APPROACHES TO URINARY DETECTION OF PROSTATE CANCER

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Abstract

Background: Prostate cancer is the most common cancer in American men that ranges from low risk states amenable to active surveillance to high risk states that can be lethal especially if untreated. There is a critical need to develop relatively non-invasive and clinically useful methods for screening, detection, prognosis, disease monitoring, and prediction of treatment efficacy. In this review, we focus on important advances as well as future efforts needed to drive clinical innovation in this area of urine biomarker research for prostate cancer detection and prognostication.

Methods: We provide a review of current literature on urinary biomarkers for prostate cancer. We evaluate the strengths and limitations of a variety of approaches that vary in sampling strategies and targets measured; discuss reported urine tests for prostate cancer with respect to their technical, analytical, and clinical parameters; and provide our perspectives on critical considerations in approaches to developing a urine-based test for prostate cancer.

Results: There has been an extensive history of exploring urine as a source of biomarkers for prostate cancer that has resulted in a variety of urine tests that are in current clinical use. Importantly, at least three tests have demonstrated high sensitivity (~90%) and negative predictive value (~95%) for clinically significant tumors; however, there has not been widespread adoption of these tests.

Conclusions: Conceptual and methodological advances in the field will help to drive the development of novel urinary tests that in turn may lead to a shift in the clinical paradigm for prostate cancer diagnosis and management.

Keywords

Prostate cancer; urine; biomarker

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Introduction

In recent decades, the most prevalent method of prostate cancer detection has been serum prostate-specific antigen (PSA) testing [1]. Elevated PSA levels in the serum can indicate prostate cancer, but, as a screening tool, PSA lacks adequate specificity, since elevation of serum PSA is not exclusive to prostate cancer [2]. Prostatitis, digital rectal examination (DRE), and benign prostate hyperplasia (BPH) all have been linked to increased serum PSA levels. PSA test results are especially problematic when values fall in the range of 4.0 to 10.0 ng/mL, leading to unnecessary procedures and potential overdiagnosis and overtreatment. In the United States, about one million men undergo prostate biopsies annually because of an elevated serum PSA, of which approximately 70% do not detect prostate cancer [3]. The biopsy procedure also may detect indolent tumors that are unlikely to cause morbidity and mortality, often resulting in needless interventions for patients with low-grade cancer.

Some patients diagnosed with prostate cancer have advanced disease at diagnosis or progress to an advanced stage over time. As PSA also is routinely used for disease monitoring purposes, a rising PSA often triggers additional aggressive local or systemic interventions. A key drawback of PSA is that it provides limited information beyond its utility as a disease indicator. Biomarkers that are more effective are needed to inform the management of prostate cancer patients, particularly those on active surveillance.

To address these issues, there has been great interest in identifying more specific and sensitive biomarkers for detecting and monitoring prostate cancer as well as treatment-specific markers that can indicate benefit or lack of benefit from clinical interventions. Urine has emerged as a promising non-invasive source of biomarkers. Recent exploration of urinary analytes, including DNA, RNA, proteins, exosomes, and cellular metabolites has revealed numerous promising candidate markers. In this review, we provide an overview on the current state of urine biomarker research for prostate cancer, discuss the approaches that have been implemented to detect potential markers, and highlight some of the ongoing challenges facing development of urinary biomarker tests. We provide our perspective on development of urinary markers with a pathway to the clinic.

Urine as a source of biomarkers

Urine is a complex medium, containing a variety of substances, some of which are filtered from the circulation, such as metabolic waste products and small proteins (<20 kDa) secreted by numerous cell types, as well as larger proteins and cells that originate from urogenital organs downstream of glomerular filtration [4]. Solid components of urine can easily be separated from the liquid fraction by low-speed centrifugation (Figure 1). The resulting pellet contains formed elements such as cells, casts, mucin, and debris; whereas, the supernatant retains the soluble components including proteins, exosomes, biochemicals, and cell-free nucleic acids. The composition of urine is highly dynamic and exhibits high variability both within and across individuals due to numerous factors such as age, diet, gender, and physical activity [5].

There are several advantages to using urine for clinical assays. Collection of urine is a noninvasive form of liquid biopsy without any risk of harm to the patient. Specimens can be obtained at frequent intervals and in large quantities, making urine amenable to repeat sampling procedures. Urine contains numerous substrates that can be used as biomarkers. Urine may contain prostatic secretions and exfoliated prostate epithelial cells due to the anatomic proximity of the prostate to the bladder and urethra (Figure 2). Various types of prostate biomarkers are released into urine, including cell-associated markers and secreted cell-free markers. Moreover, prostate material in urine can be enriched by physical manipulation of the gland during digital rectal examination. This procedure promotes mobilization of fluids and cells that drain from prostatic ducts into the urethra and then are carried out during micturition. As a source of biomarker for localized and early-stage prostate cancer, urine may be better suited than blood that contains markers from virtually all body tissues, leading to high background interference that can hinder detection ability. In contrast, urine is enriched in material coming directly from the prostate gland; it does not require crossing blood-tissue barriers; and it contains fewer confounding elements.

Clinical application of urine tests for prostate cancer

Urinary prostate biomarkers could be applied to various scenarios in which they may guide clinical decision-making (Figure 3). In the diagnostic setting, an ideal biomarker would discriminate normal and benign prostate conditions from malignancy. There has been considerable interest in urinary markers as a supplement to serum PSA measurements with the goal of reducing unnecessary biopsies, as a complementary tool to be used in conjunction with biopsy, or as a means of replacing the biopsy procedure entirely [6]. Numerous strategies have been implemented in an effort to use urine as a source of prostate cancer biomarkers that have led to the development of several clinical urine tests for prostate cancer detection (Table 1).

Reliable prognostic and predictive markers are sorely needed. Urinary markers have the potential to improve detection of clinically significant prostate cancers. For example, at least three tests have demonstrated high sensitivity (~90%, Table 1) and ~95% negative predictive value for Gleason pattern 4 tumors. Improvement in detecting significant cancer using urine biomarkers may come at the cost of over-detecting clinically insignificant prostate cancer, a scenario similar to the outcome of widespread PSA testing. A urinary prostate marker that merely detects any prostate cancer (including significant cancer) will not be sufficient to improve patient care. To optimize clinical management of prostate cancer, an ideal urine test would help to improve the detection of significant cancer and in the meantime decrease the detection of clinically insignificant cancer. For patients with early, localized prostate cancer, urine biomarkers that are associated with high-risk features may fulfill this objective by distinguishing patients harboring aggressive tumors who might benefit from interventions from those with indolent tumors that might be managed with active surveillance.

Biochemical and metabolic markers

Secreted prostate fluid is an intriguing source of biomarkers that can be obtained non-invasively via collection of urine. The composition and content of prostatic secretions have

been shown to mirror those of prostate tissue, presenting an opportunity to gain insight into the health of gland, which may be particularly useful for cancer detection since alterations in prostate secretory function and fluid composition are associated with malignancy [7, 8]. Biochemical urine tests generally require few processing steps and results can be obtained rapidly. The advantage of a simple workflow and easy interpretation associated with biochemical urine assays raises the possibility of inexpensive point-of-care testing (Figure 4).

Secreted components of prostatic fluid

The earliest demonstration that prostate fluids could be detected in urine specimens was reported in a 1942 publication by Scott & Huggins that demonstrated the prostatic origin of urinary acid phosphatase [9]. These observations were validated in subsequent studies by others [10–12], providing a foundation for future investigations into biochemical analysis of urine as a way to measure biomarkers derived from prostate secretions.

Tumors in the prostate gland disrupt the homeostatic regulation of prostate fluid composition. Under normal conditions, prostate glandular cells accumulate and secrete exceptionally high levels of zinc [13]. With prostate cancer, both tissue and secreted levels of zinc are markedly reduced compared to benign prostate tissue [14]. Numerous reports have established a strong association zinc secretory output and malignancy, indicating that decreased zinc is a hallmark characteristic of prostate cancer [15]. As a urine biomarker, low zinc levels could be used for detection of malignancy, while normal urinary zinc levels may help rule out prostate cancer for patients with PSA values in the gray zone (4-10 ng/mL). In spite of the abundant evidence supporting zinc as a prostate biomarker, only few studies have explored urinary zinc measurements for noninvasive detection of malignancy. Medarova et al [16] proposed urinary zinc concentrations could be used as a risk stratification tool [17]. They developed a novel assay that involves rapid quantification of mobile zinc in urine samples using a fluorescent Zn^{2+} sensor measured using a fluorescent spectrophotometer. "Zinc scores" were calculated by multiplying the zinc concentration (μM) by the creatinine concentration (μg/mL) to adjust for variance in urine volumes. When they applied a cutoff zinc score 7 as criteria for malignancy, only 24 (63%) samples were correctly classified as either benign or malignant. Nine of the misclassified samples were falsely identified as positives, and 5 were false-negatives. Despite the disappointing performance of zinc as a urinary biomarker in this study, additional and larger studies are necessary before drawing conclusions on its clinical utility.

Metabolites

Over the past decade, urinary metabolites have gained considerable attention as potential markers for prostate cancer. Metabolomic profiling of cancer is an attractive approach to biomarker discovery, because it provides insight into the current physiological or pathophysiological conditions through measuring end products of biochemical reactions from cells and tissues [18]. The prostate gland has a unique metabolism that is associated with the production PSA, spermine, citrate, zinc, and other components of the prostatic fluid [19]. Perturbations in the composition of prostate fluid that commonly occur in malignancy are attributed to metabolic alterations that could be captured through metabolic profiling of

urine samples for cancer detection. Proof-of-concept for this was reported in a landmark study by Sreekumar et al in 2009. They analyzed 1,126 urine metabolites in 100 urine samples and identified sarcosine as a lead candidate [20]. They reported that urinary sarcosine levels were increased in prostate cancer and could distinguish benign samples from those obtained from prostate cancer patients. However, follow-up studies reported mixed results. Some investigators were unable to validate diagnostic utility of sarcosine and reported no significant differences in urinary sarcosine between prostate cancer cases and controls [21, 22], while others reported findings supporting the initial observations [23, 24]. Several explanations for the discrepancies across studies have been proposed [25], including sample selection, urine handling, normalization methods, and analytical techniques.

Volatile organic compounds

Other investigations of urine biochemicals have included a series of studies involving the detection of volatile organic compounds (VOCs) as urinary biomarkers for prostate cancer. VOCs are end products of cellular metabolism that are excreted from cells and tissue; alterations in normal VOC production occur in pathological conditions and reflect metabolic disturbances that occur in numerous disease states [26]. Interest in VOCs as biomarkers gained momentum following a publication in 2008 by Gordon et al that trained sniffer dogs could discriminate between healthy patients and those with prostate cancer [27]. This report was followed by several subsequent studies using dogs [28–30]. Varying degrees of animal proficiency were reported across these studies, which is likely attributable to differences in study design, dog breeds, canine training methods, and specimens included for analysis (Table 2).

Research on cancer detection using trained animals has garnered much media attention despite limited scientific enthusiasm. Impracticality for large-scale clinical application, flaws in design of published studies, insufficient diagnostic accuracy, and other criticisms have been discussed elsewhere [30–34]. Although controversial, these observations have raised important questions on the nature of VOCs in biofluids and the possibility of their utility in diagnosis of cancer. In effort to further research urinary VOCs and avoid the limitations of training and using dogs as VOC detectors, some groups have begun testing instrumental methods to analyze and generate odorant signatures for biological samples [35]. Several studies reported utility of electronic nose technology to discriminate between urine from patients with prostate cancer from control urine specimens [36–38]. These preliminary studies suggest that the diagnostic potential of VOC detection using electronic noses is promising.

Cytology

Conventional cytology

Prostate cells in urine are considered to be luminal epithelial cells that have shed from the gland (Figure 2), but the precise nature of urinary prostate cells and means by which they are released into urine is not fully understood. Presumably, some shedding of prostate cells occurs during normal cell turnover. Alternatively, exfoliation of prostate cells can be achieved mechanically by performing prostate massage. The prevalence of intact prostate

cells in urine sediment has been little investigated, and as such, normal rates of prostate cell shedding into urine has not been documented for healthy or diseased prostate tissue. Nevertheless, the morphological features of prostate cells have been well defined in several cytological studies [39–41]. Prostate cells found in the urine sediment have a distinct appearance that can be identified microscopically using conventional cytology staining methods; they generally are round with a high nuclear to cytoplasm ratio, prominent nucleoli, and often present in small clusters. Despite these characteristic features, identifying prostate cells solely based on morphology is difficult even for trained cytopathologists because of their overlapping appearance with other cell types found in the urine sediment as well as their scarcity in regularly voided urine specimens.

As a clinical tool for numerous diagnostic scenarios, performance of cytology consistently demonstrates high specificity. The main advantage of a cytological approach to prostate cancer detection in urine is the ability to visualize cells of interest. This provides a distinct benefit over urine tests for prostate cancer currently under investigation, nearly all of which involve measurement of soluble markers and detection methods that do not provide information about their cellular origin (e.g. PCR-based assays). Alternatively, by using a cytological approach, single prostate cells can be examined and evaluated within a highly heterogeneous cell population, an advantage over other detection methods that allows for discrimination of cells originating in the prostate gland from those of non-prostate origin (Figure 4). In this regard, the consistently high specificity of cytological tests is likely a consequence of the need to visually identify and confirm presence of malignant cells [42, 43].

Unfortunately, urine cytology for detection of prostate cancer suffers from having a very low sensitivity [43]. The performance of cytology as a highly specific test with low detection sensitivity is a common problem in the diagnostic setting for many other conditions in addition to prostate cancer. This limitation has long been established [44] and renders cytology unacceptable for clinical use in prostate cancer detection. Several possibilities may account for the poor sensitivity. First, there may not be sufficient cellular shedding of prostate cells into urine; successful recognition of malignancy by cytology requires presence of intact prostate cells that are rare in urine, even when specimens are collected immediately following prostatic massage. Second, passage of cells from prostatic ducts into urine may be hindered by anatomical locations of tumors that are distant from the urethra. Further causes of low sensitivity may arise as a result of specimen acquisition and processing procedures. Slide preparation of urine sediment usually involves several centrifugation steps, increasing the likelihood that these rare cells are lost during processing. Reported cell recovery rates for urine sediments ranges from 30% to 85%, depending on technique [45, 46]. Cells not lost during the processing steps could be missed during analysis; urine sediment contains a number of elements and cell types that can obscure detection of rare cell populations; or prostate cells could be mistaken for other types of cells. Cytology is also limited by intra-observer variability and the need for specialized laboratory personnel and training. Moreover, urine is an inhospitable environment for cells, thus improper sample handling or prolonged storage at room temperature before processing can cause rapid deterioration of cellular components [47].

Molecular cytology

An ongoing area of investigation to overcome the shortcomings of conventional cytology is the integration of molecular techniques such as immunolabeling, in situ hybridization, and specialty stains into standard cytological methods. Molecular markers can enhance the ability to visually detect and differentiate cell types during analysis of cytology slides by providing additional disease- or tissue-specific information. Regarding urine cytology for prostate cancer detection, immunolabeling for prostate-specific markers has been investigated in a few studies. Successful immunostaining of urine slides for PSA and prostatic acid phosphatase as way to confirm the cellular origin of malignant cells during routine cytology was reported in 2 small studies [41, 48]. Although the aim of these studies was not to determine whether prostate markers could be used alone as diagnostic tools, they provided early evidence that immunostaining, a technique primarily performed using histological sections, for prostate markers could successfully be applied to urine cytology slides and may aid in clinical decision making. Expanding on these observations, Fujita et al investigated prostate cancer in urine specimens by performing multiplex fluorescent immunostaining for AMACR, NKX3-1 and nucleolin [49]. Urine samples were collected from healthy patients (n=23) and those with biopsy-confirmed prostate cancer (n=27). Sediments were scored for positivity by multiplex immunostaining followed by independent scoring after Papanicolaou staining and conventional cytology. They reported 100% specificity and an increase in detection sensitivity with immunostaining (Sens=36%) compared to the conventional method of Papanicolaou staining (Sens=15%). While there was only a modest improvement in sensitivity, this study validates the concept that performance of urine cytology for prostate cancer detection can be enhanced by incorporation of modern technologies and newly identified biomarkers.

Although low sensitivity has led to the loss of enthusiasm for urine cytology in the detection of prostate cancer, it may still hold promise. While high-throughput genomic and proteomic approaches have gained recent popularity over cytological methods, they have failed to achieve a comparably high specificity. It is conceivable that current performance deficits regarding the sensitivity of cytology could be dramatically improved through optimization and incorporation of novel technologies. For example, platforms under development for detection of rare cell populations in blood could be adapted and applied to urine, potentially enhancing the ability to find and isolate urinary populations of prostate cells. Identifying and implementing effective methodologies could allow for a more strategic cytological approach to detecting exfoliated prostate cancer cells in urine that increases sensitivity without compromising specificity. Further preclinical investigation and assessment of potential technologies is warranted.

Nucleic acid biomarkers

Nucleic acids are normal constituents of urine in both healthy individuals and those with cancer. They are found in the cellular fraction of urine after centrifugation as well as extracellularly in the supernatant as cell-free nucleic acids; or they may be contained within extracellular vesicles [50, 51]. Profiling urinary nucleic acids represents a promising means of prostate cancer detection. Qualitative and quantitative alterations in nucleic acids are

hallmark characteristics of prostate carcinogenesis, and therefore genetic, transcriptional, and epigenetic profiling of urine offers a potential method for noninvasive detection. Substantial effort has been invested in profiling urinary nucleic acids in the search for prostate cancer biomarkers. Numerous studies have demonstrated their clinical value as biomarkers for prostate cancer leading to successful development and FDA-approval of an RNA-based urine test for prostate cancer [52].

DNA and RNA

Urinary RNAs are detectable in all fractions of urine and consist of many RNA varieties including mRNAs, long non-coding RNAs, and microRNAs. To date, RNA-based biomarkers have been the most widely investigated subtype of urinary nucleic acids for prostate cancer detection, amongst which PCA3 and TMPRSS2 fusions are the most extensively studied [53]. PCA3 is a long noncoding RNA that is highly prostate specific and is overexpressed in prostate cancer [54]. The first and only FDA-approved urinary biomarker for prostate cancer is the Progensa PCA3 assay (Hologic, Inc) that measures the concentration of PCA3 and PSA mRNA levels by transcription-mediated amplification using 2.5 mL post-digital rectal examination urine. A "PCA3 Score" is generated by calculating the ratio pf PCA3 and PSA mRNA, the latter being used as a method of normalizing for the amount of prostate material within the total volume of urine. Another prominent urinary RNA marker is the TMPRSS2:ERG fusion transcript [55] that is known to be a prostate cancer-specific marker [56] that can be measured in urine [57]. Other noteworthy RNA markers include several mRNAs known to be overexpressed in prostate cancer (Table 3), such as α-methylacyl-coenzyme-A racemase (AMACR)[58, 59], golgi membrane protein 1 (GOLM1)[60, 61], human telomerase reverse transcriptase (hTERT)[62, 63], homeobox C6 (HOXC6)[64, 65], and prostate-specific membrane antigen (PSMA)[66], as well as numerous microRNAs and long non-coding RNAs [53]. Urinary DNA-based markers include single nucleotide polymorphisms (SNPs), copy number variations, loss of heterozygosity, gene amplification, microsatellite instability, and alteration in promoter-region methylation [67]. Measurement of epigenetic alterations in glutathione S-transferase pi 1 (GSTP1) is the most extensively studied urinary DNA marker for prostate cancer. Additional investigational DNA-based urine markers are listed in Table 4.

From a technical perspective, several factors need to be considered when analyzing urine for nucleic acid markers. Urine contains a high level of nucleases, including DNases I/II and RNases I/II. DNase I is the major DNA-hydrolyzing enzyme present in urine. Its activity is reported to be 100-fold higher in urine than in blood, thus providing a potential explanation for the high level of DNA fragmentation that has been observed in urine samples. It is critical that urine used for analysis of nucleic acids is immediately processed with a preservative after collection. Reverse transcriptase quantitative polymerase chain reaction (RT-qPCR) is the most commonly used method for measuring expression of candidate markers, but analytical protocols for transcript quantification vary between studies with some report performing cDNA preamplification [66, 68], while others do not [69], and some use transcription-mediated amplification (TMA) [60, 70] or droplet digital PCR (ddPCR) [71]. Additionally, lack of consistent urine sampling and processing procedures exists across different studies. The benefits of a PCR-based approach include quantitative measurements

and high reproducibility (Figure 4). Robust clinical performance, low inter- and intra-run variance, and high informative rates (>92%) have been demonstrated for the Progensa PCA3 assay [72, 73]. The greatest limitation of this approach is the inability to determine the cellular origin of biomarker expression in a nucleic acid study. Numerous cell types are found in urine, including normal prostate cells, leukocytes, urothelial cells, and renal cells, which can confound results [74]. Because of this cellular diversity, suitable markers for analysis have been limited to those that are highly prostate cancer-specific (e.g., PCA3), reducing the number of potential candidates.

Cell-free nucleic acids

Cell-free nucleic acids (cfNA) have been established as a normal constituent of urine [75]. They can originate from any tissues along the urinary tract and may be derived from apoptotic and/or necrotic cells found in nucleocomplexes or can be contained within extracellular vesicles [76]. Using urinary cfNA for analysis may overcome the limited sensitivity of biomarker detection using urine sediment, as it does not require of presence of cells. Additionally, preparing urine specimens for analysis of cfNA requires fewer initial processing steps, thus reducing errors and confounding factors. Reports of urinary cfNAs as biomarkers for prostate cancer detection are limited and have been primarily restricted to investigating DNA rather than RNA, presumably because RNA is not well preserved in urine due to its relative instability compared to DNA and the presence of RNA-hydrolyzing enzymes in urine. However, because of their small size (20-25 nucleotides), cell-free microRNAs are more resistant to nuclease degradation compared to longer chain mRNAs, and they can be detected in urine specimens. Casadio et al reported concentrations of cfDNA in urine ranging from 2-36 ng/µl that did not significantly differ between healthy controls and cancer patients, but DNA fragmentation patterns could differentiate between prostate cancer patients and controls [75].

Extracellular vesicles

In addition to cellular and cell-free forms, urinary nucleic acids can be found extracellularly enclosed within small, membrane-bound vesicles. Extracellular vesicles are secreted by most cell types. They contain secreted proteins, lipids and nucleic acids [77]. Extracellular vesicles are found in most bodily fluids, including urine, as a heterogenous mixture consisting of exosomes, microvesicles, or apoptotic bodies [78]. Urinary extracellular vesicles can originate from any epithelial cells within the urinary tract. Extracellular vesicles have gained interest as a source of biomarkers because their content reflects that of their parent cells. They are particularly valuable for investigating miRNAs and other small RNAs due to the enrichment of this subset of RNAs. As a biomarker source, isolating and evaluating vesicles originating from the prostate could provide a way screen for prostate-specific nucleic acid markers, while reducing analytical interference from other cell types in urine [79]. McKiernan et al 2016 reported development of ExoDx, a diagnostic test measuring exosome markers for prediction of high-grade prostate cancer in men with PSA 2-10 ng/mL in the initial biopsy setting [80]. Their test measures expression of three exosomal RNAs (ERG, PCA3, and SPDEF) in voided urine specimens collected without a

prior prostate massage. Other promising exosome markers with potential clinical utility include several different proteins and miRNAs [53].

Prostate-derived extracellular vesicles are a good source of biomarkers due to their stability in urine. Their contents are encapsulated by a membrane that protects against degradation, and they can withstand vortexing and multiple freeze-thaw cycles [81]. However, it is important to rapidly preserve urine specimens using standardized collection and storage procedures to preserve biomarker integrity. At present, the largest drawback of utilizing of extracellular vesicles is collection and isolation. A variety of methods have been developed for the isolation of extracellular vesicles from bodily fluids. The most established method for exosome isolation is differential centrifugation that consists of sequential centrifugation steps. Other methods include density gradient-based isolation, use of precipitating solutions, and column-based assays. Procedural differences in processing of urinary extracellular vesicles have been shown to impact isolation and detection of certain sub-populations. A comparative study by Royo et al evaluated various methods for the isolation of urinary extracellular vesicles [82]. By profiling CD exosome markers, they observed differential enrichment of exosome subpopulations, depending the isolation procedure.

Protein biomarkers

Early initiatives to identify urine protein biomarkers for prostate cancer were impeded by challenges associated with their relatively low abundance and a lack of high-throughput detection assays. However, recent advances in proteomic technologies are enabling high throughput, reproducible and quantitative profiling of urine biomarkers on a comprehensive scale [83]. Urine is well suited as a source of protein biomarkers due to minimal proteolytic activity in addition to harboring a highly diverse protein landscape from small peptides and metabolites to larger proteins and macromolecular complexes. The total protein content of urine is low (typically <150 mg per day) – approximately 1000-fold lower by comparison to blood. This makes analysis simpler in comparison to serum to plasma [84, 85]. On the other hand, the composition of urine is highly variable. Using quantitative proteomic techniques, Nagaraj and Mann demonstrated high variability in the proteome for both inter-person (47.1%) and intra-person (45.5%) urine specimens [86].

Proteins and peptides are found in all fractions of urine. They originate from cellular components, filtration of blood plasma, proteolytic cleavage of cell surface proteins, apoptosis, and secretion of extracellular vesicles [87]. Approximately 30% of the urinary proteome is derived from glomerular filtration, whereas 70% originates from the urogenital tract, indicating the urine proteome is an abundance source of biomarkers related to urogenital health and malignancy [88]. Prostate-specific protein markers in urine include cellular antigens from exfoliated prostate cells as well as proteins contained within extracellular vesicles or secreted into prostatic fluid. Secreted proteins and peptides may prove to be particularly advantageous urine analytes, as they are not limited by the presence of cellular material. Candidate protein biomarkers that have been investigated include PSA, AMACR, IL-18BP, annexin A3, engrailed-2, and ZAG (Table 5). Numerous methods for detecting urine proteins have been reported, including ELISAs, western blotting, gelatin zymography, and capillary electrophoresis-mass spectrometry.

Considerations for study design and experimental conditions

Urinary biomarkers have the potential to transform the way prostate cancer is diagnosed and treated. Yet, there are numerous inherit challenges and practical issues associated with using urine as a biospecimen that need to be addressed moving forward. In this section, we highlight some pertinent concerns facing detection that are specific to urinary prostate markers.

Digital rectal examination prior to sample collection

A prostate massage at the time of digital rectal examination is often conducted in studies for prostate biomarkers before urine collection to increase the amount of prostate-derived material. Collection of specimens following massage is generally emphasized as a critical factor when collecting urine for identification of prostate biomarkers, because it enriches for the volume of prostate secretions. This has been consistently demonstrated across numerous studies for RNA, DNA, exomes, exfoliated prostate cells, and protein-based prostate markers. Most recently, the positive effect of prostate massage on urinary biomarker levels was observed in a study comparing levels of PCA3, ERG and KLK3 in which there was approximately a 10-fold increase in expression following digital rectal examination [89]. However, some evidence suggests massage may not be necessary for detection of urinary markers. Exosome expression of PCA3 and ERG [90, 91] and cellular DNA methylation markers [71] have been successfully measured in urine samples collected without a prior digital rectal examination or massage. Given this information, whether or not a DRE must be performed prior to urine collection is unresolved. On one hand, evidence is clear that prostate massage increases the amount of the prostatic secretions detectable in urine that aids in marker detection and may be especially beneficial for low-abundance biomarkers. Conversely, this process adds an element of variability that needs to be accounted for in data analysis using validated methods of normalization. Omission of the digital rectal examination in the urine collection process (if validated and does not negatively influence detection sensitivity) would be advantageous for standardizing collection procedures and could allow for routine monitoring prostate markers at increased frequencies. It also makes the test more practical and acceptable to patients because it does not require a visit to a health-care provider to obtain the urine sample.

Study design

Despite the considerable amount of research involving urinary prostate biomarkers, guidelines for the many procedural issues for urine biomarker studies have not been defined. These include which part of the void to use, how much volume to collect, which fraction to analyze, preservative fluids to be added, and the performance of digital rectal examination. The procedures used have been highly variable across urine biomarker studies. There is no uniformity regarding specimen collection, preparation, analytical methods, or data evaluation. The lack of a standardized approach adds further complications to working with an innately complex specimen and prohibits comparisons of biomarker performance across different laboratories. The adverse consequences of this problem are evident in publications on urinary sarcosine, in which the lack of reproducibility across studies is likely due the absence of standardized procedures. Moving forward, careful attention to collection

procedures included in study design will be necessary in order to attain high quality and clinically meaningful information.

Normalization

How best to normalize urinary prostate markers is perhaps the largest unresolved problem spanning all detection platforms. Normalization is a critical step in urine biomarker studies due to wide inter- and intra-person fluctuations in urine composition and volume, in addition to variations in prostatic secretory output. Yet, currently there are no guidelines or standard methods indicated for standardizing prostate urine markers and the selection of a given methodology is at the discretion of the investigator. Consequently, there has been no consistency across publications regarding the kind of normalization method employed. PSA expression is frequently used in studies of RNA-based markers (Table 3), whereas creatinine is common for standardization in proteomic studies (Table 5). Some of the other reported strategies include normalizing with SPDEF transcript levels, osmolality, urine volume, or total protein concentration. Furthermore, sufficient evidence to support utilization of any of the commonly used normalization approaches is lacking. While some of the methods have been validated for other kinds of urine biomarkers or for tissue-based biomarker detection, there has not been verification that these are appropriate when investigating prostate markers. Ultimately, the lack of evidence-based guidelines for appropriate methods increases the likelihood of introducing bias into a study and misinterpreting the results.

Pathway to the clinic

Although numerous promising urine biomarkers have emerged in recent years (Table 1), a non-invasive assay with high sensitivity and specificity for clinically significant prostate cancer has yet to be identified and the diagnosis of prostate cancer continues to be a major clinical challenge. To improve the path to large-scale clinical implementation, careful consideration must be taken in study design of preclinical trials to adequately validate emerging urine markers. The relevant clinical question that a urinary biomarker can address should be clearly defined at the start of a study, as the nature of the question being addressed will have direct implications for sample acquisition and analysis. Standardized procedures need to be implemented to reduce potential confounding factors due to pre-analytical variables. The determination of best-suited procedures should be established after careful consideration of downstream analytical requirements and subsequently should be validated before moving into large clinical trials. Additionally, consideration should be given to optimizing a workflow that is amenable to clinical practice, both at the point-of-care setting and in the clinical laboratory.

Development of urinary tests may benefit from improved pathological risk stratification and adoption of reporting standards [92]. For example, the cribriform morphology is recognized as an aggressive pattern of prostate cancer and a contraindication for active surveillance if detected on biopsy [93]. All cribriform tumors are now categorized as Gleason pattern 4 [92]. However, cribriform Gleason pattern 4 tumors are under-detected by modern biopsy approaches [93]. It is tantalizing to speculate that urinary biomarkers may be more effective

in detecting this increasingly recognized diagnostic entity. Studies designed to address this question will certainly benefit from reporting standards endorsed by ISUP and WHO [93].

Conclusions

Clinical application of urinary biomarkers holds great promise in the management of early and late-stage prostate cancer patients. Although several challenges must be addressed before prostate urine markers reach large-scale clinical applicability, urine tests represent a valuable tool for non-invasive cancer detection that may greatly improve upon existing methods. Future implementation of urinary biomarkers in routine clinical testing has the potential to revolutionize management of prostate cancer.

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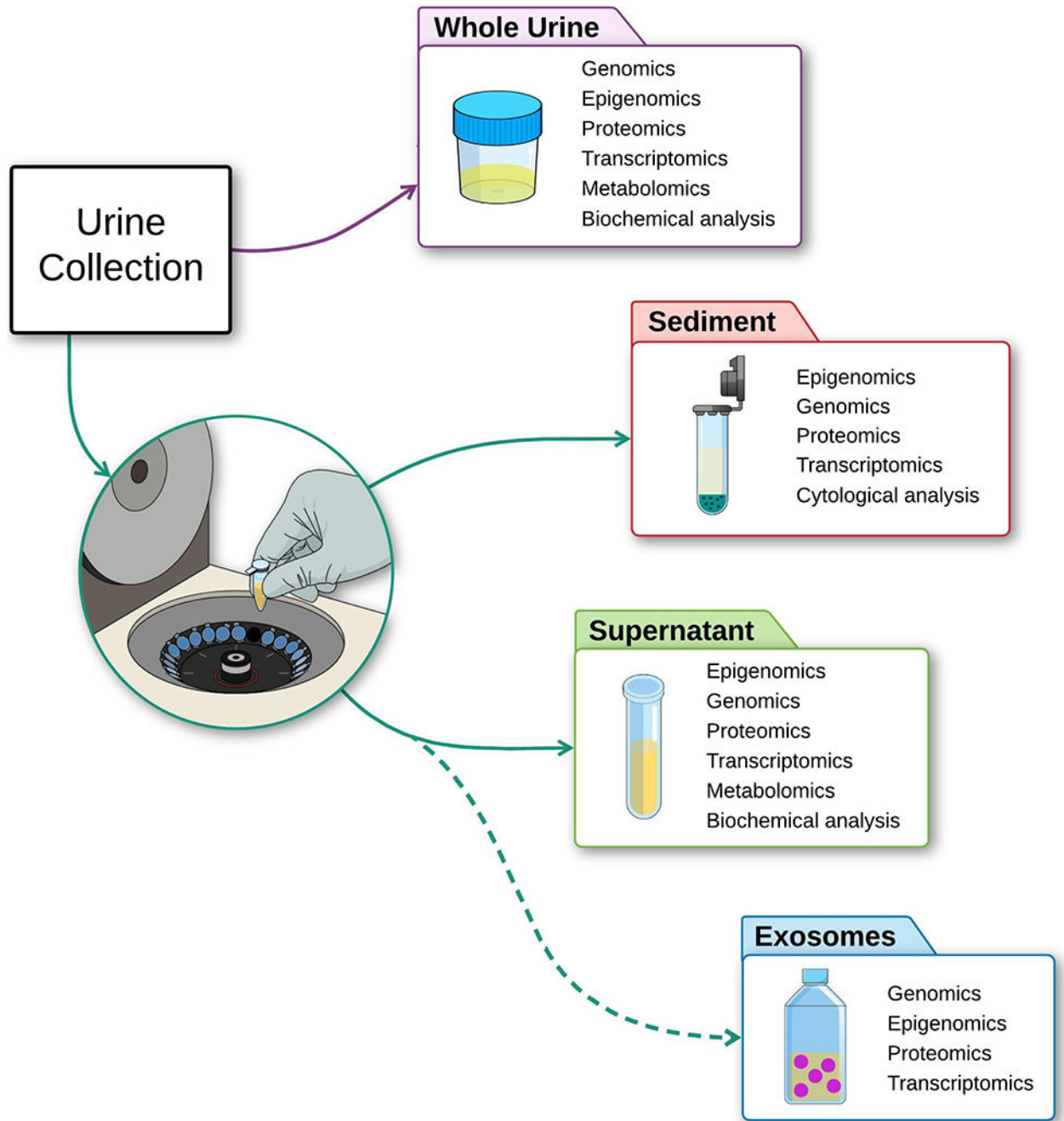
Figure 1.
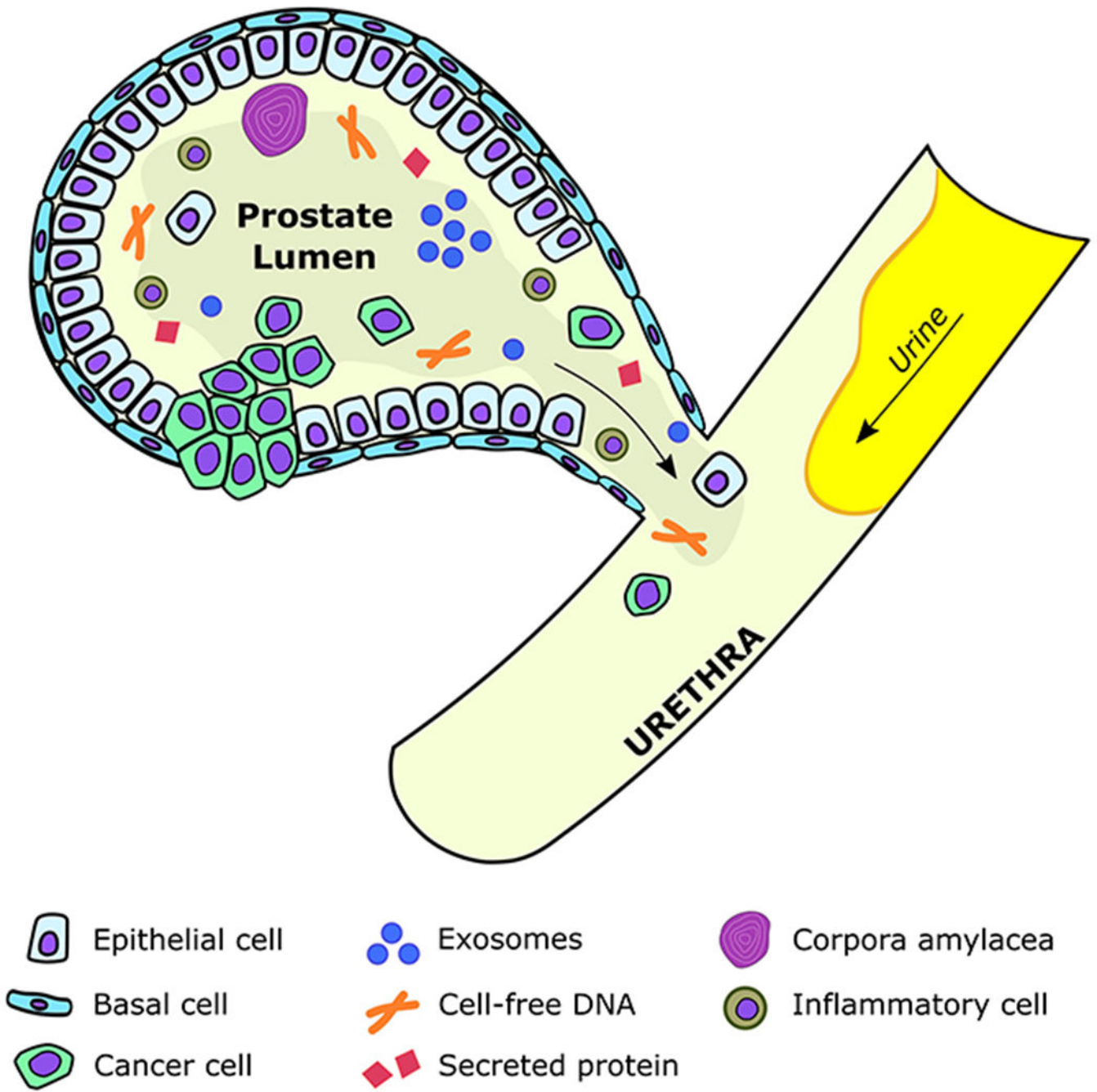Analytical approaches associated with the various fractions of urine following centrifugation.

Figure 2.
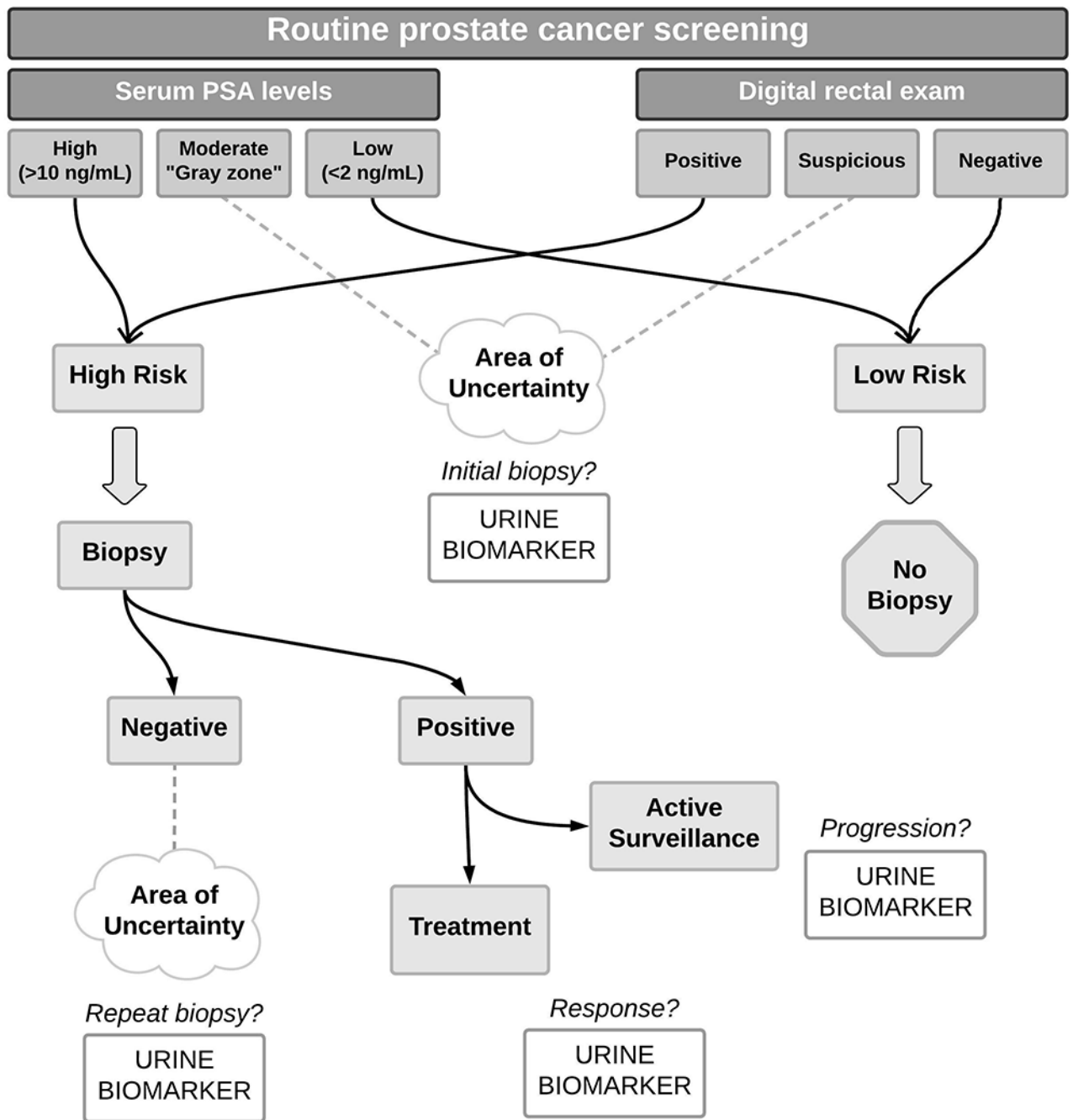Prostatic components detectable in urine.

Figure 3.
Future urine tests could be used to guide clinical decision making in detection and management of prostate cancer.
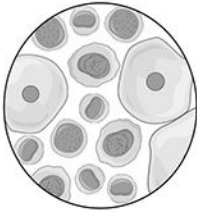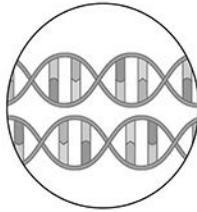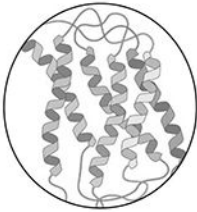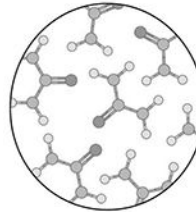
	CYTOLOGICAL	GENOMIC & TRANSCRIPTOMIC	PROTEOMIC	BIOCHEMICAL & METABOLOMIC
TECHNIQUES	• Conventional cytology • In situ hybridization • Flow cytometry • Immunostaining	• Quantitative PCR • Methylation-specific PCR • RNA-Seq • Transcription mediated amplification (TMA)	• Western blot • ELISA • Mass spectrometry	• Mass spectrometry • Dipstick tests • Electronic noses
STRENGTHS	• Visualization of normal and cancer cells • Ability to label for specific markers • High specificity • Amenable to automation • Quantitative & qualitative	• Quantitative • High analytical sensitivity • Standard procedures • Easy to perform and interpret results • Can measure levels in isolated urine fractions (exosomes, cells, etc)	• High-throughput and quantitative methods • Minimal processing steps • Abundance of soluble peptides/proteins • Less protein complexity than serum or plasma • No proteolytic degradation	• Point-of-care testing possible • Minimal processing steps required • Rapid results • Inexpensive
LIMITATIONS	• Low sensitivity • Difficult to find rare cells, requires sophisticated imaging software • No standard processing methodology • Labor intensive, requires trained personnel • Inter-observer variability	• Mixed cell population • Requires prompt sample processing • Normalization required • Cannot determine cellular origin of markers	• High dynamic range of urine proteins • Confounding by other non-cancer prostate conditions • High abundance proteins can mask low abundance proteins • High urine salt content	• Normalization required • Wide range of normal values • Contribution of systemic metabolites to urine • Lack of cancer specificity

Figure 4.
Strengths and limitations of the various approaches to urinary biomarker detection.

Table 1.

Clinical urine tests for prostate cancer

Test	Marker description	Biomaterial	Indication	Clinical value	Performance	Available as	References
Progensa PCA3	PCA3 & PSA mRNA	Post-DRE urine	Diagnosis: re-biopsy setting	Reduction in the number of prostate biopsies	Se: 42–54% Sp: 74–91%	FDA approved (Hologic)	[54, 94, 95]
ExoDx Prostate Intelliscore	ERG, PCA3, SPDEF Exosomal RNA	Pre-DRE urine	Diagnosis/ prognosis	Reduction in the number of prostate biopsies, prediction of aggressive prostate cancer (GS7+)	Se: 92% Sp: 34%	CLIA-approved LDT (Exosome Diagnostics)	[80, 90]
SelectMDx	HOXC6, DLX1, KLK3 mRNA	Post-DRE urine	Diagnosis/ prognosis	Reduction in the number of prostate biopsies, prediction of aggressive prostate cancer (GS7+)	Se: 91% Sp: 36%	CLIA-approved LDT (MDx Health)	[64, 65]
Mi-Prostate Score (MiPS)	PCA3 and TMPRSS2-ERG mRNA, serum PSA	Post-DRE urine, blood serum	Diagnosis/ prognosis	Reduction in the number of prostate biopsies	Se: 80–93% Sp: 33–90%	CLIA-approved LDT (University of Michigan MLabs)	[70, 96]
Prostarix Risk Score	Sarcosine, alanine, glycine, glutamate	Post-DRE urine	Diagnosis/ prognosis	Reduction in the number of prostate biopsies, prediction of recurrence	Se:25–90% Sp: 24–90%	CLIA-approved LDT (Metabolon)	[97, 98]

Abbreviations: CLIA, Clinical Laboratory Improvement Amendments; DLX1, distal-less homeobox 1; DRE, digital rectal exam; ERG, ETS-related gene; HOXC6, homeobox C6; LDT, laboratory developed test; PCA3, prostate cancer antigen 3; PSA, prostate specific antigen; Se, sensitivity; Sp, specificity; SPDEF, SAM pointed domain-containing ETS transcription factor

Table 2.

Volatile organic compounds

Marker	Cases	Controls	Sens (%)	Spec (%)	DRE[a]	Urine	Method	Ref
Canine olfaction	362	540	98-100	97-98	PRE	Whole urine	2 dogs (both female German Shepherds previously trained for explosive detection), no information regarding training frequency or duration	[29]
	50	67	13-25	71-75	PRE	Whole urine	2 dogs (1 Yellow Labrador and 1 Border Collie), no information regarding training frequency or duration	[30]
	33	33	91	91	POST	Whole urine	1 dog (Belgian Malinois Shepherd), training: 5 days/week, 16 month duration	[99]
	57	186	-	-	PRE	Whole urine	4 dogs (1 Chihuahua mix, 1 Miniature Goldendoodle, 1 Pembroke Welsh Corgi, 1 Border Collie), training: 2-7 days/week, 12-14 month duration	[27]
Electronic nose	12	18	-	-	PRE	Headspace, first morning urine	Electronic nose (ENQBE conducting polymers)	[36]
	6	15	-	-	PRE	Headspace, whole urine	Electronic nose (conducting polymers), partial least square discriminant analysis	[100]
	14	27	71	93	PRE	Headspace, whole urine, first catch and midstream	Electronic nose, partial least square discriminant analysis, leave-one-out as cross-validation criterion	[38, 101]
	50	24	78	67	PRE	First morning urine	Electronic nose, ChemPRO 100 eNose, leave-one-out as cross-validation criterion, linear discriminant analysis	[37]

[a] Specifies if urine samples were collected pre- or post-digital rectal exam (DRE).

Table 3.

RNA markers (other than PCA3 and TMPRSS2:ERG)

Marker	Cases	Controls	Sens (%)	Spec (%)	AUC	DRE[a]	Urine	Method	Normalization	Ref
AMACR	43	49	70	71	0.65	POST	Sediment	RT-qPCR	RNA levels of KLK3	[69]
	138	96	-	-	-	POST	Sediment	RT-qPCR	RNA levels of KLK3 and GAPDH	[60]
	7	6	-	-	-	POST	Sediment	RT-qPCR	RNA levels of KLK3	[59]
	10	9	77-88	85-100	-	POST	Sediment	RT-qPCR	RNA levels of KLK3	[102]
	91	85	65	65	0.65	POST	Sediment	RT-qPCR	RNA levels of KLK3	[68]
ERG	138	96	-	-	-	POST	Sediment	RT-qPCR	RNA levels of KLK3 and GAPDH	[60]
	97	140	31	84	0.59	POST	Sediment	RT-qPCR	RNA levels of KLK3	[103]
GalNAC-T3	44	46	-	-	0.64	POST	Sediment	RT-qPCR	RNA levels of KLK3	[104]
GOLPH2	138	96	-	-	0.66	POST	Sediment	RT-qPCR	RNA levels of KLK3 and GAPDH	[60]
	195	124	59	71	0.62	POST	Sediment	RT-qPCR	RNA levels of KLK3 and GAPDH	[61]
Hepsin	44	46	-	-	0.48	POST	Sediment	RT-qPCR	RNA levels of KLK3	[104]
MALAT1	81	135	65	67	-	POST	Sediment	RT-qPCR	RNA levels of KLK3	[105]
PSGR	57	97	63	64	0.65	POST	Sediment	RT-qPCR	RNA levels of KLK3	[66]
	73	142	59	73	0.68	POST	Sediment	RT-qPCR	RNA levels of KLK3	[106]
PSMA	57	97	81	41	0.62	POST	Sediment	RT-qPCR	RNA levels of KLK3	[66]
	44	46	-	-	0.64	POST	Sediment	RT-qPCR	RNA levels of KLK3	[104]
SPINK1	138	96	-	-	0.64	POST	Sediment	RT-qPCR	RNA levels of KLK3 and GAPDH	[60]
TFF3	138	96	-	-	-	POST	Sediment	RT-qPCR	RNA levels of KLK3 and GAPDH	[60]
TTTY15-USP9Y gene fusion	75	151	84	78	0.82	POST	Sediment	RT-qPCR	RNA levels of KLK3	[107]

[a]Specifies if urine samples were collected pre- or post-digital rectal exam (DRE).

Abbreviations: AMACR, α-methylacyl-CoA racemase; ERG, ETS-related gene; GalNAC-T3, N-acetylgalactosaminyltransferase 3; GOLPH2, golgi phosphoprotein 2; MALAT1, metastasis associated lung adenocarcinoma transcript 1; PSGR, prostate specific G-coupled receptor; PSMA, prostate specific membrane antigen; SPINK1, serine peptidase inhibitor, Kazal type 1; TFF3, trefoil factor 3; TTTY15, testis-specific transcript, Y-linked 15; USP9Y, ubiquitin specific peptidase 9 Y-linked.

Table 4.

DNA methylation markers

Marker	Cases	Controls	Sens (%)	Spec (%)	AUC	DRE [a]	Urine	Method	Normalization	Ref
APC	117	47	Pre: 17 Post: 42	Pre: 94 Post: 94	-	PRE & POST	Cells isolated by filtration	ddPCR	Copies per µL	[71, 108]
	12	5	-	-	-	POST	Sediment	Methylation specific PCR	Not specified	[108]
	113	128	36-51	83-91	0.59-0.62	POST	Sediment	Methylation specific PCR	ACTB	[109]
	178	159	-	-	0.59	POST	Sediment	Methylation specific PCR	Not specified	[110]
	232	238	-	-	0.63	POST	Sediment	Methylation specific PCR	Not specified	[111]
C1orf114	117	47	Pre: 6 Post: 26	Pre:100 Post: 100	-	PRE & POST	Cells isolated by filtration	ddPCR	Copies per µL	[71]
GSTP1	40	45 BPH + 7 PIN	73	98	-	POST	Sediment	Methylation specific PCR	Not specified	[112]
	28	0	-	-	-	PRE	Sediment	Methylation specific PCR	Not specified	[113]
	69	31 BPH	-	-	-	PRE	Sediment	Methylation specific PCR	Not specified	[114]
	18	27	58	61	-	Post-biopsy	Sediment	Methylation specific PCR	Not specified	[115]
	12	5	-	-	-	POST	Sediment	Methylation specific PCR	Not specified	[108]
	31	34 BPH	98	87	-	POST	Sediment	Methylation specific PCR	Not specified	[116]
	24	69 BPH + 8 HGPIN	75	98	-	POST	Sediment	Methylation specific PCR	Ratio of GSTP1 to ACTB	[117]
	113	128	33-36	91-95	0.64-0.65	POST	Sediment	Methylation specific PCR	ACTB	[109]
	178	159	-	-	0.66	POST	Sediment	Methylation specific PCR	Not specified	[110]
	117	47	Pre: 21 Post: 42	Pre: 77 Post: 82	-	PRE & POST	Cells isolated by filtration	ddPCR	Copies per µL	[71]
	232	238	-	-	0.68	POST	Sediment	Methylation specific PCR	Not specified	[111]
	91	51	21-78	50-95	0.69	POST	Sediment	Methylation specific PCR	Not specified	[118]
PITX2	117	47	Pre: 4 Post: 16	Pre: 100 Post: 100	-	PRE & POST	Cells isolated by filtration	ddPCR	Copies per µL	[71]
RARB2	113	128	29-40	84-91	0.59-0.64	POST	Sediment	Methylation specific PCR	ACTB	[109]
	178	159	-	-	0.71	POST	Sediment	Methylation specific PCR	Not specified	[110]
	232	238	-	-	0.64	POST	Sediment	Methylation specific PCR	Not specified	[111]
RASSF1	253	32 BPH	45	84	-	POST	Sediment	Methylation specific PCR	ACTB	[119]

Marker	Cases	Controls	Sens (%)	Spec (%)	AUC	DRE [a]	Urine	Method	Normalization	Ref
	117	47	Pre: 47 Post: 65	Pre: 41 Post: 35	-	PRE & POST	Cells isolated by filtration	ddPCR	Copies per µL	[71]
RASSF2	91	51	21-74	50-95	0.66	POST	Sediment	Methylation specific PCR	Not specified	[118]
TFAP2E	91	51	21-67	50-95	0.65	POST	Sediment	Methylation specific PCR	Not specified	[118]

[a] Specifies if urine samples were collected pre- or post-digital rectal exam (DRE).

Abbreviations: APC, Adenomatous polyposis coli; ddPCR, droplet digital polymerase chain reaction; GSTP1, Glutathione S-transferase P1; RARB2, Retinoic acid receptor beta-2; RASSF1, Ras association domain-containing protein 1; RASSF2, Ras association domain-containing protein 2; TFAP2E, Transcription Factor AP-2 Epsilon; PCR, polymerase chain reaction; ACTB, beta-actin; BPH, benign prostatic hyperplasia; HGPIN, high-grade prostatic intraepithelial neoplasia.

Table 5.

Protein markers

Marker	Cases	Controls	Sens (%)	Spec (%)	AUC	DRE [a]	Urine	Method	Normalization	Ref
5-alpha reductase	16	91	-	-	-	PRE	Not specified	Western blot, dot blot & ELISA	Not specified	[120]
ADIRF	16	15	81	100	-	PRE	Exosomes; first morning urine	Mass spectrometry	Not normalized	[121]
AMACR	12	12 + 2 atypia	100	58	-	Post biopsy	Sediment	Western blot	Not specified	[58]
Annexin A3	368	223	-	-	0.82	POST	Sediment	Western blot	Volume & density	[122]
B-2-microglobulin	90	83 BPH	-	-	0.66	PRE	Whole urine	iTRAQ, LC-MS/MS	Not specified	[123]
Bladder tumor fibronectin	21	8 + 13 BPH	-	-	-	PRE	Voided and catheterized urine	Chemiluminescent immunoassay	Creatinine	[124]
Bradeion	51	14	-	-	-	PRE	Not specified	Immuno-chromatographic membrane strip tests	Not specified	[125]
c-Met	75 (localized PCa)	81 (metastatic PCa)	-	-	0.90	PRE	Supernatant	Electrochemi-luminescent immunoassay	Creatinine	[126]
CRISP3	47 localized PCa, 39 metastatic	48 BPH	79	84	0.87	POST	Supernatant	iTRAQ, ELISA & western blot	Not specified	[127]
Delta-catenin	16	15	88	83	-	PRE	Prostasomes	Western blot	Not specified	[128]
Endoglin (CD105)	67	52	34-85	50-94	-	POST	Supernatant	ELISA	Total protein and creatinine	[129]
Engrailed-2	82	102	66	88	-	PRE	Supernatant	ELISA	Not specified	[130]
	58	67	-	-	-	PRE	Supernatant, midstream catch	ELISA	Not specified	[131]
	267	140	67	89	-	PRE	Supernatant	ELISA	Not specified	[132]
Fibronectin	8	10 + 12 BPH	75	50	-	PRE	Whole urine	LC-MS/MS	Not specified	[133]
GCNT1	35		-	-	0.76	POST	Supernatant	Dot blotting	Not specified	[134]
GOLPH2	195	124	75	72	0.79	POST	Sediment	Western blot	PSA and GAPDH	[61]
IL18 BP	67	32	69	56	0.65	POST	Supernatant	ELISA	Not normalized	[135]
Intestinal mucin 3	90	83 BPH	-	-	0.61	PRE	Whole urine	iTRAQ, LC-MS/MS	Not specified	[123]
MCM-5	12	131 + 70 BPH	92	82	-	PRE	Sediment	Immunofluorometric assay	Not specified	[136]

Marker	Cases	Controls	Sens (%)	Spec (%)	AUC	DRE[a]	Urine	Method	Normalization	Ref
MMP-9	103	86	58	82	-	PRE	Whole urine	Gelatin Zymography	Not specified	[137]
MRP-14	6	6	-	-	-	POST	Supernatant	2D-MALDI-TOF	Not specified	[138]
	32	74	38	73	-	PRE	Not specified	ELISA	Not specified	[139]
MSMB	25	27 BPH	96	26	-	PRE & POST	Supernatant	MALDI-TOF	Creatinine	[140]
PCYOX1	16	15	25	100	-	PRE	Exosomes; first morning urine	Mass spectrometry	Not normalized	[121]
Pepsinogen 3, group 1	90	83 BPH	-	-	0.62	PRE	Whole urine	iTRAQ, LC-MS/MS	Not specified	[123]
PSA	42	51 + 77 BPH	93	36	-	PRE	Whole urine	ELISA	Not specified	[141]
	9	11	-	-	-	PRE	First void and midstream urine	ELISA	Not specified	[142]
	13	18 + 8 BPH	31	100	-	PRE	24-hour urine	Radioimmunoassay	Creatinine	[143]
Fucosylated PSA	49	20	77	80	-	POST	Supernatant	ELISA	Not specified	[144]
C-terminal PSA fragment	50	19	-	-	-	POST	Supernatant	MALDI-DIT-TOF	Not specified	[145]
PSAP	37	24	-	-	-	POST	Supernatant	Mass spectrometry	Not specified	[146]
PSMA	10	0	-	-	-	PRE	Exosomes, early morning	Western blotting	Not specified	[147]
Prostatic inhibin-like peptide	25	24 + 17 BPH	84	100	-	PRE	24-hour urine	ELISA	Creatinine	[148]
	21	20	81	100	-	PRE	24-hour urine	ELISA	Creatinine	[149]
Scatter factor	49	24 + 14 BPH	-	-	-	PRE	Supernatant, midstream catch	ELISA	Creatinine	[150]
Survivin	19	45	0	91	-	PRE	Random clean catch or straight catheter	Western blot	Not specified	[151]
Tissue factor	53	20 + 37 BPH	57	75	-	PRE	Supernatants; early morning urines, midstream catch	Chromogenic uTF assay	Not specified	[152]
	106	87 + 67 BPH	65	75	-	PRE	Sediment	Chromogenic uTF assay	Creatinine	[153]
Transferrin	22	19 + 4 BPH	36	95	-	PRE	Not specified	Immunoturbidimetric assay	Creatinine	[154]
	34	36+ 28 BPH + 5 prostatitis	75	30	-	PRE	Not specified	Immunoturbidimetric assay	Creatinine	[155]
TMEM256	16	15	94	100	0.87	PRE	Exosomes; first morning urine	Mass spectrometry	Not normalized	[121]
Thymosin b15	121	221	41	92	-	PRE	Supernatant	ELISA	Total protein and creatinine	[156]
Zinc α2-glycoprotein	42	56 + 29 PIN	79	60	0.68	POST	Supernatant	Western blot	Creatinine	[157]

[a] Specifies if urine samples were collected pre- or post-digital rectal exam (DRE).

Abbreviations: ADIRF, adipogenesis regulatory factor; AMACR, α-methylacyl-CoA racemase; CRISP3, cysteine-rich secretory protein 3; GCNT1, Core 2 β-1,6-N-acetylglucosaminyltransferase-1; GOLPH2, golgi phosphoprotein 2; IL18BP, interleukin-18-binding protein; MCM-5, mini-chromosome maintenance-5; MMP, matrix metalloprotease; MRP-14, Myeloid related protein-14; MSMB, microseminoprotein beta; PCYOX1, Prenylcysteine oxidase 1; PSA, prostate specific antigen; PSAP, prostatic acid phosphatase; PSMA, prostate specific membrane antigen; TMEM256, Transmembrane Protein 256