ŁAPIŃSKI, Maciej, LEWKOWSKA, Monika and SADOWSKA, Maja. Prostate cancer diagnostics - new perspectives for salivary and urinary biomarkers. Journal of Education, Health and Sport. 2025;86:67368. eISSN 2391-8306.
https://doi.org/10.12775/JEHS.2025.86.67368
https://apcz.umk.pl/JEHS/article/view/67368

The journal has had 40 points in Minister of Science and Higher Education of Poland parametric evaluation. Annex to the announcement of the Minister of Education and Science of 05.01.2024 No. 32318. Has a Journal's Unique Identifier: 201159. Scientific disciplines assigned: Physical culture sciences (Field of medical and health sciences); Health Sciences (Field of medical and health sciences).
Punkty Ministerialne 40 punktów. Załącznik do komunikatu Ministra Nauki i Szkolnictwa Wyższego z dnia 05.01.2024 Lp. 32318. Posiada Unikatowy Identyfikator Czasopisma: 201159. Przypisane dyscypliny naukowe: Nauki o kulturze fizycznej (Dziedzina nauk medycznych i nauk o zdrowiu); Nauki o zdrowiu (Dziedzina nauk medycznych i nauk o zdrowiu).
The authors declare that there is no conflict of interests regarding the publication of this paper.
Received: 11.12.2025. Revised: 28.12.2025. Accepted: 28.12.2025. Published: 31.12.2025.

Prostate cancer diagnostics - new perspectives for salivary and urinary biomarkers

Maciej Łapiński[1] [MŁ] https://orcid.org/0009-0008-4509-8618
maciek.lapinski.99@gmail.com
[1]Medical University of Bialystok, Jana Kilińskiego 1 street, 15-089 Białystok, Poland

Monika Lewkowska[1] [ML] https://orcid.org/0009-0001-1560-5307
monika.lw19@gmail.com
[1]Medical University of Bialystok, Jana Kilińskiego 1 street, 15-089 Białystok, Poland

Maja Sadowska[1] [MS] https://orcid.org/0009-0001-5198-6848
majasadowska7@wp.pl
[1]Medical University of Bialystok, Jana Kilińskiego 1 street, 15-089 Białystok, Poland

Abstract

Background. The growing epidemiological burden of prostate cancer, projected for 2025, combined with the diagnostic limitations of prostate-specific antigen (PSA), necessitates the search for new strategies in precision oncology.

Aim. This review article analyzes the evolution of diagnostic methods, pointing to the need to move from traditional serum markers to non-invasive liquid biopsy.

Material and methods. We synthesize the current state of knowledge on established urinary biomarkers and present a novel approach using saliva as a source of information on cancer status.

Results. The paper discusses the potential of metabolic profiling (sialic acid, citrate), identification of new protein biomarkers (S100P), analysis of specific microRNA signatures

depending on the stage of the disease, and the role of the oral microbiome in pathogenesis and risk stratification.

Conclusions. We conclude that salivary and urinary biomarkers represent a rapidly developing experimental direction in prostate cancer diagnostics. Preliminary studies indicate promising diagnostic performance; however, the majority of available evidence is based on pilot and case–control studies and requires further large-scale validation before clinical implementation.

Key words: prostate cancer, liquid biopsy, biomarkers, saliva, precision oncology.

1. Introduction

Prostate cancer is one of the greatest challenges of modern oncology, being the most frequently diagnosed malignant cancer among men in the United States and worldwide, excluding non-melanoma skin cancers [1–3]. It is predicted that in the United States alone, 313,780 new cases of this disease will be recorded in 2025, making it the most frequently diagnosed cancer in the male population [1,4] . Globally, in 2020, the number of new cases was approximately 1.41 million, and current projections indicate a sustained upward trend in both incidence and prevalence through 2046 [5,6]. This disease is the second most common cause of cancer deaths in men in the USA, accounting for an estimated 35,770 deaths in 2025 [1].

Although the five-year survival rate for localized disease exceeds 99%, the prognosis drastically worsens for metastatic disease, where the rate drops to 30% [5,7].

The central element of clinical diagnosis and monitoring remains prostate-specific antigen (PSA), a serine protease secreted by the luminal epithelial cells of the prostate gland [8]. Although PSA levels are significantly higher in patients with cancer compared to healthy individuals, making it a preferred marker in diagnosis and monitoring of treatment response this marker lacks adequate specificity. Increased PSA levels can result from benign prostatic hyperplasia (BPH), prostatitis, and other non-cancerous conditions, which makes it impossible to reliably distinguish between benign and malignant changes based on this parameter alone [9–13]. A significant limitation is the fact that about 15% of men with a negative PSA result have cancer detectable in a biopsy, which underscores the need to search for markers with higher sensitivity and specificity [13,14].

In light of these limitations, modern medicine focuses on identifying biomarkers that enable early detection, precise determination of the stage of the disease, and prognosis [14,15]. Besides established tissue and urine markers, the use of salivary biomarkers such as miRNA, sialic acid, S100P protein, or spectral changes detected by spectroscopy is becoming a promising direction, which may offer a non-invasive diagnostic alternative [16–19]. In this article, we summarize the current state of knowledge on key molecular and genetic markers and present perspectives for novel diagnostic methods, including microbiome and saliva metabolite analysis, in the context of precision medicine.

1.1. Molecular landscape

While PSA remains the foundation of screening tests, its limitations in differentiating between benign and malignant changes have forced the search for more precise tools. Modern prostate cancer diagnostics is evolving towards a panel of markers that include not only proteases, but also specific membrane antigens, non-coding RNA, gene fusions, and advanced multigene tests.

1.2. Limitations and optimization of enzyme markers

Prostate-specific acid phosphatase (PAP), historically used as a routine laboratory test, like PSA, is not fully satisfactory as a standalone diagnostic marker, although it is being studied as a prostate-specific indicator alongside newer parameters [9,12,20]. To increase the diagnostic specificity of PSA itself and distinguish cancer from benign prostatic hyperplasia (BPH), clinical practice has introduced PSA isoforms, such as free PSA (fPSA) and complexed PSA (cPSA), as well as parameters related to the volume of the gland [21].

However, it should be emphasized that the PSA level is influenced by a number of factors unrelated to cancer, which complicates the interpretation of the results. Research indicates that obesity and diabetes may paradoxically lower PSA levels through mechanisms of hemodilution (increased plasma volume) and changes in steroid hormone levels, creating a risk of false negative diagnoses [22]. Additionally, the use of medications such as finasteride or metformin is also associated with a decrease in the level of this marker [22].

In response to these limitations, new-generation tests have been developed. The Prostate Health Index (PHI), which combines total PSA, free PSA, and the [-2]proPSA isoform, shows higher effectiveness in detecting clinically significant prostate cancer compared to PSA alone [23]. Similarly, the 4Kscore test, which analyzes an algorithm of four kallikreins (total PSA, free PSA, intact PSA, and human kallikrein 2 (hK2)) along with clinical data, allows for more effective stratification of the risk of aggressive disease (Gleason ≥7) [23]. Despite its clinical

utility, the test is limited by cost, availability and the lack of universal reimbursement in many healthcare systems. It should be emphasized that multiparametric prostate MRI is currently the most important complementary tool to PSA testing, significantly reducing unnecessary biopsies. Therefore, any novel biomarker should be evaluated not only against PSA alone, but also in combination with MRI-based diagnostic pathways.

1.3. Tissue specificity: PSMA, AMACR, and Galectin-3

In contrast to markers with a broad expression profile, prostate-specific membrane antigen (PSMA) laexhibits overexpression in tumor tissue compared to normal prostate or BPH [11,24]. PSMA, a transmembrane protein with glutamate carboxypeptidase activity, is considered a highly specific marker for prostate cancer, particularly in its advanced and metastatic forms [20,25]. It should be noted that PSMA currently plays a more significant role as a theranostic target in imaging and radionuclide therapy rather than as a standalone non-invasive screening biomarker. The expression of PSMA is regulated by the androgen receptor (AR) pathway and DNA repair mechanisms, and its high level correlates with resistance to castration [26]. Although the name suggests exclusive specificity for the prostate, PET/CT imaging studies using ligands such as [68Ga]Ga-PSMA-11 or [18F]-JK-PSMA-7 confirm its expression in other tissues, including salivary glands, kidneys, and the neovasculature of other solid tumors [26,27]. Concurrently, in immunohistochemical diagnostics of prostate cancer significantly increased alpha-methylacyl-CoA racemase (AMACR) plays a crucial role [11,28]. AMACR is commonly used in diagnostic panels, often in combination with basal cell markers (HMWCK, 34βE12) [15]. An interesting addition to this landscape is galectin-3 (Gal-3), a β-galactoside-binding protein that is a substrate for PSA. It has been shown that in tumor tissue, Gal-3 undergoes proteolytic cleavage by PSA, which changes its biological functions, promoting angiogenesis and resistance to apoptosis, and its level in the tissue paradoxically decreases as the cancer progresses [29].

1.4. Advancements in molecular and genetic markers

Advancements in understanding the pathogenesis of prostate cancer have led to the identification of the TMPRSS2 gene fusion, which is a significant step forward [11,30]. However, the most specific molecular marker currently recognized is prostate cancer antigen 3 (PCA3) which acts as a non-coding RNA that is overexpressed in tumor tissue [12]. PCA3, detected in urine, showed an encouraging sensitivity and specificity profile in studies [30]. Modern tests, such as ExoDx Prostate (which analyzes exosomal RNA of PCA3, ERG, and

SPDEF genes) and SelectMDx (which measures the mRNA levels of HOXC6 and DLX1 genes), utilize these findings, offering a non-invasive assessment of the risk of high-grade cancer (Gleason ≥7), which helps avoid many unnecessary biopsies [23].

1.5. Genetic and prognostic markers

In the field of genetics, although research indicates the predictive value of certain genetic markers (e.g. SNP, BRCA2), their usefulness in routine screening has historically been limited [30]. However, this situation is changing thanks to the introduction of Polygenic Risk Scores (PRS). Studies such as BARCODE1 have shown that PRS based on genetic variant analysis (SNP) from germinal DNA (collected from saliva, for example) allows for the effective identification of men with an increased risk of disease, even with low PSA values [23]. Tests such as Stockholm3 integrate clinical data, protein markers, and over 100 genetic variants (SNPs), which significantly improves the detection of clinically significant prostate cancer compared to PSA alone [23].

Changes in tumor suppressor genes (p53, p16, Rb) and overexpression of genes such as telomerase or hepsin are still being studied as potential indicators [24,31]. It is also worth noting the identification of thirteen mast cell markers associated with prognosis [32]. Despite the wide range of molecules tested, this integrated approach combining genetics with proteomics appears to be the successor to traditional diagnostics based solely on PSA.

2. Materials and methods

To prepare this review, a multi-stage analysis of scientific literature was conducted, focusing on the identification, characterization, and clinical assessment of the usefulness of new biomarkers of prostate cancer in body fluids. Electronic databases PubMed, ScienceDirect, and Google Scholar were searched using advanced search strategies based on keywords in the field of urologic oncology, molecular diagnostics, and analytical chemistry. The search included medical terms (MeSH) and key phrases such as "prostate cancer," "liquid biopsy," "saliva," "urine," "biomarkers," "S100P," "citrate," "miRNA," "exosomes," and "ATR-FTIR spectroscopy." To precisely narrow the results to the most relevant studies, individual keywords were combined using Boolean logic operators (AND, OR). This allowed for the effective combination of general cancer-related keywords with specific detection methods (e.g., "prostate cancer AND saliva AND spectroscopy" or "biomarkers OR metabolic profiling").

The time frame of the review was divided functionally, depending on the topic being discussed. Articles published before 2015, dating back to the late 1990s. In the 20th century, they were

mainly used to outline the historical background and discuss the established role and limitations of the PSA antigen. However, for innovative diagnostic methods, such as metabolic profiling, microbiome analysis, detection of S100P protein in saliva, or biospectroscopy, the analysis was limited to reports from 2015–2025, with a particular emphasis on works published after 2020, to ensure the timeliness of the technological and clinical data presented. Only full-text original and review papers in English that provided quantitative data on diagnostic effectiveness in human studies were included in the final analysis, rejecting reports that did not meet the criteria for scientific reliability.

3. Non-invasive fluid diagnostics: urine and saliva

The development of precision oncology is forcing a shift away from invasive procedures towards biomarkers available in body fluids. Liquid biopsy, which includes urine and saliva analysis, is becoming a key area of research, offering the possibility of monitoring the dynamics of cancer in real time.

3.1. Advanced urine analysis

In the context of urinary biomarkers, prostate cancer antigen 3 (PCA3) remains the cornerstone of molecular diagnostics, a non-coding RNA that is overexpressed in tumor tissue, reaching levels 66 times higher than in normal tissue [33–35]. Research has shown that PCA3 is elevated in over 95% of primary prostate cancer cases, making it a highly sensitive indicator [34]. Urinary tests based on PCA3, such as the FDA-approved PROGENSA system, offer much higher specificity than serum PSA tests [34,36]. This diagnosis is supplemented by the detection of the TMPRSS2-ERG gene fusion. This specific chromosomal aberration occurs in 30-50% of newly diagnosed patients and can be effectively detected in urine samples taken after digital rectal examination (DRE) [37,38]. Incorporating the TMPRSS2-ERG fusion into diagnostic panels significantly increases their predictive value [39].

However, diagnostics now go beyond established genetic markers, focusing on the analysis of metabolic reprogramming. Unlike healthy epithelial cells, which secrete significant amounts of citrate (due to the inhibition of the enzyme aconitase by high zinc concentrations), cancer cells reactivate the Krebs cycle (TCA) to produce energy [22]. This results in a drastic decrease in citrate concentration in tumor tissue and body fluids, including urine, which is considered a metabolic hallmark of prostate cancer [22]. The level of citrate in urine was shown to be significantly lower in patients with prostate cancer compared to those with benign prostatic

hyperplasia (BPH), making it a promising marker for differentiating the physiology of cancer from benign changes [22].

Meanwhile, researchers are focusing on extracellular vesicles (EVs) in urine, which protect miRNA from degradation [40].A review of studies indicates that the miRNA panel, including miR-21-5p and miR-375, is an effective tool for differentiating cancer patients from healthy controls, and analysis of the isomiRs of these microRNAs in urinary exosomes may offer higher sensitivity than analysis of the mature forms [40].

3.2. Saliva as a reservoir of biomarkers

Saliva, an ultrafiltrate of plasma, contains a wide spectrum of proteins that reflect the physiological state of the organism. A significant positive correlation between serum PSA concentration and its level in saliva was demonstrated in oncological patients [41,42]. However, it should be noted that although the average concentrations of free and total PSA differ between healthy and diseased individuals, the ratio of the free to total fraction (f/t) in both fluids remains similar in the control group, which limits the diagnostic usefulness of this parameter in isolation [41]. However, the potential of saliva goes beyond PSA alone. This fluid was found to contain a range of other glycoprotein biomarkers that can aid in the detection of cancer, such as c-erbB-2, cancer antigen 125 (CA 125), CA 19-9, CA 15-3, and carcinoembryonic antigen (CEA) [41,42].

However, the spectrum has been significantly expanded to include new, highly specific indicators. The calcium-binding protein S100P has been proposed as a potential experimental salivary biomarker, however current evidence remains insufficient for clinical application[18]. Clinical-control studies have shown that the concentration of S100P in saliva is significantly higher in men with prostate cancer (mean 10.2 ± 0.4 ng/mL) compared to the group with benign prostatic hyperplasia (BPH, 2.7 ± 0.2 ng/mL) [18]. This parameter shows a strong positive correlation with serum concentration (r=0.82) and has an area under the curve (AUC) of 0.928, confirming its high potential in differentiating malignant from benign changes [18].

3.3. Metabolic Profiling

In recent studies, special attention has been paid to sialic acid (SA). Analyses showed a specific concentration gradient of this metabolite in saliva: the highest values were observed in patients with confirmed prostate cancer, intermediate values in those with bone metastases, and the lowest in those with benign prostatic hyperplasia (BPH) [16]. This method has significant predictive value. The level of SA (measured in relation to PSA) was shown to be significantly

higher in high-risk patients compared to low-risk and intermediate-risk groups [16]. This parameter is characterized by high diagnostic accuracy, achieving a sensitivity of 85.7% and specificity of 95.3% [16]. The metabolic profile of saliva is supplemented by the detection of sarcosine and related metabolites using capillary electrophoresis coupled with mass spectrometry (CE-MS) [43].

Recent research has expanded this panel to include inorganic and enzymatic markers. It has been shown that concentrations of zinc, urea, creatinine, and BB creatine kinase (CK-BB) are significantly higher in the saliva of prostate cancer patients compared to the BPH group [44]. In particular, zinc, which is key to prostate physiology, shows a strong correlation between serum and saliva concentrations ($r=0.76$), suggesting that its accumulation in tissue is reflected in body fluids [44]. The opposite trend is observed for melatonin as its level in saliva is significantly lower in cancer patients (82.1 ng/L) compared to BPH (127.2 ng/L), which may reflect systemic circadian rhythm disorders [44]. Although alterations in citrate and zinc metabolism represent a well-established hallmark of prostate cancer biology, their direct application as standalone diagnostic biomarkers remains limited and requires further technological standardization.

3.4. MicroRNA

The use of modern nanotechnologies, such as nanographene oxide, has enabled the non-invasive detection of nucleic acids in saliva. Specific microRNA expression patterns have been identified that are dependent on the stage of the disease. miR-141 is significantly elevated in patients with advanced cancer, while overexpression of miR-21 is characteristic of early stages of the disease [16]. This discovery suggests that a panel-based study of salivary miRNAs can serve as a non-invasive method for early diagnosis and monitoring of progression [45,46]. Recent studies on salivary exosomes have also confirmed that lowering the levels of miR-331-3p and miR-200b allows differentiating prostate cancer from benign changes with a positive predictive value of 71% [47]. However, most studies on circulating miRNAs in prostate cancer are based on relatively small cohorts and lack prospective, multi-center validation.

3.5. Integrated concept and perspectives

An analysis of available data leads to the conclusion that the future of prostate cancer diagnostics does not lie in the search for one ideal biomarker, but in the integration of many biological signals from different body fluids. Despite the growing number of indicators discovered, no single marker has proven to be fully satisfactory for early detection, determining

the stage of the disease, or predicting its course [13–15]. Even the most established PSA fails in about 15% of men with cancer detectable in biopsy who present negative test results [13].

3.6. Biomarker synergy

It is postulated that the solution to this problem is a panel approach, combining markers with different specificities. Research has shown that the correlation between miR-21 expression and serum PSA levels allows for higher diagnostic and prognostic accuracy than using either of these markers alone [48]. Similarly, combining PCA3 with other indicators, such as PSA or TMPRSS2-ERG fusion, significantly improves diagnostic precision [36,39]. An example of such synergy is also the measurement of sialic acid levels directly on the PSA molecule, which allows for more precise stratification of risk in patients [16].

3.7. Saliva as a new diagnostic window

The prospect of using saliva as an easily accessible biological material is a promising direction for precision medicine. The stability of miRNA in extracellular fluids and the possibility of non-invasive detection of miR-141 and miR-21 using advanced nanotechnologies may revolutionize screening [16]. Moreover, the discovery of specific microbiological signatures in saliva that overlap with the bacterial flora of the prostate suggests the existence of an "oral cavity-prostate" axis. The presence of bacteria such as Pauljensenia or Oribacterium may indicate a potential causal effect or early risk marker, which requires further translational research [17,49]. Importantly, currently available data demonstrate only associative relationships and do not allow for causal inference.

A promising development in point-of-care (POC) diagnostics is the use of Fourier transform infrared spectroscopy (ATR-FTIR) [19]. A large screening study of nearly 1,000 samples used a simple dip test (dip the swab in saliva), obtaining spectra in the range of 4,000-650 cm^{-1} [19]. The use of chemometric algorithms (PCA-QDA) allowed for distinguishing patients with prostate cancer from the control group with high accuracy: 97% in the training set and 93% in the test set, with clinical sensitivity reaching 100% and specificity 92% [19]. These results originate primarily from pilot studies and experimental laboratory settings. This method is particularly sensitive to changes in the lipid profile, as confirmed by independent studies [50]. The ratio of lipid band intensities 1458/1396 cm^{-1} was shown to decrease significantly in patients with prostate cancer, while the ratio 2923/2957 cm^{-1} increased, reflecting age-dependent changes in fatty acid chain structure [50]. At the current stage, ATR–FTIR spectroscopy should be regarded as a research tool with translational potential, rather than a

clinically validated diagnostic method. Additionally, the high cost of equipment and lack of standardized clinical protocols remain significant barriers to widespread implementation.

3.8. Genetic risk stratification and microbiome

Saliva also serves as a non-invasive source of germinal DNA for calculating polygenic risk scores (PRS). The BARCODE1 study showed that using PRS based on genetic variants isolated from saliva can effectively identify men with an increased genetic risk who have clinically significant prostate cancer detected by biopsy in 40% of cases, often even with low PSA values [23].

A groundbreaking area of research is the analysis of the saliva microbiome, which revealed statistically significant associations between 42 bacterial species (belonging to 24 genera and 17 families) and prostate cancer [17]. Specific types of bacteria have been identified whose presence is associated with an increased risk of disease. They include: Oribacterium, Pauljensenia, Campylobacter A, Catonella, Lachnoanaerobaculum, and RUG343 [17]. In contrast, species that exhibit potential protective or inhibitory effects (odds ratio <1) include: Aggregatibacter, Solobacterium, Streptococcus, and Gemella [17]. The hypothesis of the existence of the "oral cavity-prostate" axis is supported by research showing the presence of the same bacterial species in the oral cavity and prostate secretion in 70% of patients suffering from chronic prostatitis or BPH, suggesting a potential causal relationship [49]. However, one should be cautious when interpreting these relationships. Large-scale population studies, such as those conducted on the Atlantic PATH and ATP cohorts, suggest that while there are some taxonomic associations (e.g., an increase in the relative abundance of Alloprevotella rava), the overall diversity of the oral microbiome does not show drastic changes in the case of prostate cancer, unlike the strong signals observed in colorectal cancer. This suggests that the salivary microbiome may be a secondary marker rather than a standalone screening tool [49]. The oral–prostate cancer link remains highly speculative and requires mechanistic confirmation.

3.9. Clinical implications: PSMA and salivary gland metastases

In terms of clinical and teranostic aspects, the presence of a specific prostate membrane antigen (PSMA) in salivary glands is significant. Patients with prostate cancer who underwent a study using the systemically administered radiolabel [18F]DCFPyL showed high uptake in the salivary glands and increasing secretion of the radiolabel into saliva over time [51]. The physiological uptake in the salivary glands has also been confirmed for newer tracers, such as [18F]-JK-PSMA-7, which is crucial for planning radioligand therapy to avoid gland damage

[26,27]. Although this phenomenon is mainly relevant for radiotherapy planning, literature also notes rare cases of prostate cancer metastases directly to the parotid gland [52].

4. Discussion

An analysis of available data leads to the conclusion that the future of prostate cancer diagnostics does not lie in the search for one ideal biomarker, but in the integration of many biological signals from different body fluids (Table 1). Despite the growing number of discovered indicators, no single marker has proven to be fully satisfactory for early detection, determining the stage of the disease, or predicting its course [13–15]. Even the most established PSA fails in about 15% of men with cancer detectable in biopsy who present negative test results [13].

It is postulated that the solution to this problem is a panel approach, combining markers with different specificities. A promising direction is combining PSA with metabolic markers. Because the level of citrate decreases in the tumor (due to the reactivation of the TCA cycle), and the level of PSA increases, their simultaneous analysis in body fluids can drastically increase diagnostic specificity, eliminating false-positive results resulting from, for example, BPH [22]. Also at the molecular level, combining PCA3 with other indicators, such as PSA or TMPRSS2-ERG fusion, significantly improves diagnostic precision [36,39]. New research indicates that incorporating saliva-derived polygenic risk scores (PRS) into these panels allows for more precise stratification of patients before referral for MRI or biopsy [23].

The prospect of using saliva and urine as easily accessible biological materials is a promising direction for oncology. Standardization of extracellular vesicle (EV) isolation methods will play a key role, as EVs are a stable carrier for a panel of miRNAs (e.g., miR-21, miR-375) and allow for the assessment of tumor aggressiveness without the need for invasive tissue biopsy [40]. The implementation of ATR-FTIR biospectroscopy as a rapid screening test in primary care may also prove highly valuable. The possibility of obtaining an immediate result based on a biochemical saliva "fingerprint," with sensitivity exceeding traditional methods, paves the way for inexpensive and mass screening [19].

The major limitations of currently available studies include small sample sizes, heterogeneity of analytical methods, lack of prospective multi-center validation, limited cost–effectiveness analysis and insufficient comparison with MRI-based diagnostic strategies.

Table 1. Comparative characteristics of key prostate cancer biomarkers in various body fluids.

* Indicates presence detected in research but not yet used routinely in clinical practice.

Biomarker	Molecule Type/ Method	Diagnostic Material	Clinical significance and characteristics	Source
S100P	Calcium-binding protein	Saliva, Serum	Significantly elevated in prostate cancer vs BPH. Strong saliva-serum correlation (r=0.82). AUC > 0.92.	[18]
Citrate	Metabolite	Urine, Semen, Tissue	Significant decrease in cancer (TCA cycle reactivation). Specific marker for cancer cell metabolism.	[22]
Spectral Signature	ATR-FTIR Spectroscopy	Saliva (swab)	Biochemical "fingerprint" analysis. 93% accuracy in detecting prostate cancer (test set).	[19]
Lipid Profile	Lipids (IR band ratios)	Saliva	Decrease in 1458/1396 cm^{-1} ratio and increase in 2923/2957 cm^{-1} in prostate cancer. Age-dependent.	[50]
Zinc	Trace element	Saliva, Serum	Elevated levels in saliva of prostate cancer patients vs BPH.	[44]
Melatonin	Hormone	Saliva, Serum	Decreased levels in saliva and serum of prostate cancer patients vs BPH.	[44]
CK-BB	Kinase isoenzyme	Saliva, Serum	Higher concentration in saliva in the prostate cancer group compared to BPH.	[44]
PSA	Serine protease	Serum, (Saliva*)	Gold standard, but low specificity; does not distinguish BPH from cancer. In saliva, f/t ratio is close to normal.	[44]
PCA3	Non-coding RNA	Urine	Highly specific for tumor (up to 66-fold overexpression); not present in normal tissue.	[40]
miR-21/miR-375	MicroRNA (EVs)	Urine, Saliva	Present in extracellular vesicles. Isoforms (isomiRs) improve diagnostic sensitivity.	[40]
miR-331-3p/miR-200b	MicroRNA (EVs)	Saliva	Decreased in prostate cancer. Positive predictive value of 71%.	[53]
Sialic Acid (SA)	Metabolite	Saliva	Level correlates with stage. Sensitivity: 85.7%, Specificity: 95.3% (on PSA).	[16]
PRS (Polygenic Risk Score)	Germline DNA	Saliva	Identification of high genetic risk group; 40% biopsy accuracy in this group.	[23]
Microbiome	Bacterial DNA	Saliva, Tissue	Genera *Pauljensenia*, *Oribacterium* linked to risk; oral-prostate axis.	[49]

5. Conclusions

Prostate-specific antigen (PSA) remains the standard for screening but suffers from low specificity, leading to overdiagnosis and unnecessary biopsies. The rising epidemiological burden of prostate cancer necessitates new, non-invasive diagnostic strategies, such as liquid biopsies using saliva and urine. Saliva has emerged as a valuable diagnostic material containing

proteins like S100P, metabolites including citrate and sialic acid, and microRNAs. S100P, a calcium-binding protein, is significantly elevated in the saliva of patients with prostate cancer compared to those with benign prostatic hyperplasia (BPH) and shows a strong correlation with serum levels. Metabolic reprogramming in cancer cells leads to the reactivation of the TCA cycle and a significant decrease in citrate levels in tissue and fluids, serving as a metabolic hallmark of the disease. Specific salivary microRNA signatures, including miR-21, miR-141, and exosomal miR-331-3p/miR-200b, allow for the differentiation of cancer stages and distinction from benign conditions. ATR-FTIR biospectroscopy offers a rapid, non-invasive method to detect biochemical "fingerprints" in saliva, distinguishing prostate cancer patients with high accuracy. Saliva-derived DNA enables the calculation of Polygenic Risk Scores (PRS) to identify men at high genetic risk, improving patient stratification before biopsy. While the oral microbiome shows some associations with prostate cancer risk (e.g., specific bacterial genera), overall diversity changes are less prominent than in other cancers like colorectal cancer. Salivary and urinary biomarkers should be considered promising adjunct tools that may complement, but not replace, existing diagnostic pathways pending further validation.

Disclossure

Author Contributions

Conceptualization, M.Ł. and K.Ż.; methodology, M.Ł., M.L. and M.S.; validation, M.Ł., M.L., M.S. and K.Ż; formal analysis, M.Ł. and M.L; investigation, M.Ł., M.L. and M.S.; resources, M.Ł.; data curation, M.Ł., M.L. and M.S.; writing- original draft preparation, M.Ł. and K.Ż.; writing- review and editing, M.Ł. M.S., M.L. and K.Ż.; supervision, M.Ł.

All authors have read and agreed to the published version of the manuscript.

Funding

The study did not receive special funding.

Institutional Review Board Statement

Not applicable.

Informed Consent Statement

Not applicable.

Data Availability Statement

Not applicable.

Acknowledgements

Conflicts of Interest

The authors declare no conflict of interest.

References

[1] Suartz CV, Santos LA, Matalani CFA, DallAqua V, Schumacher LB, Lepine HL, et al. Transperineal Versus Transrectal Prostate Biopsy: A Systematic Review and Meta-Analysis of Randomized Controlled Trials. Clin Genitourin Cancer 2025. https://doi.org/10.1016/j.clgc.2025.102467.

[2] Wang H, Zhao Z, Xu X, Guo Z, Liu G. Ultra-low-dose radioimmunotherapy improves prostate cancer treatment efficacy and safety. Chinese Chemical Letters 2025. https://doi.org/10.1016/j.cclet.2025.111799.

[3] Abramczyk E, Nisar MU, Nguyen JK, Austin N, Ward RD, Weight C, et al. The Role of Prostate-Specific Membrane Antigen-Radioligand and Magnetic Resonance Imaging in Patients with Prostate Cancer Biochemical Recurrence. Seminars in Ultrasound, CT and MRI 2025;46:71–82. https://doi.org/10.1053/J.SULT.2024.11.005.

[4] Li X, Zhang X, Fu X, Wu H, Ye X, Huang X, et al. Urokinase-type plasminogen activator deficiency enhances CD8 + T cell infiltration and anti-PD-1 therapy efficacy in prostate cancer. Front Immunol 2025;16. https://doi.org/10.3389/fimmu.2025.1625226.

[5] Baron D, Pasquier D, Pace-Loscos T, Vandendorpe B, Schiappa R, Ortholan C, et al. Systemic therapy escalation after stereotactic body radiation therapy for oligometastatic hormone-sensitive prostate cancer. Clin Transl Radiat Oncol 2023;43. https://doi.org/10.1016/j.ctro.2023.100673.

[6] Zhao X, Liu S, Zou Z, Liang C. Global, regional, and national prevalence of prostate cancer from 1990 to 2021: a trend and health inequality analyses. Front Public Health 2025;13. https://doi.org/10.3389/fpubh.2025.1595159.

[7] Kessel A, Kohli M, Swami U. Current management of metastatic castration-sensitive prostate cancer. Cancer Treat Res Commun 2021;28:100384. https://doi.org/10.1016/J.CTARC.2021.100384.

[8] Hurlstone A, Black DM. Prostate cancer: New therapies in the pipeline? Current Biology 1998;8. https://doi.org/10.1016/S0960-9822(98)70270-2.

[9] Majd Shokorlou Y, Heidarzadeh H. Multispectral plasmonic biosensors based on a Penta-supercell metamaterial for detection of prostate-specific antigen: Ultrasensitive in LC resonance mode. Biosens Bioelectron 2022;217. https://doi.org/10.1016/j.bios.2022.114722.

[10] Lowes LE, Goodale D, Keeney M, Allan AL. Image Cytometry Analysis of Circulating Tumor Cells. vol. 102. 2011. https://doi.org/10.1016/B978-0-12-374912-3.00010-9.

[11] Tao Z, Shen M, Zheng Y, Mao X, Chen Z, Yin Y, et al. PCA3 gene expression in prostate cancer tissue in a Chinese population: Quantification by real-time FQ-RT-PCR based on exon 3 of PCA3. Exp Mol Pathol 2010;89. https://doi.org/10.1016/j.yexmp.2010.01.008.

[12] Satapathy BS, Mishra A, Behera S, Mohanty M, Kundu AK. Biosensors as emerging tools in cancer theranostics: Recent progress. 2025. https://doi.org/10.1016/B978-0-443-21592-6.00032-X.

[13] Tacke F, Kanig N, En-Nia A, Kaehne T, Eberhardt CS, Shpacovitch V, et al. Y-box protein-1/p18 fragment identifies malignancies in patients with chronic liver disease. BMC Cancer 2011;11. https://doi.org/10.1186/1471-2407-11-185.

[14] Grossfeld GD, Carroll PR, Lindeman N, Meng M, Groshen S, Feng A-C, et al. Thrombospondin-1 expression in patients with pathologic stage T3 prostate cancer undergoing radical prostatectomy: association with p53 alterations, tumor angiogenesis, and tumor progression. Urology 2002;59. https://doi.org/10.1016/S0090-4295(01)01476-5.
[15] O'Hurley G, Busch C, Fagerberg L, Hallström BM, Stadler C, Tolf A, et al. Analysis of the Human Prostate-Specific Proteome Defined by Transcriptomics and Antibody-Based Profiling Identifies TMEM79 and ACOXL as Two Putative, Diagnostic Markers in Prostate Cancer. PLoS One 2015;10. https://doi.org/10.1371/journal.pone.0133449.
[16] Cui Y, Yang M, Zhu J, Zhang H, Duan Z, Wang S, et al. Developments in diagnostic applications of saliva in human organ diseases. Med Nov Technol Devices 2022;13. https://doi.org/10.1016/j.medntd.2022.100115.
[17] Feng K, Ren F, Wang X. Association between oral microbiome and seven types of cancers in East Asian population: a two-sample Mendelian randomization analysis. Front Mol Biosci 2023;10. https://doi.org/10.3389/fmolb.2023.1327893.
[18] Amri J, Goodarzi D, Alaee M, Zarei M, Salehi M. Salivary Biomarkers for Prostate Cancer: A Case-Control Study of PSA and S100P in Iranian Men. Acta Biochimica Iranica 2025. https://doi.org/10.18502/abi.v2i3.19097.
[19] Martin FL, Morais CLM, Dickinson AW, Saba T, Bongers T, Singh MN, et al. Point-of-Care Disease Screening in Primary Care Using Saliva: A Biospectroscopy Approach for Lung Cancer and Prostate Cancer. J Pers Med 2023;13. https://doi.org/10.3390/jpm13111533.
[20] Laidler P, Dulińska J, Lekka M, Lekki J. Expression of prostate specific membrane antigen in androgen-independent prostate cancer cell line PC-3. Arch Biochem Biophys 2005;435. https://doi.org/10.1016/j.abb.2004.12.003.
[21] Eskicorapci SY, Özkara HA, Önder E, Akdogan B, Erkan İ, Ciliv G, et al. Serum ribonuclease activity in the diagnosis of prostate cancer in men with serum prostate-specific antigen levels between 2.5 and 20 ng/mL. Clin Biochem 2006;39. https://doi.org/10.1016/j.clinbiochem.2005.11.020.
[22] Galey L, Olanrewaju A, Nabi H, Paquette JS, Pouliot F, Audet-Walsh É. PSA, an outdated biomarker for prostate cancer: In search of a more specific biomarker, citrate takes the spotlight. J Steroid Biochem Mol Biol 2024;243:106588. https://doi.org/10.1016/J.JSBMB.2024.106588.
[23] Haider M, Leow JJ, Nordström T, Mortezavi A, Albers P, Heer R, et al. Emerging tools for the early detection of prostate cancer. BJUI Compass 2025;6. https://doi.org/10.1002/bco2.70081.
[24] Gomella LG, Raj G V., Moreno JG. Reverse Transcriptase Polymerase Chain Reaction for Prostate Specific Antigen in the Management of Prostate Cancer. J Urol 1997;158. https://doi.org/10.1016/S0022-5347(01)64472-0.
[25] Iczkowski KA, Bostwick DG. PROSTATE BIOPSY INTERPRETATION: Current Concepts, 1999. Urologic Clinics of North America 1999;26. https://doi.org/10.1016/S0094-0143(05)70193-2.
[26] Fendler WP, Eiber M, Beheshti M, Bomanji J, Calais J, Ceci F, et al. PSMA PET/CT: joint EANM procedure guideline/SNMMI procedure standard for prostate cancer imaging 2.0. Eur J Nucl Med Mol Imaging 2023;50:1466–86. https://doi.org/10.1007/s00259-022-06089-w.

[27] Hohberg M, Kobe C, Krapf P, Täger P, Hammes J, Dietlein F, et al. Biodistribution and radiation dosimetry of [18F]-JK-PSMA-7 as a novel prostate-specific membrane antigen-specific ligand for PET/CT imaging of prostate cancer. EJNMMI Res 2019;9:66. https://doi.org/10.1186/s13550-019-0540-7.
[28] Garg M, Kaur G. Histopathological spectrum of 364 prostatic specimens including immunohistochemistry with special reference to grey zone lesions. Prostate Int 2013;1. https://doi.org/10.12954/PI.13026.
[29] Lima T, Perpétuo L, Henrique R, Fardilha M, Leite-Moreira A, Bastos J, et al. Galectin-3 in prostate cancer and heart diseases: a biomarker for these two frightening pathologies? Mol Biol Rep 2023;50:2763–78. https://doi.org/10.1007/s11033-022-08207-1.
[30] Roobol MJ, Schröder FH, Crawford ED, Freedland SJ, Sartor AO, Fleshner N, et al. A Framework for the Identification of Men at Increased Risk for Prostate Cancer. J Urol 2009;182. https://doi.org/10.1016/j.juro.2009.07.018.
[31] Reynolds MA, Kastury K, Groskopf J, Schalken JA, Rittenhouse H. Molecular markers for prostate cancer. Cancer Lett 2007;249. https://doi.org/10.1016/j.canlet.2006.12.029.
[32] Maimaitiyiming A, An H, Xing C, Li X, Li Z, Bai J, et al. Machine learning-driven mast cell gene signatures for prognostic and therapeutic prediction in prostate cancer. Heliyon 2024;10. https://doi.org/10.1016/j.heliyon.2024.e35157.
[33] Wu D, Ni J, Beretov J, Cozzi P, Willcox M, Wasinger V, et al. Urinary biomarkers in prostate cancer detection and monitoring progression. Crit Rev Oncol Hematol 2017;118:15–26. https://doi.org/10.1016/j.critrevonc.2017.08.002.
[34] Bae J, Yang SH, Kim A, Kim HG. RNA-based biomarkers for the diagnosis, prognosis, and therapeutic response monitoring of prostate cancer. Urologic Oncology: Seminars and Original Investigations 2022;40:105.e1-105.e10. https://doi.org/10.1016/J.UROLONC.2021.11.012.
[35] Mitobe Y, Takayama K ichi, Horie-Inoue K, Inoue S. Prostate cancer-associated lncRNAs. Cancer Lett 2018;418:159–66. https://doi.org/10.1016/J.CANLET.2018.01.012.
[36] Wu M, Zhang X, Han X, Pandey V, Lobie PE, Zhu T. The potential of long noncoding RNAs for precision medicine in human cancer. Cancer Lett 2021;501:12–9. https://doi.org/10.1016/J.CANLET.2020.11.040.
[37] Zhang C, Yang Q, Li W, Kang Y, Zhou F, Chang D. Roles of circRNAs in prostate cancer: Expression, mechanism, application and potential. Int J Biochem Cell Biol 2021;134:105968. https://doi.org/10.1016/J.BIOCEL.2021.105968.
[38] Mahalanobis S, Apoorva, Sharma N, Luthra R, Menon T, Das A. Cancer prognosis and immune system. Microbial Crosstalk with Immune System: New Insights in Therapeutics 2022:75–144. https://doi.org/10.1016/B978-0-323-96128-8.00004-3.
[39] Martignano F, Rossi L, Maugeri A, Gallà V, Conteduca V, De Giorgi U, et al. Urinary RNA-based biomarkers for prostate cancer detection. Clinica Chimica Acta 2017;473:96–105. https://doi.org/10.1016/J.CCA.2017.08.009.
[40] Jain G, Das P, Ranjan P, Neha, Valderrama F, Cieza-Borrella C. Urinary extracellular vesicles miRNA—A new era of prostate cancer biomarkers. Front Genet 2023;14. https://doi.org/10.3389/fgene.2023.1065757.
[41] Liu J, Duan Y. Saliva: A potential media for disease diagnostics and monitoring. Oral Oncol 2012;48. https://doi.org/10.1016/j.oraloncology.2012.01.021.
[42] Trevisan França de Lima L, Müller Bark J, Rasheduzzaman M, Ekanayake Weeramange C, Punyadeera C. Saliva as a matrix for measurement of cancer biomarkers. 2022. https://doi.org/10.1016/B978-0-12-824302-2.00008-4.

[43] Godzien J, López-Gonzálvez Á, García A, Barbas C. Metabolic Phenotyping Using Capillary Electrophoresis Mass Spectrometry. 2019. https://doi.org/10.1016/B978-0-12-812293-8.00005-0.
[44] Farahani H, Alaee M, Amri J, Baghinia MR, Rafiee M. Serum and Saliva concentrations of biochemical parameters in men with prostate cancer and benign prostate hyperplasia. Lab Medicine 2020;51:243–51. https://doi.org/10.1093/LABMED/LMZ053.
[45] Zhang C-Z, Cheng X-Q, Li J-Y, Zhang P, Yi P, Xu X, et al. Saliva in the diagnosis of diseases. Int J Oral Sci 2016;8. https://doi.org/10.1038/ijos.2016.38.
[46] Eftekhari A, Maleki Dizaj S, Sharifi S, Salatin S, Khalilov R, Samiei M, et al. Salivary biomarkers in cancer. vol. 110. 2022. https://doi.org/10.1016/bs.acc.2022.06.005.
[47] Luedemann C, Reinersmann JL, Klinger C, Degener S, Dreger NM, Roth S, et al. Prostate Cancer-Associated miRNAs in Saliva: First Steps to an Easily Accessible and Reliable Screening Tool. Biomolecules 2022;12. https://doi.org/10.3390/biom12101366.
[48] Ahmed SI, Sarwar S, Verma H, Verma R, Lakkireddy C, Adil MAM, et al. Implication of circulating microRNA-21 as a potential non-invasive diagnostic predictor of prostate cancer patients. Human Gene 2022;34:201081. https://doi.org/10.1016/J.HUMGEN.2022.201081.
[49] Nearing JT, DeClercq V, Langille MGI. Investigating the oral microbiome in retrospective and prospective cases of prostate, colon, and breast cancer. NPJ Biofilms Microbiomes 2023;9. https://doi.org/10.1038/s41522-023-00391-7.
[50] Bel'Skaya L V., Sarf EA, Kosenok VK. Analysis of saliva lipids in breast and prostate cancer by IR spectroscopy. Diagnostics 2021;11. https://doi.org/10.3390/diagnostics11081325.
[51] Fernandes B, Roy J, Basuli F, Warner BM, Lindenberg L, Mena E, et al. Salivary excretion of systemically injected [18 F]DCFPyL in prostate cancer patients undergoing PSMA scans. Front Oncol 2024;14. https://doi.org/10.3389/fonc.2024.1367962.
[52] Hrebinko R, Taylor SR, Bahnson RR. Carcinoma of prostate metastatic to parotid gland. Urology 1993;41. https://doi.org/10.1016/0090-4295(93)90573-S.
[53] Luedemann C, Reinersmann J-L, Klinger C, Degener S, Dreger NM, Roth S, et al. Correction: Luedemann et al. Prostate Cancer-Associated miRNAs in Saliva: First Steps to an Easily Accessible and Reliable Screening Tool. Biomolecules 2022, 12, 1366. Biomolecules 2023;13:628. https://doi.org/10.3390/biom13040628.