through classical signaling pathways, thereby influencing tumor behavior and immune modulation [3,5,7,8,11,12].

Moreover, in recent years, urinary biomarkers appeared as a promising alternative to prostate-specific antigen (PSA) for the detection of prostate cancer (PCa). These predominantly non-invasive biomarkers provide enhanced specificity regarding both the presence and biological aggressiveness of PCa, thereby improving diagnostic accuracy, optimizing clinical decision-making, and significantly reducing the incidence of unnecessary invasive interventions, particularly prostate biopsies. Among the most extensively investigated urinary biomarkers are Prostate Cancer Antigen 3 (PCA3) and the TMPRSS2-ERG gene fusion [13].

Urinary prostate biomarkers

PCA3 is a prostate-specific long non-coding RNA that is markedly overexpressed in prostate cancer cells, rendering it a robust molecular target for PCa detection. Urinary quantification of PCA3 has demonstrated particular clinical utility in men presenting with persistently elevated PSA levels and prior negative biopsy results, offering improved risk stratification in this diagnostically challenging population. Similarly, the TMPRSS2-

ERG fusion gene, a molecular hallmark frequently identified in PCa, serves as a complementary biomarker that further enhances diagnostic sensitivity and specificity when combined with PCA3.

Beyond isolated biomarkers, the development of multimodal assay platforms has further refined the diagnostic landscape of PCa. This narrative review focuses on three widely adopted diagnostic tests: the ExoDx Prostate (EPI) test, the SelectMDx test, and the MyProstateScore (MPS) test. The ExoDx Prostate test analyzes urinary exosomes to quantify the expression of PCA3, ERG (TMPRSS2-ERG), and SPDEF (SAM Pointed Domain-Containing ETS Transcription Factor), thereby providing a risk assessment for the presence of high-grade, clinically significant PCa. The SelectMDx assay evaluates post-digital rectal examination urinary mRNA expression levels of DLX1 and HOXC6, enabling identification of patients with an elevated likelihood of harboring aggressive disease and aiding in biopsy decision-making. The MyProstateScore test integrates urinary PCA3 and TMPRSS2-ERG measurements with serum PSA concentrations to generate a composite risk estimate for high-grade tumors, thereby enhancing diagnostic precision and contributing to the reduction of unnecessary biopsies while improving individualized patient management.

Collectively, these urinary biomarkers and their corresponding diagnostic platforms represent a substantial advancement in the early detection and risk stratification of prostate cancer, facilitating a more personalized, accurate, and less invasive diagnostic approach [14]. Furthermore, recent evidence underscores that the combined application of multiple biomarker modalities, including both blood-based and urine-based markers, provides a more comprehensive and nuanced assessment of PCa biology and disease burden, further improving diagnostic and prognostic accuracy [15, 16].

Blood-based biomarkers in prostate cancer

Prostate Specific Antigen (PSA) is the most commonly utilized biomarker for assessing prostate gland status. It is a glycoprotein enzyme detectable in the bloodstream, synthesized by both normal and malignant epithelial cells of the prostate. PSA is secreted into the seminal fluid, where it functions to liquefy the seminal coagulum. Its synthesis is regulated by circulating androgens via androgen receptors expressed on the glandular epithelium [17-19]. In this context, PSA represented for years, the most reliable indicator of the prostate cancer beside digital rectal examination [19]. However, PSA has some limitations with false negative outcomes in large benign prostatic hyperplasia or in prostatitis, meanwhile a low-value does not exclude a prostate cancer [18]. Thus, a new biomarker named PHI (Prostate Health Index) was developed. Studies showed that in patients with PSA concentrations below 10 ng/mL, PHI demonstrated superior diagnostic performance, achieving an area under the curve (AUC) of 0.789 (95% CI: 0.737–0.835), compared with an AUC of 0.577 for PSA alone [20-22]. In addition, PHI effectively differentiates prostate cancer (PCa) from healthy individuals (AUC: 0.887; 95% CI: 0.825–0.948) as well as from benign prostatic hyperplasia (AUC: 0.639; 95% CI: 0.546–0.730). The clinical utility of PHI is further strengthened when combined with imaging techniques, including magnetic resonance imaging (MRI) and transrectal ultrasound (TRUS), particularly in patients presenting with PI-RADS 3–5 lesions. In these patient groups, the integration of PHI with imaging modalities significantly enhanced diagnostic accuracy, yielding AUCs of 0.884 (95% CI: 0.792–0.976) for PI-RADS 3 lesions and 0.792 (95% CI: 0.707–0.877) for PI-RADS 4/5 lesions [23–25]. Collectively, these results highlight PHI as a promising diagnostic biomarker that offers improved specificity and accuracy for PCa detection, thereby potentially reducing diagnostic uncertainty and supporting more informed clinical decision-making. Another validated blood-based biomarker is the 4Kscore test, developed by OPKO Health (Nashville, TN, USA), which has received FDA approval and has been incorporated into the NCCN guidelines since 2015. The 4Kscore integrates quantitative measurements of four kallikrein proteins—total PSA, free PSA, intact PSA, and human kallikrein-2 (hK2)—with essential clinical variables, including patient age, findings from digital rectal examination (DRE), and a history of prior negative

biopsy. These inputs are combined within an algorithm to generate an individualized risk estimate, expressed as a percentage (0–100%), for the presence of clinically significant prostate cancer. Among these biomarkers, hK2 has demonstrated a strong correlation with poorly differentiated tumors, particularly those with a Gleason grade of ≥3 [26]. Multiple large-scale retrospective studies conducted in both the United States and Europe have confirmed the clinical utility of the 4Kscore in predicting aggressive prostate cancer (PCa). These investigations demonstrated that the test can reduce unnecessary biopsies by 30%–58% while maintaining low rates of delayed diagnosis, ranging from 1.3% to 4.7% for cancers with a Gleason score ≥7 [27-29]. Among patients managed with active surveillance, the 4Kscore has shown particular value in predicting tumor upgrading, with a reported sensitivity of 89% and specificity of 29%, in addition to decreasing the number of unnecessary biopsies by 27% [27,30].

Another promising biomarker for prostate cancer (PCa) detection is PCA3, also known as DD3. PCA3 is a non-coding mRNA that is markedly overexpressed—by approximately 10- to 100-fold—in prostate cancer tissue. Higher PCA3 expression is associated with significantly increased Ki-67 levels compared to tumors classified as ISUP grades 1–3 ($p < 0.0001$, $p = 0.0046$, $p = 0.0027$) [31]. In addition, Ki-67 expression is positively correlated with Gleason score, extraprostatic extension, and seminal vesicle invasion. When combined with Bcl-2 overexpression, its ability to predict tumor progression and poor clinical outcomes is further enhanced [32]. But, despite its' promising outcomes, it was not applied as clinical standard. In addition to Bcl-2 and Ki-67, several other molecular markers have been implicated in PCa progression. Among these is Enhancer of Zeste Homolog 2 (EZH2), a polycomb group protein involved in epigenetic regulation and cell-cycle control. EZH2 is frequently overexpressed in metastatic disease and in poorly differentiated localized tumors, suggesting a contribution to aggressive tumor behavior [33]. Another emerging class of biomarkers includes the minichromosome maintenance (MCM) proteins, particularly MCM7, which play a key role in DNA replication. Increased MCM expression has been linked to greater tumor aggressiveness and reduced progression-free survival in patients undergoing radical prostatectomy [34, 35]. Genomic analyses have also revealed recurrent genetic alterations in PCa, most notably gain of chromosome 8q, which is strongly associated with poor prognosis in localized disease. Furthermore, amplification of the EIF3S gene, located on chromosome 8q and encoding a subunit of eukaryotic initiation factor 3, correlates with higher Gleason scores, more advanced tumor stage, and unfavorable clinical outcomes [35–37].

Future perspectives in the development of new biomarkers

Artificial intelligence (AI) and machine learning (ML) are increasingly recognized as critical tools for biomarker discovery and clinical risk prediction across a broad spectrum of diseases, including prostate cancer (PCa). A notable example is the study by Huang et al., who developed a machine learning–based framework to construct a DNA replication stress model designed to predict clinical outcomes and therapeutic vulnerabilities in primary PCa [38]. This work underscores the capacity of ML approaches to generate robust molecular signatures, such as the replication stress signature (RSS), which can enhance risk stratification, uncover novel therapeutic targets, and support the development of personalized treatment strategies in prostate cancer [Fig. 1]. Overall, the integration of AI and ML into oncologic research holds substantial promise for improving patient outcomes and advancing precision medicine in Pca [38].

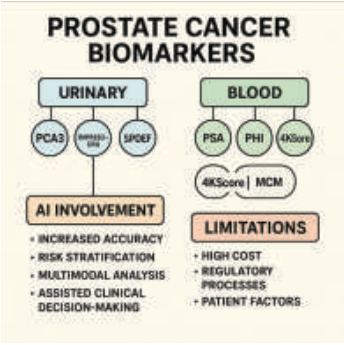


Fig. 1. A scheme of the most frequent prostatic biomarkers and the AI role.

Conclusions

Despite the growing number of available biomarkers, several obstacles as, the high cost, the regulatory approval processes, the patient-related factors continue to limit their widespread implementation in clinical practice. However, there are encouraging premises that using the newest tools could help in the faster development and testing new reliable tests for the prostate cancer.

References

1. Awedew, A.F.; Han, H.; Abbasi, B.; Abbasi-Kangevari, M.; Ahmed, M.B.; Almidani, O.; Amini, E.; Arabloo, J.; Argaw, A.M.; Athari, S.S.; et al. The global, regional, and national burden of benign prostatic hyperplasia in 204 countries and territories from 2000 to 2019: A systematic analysis for the Global Burden of Disease Study 2019. Lancet Healthy Longev. 2022, 3, e754–e776.
2. National Cancer Institute. SEER Stat Fact Sheets: Prostate; National Cancer Institute: Bethesda, MD, USA.
3. Nisticò, P.; Ciliberto, G. Biological mechanisms linked to inflammation in cancer: Discovery of tumor microenviron-ment-related biomarkers and their clinical application in solid tumors. Int. J. Biol. Markers 2020, 35 (Suppl. S1), 8–11.
4. Reinfeld, B.I.; Madden, M.Z.; Wolf, M.M.; Chytil, A.; Bader, J.E.; Patterson, A.R.; Sugiura, A.; Cohen, A.S.; Ali, A.; Do, B.T.; et al. Cell-programmed nutrient partitioning in the tumor microenvironment. Nature 2021, 593, 282–288.
5. Ene, C.D.; Anghel, A.E.; Neagu, M.; Nicolae, I. 25-OH Vitamin D and Interleukin-8: Emerging Biomarkers in Cutaneous Mela-noma Development and Progression. Mediators Inflamm. 2015, 2015, 904876.
6. Jang, J.H.; Kim, D.H.; Surh, Y.J. Dynamic roles of inflammasomes in the inflammatory tumor microenvironment. NPJ Precis. Oncol. 2021, 5, 18.
7. Sfanos, K.S.; Yegnasubramanian, S.; Nelson, W.G.; De Marzo, A.M. The inflammatory microenvironment and microbiome in prostate cancer development. Nat. Rev. Urol. 2018, 15, 11–24.
8. Bahmad, H.F.; Jalloul, M.; Azar, J.; Moubarak, M.M.; Samad, T.A.; Mukherji, D.; Al-Sayegh, M.; Abou-Kheir, W. Tumor Mi-croenvironment in Prostate Cancer: Toward Identification of Novel Molecular Biomarkers for Diagnosis, Prognosis, and Therapy Development. Front. Genet. 2021, 12, 652747.
9. Matsuoka, Y.; Nakayama, H.; Yoshida, R.; Hirosue, A.; Nagata, M.; Tanaka, T.; Kawahara, K.; Sakata, J.; Arita, H.; Nakashima, H.; et al. IL-6 controls resistance to radiation by suppressing oxidative stress via the Nrf2-antioxidant pathway in oral squa-mous cell carcinoma. Br. J. Cancer. 2016, 115, 1234–1244.
10. Ene, C.D.; Penescu, M.N.; Nicolae, I. Sialoglyco-Conjugate Abnormalities, IL-6 Trans-Signaling and Anti-Ganglioside Immune Response-Potential Interferences in Lupus Nephritis Pathogenesis. Diagnostics 2021, 11, 1129.
11. Yu, S.H.; Maynard, J.P.; Vaghasia, A.M.; De Marzo, A.M.; Drake, C.G.; Sfanos, K.S. A role for paracrine interleukin-6 signaling in the tumor microenvironment in prostate tumor growth. Prostate 2019, 79, 215–222.
12. Archer, M.; Dogra, N.; Kyprianou, N. Inflammation as a Driver of Prostate Cancer Metastasis and Therapeutic Resistance. Cancers 2020, 12, 2984.
13. Salagierski, M.; Schalken, J.A. PCA3 and TMPRSS2-ERG: Promising Biomarkers in Prostate Cancer Diagnosis. Cancers 2010, 2: 1432–1440.
14. Chen, J.-Y.;Wang, P.-Y.; Liu, M.-Z.; Lyu, F.; Ma, M.-W.; Ren, X.-Y.; Gao, X.-S. Biomarkers for Prostate Cancer: From Diagnosis to Treatment. Diagnostics 2023, 13, 3350.
15. Wong, C.H.-M.; Ko, I.C.-H.; Ng, C.F. Liquid Biomarkers in Prostate Cancer: Recent Advancements and Future Directions. Curr. Opin. Urol. 2025, 35, 3–12.
16. Liu, Y.; Hatano, K.; Nonomura, N. Liquid Biomarkers in Prostate Cancer Diagnosis: Current Status and Emerging Prospects. World J. Mens Health 2025, 43, 8.
17.Balk SP, Ko YJ, Bubley GJ. Biology of prostate-specific antigen. J Clin Oncol Jan. 2003;21(2):383–91.
18. Liu Y, Hatano K, Nonomura N. Liquid biomarkers in prostate cancer diagnosis: current status and emerging prospects. World J Mens Health 2025;43(1).
19. Borbiev T, Kohaar I, Petrovics G. Clinical biofluid assays for prostate cancer. Cancers (Basel) Jan. 2023;16(1).
20. Jansen FH, et al. Prostate-specific antigen (PSA) isoform p2PSA in combination with total PSA and free PSA improves diagnostic accuracy in prostate

cancer detection. Eur Urol Jun. 2010;57(6):921–7.
21. Boehm BE, York ME, Petrovics G, Kohaar I, Chesnut GT. Biomarkers of aggressive prostate cancer at diagnosis. Int J Mol Sci Jan. 2023;24(3):2185. https://doi.org/10.3390/IJMS24032185. 2023, Vol. 24, Page 2185.
22. Rajwa P, Syed J, Leapman MS. How should radiologists incorporate non-imaging prostate cancer biomarkers into daily practice? Springer; Dec. 01, 2020.
23. Loeb S, et al. The prostate health index selectively identifies clinically significant prostate cancer. J Urol Apr. 2014;193(4):1163.
24. Meehan J, Gray M, Martínez-P´erez C, Kay C, McLaren D, Turnbull AK. Tissue- and liquid-based biomarkers in prostate cancer precision medicine. J Personal. Med Jul. 2021;11(7).
25. Schmid M, Trinh QD, Graefen M, Fisch M, Chun FK, Hansen J. The role of biomarkers in the assessment of prostate cancer risk prior to prostate biopsy: which markers matter and how should they be used? World J Urol 2014;32(4): 871–80.
26. Sotomayor PC, et al. Active surveillance in prostate cancer: current and potentially emerging biomarkers for patient selection criteria. S. Karger AG; Dec. 01, 2022.
27. Ferro M, Rocco B, Maggi M, Lucarelli G, Falagario UG, Del Giudice F, Crocetto F, Barone B, La Civita E, Lasorsa F, Brescia A, Catellani M, Busetto GM, Tataru OS, Terracciano D. Beyond blood biomarkers: the role of SelectMDX in clinically significant prostate cancer identification. Expert Rev Mol Diagn 2023;23(12): 1061–70.
28. Bryant RJ, et al. Predicting high-grade cancer at ten-core prostate biopsy using four kallikrein markers measured in blood in the ProtecT study. J Natl Cancer Inst Jul. 2015;107(7).
29. Punnen S, et al. A multi-institutional prospective trial confirms noninvasive blood test maintains predictive value in african American men. J Urol Jun. 2018;199(6): 1459–63.
30. Parekh DJ, et al. A multi-institutional prospective trial in the USA confirms that the 4Kscore accurately identifies men with high-grade prostate cancer. Eur Urol Sep. 2015;68(3):464–70.
31. Rubio J, et al. Immunohistochemical expression of Ki-67 antigen, Cox-2 and Bax/ Bcl-2 in prostate cancer; prognostic value in biopsies and radical prostatectomy specimens. Eur Urol Nov. 2005;48(5):745–51. https://doi.org/10.1016/j.eururo.2005.06.014.
32. Ferro M, Crocetto F, La Civita E, Fiorenza M, Jannuzzi G, Carbone G, Sirica R, Sicignano E, Pagano G, Imbimbo C, Terracciano D. Serum (-2)proPSA/freePSAratio, (-2)proPSA/freePSA density, prostate health index, and prostate health index density as clues to reveal postoperative clinically significant prostate cancer in men with prostate-specific antigen 2-10 ng/mL. Prostate 2024;84(12): 1157–64.
33. Wolters T, Vissers KJ, Bangma CH, Schr¨oder FH, van Leenders GJ. The value of EZH2, p27(kip1), BMI-1 and MIB-1 on biopsy specimens with low-risk prostate cancer in selecting men with significant prostate cancer at prostatectomy. BJU international 2010;106(2):280–6.
34. Laitinen S, Martikainen PM, Tolonen T, Isola J, Tammela TLJ, Visakorpi T. EZH2, Ki-67 and MCM7 are prognostic markers in prostatectomy treated patients. Int J Cancer Feb. 2008;122(3):595–602.
35. Ren B, et al. MCM7 amplification and overexpression are associated with prostate cancer progression. Oncogene Feb. 2006;25(7):1090–8.
36. Strohmeyer DM, et al. Genetic aberrations in prostate carcinoma detected by comparative genomic hybridization and microsatellite analysis: association with progression and angiogenesis. Prostate Apr. 2004;59(1):43–58.
37. Padmanabhan V, Callas P, Philips G, Trainer TD, Beatty BG. DNA replication regulation protein Mcm7 as a marker of proliferation in prostate cancer. J Clin Pathol Oct. 2004;57(10):1057–38. Sung H, Ferlay J, Siegel RL, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2021;71(3):209–49.