Urine Biomarkers for Prostate Cancer Diagnosis and Progression

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Abstract

Prostate cancer (PCa) can be highly heterogeneous and multifocal, and accurate assessment of the volume, grade, and stage of PCa in situ is not a simple task. Urine has been investigated as a source of PCa biomarkers for over 70 years, and there is now strong evidence that analysis of urine could provide more accurate diagnosis and a better risk stratification that could aid clinical decisions regarding disease surveillance and treatment. Urine diagnostics is a developing area, moving towards multi-omic biomarker integration for improved diagnostic performance. Urine tests developed by strong collaborations between scientists and clinicians have the potential to provide targeted and meaningful data that can guide treatment and improve men's lives.

1. Introduction: Urine as a Source of Prostate Cancer Biomarkers

Prostate cancers (PCa) can be highly heterogeneous[1,2] and multifocal[2,3]. Accurate assessment of the volume, grade, and stage of prostate cancer in situ is not a simple task. Significant amounts of biopsy results can be upgraded or downgraded on prostatectomy analysis[4,5]. Multi-parametric MRI has improved enormously but has inter-operator inconsistencies[6], can miss significant cancers (Gleason > 4), and has a false positive rate of around 50%[7]. Urine has been investigated as a source of PCa biomarkers for over 70 years[8–10], and there is now strong evidence that urine analysis could provide a better assessment of disease diagnosis and prognosis that could aid clinical decisions regarding disease surveillance and treatment.

Prostatic secretions make up 30% of the volume of semen, and its composition can reflect pre-neoplastic or malignant changes[11]. The prostate is continually secreting, and these secretions flow from all areas of the prostate where PCa is found[12,13]. These secretions flow into the urethra whence they are flushed out of the body on urination[12]. When a cancer is present, tiny bits of tumour (cells, extracellular vesicles, and molecules) can also be carried in the secretions and these can be detected in urine[8,9]. Urine is advantageous as a source for liquid biopsy because it can be collected at low cost, is completely non-invasively, and has the potential to sample all secretory areas of the prostate at the same time.

Key Words

Urine, biomarkers, prostate cancer

Competing Interests

Dr Clark and Professor Cooper have a patent GB1905111.9 issued in relation to the PUR (prostate urine risk) signatures discussed in this review. Professor Pandha and Professor Morgan have a patent issued for EN2 as a diagnostic marker. Dr Perry reports grants from Enterprise Ireland, Movember, Prostate Cancer Foundation, and Science Foundation Ireland during the conduct of the study. Dr Perry has a patent EP15831140.7 issued, and a patent 15/538928 pending. The remaining authors declare no competing interests.

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Abbreviations

DRE digital rectal examination
EVs extracellular vesicles
Gs Gleason score
MiPS Mi-Prostate score
PCa prostate cancer
PCA3 prostate cancer antigen 3
PCPT-RC prostate cancer prevention trial risk calculator
PUR prostate urine risk
VIP vasoactive intestinal peptide

Urine samples have been analysed for promising cancer biomarkers in the form of cells, DNA, RNA, proteins, and metabolites. The relative proportions of biomarkers vary between whole urine, cell sediment, and supernatant which will be discussed separately. The majority of the research presented here has been performed on small cohorts from which limited conclusions can be made. The current state and future directions of urine analysis for prostate cancer diagnosis and prognosis are described herein. **Table 1** provides an overview of the biomarkers discussed in this review.

2. Analysis of Whole Unfractionated Urine

Elevated levels of C3, C4, and transferrin proteins were found in prostatic fluid from PCa patients by Greyhack et al. in 1979[11], and in 1982 PCA-1 (prostate cancer antigen-1) protein was detected in urine from PCa patients but not in urine from age-matched non-PCa men[10]. However, it was not until 2002, when PCA3 (prostate cancer antigen 3) RNA transcripts in urine were found that the potential for urine molecular diagnostics in clinical urological practice was realised.

PCA3 is a prostate-specific long non-coding RNA overexpressed in ≥ 95% of prostate cancers that was first investigated as a urinary PCa marker by de Kok et al. in 2002[14]. In a multicentre validation study[15,16] it was shown to predict Gleason score ≥ 7 cancer with an 80% negative predictive value.

The FDA-approved Progensa *PCA3* urine test was approved in 2012. Whole urine for this test is collected after prostate massage with the intent of predicting the likelihood of detecting PCa on repeat prostate biopsy[17,18]. The *PCA3* score is calculated as the ratio of 2 mRNAs: *PCA3/KLK3* x1000[17]. A threshold score of 35 provided a sensitivity and specificity of 58% and 72%, respectively for presence of significant PCa on rebiopsy[19,20]. Investigations into a direct relationship between the *PCA3* test and PCa volume or Gleason pattern have been unclear, yielding opposing results in different studies[14,21,22]. Metanalysis by Luo et al. found great heterogeneity among published data sets with *PCA3* test sensitivity ranging from 47% to 82%[23]. The reasons for this were unknown, and they may underlie the poor uptake of the *PCA3* test in the clinic. However, the *PCA3* test is important as it was first to demonstrate that collection, transport, and centralised laboratory analysis of urine was a viable means of PCa biomarker analysis.

The *TMPRSS2:ERG* fusion gene is found in ~50% of PCa foci; however, as *TMPRSS2:ERG*-positive and negative tumour foci can be found in individual prostates[24], a *TMPRSS2:ERG* may be present in ~70% of PCa-radical prostatectomies[25], making its detection more useful than was initially apparent. Urine transcript levels of *TMPRSS2:ERG* correlated with *ERG* expression in PCa tissue and aided prediction of PCa by *PCA3*. The Mi-Prostate score (MiPS) combined detection of *PCA3*, *TMPRSS2:ERG* and serum PSA levels[26], providing significantly improved detection of any PCa and Gleason score (Gs) ≥ 7 on biopsy compared with PSA or the prostate cancer prevention trial risk calculator (PCPT-RC)[27].

Further gene transcripts have been investigated for additional improvements. van Neste et al. combined RT-PCR data from *HOXC4, HOXC6, TDRD1, DLX1, KLK3*, and *PCA3* with clinical information from 2 independent multicentre prospective collections (n = 906)[28].An optimal model (SelectMDX) required only a combination of PSAD, DRE result, *HOXC6* and *DLX1*, with *KLK3* used for relative biomarker quantitation[28]. SelectMDX had a strong net benefit, potentially reducing unnecessary biopsies over the *PCA3* test, PSA and the PCPT-RC with a validation cohort AUROC of 0.9 for detection of Gs ≥ 7 cancer. SelectMDX has been reported to be able to reduce diagnostic costs in a study covering 5 European countries, the degree of benefit varying with the amount of overtreatment in each country's clinical procedures[29].

3. Analysis of Urine Cell Sediment

3.1 PCa cells in urine

Urine can contain many different cell types, including bladder urothelial cells, squamous cells, seminal vesicle cells, prostate cells, red blood cells, and white blood cells[30], up to 80% of which can originate from the prostate[31,32]. Prostate cancer cells were first detected in urine samples by microscopy in 1947[9] and are associated with higher risk and advanced cancers[31]. The relative proportions of the different cell types in urine can alter with a DRE[31,33] or disease state such as prostatitis[34], prostate/urinary tract problems, or PCa[30,35].

TABLE 1.

Overview of relevant biomarkers

Biomarker type	Use of test	Urine fraction or source	Detection method	Largest cohort size	Results	References
PCa cells						
AMACR, Nkx3.1, nucleolin, ERG and prostein	PCa	Urine sediment	Antibody, microscopy	63	Sensitivity 64%, specificity 69%	35, 36*
Chromosome alterations	PCa	Urine sediment	FISH microscopy	100	AUROC 0.83, 81% accuracy	32
VPAC receptors	PCa	Urine sediment	Fluorescent peptide, microscopy	176	> 98% sensitivity and specificity	37
Protein						
C3, C4 transferrin	PCa	Prostatic fluid	Immunoelectrophoresis, radial immunodiffusion	10	Significantly elevated in PCa	11
PCa-1	PCa	Whole urine	2D gel electrophoresis	17	16/17 PCa positive	10
ITGA3, ITGB1	Metastasis	Supernatant	Mass spectrometry, Western blot	13	More abundant in 3 urines from metastatic patients	92
EN2	PCa, higher tumour stage (T1 v T2)	Supernatant	Antibody, ELISA, graphene-based biosensor	184	PCa AUROC 0.8, sensitivity 66%, specificity 88%	99*–101, 104

AS: active surveillance; AUROC: area under receiver operating characteristic curve; FISH: fluorescent in situ hybridisation.

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Urine cell pellet staining for AMACR, Nkx3.1, nucleolin, ERG, and prostein[35,36] can detect prostate cancer cells but overall lacked sensitivity compared with biopsy[36]. Two fluorescent approaches have shown promise: OligoFISH probes to detect alterations in chromosomes 7, 16, 18, and 20 has been shown to have an 80% specificity compared with biopsy data[32], while a fluorescent peptide detected VPAC receptors with > 98% sensitivity and specificity[37]—VPAC receptors bind VIP, a neuropeptide linked to development, growth, immune system and cancer.

3.2 RNA in urine sediment

A disadvantage with urine sediment analysis is that the cell transcriptome is likely to alter on becoming detached and/or on contact with urine[38,39]. However, urine cell sediment has been found to be useful for PCa diagnosis.

PCA3 has a reported sensitivity of detection of PCa in urinary sediment of 62%, boosted to 73% by co-detection of *TMPRSS2:ERG*[40,41]. Other combination markers used with *PCA3* have been found to aid PCa detection in cell sediment: (1) *AMACR, TRPM8, MSMB*[42], (2) *TMPRSS2:ERG, GOLPH, SPINK1*[43], and (3) *HIST1H2B, SPP1, ELF3*[44]. However, Leyten et al. found that *PCA3* was unnecessary when *HOXC6, DLX1*, and *TDRD1* were used[45], *TDRD1* being a direct target of *ERG* and co-expressed with *ERG* in PCa[46]. In combination with the European Randomised Study of Screening for PCa (ERSPC) risk calculator[47], Leyten et al. noted that *TMPRSS2:ERG* added significant predictive value to the ERSPC calculator to predict biopsy Gleason whereas *PCA3* did not. *TMPRSS2:ERG* has been reported to be less common in Chinese populations[48], and detection of *TTTY15:USP9Y* gene fusion transcripts found in 35% of Chinese patients PCa[48] has improved PCa detection in urine sediments in that population (n = 226, AUROC 0.83)[49]. Other probe combinations excluding *PCA3* include a panel of 6 genes overexpressed in PCa tissue (*CCND1, LMTK2, FN1, GSTP1, HPN*, and *MYO6*), used in the analysis of 156 PCa patients' urine sediments (n = 67), which had a sensitivity of 80.6% and specificity of 62.9% for PCa detection (AUROC of 0.80)[50].

TABLE 1.

Overview of relevant biomarkers, *Cont'd*

Biomarker type	Use of test	Urine fraction or source	Detection method	Largest cohort size	Results	References
RNA						
PCA3	PCa on repeat biopsy Gs≥7	Whole urine, supernatant	qRT-PCR, NanoString, quantitative nucleic acid amplification	809	AUROC 0.66-0.8. Sensitivity 47%–82%	15, 16*–23, 69, 80–84, 97, 105
PCA3, TMPRSS2:ERG fusion gene	PCa detection Gs≥7, higher vol PCa	Whole urine, urine sediment, supernatant	qRT-PCR, quantitative nucleic acid amplification	497	AUROC 0.77-0.8.	26, 40, 41, 47*, 69, 80–84, 105
HOXC6, DLX1 (SelectMDx)	PCa detection Gs≥7	Whole urine	qRT-PCR	358	AUROC 0.77	29, 45*
KLK3	Control probe in analyses	Whole urine, sediment, supernatant	qRT-PCR, NanoString linear amplification	NA	NA	14-23, 28, 29, 40, 41, 43, 69, 80–84, 87
AMACR, TRPM8, MSMB	PCa	Cell sediment	qRT-PCR	104	AUROC 0.74	42
GOLPH, SPINK1	PCa	Cell sediment	qRT-PCR	235	AUROC 0.76, sensitivity 66%, specificity 76%	43
HIST1H2B, SPP1, ELF3, PCA3	PCa	Cell sediment	qRT-PCR	224	AUROC 0.76, sensitivity 77%, specificity 67%	44
TTTY15:USP9Y	PCa	Cell sediment	qRT-PCR	226	AUROC 0.83	49
CCND1, LMTK2, FN1, GSTP1, HPN and *MYO*	PCa	Cell sediment	qRT-PCR	67	AUROC of 0.80	50
AGR2	PCa	Supernatant	qRT-PCR	32	AUROC 0.96	85
Birc5	PCa	Supernatant	qRT-PCR	207	AUROC 0.67	81
CDH3	PCa	Supernatant	qRT-PCR	53	Significantly decreased in PCa, sensitivity 0.69	86
PUR signatures 39 gene probes	High risk, AS monitoring to treatment intervention	Supernatant	NanoString	535	AUROC 0.77 for high risk, HR 8.2 for AS monitoring	70

AS: active surveillance; AUROC: area under receiver operating characteristic curve; HR: hazard ratio; qRT-PCR: quantitative reverse transcribed and polymerase chain reaction.

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TABLE 1.

Overview of relevant biomarkers, *Cont'd*

Biomarker type	Use of test	Urine fraction or source	Detection method	Largest cohort size	Results	References
miRNA						
miR-125b	PCa, high risk	Cell sediment	Exiqon miRNA RT-PCR platform	415	AUROC 0.76	53
miR-24, mir-30c	PCA	Cell sediment	Exiqon miRNA RT-PCR platform, miRCURY LNA miRNA SYBR Green PCR	415	AUROC 0.89	53,* 55
miR-148a, miR-375	PCA	Cell sediment	Taqman low density array	72	AUROC 0.79	52
miR-3195, let-7b-5p, miR-144-3p, miR-451a, miR-148a3p, miR-512-5p, miR-431-5p	PCA	Cell sediment	NanoString	149	AUROC 0.74	54
DNA						
c-Myc, BCAS1, HER2, AR, PTEN, TMPRSS2:ERG	PCa	Supernatant	qRT-PCR for copy number and mutations	10	AUROC 0.8	91
MethDNA						
epiCaPture: GSTP1, SFRP2, IGFBP3, IGFBP7, APC, PTGS2	PCa Gs≥8	Cell sediment	Quantitative methylation-specific polymerase chain reaction	463	AUROC 0.83	62
ProCUrE: HOXD3 and *GSTP1*	PCa Gs≥7	Cell sediment	Quantitative MethyLight	408	AUROC 0.8	63
APC, CRIP3, GSTP1, HOXD8	PCa Gs≥7	Cell sediment	Multiplex quantitative MethyLight	153	OR 2.6	64

AS: active surveillance; AUROC: area under receiver operating characteristic curve; MethDNA: methylated DNA

3.3 miRNA in urine sediment

miRNA dysregulation is frequently observed in cancer[51], and a number of diagnostically useful miRNAs are detectable in urine[52–55]. *miR-21* and *miR-125b* are controlled by the androgen receptor (AR), and are overexpressed in PCa and associated with apoptotic resistance[53,54]. In contrast, *miR-205* is a tumour suppressor miRNA, promoting apoptosis, and its loss is associated with the early stages of PCa development[56]. Despite *miR-205* being down-regulated in PCa, it is a constituent of several miRNA urinary biomarker panels. AUROCs vary from 0.6 to 0.85 for detection of PCa using multiple combinations of miRNAs[52,53], and 0.74 for distinguishing low-risk from high-risk disease[54].

3.4 DNA-methylation in urine sediment

Epigenetic alterations are heritable changes in gene expression with no change to the DNA code. In cancer, DNA-hypermethylation silences tumour suppressors and other important regulatory genes[57]. It is easily detectable by PCR and it occurs early in tumorigenesis making it an ideal biomarker for early detection as well as disease progression monitoring and risk stratification of patients[58,59].

Pioneering work in the detection of PCa and significant PCa (Gs ≥ 7) was performed by Cairns et al. in 2001, who showed that methylation of the *GSTP1* gene was detectable in urine of men with PCa but at a low sensitivity (27%)[60]. *GSTP1* is hypermethylated in > 90% of PCa[60] and is relatively PCa-specific, it

typically being overexpressed in most other cancers. For these reasons, it is a stalwart of PCa-methylation analysis. Gene panels improved performance, and a combination of *APC, RARbeta, RASSF1A, PTGS2, ABCB1* methylation was detectable in > 85% of cases[61]. Notable examples include epiCaPture, a 6-gene DNA-methylation panel (*GSTP1, SFRP2, IGFBP3, IGFBP7, APC, PTGS2*) that can detect 85% of aggressive PCa (Gs ≥ 8) with a 70% improvement in the specificity of PSA[62] and ProCUrE, a 2-gene DNA-methylation panel (*HOXD3* and *GSTP1*) with a sensitivity of 57.1% and specificity of 97% for significant PCa[63]. Zhao et al. established a 4-gene panel (*APC, CRIP3, GSTP1, HOXD8*) with some ability to predict cancer progression in patients on active surveillance (OR 2.559; 95% CI 1.257 to 5.212) from post-DRE urine[64]. They subsequently incorporated microRNAs and reported that *miR-24, miR-30c* and *CRIP3* methylation could predict reclassification of AS patients[55].

Currently, no commercially available standardised DNA-methylation-based urine tests for PCa are available[60], which presents an obstacle to clinical uptake[65]. Sample storage conditions affect results as methylated-DNA is only stable for up to 28 days in urine stored at –20/–80°C and a preservative is required at room temperature[66]. Most urine assays use bisulfite conversion of unmethylated cytosines to uracil, leaving hypermethylated cytosines preserved for detection. However, a study of 12 different bisulfite kits discovered that conversion efficiency varied greatly[67], and storage of the less stable single-stranded bisulfite converted DNA may also be an issue[68]. Target sequence choice is critical, proximity to the transcription start site, transcription factor binding motifs, and DNase-hypersensitivity are all factors that can affect sensitivity and specificity[59]. Large, multicentre, standardised urine collections and clinical follow-up are needed to reduce the unknowns and bring PCa methylation biomarkers to fruition.

4. Urine Supernatant

4.1 RNA in urine extracellular vesicles

Large numbers of extracellular vesicles (EVs) can be found in urine[69], the majority of which in first-catch adult male urine originate from the prostate[69,70]. EVs are lipid-bound vesicles produced by a wide range of cell types[71]. EVs function as inter-cellular messengers that can bind to and influence the phenotype of cells they come into contact with[72,73]. Cancer cells produce EVs, which can enhance vasculature[74], increase metastasis[75], and influence the immune system[76] and can contain PCa-specific mRNAs such as *TMPRSS2:ERG* fusion gene transcripts[40]. EVs contain lipids, RNA, DNA, and proteins including membrane receptors[72,77,78] which are protected from degradation by, for example, RNAses by the EV lipid membrane[79].

The majority of publications refer to analysis of only small numbers of gene transcripts in EVs, namely *PCA3, ERG, TMPRSS2:ERG, KLK3*, which have been found to be useful in PCa diagnosis and detection of Gleason ≥ 4 cancer[69,80–84]. Additional genes with diagnostic potential are *AGR2* splice variants[85], *Birc5*[81], and decreased expression of *CDH3*[86]. In contrast, Connell et al. used a NanoString panel of 167 gene probes, mostly selected from published evidence of over-expression in PCa tissue[70]. Analysis of 535 urine EV samples from patients with and without PCa led to the prostate urine risk (PUR) signatures constructed from a subgroup of 39 gene probes. In contrast to all other urine analyses, instead of a single cancer signature they constructed 4 PUR signatures, which were built around samples categorised as non-cancer (PUR-1), plus the 3 D'Amico risk groups for cancer aggression, namely low-risk (PUR-2), intermediate-risk (PUR-3), and high-risk (PUR-4). Each sample could have representation from all 4 signatures and the sum of the 4 PUR signatures in each sample was '1'. Connell et al. found that PUR-4 could predict the presence of significant cancer on TRUS biopsy (AUROC 0.77). On examination of an active surveillance cohort (n = 87) PUR-4 could be used to divide patients into 2 groups with rates of progression to treatment intervention of 10% and 60% up to 5 years after urine collection (HR 8.23). A strong PUR-1 signature correlated with stability of low-grade disease that did not progress in the 5-year follow-up. The PUR-2 and PUR-3 signatures had less utility but were hypothesised as integral to the creation of a clearer signature for higher grade Gleason cancer detectable by PUR-4.

A few studies have compared PCa mRNA transcripts in both cell and EV urine fractions. Prostatic transcripts appear to be higher in EV fractions[69,80,87], but may have better diagnostic utility in the cell sediment[88] with a caveat that ~10% of cell sediments may not be analysable. Hendriks et al. reported that *PCA3* transcripts were expressed significantly higher in PCa patients than in non-PCa patients in both the whole-urine and cell-sediment fractions but not in the EV fraction[87]. Webb et al. compared RNA yields from cell sediment and EVs in 200 patients and found them to be highly variable with no apparent correlation. This observation suggests that examination of RNA biomarkers in whole urine could be obfuscated by the unknown relative contribution of transcripts from the different urine fractions and suggests that separate analysis of the 2 fractions is to be recommended[80].

4.2 Cell-free DNA in urine

Cell-free urine DNA (cfDNA) has been found both inside EVs and bound outside EV membranes[79], the source of which has been hypothesised to be from apoptotic cells[79] and mitochondria[89]. cfDNA yields from EVs are low (18pg/mL urine[90]) but have been used to detect methylated *GSTP1* in men with PCa that was not present in urine from men with BPH[78]. Casadio et al. used copy number analysis of *c-Myc, BCAS1,* and *HER2* by qRTPCR to distinguish PCa from non-PCa men with an AUROC of 0.8, while copy number gains of AR, genomic deletions including PTEN, and *TMPRSS2:ERG* fusion sequences have been detected in a small cohort of men with castrate-resistant cancer (n = 10)[91].

4.3 Supernatant proteins

Thousands of proteins on or encapsulated within EVs have been identified by mass spectrometry analysis, with for example ITGA3 and ITGB1 being linked to metastasis[92]. For a thorough review see recent papers by Pang et al.[93] and Wu et al.[94].

Possibly the most thoroughly investigated urine protein biomarker is the transcriptional repressor EN2[95]. Unusually for a transcription factor, EN2 can be secreted from normal and PCa cells and then be internalised by other cells to effect transcriptional changes in, for example, stroma[96]. EN2 is involved with embryonic brain development and is inappropriately expressed in a range of cancers including bladder and prostate where EN2 may regulate androgen-receptor activity in androgen-sensitive prostate cancer cells[97,98]. In a 2011 study by Morgan et al., men with prostate cancer had a 10-fold higher level of EN2 in their urine versus non-cancer controls, and EN2 was identified in 66% of urine samples from biopsy-proven PCa patients, some of whom had undetectable levels of serum PSA[99]. This was in contrast to < 15% positivity in control groups (some of whom would have been expected to harbour occult prostate cancer), giving a specificity of 88.2% (AUROC 0.8; $P < 0.001$). Higher EN2 levels correlated with advancing tumour stage, eg, pT3a versus pT2b ($P = 0.027$), positive margins ($P = 0.008$), increasing tumour volume[100,101], and subsequent diagnosis of PCa in BRCA1/2 mutation carriers[102].

There have, however, been no large-scale EN2 trials because there is no robust commercially available test for EN2 protein in urine, which may be due to its very high net-charge causing non-specific attachment to some plastic surfaces (personal communication from H. Pandha [co-author], 2019). Indeed, a recent study looking at commercially available ELISA kits for EN2 found no significant diagnostic value for urinary EN2 in PCa patients[103]. Novel approaches are in development, such as a graphene-based biosensor[104] and examining urine cfRNA EN2 transcripts[70].

5. Urine Biomarkers and the DRE

A problem with urine is the inconsistency in the amounts of prostatic material between samples. The digital rectal examination (DRE) of the prostate is one source of variation. When men present at a hospital, nerves very often mean that they would urinate before seeing the doctor and flush out all the prostatic secretions from the urethra. To replenish the prostatic biomarkers in the urethra, urine has usually been collected after a DRE whereby the doctor would stroke the prostate with a finger pushing prostate secretions into the urethra shortly before urination. However, urine cfRNA yields correlate with the clinician performing the DRE, with 10-fold differences being found between clinicians, differences which were hypothesised as being linked to the clinician's DRE technique, finger length and prostate position[80].

A number of studies indicate that RNA yields from urine collected in the clinic without a DRE are less than a tenth of post-DRE samples[69,80] and levels of prostate markers such as KLK3 were also reduced approximately 10-fold[87]. However, studies by Donovan et al. and McKiernan et al., using non-DRE urine found AUROCs of 0.8 and 0.77, respectively for detection of Gs >7 using *PCA3* and *ERG* combined with clinical parameters[82,83,105], strongly suggesting that non-DRE urine has utility. Webb et al. took this one step further[80]: their hypothesis centred on the finding by Huggins et al. in 1945 that the prostate was constantly secreting[12], indicating that time since previous urination was key. Urine samples collected at home from the first urination of the day were found to have RNA yields comparable to samples collected post-DRE from the same patients in the clinic a week earlier. Significantly, Webb et al. found that detection of *PCA3* and *TMPRSS2:ERG* by RT-PCR proved to be much more sensitive in these morning samples than in the post-DRE samples. While this study was limited by the low number of men (n = 14) it does suggest that urine collections could be performed by mail, could enable mass screening, and could simplify disease monitoring of, for example, active surveillance cohorts. Webb et al. also suggested that inter-sample consistency could be further improved by collecting a second urine sample at a fixed interval of 1-hour later.

Conclusions

The extensive interconnecting luminal structures of the prostate that carry prostatic secretions to the urethra make urine a valuable non-invasive resource to examine all parts of the prostate where PCa arises. Urine has proven utility in predicting disease load and monitoring disease progression, and its use could result in the development of a PCa screening test. However, the translation of biomarkers from research to clinical practice is littered with failure[106]. The heterogeneity of PCa and analysis of cohorts with different ranges of disease severity make data difficult to inter-compare. A further layer of obfuscation is provided by variabilities in sample collection, extraction and specifics of analysis compounded by inaccurate estimates of PCa disease status by standard clinical means. However, urine diagnostics is a developing area, moving towards multi-omic biomarker integration for improved diagnostic performance. Urine tests developed by strong collaborations between scientists and clinicians have the potential to provide targeted and meaningful data that can guide treatment and truly improve men's lives.

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