REVIEW ARTICLE

Prostate cancer detection: a systematic review of urinary biosensors

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BACKGROUND: Current diagnostic methods for prostate cancer are invasive and lack specificity towards aggressive forms of the disease, which can lead to overtreatment. A new class of non-invasive alternatives is under development, in which urinary biomarkers are detected using biosensing devices to offer rapid and accurate prostate cancer diagnosis. These different approaches are systematically reviewed and their potential for translation to clinical practice is evaluated.
METHODS: A systematic review of the literature was performed in May 2021 using PubMed Medline database, Embase, and Web of Science. The objective was to review the structural designs and performance of biosensors tested on urine samples from patients with prostate cancer.
RESULTS: A total of 76 records were identified. After screening and eligibility, 14 articles were included and are discussed in this paper. The biosensors were discussed based on the target biomarkers and detection technologies used, as well as the results of the clinical studies. Most of the works reported good discrimination between patients with prostate cancer and controls.
CONCLUSIONS: This review highlights the potential of urinary biosensors for non-invasive prostate cancer detection. However, clinical studies have so far only been conducted on small cohorts of patient, with large scale trials still needed to validate the proposed approaches. Overall, the consensus arising from the proof of concepts studies reviewed here, is that an adequate combination of biomarkers into multiplex biosensor platforms is required to achieve accurate diagnostic tests. Furthermore, whether such devices can discriminate between aggressive and indolent cancer has not yet been addressed, because it entails optimized biomarkers panels and long-term clinical trials.

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INTRODUCTION

Prostate cancer is one of the most frequently diagnosed cancers. In 2020, there were over 1.4 million new cases and 375,000 deaths [1]. Forecasts predict over 2.2 million new cases and over 700,000 deaths from prostate cancer globally in 2040. Prostate cancer is already leading cancer for males [2] and considering the aging population, is set to become an even more serious public health issue in the future.

There is currently no widely adopted routine screening test for prostate cancer. Prostate-specific antigen (PSA) blood testing and digital rectal examination (DRE) are commonly undertaken to detect prostate cancer. However, PSA testing is controversial because of its high rates of false positives and DRE is an uncomfortable invasive procedure with very limited sensitivity and specificity [3]. A screening program using these methods would result in overdiagnosis and lead to overtreatment [4] with high rates of complications, risks, and costs [5, 6]. In order to reduce medical expenses and improve patient quality of life, a non-invasive diagnostic method with high accuracy is needed.

Urine is a potential window into the diagnosis of urogenital cancers (e.g. cancer of the kidneys, prostate, and bladder) [7–9]. Urine fractions; namely whole urine, urine sediments (circulating tumor cells), or supernatant (extracellular vesicles, exosomes); can be used to detect a variety of biomarkers which can be protein, metabolite, or nucleic acid based. Four rapid and non-invasive urinary biomarker tests are currently available for prostate cancer diagnosis. These tests can have high sensitivity (three assays achieving 93% [10–12] and PCA3 (Progensa) 78% [13]) but they tend to be less specific than tissue biopsies. To date, only the Progensa PCA3 assay has been approved by the FDA to help determine the need for repeat prostate biopsies in men who have had a previous negative biopsy. The main shortcoming of the four urinary commercial tests is their low specificity (33 to 57%). Therefore, these assays generate high rates of false-positive tests with overdiagnosis of low risk or slow-growing prostate cancer. Overdiagnosis in this context can lead to overtreatment (radiation/hormone therapy or radical prostatectomy) and complications such as incontinence, impotence, and bowel problems. The particular challenge with a prostate cancer diagnosis is to specifically detect aggressive cancers, as opposed to indolent ones.

Therefore, more reliable and specific strategies for biomarker detection are required to diagnose clinically relevant prostate

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cancer. Biosensor technology provides a promising method for biomarker detection through rapid, low-cost, simple-to-use applications. The sensitivity and specificity of biosensing platforms can be improved by surface functionalisation, system miniaturization, target biomarkers selectiveness, as well as nanomaterials-assisted signal enhancement.

Biosensors are devices combining biochemical recognition elements, transducers and detectors. The interaction between the targeted molecule and the biochemical recognition components (receptors) on the biosensor generates an optical or electrochemical signal for quantification. The advantages of small sample volume and versatility of the recognition components increase selectivity. Biosensors are already applied in medical diagnostic and point of care analysis, for many other diseases [14–17]. In this systematic review, we report specifically on new urine-based biosensors for prostate cancer diagnostics and their possible use in clinical practice.

METHODS

Definitions

Sensors are defined as devices used to detect and quantify physical, chemical, or biological elements. Biosensors are a subclass of sensors, set apart by the biological nature of the sample, target analyte, or both. Here, the term biosensor is used to refer to devices that detect the presence or concentration of an analyte of interest in a biological sample. Often, the analyte of interest is a biomarker: a biological molecule that is an indicator of particular biological processes or pathological conditions. Biosensors used for cancer diagnosis typically measure and evaluate the presence or concentration of one or more cancer biomarker. In the following results section, we review sensor types that are considered biosensors because they were developed with the aim of detecting urinary analyte.

Search strategy

A systematic review of the literature was performed in May 2021 using PubMed Medline database, Embase, and Web of Science. The search included articles published from January 2007 to March 2021 with the following terms: 'prostate cancer' OR 'prostatic neoplasms' AND 'urinary biomarker' OR 'urine' AND 'detection' OR 'diagnosis' OR 'diagnostic' OR 'screening' AND 'biosensor' OR 'microfluidic' OR 'nano' AND 'non-invasive' OR 'noninvasive' OR 'non-invasive'.

Studies that were duplicates and publication types other than research articles were excluded. The titles and abstracts of the remaining research articles were reviewed for inclusion, with the full articles only reviewed when the titles and abstract were unclear. The search strategy was registered in Open Science Framework: 10.17605/OSF.IO/H6URA. The initial screening and full-text reviews were performed by two authors independently. The discrepancies were resolved after discussion by consensus. A search was also conducted on the clinical trial registry website (clinicaltrials.gov) in Oct 2021, using the same search terms to identify clinical trials with results of diagnostic/device interventions.

Selection criteria

Studies included in this review satisfied the following two criteria: (1) a sensor device used for prostate cancer diagnosis and (2) the sensor was tested on clinical urine samples. Research studies that did not include prostate cancer patients, or did not test urine samples, and those with objectives other than diagnosis or detection of prostate cancer, were excluded.

Data extraction

For all included studies, the following data were extracted: biosensing approach, targeted biomarker, detection limit, techniques used to immobilize and detect the targeted biomarker, sensitivity, and specificity results from clinical patient samples. The studies were categorized according to the targeted biomarker type and compared in terms of sensitivity and specificity.

RESULTS

The literature search was conducted without a time limit and seventy-six articles were identified. No additional records were identified from the clinical trial registries search. Fourteen relevant articles with full text were included and are discussed. The flow diagram for study selection is shown in Fig. 1. The key characteristics of the included studies are summarized in Tables 1–3.

Biochemical sensors

Volatile organic compounds (VOCs) present in the urine can serve as potential biomarkers because they have unique signatures corresponding to specific diseases [18, 19]. Solid phase microextraction (SPME) is the main technique used to collect VOCs. SPME is compatible with gas chromatography–mass spectrometry (GC–MS) for VOCs detection in urine [20]. Aggio et al. used a GC-sensor system to measure differences in urinary VOCs levels between prostate cancer patients and controls. The data was transformed and important features were identified in order to best differentiate between cancer and control groups. The resulting dataset provided a computer algorithm for the development and classification of different medical conditions [21]. This pilot study ($n = 131$) achieved sensitivity and specificity of over 90%.

Another VOCs detection technique is canine olfactory detection, an approach where trained sniffer dogs are used to distinguish the urine of prostate cancer patient based on their distinctive scent. The first study which applied this method was performed by Cornu et al. and showed very promising results [22]. Guest et al. adopted a multiparametric (trained canine detection, volatiles analysis, urinary microbiota profiling) approach to further train an artificial neural network (ANN) on GC–MS data using the canine diagnosis [23] (Fig. S1A). Canine olfaction (two dogs) alone achieved 71% specificity and 70–76% sensitivity. However, this research only recruited men diagnosed with a Gleason score of 9, limiting the overall clinical relevance.

Sarcosine, an amino acid derivative, is another organic compound that has been identified as a prostate cancer biomarker. Specifically, Sreekumar et al. and Khan et al. found that sarcosine levels were elevated in prostate cancer tissues and

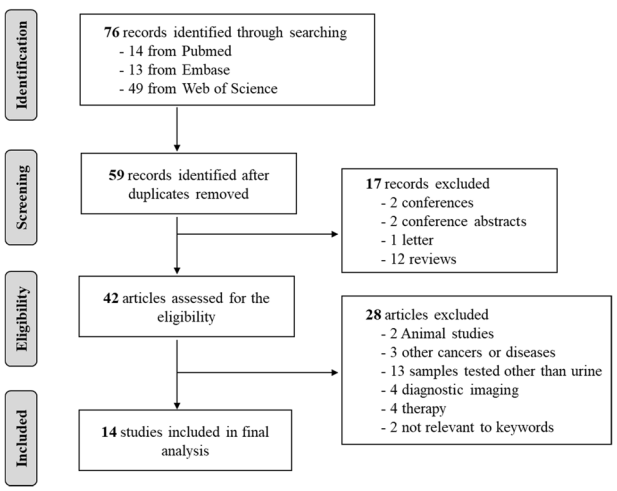

Fig. 1 Study Selection. Flow diagram illustrates the study selection process. Fourteen studies were included in the final analysis.

Table 1. Study characteristics of biochemical sensors.

Sensing approach	Target	Detection limit	Biomarker immobilization techniques	Biomarker detection techniques	Clinical patient samples		Limitations	Ref.
					Cohort	Result		
Column system coupled to a metal-oxide GC-sensor and a computer algorithm	VOCs	2.5 ppm and below	SPME	GC	58 PCa 73 controls	95% sensitivity and 96% specificity after leave-one-out cross-validation; 87 and 99% after Double cross-validation	• Data normalization required • Fail to identify the key VOC metabolites	[21]
Trained canine olfaction with GC–MS ANN-assisted examination and microbial profiling	VOCs	–	Trained dogs, HS-SPME	GC–MS 16 s rRNA sequencing	12 PCa 38 controls	Canine olfaction system was 71% sensitivity and 70–76% specificity. ROC of VOC analysis by GC–MS is 0.935	• Limited sample (control & GS = 9) size for canines training	[23]
Quantification of sarcosine via cross coupled chemical and electrochemical reactions	Sarcosine	0.4 nM	PAA/SOX functionalised GCE	Chrono-amperometry	5 PCa, 5 controls	Sarcosine concentration of urine in PCa patients is 5.2 times higher than in healthy men' urine.	• Small cohort • Limited sample information	[26]
3D stacked AgNWs-GFF SERS sensor	Metabolites	1.77 nM	AgNWs with polyvinylpyrrolidone coating-GFF	SERS	22 PCa 30 controls[a]	Good discrimination between PCa and healthy controls (100% sensitivity and 100% specificity)	• Fail to identify the key cancer-related metabolites	[27]
Potentiometric multisensor array system	Biomolecules	10^{-6} M	28 sensor arrays covering both lipophilic & hydrophilic cations & anions	Potentio-metry	15 PCa 15 controls	100% sensitivity and 93% specificity were obtained in 'Optimized Sensor set'	• Sensor optimization required • Limited cohort diversity	[28]

VOCs volatile organic chemicals, *SPME* solid-phase microextraction, *GC* gas chromatography, *PCa* prostate cancer, *ANN* artificial neural network, *HS-SPME* headspace solid-phase microextraction, *GC–MS* gas chromatography-mass spectroscopy, *rRNA* ribosomal ribonucleic acid, *ROC* receiver operating characteristic, *PAA* polyamic acid, *SOX* sarcosine oxidase, *3D* three dimensional, *AgNWs* silver nanowires, *GFF* glass fiber filter, *SERS* surface-enhanced Raman spectroscopy.

[a]22 pancreatic cancer patients included in this study (results now shown).

Table 2. Study characteristics of protein sensors.

Sensing approach	Target	Detection limit	Biomarker immobilization techniques	Biomarker detection techniques	Clinical patient samples		Limitations	Ref.
					Cohort	Result		
A spiral microfluidic chip capture tumor cells via immunocytochemistry	Protein (GPC-1)	85 (±6)% efficiency	MIL-38 anti-GPC-1 mouse primary antibody	immunoassay	14 PCa, 14 controls	79% efficiency in urine of PCa patients	• Relatively low sensitivity and specificity	[31]
FET-based ion-responsive urine sensor	Protein (ANXA3)	<1 fg/mL	anti-ANXA3 immobilized sensing membrane	FET	34 PCa	True positive rate: 82.36%; false negative rate: 17.64% within the detectable range of the sensor	• No control sample included	[35]
ML-assisted DGFET multimarker sensor system	Proteins (ANXA3, ENG, ERG, ENG)	–	anti-ANXA3/ENG/ERG/PSMA antibodies immobilised sensing channels	4-channel extended gates DGFET	25 PCa (pre & post DRE urine) 26 controls	97.1% accuracy in terms of multimarker panels in 'Test set' (30% of the total data set)	• Biomarker panel optimization required	[37]

GPC-1 glypican-1, *PCa* prostate cancer, *FET* field-effect transistor, *ANXA3* annexin A3, *ML* machine leaning, *DGFET* dual-gate field-effect transistor, *ENG* endoglin, *ERG* erythroblast transformation-specific related gene protein, *PSMA* prostate-specific membrane antigen, *DRE* digital rectal examination.

urine sediment compared to controls. [24, 25]. An enzyme-based electrochemical sensor was fabricated on a glassy carbon electrode (GCE) functionalized with multilayers of polyamic acid (PAA), glutaraldehyde, and then sarcosine oxidase (SOX) [26]. The SOX enzyme converts sarcosine to acetaldehyde and hydrogen peroxide (H_2O_2), which in turn breaks down the carboxylic acid groups of PAA on the GCE surface (Fig. S1B). This sensor exhibited a detection limit of 0.4 nM. The sensor detected a concentration of sarcosine 5.2 times higher in urine samples from prostate cancer patients than from controls, however, these results were obtained in a very small number of patients and require validation in a larger cohort.

Recently, a three-dimensional (3D) silver nanowires (AgNWs) coated glass fiber filter (GFF) biosensor was employed for multiplex detection of metabolites in prostate cancer urine samples [27]. The metabolites adsorbed on the AgNWs were measured by surface-enhanced Raman spectroscopy (SERS) with a laser at 633 nm (Fig. S1C). This study included prostate and pancreatic cancer patients, and the sensor performed well with clear discrimination between all tested groups (control, pancreatic cancer, and prostate cancer) and 100% accuracy for prostate cancer detection. A shortcoming of this metabolomic multivariate approach is the difficulty in identifying the exact urinary compounds responsible for the test specificity from the SERS peaks spectra. Both cancer groups showed SERS peaks caused by xanthopterin, inosine, hypoxanthine, xanthine that were lower or absent in the control group, but the only principal component analysis could discriminate between prostate and pancreatic cancers.

Solovieva et al. described a multisensor system comprised of 28 potentiometric sensors (PVC-plasticized, chalcogenide glass, and polycrystalline membranes) to generate charges from molecules present in the urine of prostate cancer patients [28] (Fig. S1D). This multivariate potentiometric approach distinguished the fingerprint of urine samples based on their composition of anionic, cationic, and redox species. A validation test using a set of 30 urine samples (15 with prostate cancer and 15 without prostate cancer) achieved 100% sensitivity and 93% specificity. This result was obtained with the optimized sensor set (19 sensors instead of all 28 sensors) and logistic regression analysis. Factors to consider in further studies include the confounding effects of diet, age, other diseases, and overall health status of both prostate cancer patient and control groups, as well as the need for larger sample set for multivariable data calibration.

Protein sensors

Glypican-1 (GPC-1) has been reported as a potential biomarker of prostate cancer [29, 30]. A fluorescently labeled immunoassay-based spiral microfluidic chip for GPC-1 detection has been reported [31]. The sensor successfully confirmed more than 79% of the prostate cancer cases by capturing GPC-1^+ prostate cancer cells and the sensor performance could be improved by modifying the selection of biomarkers used and fine-tuning the chip by an additional waste outlet to remove a large amount of urine crystals (Fig. S2A).

Another protein biomarker for prostate cancer detection is Annexin A3 (ANXA3), a member of the calcium/phospholipid-binding protein family that regulates cellular growth and signal transduction pathways [32]. ANXA3 is associated with prostate cancer and hence is a potential biomarker in tissue and urine samples [33, 34]. ANXA3 was quantified in untreated urine samples using a dual-gate field-effect transistor (DGFET) based ion-responsive urine sensor (IRUS). In this device, the anti-ANXA3 was immobilized on disposable sensing gates [35] (Fig. S2B) which detect urinary ANXA3. A field-effect transistor (FET) then measures the ion concentrations and converts them into an electrical signal. Although the authors claimed that the sensing platform achieved good accuracy, there are no control groups reported in their study.

Table 3. Study characteristics of nucleic acid sensors.

Sensing approach	Target	Detection limit	Biomarker immobilization techniques	Biomarker detection techniques	Clinical patient samples		Limitations	Ref.
					Cohort	Result		
Microfluidic device using sandwich immunoassay on the paramagnetic beads and electrochemical detection of the product of immunoassay	DNA (8-OHdG)	5 pg/mL	Paramagnetic particles with an alkaline phosphatase conjugated immune-complex	SFIA-ED	14 PCa	13–20 ng/mL ($n = 5$); 21–30 ng/mL ($n = 6$); >31 ng/mL ($n = 3$)	• No control samples included • Highly complex operating system	[39]
A ligation-based isothermal RPA assay for real time fluorescence identification via DNA-intercalating dye	mRNAs (TMPRSS2-ERG T1E4, T1E5)	2 amol (1000 copies)	T4 DNA ligase-based ligation of targeted probes	RPA	9 PCa, 2 controls	Both T1E4 and T1E5 positive (3 Pca); only T1E4 positive (5 PCa)	• Small cohort • Cut off values determined by control samples	[42]
DNA-directed assembly of CuNBs target on multiple RNA species	RNAs (TMPRSS2-ERG T1E4, miR-107, *SChLAP*1)	Electrochemical readout: 100 fM (T1E4 & *SChLAP*1), 10 fM (miR-107)	Magnetic beads with target-specific probes	Potentio-metry	10 PCa, 2 controls	Electrochemical detection: All 3 biomarkers positive (4 PCa); 2 biomarkers positive (4 PCa); only 1 biomarker positive (2 PCa)	• Small cohort • Cut off values determined by control samples	[43]
Adjustable ac-EHD nanomixing device to enhance probe-target hybridization	RNAs (TMPRSS2-ERG, miR-107, *SChLAP*1)	Electrochemical readout: 500 fM (TMPRSS2-ERG & *SChLAP*1), 50 fM (miR −107)	Mixing microwell with biotinylated capture probes	Potentio-metry	18 PCa, 3 controls	Electrochemical detection: Higher expression for all 3 biomarkers (8 PCa)	• Small cohort	[44]
Nanowire-based microfluidic device to collect EV-encapsulated miRNAs for sequencing analysis	EV-miRNAs	–	ZnO nanowires	EDS, FESEM, Microarray analysis	3 PCa, 3 controls[a]	Compared to ultracentrifugation, the device showed a 5-fold higher miRNA expression level and a greater variety of extracted species of miRNAs	• Small cohort	[46]
Lab-on-a-disc integrated with independent nanofiltration	EV-mRNAs (AR-V7, AR-FL)	$1–50 \times 10^8$ particles/mL	Injection-molded and ultrasonic-bonded disc, with six AAO filters with 20 nm pore size	ddPCR	36 PCa 11 controls	Provides higher EV mRNA expression than conventional UC method, ratio of AR-V7/AR-FL can used to differentiate HSPC and CRPC	• Choice of therapy may affect the test results	[47]

ac-EHD alternating current electrohydrodynamic, *PCa* prostate cancer, *EV* extracellular vesicle, *EDS* energy dispersive x-ray spectroscopy, *FESEM* field-emission scanning electron microscopy, *ZnO* zinc oxide, *miRNA* micro RNA, *AR-V7* androgen receptor splice variant 7, *AR-FL* androgen receptor full length, *ddPCR* droplet digital polymerase chain reaction, *AAO* anodic aluminium oxide, *UC* ultracentrifugation, *HSPC* hormone-sensitive prostate cancer, *CRPC* castration-resistant prostate cancer, *DNA deoxyribonucleic acid*, 8-OHdG 8-hydroxy-2′-deoxyguanosine, *SFIA-ED* stopped flow injection analysis- electrochemical detector, *PCa* prostate cancer, *mRNAs* messenger RNAs, *RPA* recombinase polymerase amplification, *TMPRSS2-ERG* transmembrane protease serine 2-ERG, *CuNBs* copper nanoblocks.

[a]Other cancer patients included in this study (results now shown).

Furthermore, ANXA3 is not specific to prostate cancer and can also be elevated in urine from bladder cancer patients [36]. In order to overcome this limitation, the authors proposed utilizing this sensor with multipanel biomarkers such as serum PSA level to improve diagnostic accuracy.

In another study from the same group, the DGFET (Fig. S2C) consisted of a disposable chip with four sensing channels, each functionalized with different antibodies (ANXA3, prostate-specific membrane antigen (PSMA), erythroblast transformation-specific related gene protein (ERG), and endoglin (ERG)) [37]. Machine learning (ML) algorithm analysis demonstrated that a better sensing performance was achieved with an increased number of combined biomarkers and the overall performance depended heavily on the biomarker combination chosen. Surprisingly, however, the inclusion of ANXA3 in the multimarker panel reduced the sensors' accuracy.

Nucleic acid sensors

Tumor-derived DNA and RNA can be found in urine and have been assessed as biomarkers for cancer detection [38]. This includes detection of 8-hydroxy-2′-deoxyguanosine (8-OHdG) which is a biomarker of oxidative stress. An immunocapture microfluidic device successfully detected 1-naphthol using modified paramagnetic particles and stopped flow injection analysis with electrochemical detection (SFIA-ED) (Fig. S3A) [39]. This assay is highly complex and in the absence of a control group, it is impossible to evaluate the efficiency of the sensor.

Specific nucleic acid transcripts such as TMPRSS2-ERG fusion transcripts are overexpressed in clinically significant prostate cancer (Gleason scores ≥7, tumor stage T3–T4) and present in half of the tumors [40, 41]. Koo et al. have designed a DNA ligase-based ligation recombinase polymerase amplification (RPA) assay to detect TMPRSS2-ERG fusion mRNA variants (Fig. S3B) [42]. The same group has also explored a nanosensor for multiple RNA species using DNA-directed copper nanoblocks (CuNBs). The detection was carried out by fluorescence/electrochemical RNA measurement [43]. To achieve better diagnostic performance, combination of different biomarkers into the same assay was suggested. A further study reported on a nanodevice using alternating current electrohydrodynamic (ac-EHD) nanomixing for multiple RNA targets detection from urine (Fig. S3C) [44]. The device required numerous processing steps that could potentially be streamlined with automation.

Extracellular vesicles (EVs) are vesicles containing various DNA, RNA, and protein from their cells of origin. They have shown great potential as cancer biomarkers [45]. ZnO nanowires were fabricated to collect urinary EVs followed by microarray analysis of miRNA expression (Fig. S3D) [46]. The nanowires device was superior to conventional ultracentrifugation or commercially available kits in term of EV collection efficiency and miRNA yield. The expression level and relevant species of miRNAs extracted from the device were five and four times higher, respectively than with ultracentrifugation or commercially available kits. It identified other urinary miRNAs as biomarker candidates in patients with a range of cancers but these are yet to be validated.

A "lab-on-a-disc" ExoHera device (Fig. S3E), quantified urinary EV androgen-receptor splice variant 7 (AR-V7) and androgen receptor full-length (AR-FL) mRNA expression in castration-resistant prostate cancer (CRPC) and hormone-sensitive prostate cancer (HSPC) patients. Using droplet digital polymerase chain reaction (ddPCR) [47], these workers recorded higher level of AR-V7 and AR-V7/AR-FL ratio in CRPC groups when compared with HSPC groups. However, large clinical trials are required to validate the approach, and how chemotherapy drugs affect the AR variants will need to be carefully considered [48, 49].

DISCUSSION

The focus of this review was prostate cancer detection in asymptomatic men, however, these tools could be modified for application in the setting of disease monitoring after treatment. Each of the different sensors described in this review has its own advantages and disadvantages (Table 4). Generally, biochemical sensors tended to be low cost with high sensitivity, however, their use was complex. Protein sensors were often simple, but high cost; and nucleic acid tests were cost-effective and rapid, but required specific sample preparations. Most have been applied to more than one diagnostic biomarker to improve accuracy and a panel of biomarkers appears to be necessary for the effective detection of prostate cancer.

A wide range of target biomarkers and/or variables are usually measured in the biochemical sensors approaches. For VOCs and metabolites measurement in biochemical sensors, a broad panel of biomarkers is needed to distinguish positive from negative. In contrast, the target biomarker measured in protein and nucleic acid sensors, usually appears to be more specific. These sensors typically target single or a small combination (less than five usually) of biological moieties for specific detection. Some studies used a single biomarker in the initial report and showed some utility. However, a combination of multiple biomarkers was often tested in follow-up studies. For instance, a single biomarker was detected in Jeun et al. [35] in a preliminary study, and a multiple biomarkers application was developed by Kim et al. [37]. Trau's group [42–44] explored different techniques to detect prostate cancer with the same biomarker TMPRSS2-ERG (see Table 3). Subsequently, multiple biomarkers associated with aggressive prostate cancer were included [43, 44] to improve sensor performance. The choice of biomarkers and their prognostic value are critical when designing a multiplex sensor. Indeed, the multiple biomarker approach utilizes a variety of biomarkers associated with different mechanisms of prostate cancer progression. By increasing the number of biomarkers, the sensitivity may be improved but specificity may suffer leading to the identification of false positives.

Biomarker combinations should be tested in urine samples from prostate cancer patients at different tumor stages, so that the data can provide an improved understanding of prostate cancer detection. It is vital to test sensor performance in large cohorts of patients with relevant clinical conditions and distinguish between studies of men with known prostate cancer versus those who are being screened for prostate cancer. Studies need to incorporate clinically relevant pre-test probabilities of cancer in order to accurately determine real-world positive and negative predictive values for cancer detection.

Several gaps in knowledge were identified in current evidence. Most studies of the sensors are underpowered and have used much less than a hundred patients. Additionally, they often did not include patient details such as tumor stage and Gleason score for comparison. It is important to emphasize that what is required in the detection of prostate cancer is not simply whether cancer is present or not but whether the cancer is aggressive and will ultimately cause morbidity and mortality in the individual. This requires biospecimen banking and long-term patient follow-up. Some of the devices reviewed herein may have the capacity to predict outcomes but this has not been tested.

Further evaluation of the biosensor candidates is required before clinical translation. Priority should be given to optimizing the novel methods proposed in relevant patient cohorts. Then, to improve the sensitivity and specificity of these biosensors, research efforts should focus on providing comprehensive evaluations of the clinical detection limits and performance parameters, including cancer progression. In addition, it will be essential to simplify the complex workflow associated with the sequential use of multiple techniques for any of these biosensors to, one day, become applicable to point-of-care diagnostics.

Table 4. Comparison of the advantages and disadvantages of sensors in this study.

Sensors		Advantages	Disadvantages
Biochemical	[21, 23]	• Low cost • Relatively low maintenance • Allows for mass spectral detection	• Normalization required • Lack of specificity • Complex operation
	[26]	• Low cost • Simple	• Risk of enzyme denaturation during the immobilization process
	[27]	• Multiplex detection • High sensitivity	• Sophisticate practice required
	[28]	• Multiplex detection	• Optimization required
Protein	[31]	• Simple	• High cost • Time consuming
	[35]	• Simple • Minimal processing	
	[37]	• Simple • Minimal processing • Multiplex detection	• Optimization required
Nucleic acid	[39]	• Cost effective • High specificity	• Complex operation
	[42]	• Rapid • Cost effective • Multiplex detection	• Extensive primers optimization required
	[43]	• Rapid • High specificity • Multiplex detection	• Nucleic acid preparation needed
	[44]	• Rapid • Multiplex detection	• Nucleic acid preparation needed
	[46]	• Allow large-scale transcriptional profiling	• High cost
	[47]	• Absolute quantification • High sensitive	• Not capable with high abundance samples

CONCLUSIONS

This review assessed the potential of biosensing platforms for urinary prostate cancer diagnosis. Some strategies have shown promising results, however, the main drawback is the lack of validation with large cohort studies and the absence of long-term follow-up so that prediction of tumor aggression and patient outcomes can be analysed. Advanced development of sensor devices should include multimarker capability for accurate diagnosis. Moreover, further investigations are needed to identify the best combination in multimarker approach and to classify the diagnostic threshold for each biomarker, in order to improve their efficacy in clinical practice.

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AUTHOR CONTRIBUTIONS

Conceptualization, investigation, results interpretation, writing – original draft preparation, KMC and MM; contribution to an oversight of the overall study, JMG and MO; writing – review and editing, MM, JMG, MO and KV; supervision, project administration, MM, JMG and KV All authors have read and agreed to the published version of the manuscript.

COMPETING INTERESTS

The authors declare no competing interests.

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