Prostate cancer (PCa) is the second most common cancer worldwide, and it is ranked as the fifth cause of death in men.\cite{1} The incidence pattern varies significantly between countries, with the highest rates of cases reported in developed countries. This may be attributed to the increased life expectancy and, possibly to an even higher degree, to a more frequent screening of patients using the prostate-specific antigen (PSA) test, rather than to a real difference in cancer incidence.\cite{2} The latter is further supported by the common failure to detect at the biopsy level, following a positive result of the PSA screening test.\cite{3} Both genetic and environmental factors are reported to affect the risk of PCa onset. A familial history of PCa,\cite{4} increased body weight \cite{5} or specific genetic variants \cite{6} were all described as risk factors for PCa. Moreover, several molecular changes occur and are associated with PCa initiation and progression, as outlined in the schematic representation (Figure 1).

Prostate cancer (PCa) is fifth leading cause of cancer-associated deaths in men worldwide. Although the application of the serum prostate-specific antigen (PSA) screening test resulted in an increase in the PCa diagnosed cases, it demonstrated a negligible benefit regarding the associated mortality. Treatment options vary, with active surveillance to be preferable for patients with low-risk PCa and therapy of advanced castration-resistant PCa to rely on α-emitters and cytotoxic chemotherapy. Although recent developments have led to the approval of novel drugs for the treatment of castration-resistant PCa, the optimal sequence and timing of medication have not been yet determined. New screening modalities could improve the discriminatory accuracy between tumors with favorable clinical prognosis. Implementation of proteomic-based biomarkers appears to be a promising improvement, which could enable a more accurate diagnosis, guide treatment and improve patient outcome. Reviewed here are urinary proteome-based approaches for detection of PCa and patient management.

**KEYWORDS:** Biomarker • diagnosis • clinical proteomics • prostate cancer • proteome • urine

PCa, on average, has a very low mortality rate, with higher likelihood of dying with PCa rather than as a result of the disease. However, more aggressive PCa tumors and presence of distant metastasis results in a notable decrease of the survival rate, falling to 30%.\cite{7} Therefore, the unmet clinical need in PCa is to distinguish between indolent disease, which is probably best left alone (e.g., by watchful waiting or active surveillance), and aggressive PCa, which requires immediate, effective treatment. For indolent disease, the benefit of detection is questionable for the patient because, if no therapeutic intervention is required, then the PCa diagnosis only generates anxiety, without any beneficial consequence.

PCa (early) diagnosis is generally based on PSA detection in blood along with digital rectal exploration (DRE) and transrectal ultrasound of the prostate gland for men over 50 years of age with a life expectancy of >10 years. This screening modality improves the detection rate of PCa
in patients without any apparent symptoms and has contributed to an enormous increase in PCa incidence. However, high levels of blood PSA (>4 ng/ml) can also be a result of infection, inflammation (prostatitis), benign hyperplasia of the prostate (BPH) or even cycling, rather than PCa.[8] Other factors that account for high PSA levels are age and sexual activity, leading to false positive results. In addition, high number of patients have a PSA level <4 ng/ml and are negative for a digital rectal examination, yet harbor PCa.[8,9] As a result of these shortcomings, the value of PSA measurement in PCa diagnosis has become controversial. Moreover, while recent studies have revealed that PSA testing reduced PCa mortality rate by 20%, most detected PCa are indolent cancers not requiring therapeutic intervention. Consequently, there is an enormous number of over-diagnosed cases, which is a serious concern.[10] While the majority of the examined patients suspicious for PCa present benign histology, [11] recent reports indicate that the diagnostic process using needle biopsy is associated with an increasing rate of infections, other related complications (like sepsis) and patient morbidity.[12] Apart from the effects on patient health and quality of life, implications from the transrectal ultrasound-guided prostate needle biopsy account for increased costs in the health-care system.[12] 

These figures highlight the actual need in PCa: to distinguish between aggressive and indolent disease, thereby leading to the treatment of patients with aggressive disease. PCa treatment varies depending on clinicopathologic parameters, such as stage and grade as well as age. To date, a dominant grading system is based on histological analysis of tissue biopsies (H&E) using light microscopy.[13] The histological scoring system relies on the analysis of primary and secondary grade patterns. The primary (predominant) and secondary (the second most common pattern) grade pattern, combined together, give scores that range from 2 to 10.[13] For low-grade PCa (low Gleason scores) in elderly (≥70 years), watchful waiting or active surveillance is the standard-of-care approach. For younger patients with a low Gleason score, active surveillance or active treatment with prostatectomy[14] is the usual treatment, although the latter might be associated with significant disadvantages, such as risk of incontinence and impotence.[15] The standard treatment for aggressive disease involves radical prostatectomy, hormonal therapy, radiation therapy or early chemotherapy. Surgical or pharmacologic castration result in tumor regression in 75% of the cases; however there are a number of side effects (osteoporosis, cognitive decline, cardiovascular morbidity, obesity, insulin resistance, fatigue and sexual dysfunction) that should be considered before therapy administration. Although treatment guidelines for low- and high-grade PCa exist, unfortunately, no specific biomarkers are available to distinguish between these 2 pathological states. The major challenge, in this regard, is to find novel biomarkers able to distinguish the indolent from aggressive disease, and/or to provide guidance on the most adequate treatment. As already outlined in the past, the most suitable source of biomarkers would be the actual tissue, or the body fluid that is in the closest contact with it.[16] In the case of PCa, the latter would be seminal fluid. While several studies have already resulted in the identification of putative biomarkers for PCa from seminal fluid, the shortcomings of this biofluid are evident, as it is frequently impossible to obtain it from patients of advanced age. 

When looking at the anatomy (Figure 2), it appears that urine represents an alternative source of biomarkers for PCa: as the prostate is connected to the urethra, molecular changes occurring

**Figure 1. Overview of the molecular changes that are associated with prostate cancer initiation and progression.**
in the prostate should also be reflected in urine. Although the prostate is also in contact with blood, blood (plasma or serum) carries several disadvantages that have been reviewed in multiple publications.[11,17] Major issues are the enormous dynamic range in protein concentration of 10 orders of magnitude [18] and the fact that blood theoretically contains information from all organs. Therefore, it is challenging to identify the molecular changes associated with a specific organ, especially if it is quite small like the prostate.

Proximal fluids such as prostatic secretion and seminal plasma contain prostate-derived proteins at high concentrations, which appear highly relevant in the search for prostate-specific cancer biomarkers. Tumor-specific proteins are expected to display indicative changes in biological fluids, being a result of quantitative changes in protein expression in the primary tissue, due to mutation, translocation, gene amplification, increased transcriptional activity or ligand-induced/paracrine–autocrine activation in tumor cells. Tumor invasion and reorganization of the extracellular matrix may cause additional release of proteins into the interstitial fluid and, from there, into the blood or urine.

Biological mechanisms underlying cancer development and progression are very complex and are associated with high intra- and inter-tumor heterogeneity. It is now widely accepted that a single biomarker is not able to reach the required sensitivity and specificity for a clinical screening test, due to the clinical heterogeneity of cancer and the need to distinguish the disease from much more highly prevalent inflammatory and benign conditions.[11] For this reason, studies have begun testing the efficiency of assays based on multiple molecular biomarkers.

**Proteomic platforms for biomarker development**

Proteomic technologies have been found to be applicable throughout all phases of the biomarker development workflow – from discovery to validation. This is attributed to the good analytical performance and capability to analyze and quantify the proteins at both global (global proteomic profiling) and individual protein levels (targeted analysis). In Figure 3, we summarize the proteomic technologies that are most commonly used in biomarker discovery and validation. The general analytical approach consists of a separating step, coupled
to protein identification and/or quantification by a mass spectrometer. Analytical performance (reproducibility, resolution, etc.) and other requirements (absolute or relative quantification, multiplexity) are typically considered for the selection of proteomics platforms for discovery, verification and validation purposes, respectively. Detailed characteristics of the proteomics technologies applied during the different phases of the biomarker development have been covered, to a large extent, in several recent publications.[19–21] A technical introduction along with the consideration on the applicability of the proteomics technologies at the different stages of biomarker development workflow are presented further.

Biomarker discovery

MS-based proteomics technologies offer the advantage of untargeted identification of differentially expressed proteins under the studied conditions, thus facilitating un-biased/non-targeted biomarker discovery.

As outlined earlier, the general principle in proteome analysis for biomarker discovery involves fractionation prior to MS analysis, mainly due to the high complexity of the human proteome. This can be performed by using gel-based (two-dimensional gel electrophoresis) or gel-free approaches (liquid chromatography or capillary electrophoresis).

Two-dimensional gel electrophoresis (2-DE) followed by MS is the conventional approach to separate and analyze differentially expressed proteins.[22,23] In this method, 2 separation steps – isoelectric focusing (IEF) and SDS-PAGE – are performed with the initial separation of the proteins according to their charge, subsequently followed by an additional separation based on the molecular mass.[22–24] Protein spots of interest are excised from the gel, digested with trypsin and identified using MS/MS (see further). 2-DE technology enables separation of typically ~1,000 proteins,[25] as well as the detection of protein isoforms and post-translational modifications[22,23], and allows for relative quantification of the proteins by comparison of protein spot intensity between analyzed groups. However, some drawbacks do exist, particularly with regard to the detection of hydrophobic membrane proteins as well as low-abundance proteins.[23] An improvement with respect to comparative analysis was introduced with the difference gel electrophoresis (DIGE), which utilizes fluorescent cyanine dyes (Cy2, Cy3 and Cy5). Protein labeling using distinct dyes (similar charge and molecular weight, but different fluorescent properties) enables simultaneous analysis of differently labeled samples on the same gel, significantly reducing variability.[26]

LC-MS/MS proteomics analysis can be employed for the separation and consecutive sequencing analysis of native or protease-derived peptides, by application of MS/MS. Typically, reversed-phase chromatographic separation is directly interfaced with an MS/MS instrument. Additional pre-fractionation strategies (Multidimensional Protein Identification Technology [MudPIT]) prior to LC-MS/MS analysis can improve the proteome coverage. Several pre-fractionation strategies are used, including chromatography (ion exchange, reverse phase, etc.), isoelectric focusing (IEF) or free-flow electrophoresis.[27] LC-MS/MS can be applied for global characterization of quantitative changes at the protein level. Two major quantification approaches can be employed: label-based (e.g., iTRAQ, iCAT, super-SILAC) or label-free quantification strategies. Both approaches, along with their advantages and limitations, were extensively reviewed by DeSouza et al. [28]

Application of high-resolution LC-MS/MS analysis enables identification and quantification of a significantly higher number of proteins in comparison to 2-DE analysis. Thus, LC-MS/MS is currently the most widely applied technique for biomarker discovery. However, sample preparation, protein/peptide fractionation and MS analysis are lengthy processes that limit the throughput capability of this approach. Moreover, the large amount of generated data (as aforementioned) as well as the complexity of data processing and analysis further increase the time required for biomarker discovery.

Toward identification of biomarkers at the peptidomics level, CE-MS has been applied in several clinical studies.[29] During this analytical procedure, small proteins and peptides are separated in an electric field according to their charge and size. Currently, separation in an un-coated, bare-fused silica capillary column at low pH is the best practical solution for clinical applications.[30] After electrophoretic separation, the analyte is ionized by electrospray ionization, followed by MS analysis. The detected peptides are characterized by the CE migration time, signal intensity and molecular mass.[31] CE-MS is a high-throughput technology, which enables analysis of an individual sample within 60 min. Like LC, CE can also be interfaced with MS/MS instruments, enabling peptide identification.[32] The analytical performance of CE-MS was evaluated recently and several issues, including precision, stability, limits of detection, reproducibility and intra-variability, were addressed.[33]

Biomarker verification/validation

During the discovery phase, a limited number of samples is analyzed, implying a low statistical power. Therefore, the detected differentially expressed proteins might reflect intra- or inter- individual variability rather than true biological change. Therefore, further validation of the findings in a larger cohort is required.[34]

Immuno-based approaches (ELISA, western blotting) are classical assays that have been applied for verification and validation of proteomics-derived biomarker candidates. Currently, ELISA is the most commonly applied method for absolute protein quantification in body fluids,[35] and may enable fast, sensitive and specific quantification of a selected protein in a complex sample. To address the need for simultaneous analysis of multiple targets, multiplex immunoassays and protein microarrays have been developed.[20] However, the classical antibody-based assays present several limitations; the most prominent ones are lack of specificity, sensitivity toward matrix effects and limited multiplexity. Although there was an attempt to overcome the last issue by using protein arrays, the associated high cost, requirements for high-throughput antibody and
difficulties in establishing multiplexing certainly hamper its broad application.

High-throughput MS-based approaches such as multiple reaction monitoring (MRM) and CE-MS (also used for biomarker discovery at the peptide level) seem to be a promising alternative for routine biomarker assessment. MRM focuses on quantifying selected peptides exemplifying the relevant protein ("proteotypic peptides"), typically using triple-quadrupole instruments. Sequential selection of precursor ions and specific products of their fragmentation leads to precise and more accurate quantification. Utilization of stable-isotope-labeled peptide standards gives an opportunity to perform absolute quantification. However, the latter increases cost, because the price of proteotypic peptides is rather high. In case of peptidomic analysis, CE-MS is a well-established platform, which has been proven to be efficient in a wide range of urinary peptide profiling applications. In the next part of the article, the applicability of some of those proteomic platforms in the context of PCa biomarker research are presented.

**Urinary proteome evaluations**

In this article, we aim at reviewing the most recent advancements of urinary proteomics in the field of PCa biomarker. Manuscripts were retrieved by systematic literature searches using the Web of Science database according to the following criteria: (TITLE: prostate, TOPIC: proteom* OR protein) AND NOT TOPIC: (rat OR mouse OR methylation) AND TOPIC: (urin*). Only original articles published within last 10 years were taken into consideration. Applying these criteria, 311 papers were retrieved (the full list of relevant articles along with their abstracts is given in Table S1). The articles were examined by the authors. Forty-seven of the 311 papers were selected as being relevant to the topic by at least 2 of the authors (Table S2). Manuscripts describing individual urinary protein biomarkers or marker panels consisting of multiple peptide and/or protein biomarkers were included. These include reports on urinary biomarkers, as well as reports of proteins derived from exosomes as biomarker candidates. To better place the report in the context of the field and state of the art, we also reviewed the recently described protein biomarkers, as well as studies investigating seminal fluid. Numerous studies have been investigating and describing PCa biomarkers derived from proteomic analysis. A list of the most promising candidates is shown in Table 1.

**Gel-based studies**

Kiprijanovska et al. analyzed urine from 8 patients with PCa using 2-D PAGE and identified 45 distinct proteins derived from the 125 most prominent spots. Upon comparison with urinary proteome profiles from healthy volunteers, 11 proteins were uniquely identified in PCa. Using Ingenuity Pathways Analysis, 3 of those proteins (E3 ubiquitin-protein ligase ruffylin, tumor protein D52 and thymidine phosphorylase) were reported as associated with cellular growth and proliferation. This initial urinary proteome map of PCa patients was followed by further investigation of biomarkers in urine samples from patients with PCa and BPH by applying 2-D DIGE coupled with MS. Thus, 41 spots, corresponding to 23 proteins, were found to be significantly altered in abundance. Pathway analysis indicated an association with the acute phase response signaling pathway. Nine proteins with differential abundances were included in this pathway: inter-alpha-trypsin inhibitor protein AMBP (AMBP), apolipoprotein A1 (APOA1), fibrinogen alpha chain (FGA), fibrinogen gamma chain (FGG), haptoglobin (HP), inter-alpha-trypsin inhibitor heavy chain H4 (ITIH4), alpha-1-antitrypsin (SERPINA1), tissue factor (TF) and transthyretin (TTR). The urinary levels of TF, AMBP and HP were measured by immunoturbidimetry in an independent validation set. The concentration of AMBP in urine was significantly higher in PCa whereas urinary levels of TF and HP were found to be decreased. The AUC value for the individual proteins ranged between 0.723 and 0.754. Further combination of HP and AMBP yielded the highest accuracy (AUC = 0.848).

Geiser et al. analyzed prostate tissue samples by 2-D DIGE MS and confirmed downregulation of secretin-1 and upregulation of vinculin in PCa. Urinary vinculin levels in PCa patients were significantly higher than in urine from patients negative for PCa (P = 0.006). Using MRM-MS analysis as an approach to validate the proteomics findings, prostatic acid phosphatase (PAP) was found to be significantly increased in the urine from patients with PCa compared to the control population (p = 0.012), while galectin-3 showed significant lower levels in the urine of PCa patients with biochemical relapse, compared to those without relapse. Jayapalan et al. aimed at the identification of proteins that are differentially expressed in the urine of patients with PCa, in comparison to patients with BPH and healthy controls. Using a combination of 2-DE and MS/MS, significantly lower levels of urinary saposin B and 2 different fragments of inter-alpha-trypsin inhibitor light chain (ITIL) were found in the PCa patients compared to the controls, the ITIL fragments also being significantly different between the PCa and BPH patients. Image analysis was further conducted on urinary proteins that were transferred to membranes and detected using lectin binding to O-glycans. A truncated fragment of the inter-alpha-trypsin inhibitor heavy chain 4 was found to be significantly enhanced in the PCa patients compared to the controls. Unfortunately, further validation of these candidates remains to be completed.

**Non-gel-based studies**

Using the LC-MS/MS, Haj-Ahmad et al. investigated protein expression profile of urine from healthy males (n = 10), patients with BPH (n = 12) and with PCa (n = 8) by performing shotgun LC-MS/MS analysis. This was followed by validation of selected findings at the mRNA level using quantitative real-time-PCR. This approach revealed fibronectin and tumor protein p53-inducible nuclear protein 2 (TP53INP2) to be significantly downregulated PCa patients. Jednak et al. employed quantitative iTRAQ LC-MS/MS analysis aiming at identifying urinary proteins that are
| Protein biomarker | No of patients | Context of use | Platform | Results | Reference Studies |
|------------------|----------------|---------------|----------|---------|-------------------|
| **Fibronectin**  | n = 8 PCa patients | Discrimination between PCa and BPH | Discovery LC-MS/MS Validation | Fibronectin decreased levels: 75% sensitivity, 50% specificity | Haj-Ahmad et al. [42] |
| **TP53INP2**     | n = 12 BPH patients |                           | Q-PCR   |             |                   |
| **MUC3**         | n = 10 healthy volunteers |                           |         |             |                   |
| **Beta-2-M (B2-M)** | n = 90 PCa patients | Discrimination between localized PCa and BPH | Discovery Quantitative iTRAQ LC-MS/MS Validation Immunoblot | Increased levels of urinary B2 M (p < 0.001), PGA3 (p = 0.006), and MUC3 (p = 0.018) were reported in PCa patients. AUC value of 0.618 for MUC3 (p = 0.009), 0.625 for PGA3 (p < 0.008), and 0.668 for B2-M (p < 0.001). Combination of markers: AUC of 0.710, p < 0.001 | Jedinak et al. [43] |
| **PGA3**         | n = 83 BPH patients  |                           |         |             |                   |
| **MUC3**         | n = 10 healthy volunteers |                           |         |             |                   |
| **Stratifin (SFN)** | n = 16 PCa patients (EPS pooled) | Discrimination between indolent and aggressive PCa | Discovery MudPIT LC-MS/MS Validation Western blot SRM-MS-based relative quantification | Increased expression levels of MME, PARK7, and TIMP1 were confirmed by Western blot | Kim et al. [45] |
| **Membrane metalloendopeptidase 7 (MME)** | n = 8 extracapsular tumor |                           |         |             |                   |
| **Parkinson protein 7 (PARK7)** | n = 8 organ-confined tumor |                           |         |             |                   |
| **Tissue inhibitor of metalloproteinase 1 (TIMP1)** | n = 5 PCa patients (Biochemical recurrence) |                           |         |             |                   |
| **Transglutaminase 4 (TGM4)** | n = 10 (no evidence of recurrence) |                           |         |             |                   |
| **Transferrin (TF)** | n = 8 PCa patients | Detection of aggressive tumors | Discovery 2D-DIGE mass spectrometry Validation Immunoturbidimetry | AMPB showed increased urinary levels in PCa, while TF and HP levels were decreased (p < 0.05). Combination of HP and AMPB showed an AUC value of 0.848. | Davaliieva et al. [39] |
| **α-1-microglobulin (AMPB)** | n = 16 BPH patients |                           |         |             |                   |
| **Haptoglobin (HP)** | n = 16 BPH patients |                           |         |             |                   |
| **Secernin-1**   | n = 12 PCa patients | Detection of PCa relapse | Discovery 2D-DIGE mass spectrometry Validation | Significant downregulation of secernin-1 (p < 0.044) and significant upregulation vinculin was reported (p < 0.001). PAP showed significantly increased urinary levels in PCa compared to controls (p = 0.012). Galectin-3 showed significant lower levels in PCa patients with biochemical relapse, compared to those without relapse (p = 0.017). | Geisler et al. [40] |
| **Vinculin**     | n = 11 PCa patients with relapse |                           |         |             |                   |
| **Prostatic acid phosphatase (PAP)** | n = 14 negative for PCa |                           |         |             |                   |
| **Galectin-3**   | n = 12 PCa patients after prostatectomy specimens without relapse |                           |         |             |                   |
| **Osteopontin (SPP1)** | n = 28 PCa patients | Discrimination between PCa and BPH | LC-MS/MS | Diagnostic accuracies of SPP1 and F2 peptides were reported to be 0.65–0.77 and 0.68–0.72 | Li et al. [46] |
| **Prothrombin (F2)** | n = 20 BPH |                           |         |             |                   |
| **Pyridinoline deoxypyridinoline** | n = 6 healthy volunteers |                           |         |             |                   |
Table 1. (continued).

| Protein biomarker | No of patients | Context of use | Platform | Results | Reference Studies |
|-------------------|----------------|---------------|----------|---------|-------------------|
| 12 biomarkers CE-MS derived urinary profile | | | | | |
| Discovery n = 51 PCa Patients n = 35 negative for PCa Validation n = 118 PCa Patients n = 95 negative for PCa n = 138 healthy volunteers | | Discrimination between PCa and BPH | CE-MS | PCa was detected with 89% sensitivity, 51% specificity Including of age and percent free PSA 91% sensitivity, 69% specificity was achieved. | Theodorescu et al. [47] |
| CE-MS derived urinary peptide panel (12 biomarkers) | n = 49 PCa Patients n = 135 BPH Patients | | CE-MS | 86% sensitivity, 59% specificity Negative and positive predictive values were 92% and 43%, respectively. AUC value 0.72. | Schiffer et al. [49] |
| MALDI derived peptide profiling | n = 89 PCa patients n = 52 patients with high-grade prostatic intraepithelial neoplasia (HGPIN) n = 125 BPH | | MALDI-TOF | PCa vs. BPH: 67.4% sensitivity, 71.2% specificity BPH vs. HGPIN: 69.2% sensitivity, 73.6% specificity PCa vs. HGPIN: 81.0% sensitivity, 80.8% specificity | M’Koma et al. [50] |

BPH: Benign hyperplasia of the prostate; EPS: Expressed prostatic secretions; PCa: Prostate cancer.

differentially excreted in men with BPH, compared with those who have localized PCa.[43] Beta-2-microglobulin, pepsin A-3 (PGA3) and mucin-3 (MUC3) were identified as potential biomarkers and validated by immunoblot analyses in 173 urine samples from patients diagnosed with BPH (N = 83) and PCa (N = 90). Univariate analysis demonstrated significant elevations in urinary beta-2-microglobulin (p < 0.001), PGA3 (p = 0.006) and MUC3 (p = 0.018) levels in PCa patients. Multivariate logistic regression analysis revealed AUC values ranging from 0.618 for MUC3 (p = 0.009), 0.625 for PGA3 (p = 0.008) and 0.668 for beta-2-microglobulin (p < 0.001). The combination of all 3 biomarkers demonstrated an AUC of 0.710 (95% confidence intervals [CI]: 0.631–0.788, p < 0.001). Diagnostic accuracy improved further when these data were combined with PSA categories (AUC = 0.812, 95% CI: 0.740–0.885, p < 0.001).

Expressed prostatic secretions (EPS) are widely analyzed for the identification of PCa-specific biomarkers in urine.[44,45] EPS-urine is proximal tissue fluid that is collected after DRE. Therefore, EPS-urine may be a rich source of prostate-derived proteins. Applying MudPIT, Principe and co-authors [44] identified 1,022 unique proteins in a heterogeneous cohort of 11 EPS-urines derived from patients confirmed with BPH and low-grade PCa.[44] Integration of proteomics datasets, transcriptomics data and immunohistochemistry data derived from the Human Protein Atlas database introduced a panel of 49 prostate-derived proteins in EPS-urine.[44] The expression of 7 of these proteins was further verified by using western blotting.[44] In a similar approach, Kim et al. investigated direct-EPS samples from 16 individuals with extracapsular (n = 8) or organ-confined (n = 8) PCa using MudPIT,[45] and 624 unique proteins were identified by at least 2 unique peptides.[45] A semi-quantitative spectral counting algorithm identified 133 potentially differentially expressed proteins in the discovery cohort. Integrative data mining prioritized 14 candidates, including 2 known PCA biomarkers: PSA and prostatic acid phosphatase (PAP), both of which were significantly elevated in the direct-EPS from the organ-confined cancer group.[45] These and 5 other candidates (SFN, MME, PARK7, TIMP1 and TGM4) were verified by western blotting in an independent set of direct-EPS from patients with biochemically recurrent disease (n = 5) versus patients with no evidence of recurrence upon follow-up (n = 10).[45] Further verification of those 5 candidates was performed using SRM-MS-based relative quantification of the 5 candidates in pools of EPS-urines from men with extracapsular and organ-confined prostate tumors.[45]

Li and collaborators investigated first-voided urine samples after prostate massage to identify potential biomarkers for PCa.[46] LC-MS/MS profiling was performed from 28 confirmed PCa patients, 20 patients with BPH and 6 healthy volunteers. Multiple peptides derived from osteopontin (SPP1) and prothrombin (F2) were found to be decreased in PCa in comparison to BPH patients. Diagnostic accuracies of SPP1 and F2 peptides ranged between 0.65–0.77 and 0.68–0.72, respectively. Additional findings support significant differences in pyridinoline (PYD) and deoxypyridinoline (DPD) between PCa and BPH patients (p = 0.001).
By applying the CE-MS platform, Theodorescu et al. [47] investigated urine samples from patients with PCa and relevant controls to identify biomarkers indicating the absence of PCa in the population of individuals with a PSA level >4 ng/ml. This would enable reducing the numbers of unnecessary, negative biopsies. The authors identified a panel of 12 biomarkers for PCa, using CE-MS. [47] The biomarkers were identified by comparing first-voided urine of 51 men with PCa and of 35 subjects with negative prostate biopsy. In this study, midstream urine samples did not allow the identification of discriminatory molecules, suggesting that prostatic fluids may be the source of the defined biomarkers. Consequently, first-voided urine samples were tested for sufficient amounts of prostatic fluid, using a prostatic fluid indicative panel ("informative" polypeptide panel [IPP]). [47] A combination of IPP and the urinary PCa biomarker panel was applied to predict positive prostate biopsy in a blinded prospective study. Two hundred and thirteen of 264 samples matched the IPP criterion. PCa was detected with 89% sensitivity at 51% specificity. Including age and percent-free PSA to the proteomic signatures resulted in 91% sensitivity at 69% specificity. [47] The findings were discussed controversially. In a study on 18 samples, a significant association of the biomarkers with PCa could not be detected. [48] However, as also concluded by the authors, the study was vastly underpowered and, by far, was too small to assess performance. In a more recent and appropriately powered study, [49] CE-MS-based urinary proteome analysis was applied for the detection of PCa in 211 patients from different outpatient centers. In 184 participants, data on follow-up and test results were available for analysis, and PCa was detected in 49 cases. The test correctly recognized 42 out of 49 tumor patients, showing a sensitivity of 86%. In 79 of 135 PCa-negative patients, the urinary proteome analysis correctly predicted absence of PCa. Negative and positive predictive values were 92% and 43%, respectively. A statistically significant improvement in terms of diagnostic accuracy was observed compared to that with serum PSA and percent-free PSA. Whereas the urinary proteome analysis for PCa test results agreed with 65.7% of the follow-up reference results, PSA achieved 33.3% and percent-free PSA achieved 42.7%. Cost–effectiveness analysis showed that the urinary proteome analysis would result in a significant benefit. The authors concluded that CE-MS-based urinary proteome analysis appears to be a valuable addition for the management of patients with suspicious PSA and/or DRE. In comparison to the other proteomics studies, the CE-MS-based approach appears to be substantially advanced, has been evaluated in several studies in a total of over 500 patients and its health economic calculations indicate a benefit. M’Koma et al. employed MALDI-TOF to identify potential biomarkers for PCa. [50] Urine samples were fractionated using reverse-phase resin, and subsequently analyzed using MALDI-TOF; 130 signals with a mass range between 1,000 and 5,000 m/z resulted in urinary peptide patterns with 71.2% specificity and 67.4% sensitivity in discriminating PCa from BPH. When attempting to compare PCa with high-grade prostatic intraepithelial neoplasia (HGPIN), 73.6% specificity and 69.2% sensitivity was reported. A major shortcoming of the study is the general lack of identification of discriminatory peptides. This may be the reason why, although the findings appear promising, further validation to support the validity was never reported. Now, almost 10 years later, it appears that this approach may not have the resolution required to assess urinary biomarkers for PCa.

Single protein biomarkers

An expansive bibliography about the putative PCa biomarkers in biological fluids has been accumulated over the time. The most prominent results regarding the candidate biomarkers, the study design and their specific context of use are summarized in the Table 2. In most of the studies, classical immunological assays (e.g., ELISA, western blot) were applied to determine the urinary concentration of the biomarkers. Although promising, most of the identified biomarker candidates, in fact, still need to be extensively validated in large clinical cohorts.

Among the biomarker candidates, CD90, a cell membrane molecule was reported to be increased in different cancers, including PCa. This molecule was also detected in the supernatant of tumors after collagenase digestion. [51] Immunohistochemical analysis revealed increased expression levels of CD90 in cancer-associated stroma compared with non-cancer stroma tissues. [51] When testing voided urine from patients prior to and after prostatectomy for the presence of CD90 fragments, a CD90 peptide was uniquely identified in pre-prostatectomy patients. This urinary CD90 protein was identified as a variant CD90 protein not known to be expressed by such cells as lymphocytes that express CD90. Unfortunately, it appears that these promising results could not be validated in a larger cohort. [51] Based on the significant role of emmprin (basigin/CD147) and disintegrin and metalloproteinase domain-containing protein 12 (ADAM12) in tumor invasion and metastasis, the association of these 2 proteins in urine with PCa was investigated by Dogru and collaborators. [52] In a small study including 66 patients and 14 control subjects, PCa was found to be associated with a significant increase of urinary ADAM12, but the correlation of urinary emmparin with PCa was not confirmed. [52] Considering the observation that deregulated expression of minichromosome maintenance 2–7 proteins (Mcm 2–7) is an early event in epithelial carcinogenesis, Mcm-5 was investigated in the urine of 88 men with PCa and in 359 control patients. [53] A significant increase of urinary Mcm-5 in patients with PCa was observed, and enabled the detection of PCa with 82% sensitivity, at a specificity level of 73%. [53] Endoglin (CD105) was reported to be increased in abundance in prostatic fluid of men with large-volume PCa, which initiated the investigation of a possible association of urinary endoglin with PCa in 99 individuals (after DRE). [54] Detection of PCa based on the increased urinary levels of endoglin was accomplished with an AUC of 0.72. [54] Following the same principle, Fujita and collaborators investigated the urinary levels of interleukin-18 binding protein (IL-18BP). [55] Similar to the previous investigation, the authors were able to demonstrate a significant increase in IL-18BP in PCa, although with a
| Protein biomarker                  | No of patients                                                                 | Context of use                                                                 | Platform       | Results                                                                 | Reference Studies       |
|-----------------------------------|-------------------------------------------------------------------------------|--------------------------------------------------------------------------------|----------------|------------------------------------------------------------------------|-------------------------|
| • EMMPRIN                         | n = 66 PCa patients (40 local, 20 locally advanced, 6 metastatic) n = 14 healthy controls | Diagnosis of PCa                                                              | ELISA          | The urinary ADAM12 levels were significantly higher in PCa patients (p = 0.013). Positive correlation between urine EMMPRIN-urine ADAM12 tests was found (r = 0.683, p = 0.001) | Bilgin Dogru et al. [52] |
| • ADAM12                           |                                                                                   |                                                                              |                |                                                                        |                         |
| Mcm 2–7                           | n = 88 PCa patients n = 28 patients with serum PSA <2 ng/ml n = 331 benign controls | Diagnosis of PCa                                                             | Immunofluorometric assay | Mcm5 test detected prostate cancer with 82% sensitivity and specificity ranging from 73 to 93% | Dudderidgeet et al. [53] |
| Endoglin (CD105)                   | n = 99 PCa Patients, n = 20 individuals at low risk for PCa n = 69 after radical prostatectomy (30 pT2, 39 pT3). | Diagnosis of PCa and correlation with advanced disease                      | ELISA          | Increased urinary endoglin levels in patients with PCa (p = 0.0014), AUC value of 0.72 | Fujita et al. [54]     |
| Annexin A3                        | n = 23 PCa patients n = 31 BPH patients                                          | Development of 2 ANXA3 immunoassay prototypes                                 | ELISA          | Lack of correlation between the two assays                              | Hamelin-Peyron et al. [79] |
| Zinc a 2-glycoprotein (ZAG)        | n = 42 PCa patients n = 56 negative for PCA n = 29 patients with prostatic intraepithelial neoplasia (PIN) | Diagnosis of PCa                                                             | Western blot   | AUC value of 0.68. Combination of ZAG with PSA revealed AUC value of 0.75 (p = 0.010). | Katafigiotis et al. [57] |
| Engrailed-2                        | n = 76 BPH patients (38 before and 38 after prostate massage) n = 66 PCa patients (33 before and 33 after prostate massage) n = 82 PCa patients n = 102 controls n = 125 PCa patients | Assess EN2 urinary levels protein before and prostate massage Association with cancer volume | ELISA          | The specificity and sensitivity of the protocol are highly dependent on the prostate massage process Sensitivity of 66% and a specificity of 88.2% was reported Urinary EN2 significantly correlates with and prostate cancer volume (p = 0.006). | Marszall et al. [60]     |
| The C-Terminal Fragment of Prostate-Specific Antigen, a 2331 Da Peptide | n = 50 PCa patients n = 19 negative for PCa patients | Diagnosis of PCa                                                             | MALDI-TOF/MS   | AUC value of 0.747 86% sensitivity, 58% specificity | Nakayama et al. [62]    |

(continued)
lower diagnostic accuracy (AUC of 0.66), compared to that published for urinary endoglin.

An increased expression of annexin 3 was reported in PCa tissue compared to adjacent normal tissue, and these results were further supported by urinary analysis. Furthermore, Hamelin-Peyron et al. investigated the value of annexin 3 as a urinary biomarker for PCa but, inconsistencies in the assays precluded any conclusive results. However, the collected data support the potential of annexin 3 as a urinary PCa biomarker. The value of Zinc alpha 2-glycoprotein as biomarker for PCa was studied in urine samples obtained by 127 men. The authors could show a moderate, yet significant, value (AUC of 0.68), which could be improved upon in combination with PSA (combined AUC = 0.75). Engrailed-2 (EN2), a homeo-domain-containing transcription factor – expressed in PCa cell lines and PCa tissues, but not in normal prostate tissues – has been proposed as urinary candidate biomarker for PCa. Urinary EN2 was detectable by western blot in men affected by prostate cancer.

| Protein biomarker | No of patients | Context of use | Platform | Results | Reference Studies |
|-------------------|----------------|----------------|----------|---------|--------------------|
| HGF               | n = 65 PCa patients (localized PCa) | Diagnosis of PCa | ELISA | HGF and IGFBP3 increased levels were significantly identified in PCa patients (p = 0.0006 and p = 0.0012). AUC values for HGF and IGFBP3 was 0.75 and 0.74, respectively. OPN levels was significantly higher in metastatic groups (p = 0.0060) with AUC value of 0.68 | Prager et al. [63] |
| IGFBP3            | n = 36 metastatic PCa | | | | |
| OPN               | n = 19 healthy volunteers | | | | |
| Met               | n = 75 Patients with localized PCa | Detection of progression | ELISA | Met urinary levels were significantly increased in PCa (P < 0.0001). AUC value of 0.90 was reported for distinguishing localized from metastatic PCa. | Russo et al. [64] |
| Golgi Protein GOLM1 | n = 52 PCa patients | Diagnosis of PCa | Western blot | 72% sensitivity, 75% specificity AUC = 0.785, P < .0001 | Varambally et al. [65] |
| Protein Glycosylation Profiling Asparagine-linked glycan (N-glycan) profiling | n = 74 PCa Patients | Diagnosis of PCa | CE-MS | A significant difference was found in core-fucosylation of biantennary structures and overall core-fucosylation of multiantennary structures between Gleason < 7 and Gleason > 7 (P = 0.010 and P = 0.020, respectively) and between Gleason = 7 and Gleason >7 (p = 0.011 and p = 0.025, respectively) N-glycans of PCa compared to BPH were characterized by a significant decrease in fucosylation (p = 0.026). AUC value 0.71 46% sensitivity, 84% specificity: 84% | Vermassen et al. [80] Vermassen et al. [81] |
| Microseminoprotein-beta (MSMB) | n = 89 PCa patients | Diagnosis of PCa | ELISA | AUC value of 0.77 for discriminating PCa from control patients. | Whitaker et al. [67] |
| n = 215 normal/benign patients | | | | | |

BPH: Benign hyperplasia of the prostate; EPS: Expressed prostatic secretions; PCa: Prostate cancer.
PCa, but not in those individuals suffering from high-grade prostatic intraepithelial neoplasia (HGPIN) or BPH. EN2 was also measured by ELISA in urine from patients with PCa (n = 82) and control individuals (n = 102). In this study, a good performance of EN2 was demonstrated, in being able to distinguish patients from controls with a sensitivity level of 66% and a specificity level of 88.2% (AUC = 0.81). Marszall et al. also investigated the value of Engrailed-2 protein as a potential urinary PCa marker in 71 individuals. Although no significant association of EN2 could be detected in voided urine, a significant increase in urinary EN2 was observed in PCa after DRE. This may, however, be a result of differences in prostate volume. As already shown previously, EN2 is associated with tumor volume. Nakayama et al. identified a 2331-Da PSA-ERG derived peptide as a potential biomarker for PCa in urine. Evaluation in a small cohort of 69 subjects showed significant association with PCa (AUC 0.747). Prager et al. assessed the value of urinary active hepatocyte growth factor (aHGF), IGF-binding protein 3 (IGFBP3) and osteopontin (OPN) as diagnostic and prognostic biomarkers for PCa. In a cohort of 120 individuals, all 3 biomarkers displayed AUC values in the range between 0.68 and 0.75, superior to PSA.

c-Met was tested as a urinary biomarker to distinguish between localized and metastatic PCa. In a cohort of 156 individuals, urinary c-Met showed an AUC estimate of 0.9, clearly indicating its value in identifying metastatic PCa. Surprisingly, these data apparently have not been reproduced and, to date, it is not widely accepted and used as a biomarker for this specific purpose. Golgi membrane protein 1 (GOLM 1) was found to be associated with PCa based on meta-analysis of expression data. When investigating abundance in 102 urine samples, significant association with PCa (AUC 0.785) could be detected. Another protein – anterior gradient protein 2 (AGR2) – was assessed by Wayner and co-workers in the urine of a very small cohort of 11 patients. AGR2 was found to be increased in PCa. However, the limited sample size could not lead to any convincing conclusion. Microseminoprotein-beta (MSMB) protein regulates apoptosis, and an SNP in the MSMB gene promoter has been linked to an increased risk of developing PCa. Taken this observation into consideration, MSMB has been investigated in urine samples collected from 304 individuals, and significant association with PCa was described. However, the selection of samples appears moderately transparent; therefore, a confirmation of the results in an independent study seems highly desirable.

A consistent biomarker for PCa is ERG (TMPRSS2-ERG fusion gene), generally assessed using RT-PCR. Urinary analysis of ERG protein was possible due to the development of an MS (MRM)-based assay by He et al., and demonstrated high sensitivity and accuracy. The authors applied a multiplexed-SRM technology by using 7 labeled proteotypic peptides of ERG protein and by spiking the recombinant TMPRSS2-ERG protein in human urinary specimens. The developed assay was proposed as a highly sensitive, specific and reproducible assay to be implemented in clinical practice. However, a well-powered validation study should be first conducted to prove the stability of the performance of the urine-based test.

The results of multiple studies were negative and did not enable the verification of the potential biomarkers under investigation. However, as the information that a certain protein is not suited as biomarker is valuable as well, we include these studies. No significant association with PCa was found when urinary myeloid related protein-14 was assessed in a study on 104 subjects. Sardana and Diamandis tested 14 urinary kalikrein proteins to distinguish between PCa and controls in a cohort of 40 samples. Unfortunately, the authors could not detect any significant value of any of the urinary kalikreins.

In several of the studies, better performance of the biomarker after DRE/prostate massage was reported. While this is to be expected, at the same time a requirement for such a procedure appears worrisome, as reproducibility will be compromised by a procedure that just is not reproducible. Further, most of the studies are based on a small sample size, and it is not certain whether the biomarkers reflect prostate volume rather than PCa.

It also becomes increasingly evident in the studies that the performance of PSA is very poor; in some reports the AUC was 0.5, equivalent to guessing, and the value of this “biomarker” should certainly be carefully reassessed. Most, if not all of the additional single biomarkers, as presented in this section, displayed a limited performance. On the other hand, several of the above-cited reports have demonstrated the added value derived from combination of single-protein biomarkers, with each other and/or with PSA. This, moreover, indicates that the solution to the aforementioned problems (biological variability, tumor heterogeneity, etc.) may lie in the application of panels composed of multiple biomarkers. This concept, that is increasingly advocated, appears to represent a promising approach toward detection of PCa in a noninvasive manner with high accuracy (AUC > 0.8) and, of probably more importance, correctly identifying truly relevant disease.

Data mining
Chen et al. presented a strategy to identify candidate urinary markers for PCa by mining cancer genomic profiles from public databases. To identify urinary markers specific for PCa, upregulated entities that might be shed in exosomes of bladder cancer and kidney cancer were first excluded. Through the ontology-based filtering and further assessment, a reduced list of 19 urinary candidate biomarkers for PCa were selected that included apolipoprotein D (APOD), lectin galactoside-binding soluble 3 (LGALS3), complement factor B (CFB) and retinol binding protein 4 (RBP4). As highlighted by the authors, LGALS3 has the largest fold-change value of 4.121 in cancerous conditions compared to normal condition among the 19 entities, rendering it a promising molecule for the diagnosis and prognosis of PCa. Ten proteins were closely associated with tumor cell growth and development by pathway enrichment analysis. Using these 10 entities as seeds, a protein–protein interaction (PPI) subnetwork was constructed that suggested a few urinary markers as preferred prognostic markers to monitor...
the invasion and progression of PCa. The study highlights a promising approach to exploit publicly available proteomic datasets by interrogating them in detail, employing appropriate bioinformatics tools. Unfortunately, a major shortcoming of the study is the complete lack of validation of the proposed candidates. Therefore, the validity of this approach cannot be assessed.

Discussion

Despite the large number of proteomic studies on PCa biomarkers and the multiple candidates identified, only very few have been verified and none is routinely implemented in clinical practice. This is, at least in part, attributed to the challenges that are associated with biomarker development. To facilitate successful translation of biomarker findings into clinical practice, several guidelines for quality assessment [18] study design and reporting of novel biomarker candidates have been introduced.[17,73,74] The latter includes the requirements for epidemiological studies (STROBE-ME project) [17] for the development of predictive (PROBE standards [73]) and prognostic biomarkers (REMARK [74]). Additionally, McGuire et al. [75] and Mischak et al. [32] provided practical guidelines and standardized protocols for proteomic (in general) and urinary proteomics studies, respectively.

A number of significant barriers also exist between the discovery and the validation phases of a biomarker project. Although these barriers have been outlined recently in detail, [11] they are frequently not taken into consideration, which results in many studies falling short to actually delivering a relevant outcome. The most frequent shortcoming is that the “potential” biomarkers are not verified in an appropriate sample type and size. As evident from the manuscripts published, most of the studies suffer from an extremely low number of subjects in the study. A major additional issue appears to be biological variability. PCa is a very heterogeneous disease with complex tumor microecology/pathology and a broad range of clinical outcomes. The complexity of the sample and inter- and intra-patient variability render the identification of valid biomarkers even more challenging, especially when investigating a low number of samples. In light of these issues, we have to assume that many of the reported potential biomarkers that have not been verified in the presented studies will, in fact, fail during validation.

The abovementioned issues, underpowered studies and failure to verify the findings could be significantly reduced if such attempts were consistently found unfundable and unacceptable for publication. At the same time, it would be desirable if the aim toward appropriately powered studies could be better supported by warranting access to high-quality, well-annotated clinical cohorts. Unfortunately, this is not the case yet; biobanks have generally failed to deliver on this expectation [34] and the required investment in time and resources to collect the required high-quality samples from well-phenotyped individuals with appropriate follow-up are typically vastly underestimated.

Given the complexity of PCa tumorigenesis and the variability of urinary peptides and proteins, a promising strategy may be to assess proteomic changes in the tissue and subsequently investigating the potential value of these proteins in body fluids like urine. Tissue proteomics may better reflect the disease pathophysiology, providing a high number of potential biomarkers. However, even in the case of tissue where the peptide/protein variability is lower, a relatively high number of samples is required to account for the issue of multiple testing.[76] As tissue is not easily available, investigation of seminal plasma appears as valid alternative. In several recent articles, potential biomarkers for PCa were described in seminal plasma.[77,78] It may be worthwhile to investigate the distribution of these potential biomarkers in urine, using targeted approaches like MRM-based assays.

An important current and future aspect of PCa biomarker research concerns diagnosis and treatment of bone metastasis. Bone is the preferential site of PCa metastasis for castration-resistant cancer and is a significant cause of morbidity for these patients. This type of metastasis determines the disruption of bone remodeling processes, resulting in lesions that cause serious pain. So far, the approved systemic chemotherapy gave only limited benefits and docetaxel – an inhibitor of microtubule function – remains the standard first-line treatment.[75] Preliminary data from our laboratories indicate that mineral-bone disease is displayed very well by specific urinary biomarkers, although in a completely different context: chronic kidney disease. However, these data indicate that urinary biomarkers may inform on bone metastases at an early point in time. As bone metastasis develops, factors derived from bone metabolism (bone-specific alkaline phosphatase and collagen type 1) are released into urine and blood, and high levels of bone biomarkers in urine may be related to an increased risk of bone metastasis.

It becomes evident that proteomics will need to move from biomarker discovery to rigorous validation and application of the findings in clinical trials. An approach similar as the one suggested for bladder cancer by Vlahou [32] seems also applicable in the case of PCa: verifying in parallel the multiple biomarkers and classifiers currently proposed in a sufficiently large cohort (n ~ 1,000) that also allows addressing the value of the biomarkers in the relevant context of use. Based on the data available, it appears that a single biomarker will not suffice to appropriately guide patient management in PCa. However, a study such as the one mentioned earlier would allow assessing the combined value of the different biomarkers in the management of PCa. Unfortunately, currently neither the funding nor the required (urine) samples with the relevant associated clinical information appear to be available. However, we hope that this article will initiate a concerted, international effort along these lines, in order to improve management of PCa in the near future.

Expert commentary

Accurate diagnosis of PCa is not possible based on serum PSA levels, resulting in significant overtreatment and unnecessary biopsy procedures. The implementation of noninvasive biomarkers could be highly beneficial in guiding invasive diagnostic
procedures and therapeutic intervention. Although increased efforts in urinary proteomics in the context of PCa are evident, most of the studies are not planned to accommodate a proper validation of the findings and future actual clinical implementation. Moreover, access to high-quality, well-annotated clinical cohort and the high cost of biomarker validation is a major limitation. Collectively, the studies support the view that urine, and particularly urine obtained after DRE, holds significant and sufficient information for reliable PCa biomarkers. What appears to be required now is a concerted effort to assess the value of the multiple biomarker candidates in an appropriately powered blinded study on prospectively collected samples. Such a study would not only allow unbiased assessment of the biomarker candidates, but also enable development and evaluation of multi-marker panels, which appear to be the optimal choice in the context of management of PCa.

Five-year view

A major requirement in the application of proteins as clinical biomarkers is a highly sensitive and specific performance in disease diagnosis and/or prognosis. As single biomarkers are of limited accuracy, a panel of biomarkers derived from proteome profile analysis appears most appropriate, supported by a robust and sensitive analytical platform. We foresee that an appropriate study perspective, including a high number of patients (in the range of 1,000), for the validation of the biomarkers and, ideally, to develop biomarker panels for different contexts of use in a prospective cohort will lead to the application of new biomarkers in the clinical practice regarding PCa. Provided such study can be conducted, we expect that a urinary multi-marker panel, possibly applied onto EPS-urine, will be routinely used in the assessment and management of PCa.

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Key Issues

- Prostate cancer (PCa) is a serious cause of increased cancer-related mortality in men.
- While indolent PCa cases are associated with low mortality rate, advanced castration-resistant PCa is associated with poor disease outcome.
- Accurate non-invasive assays to discriminate between indolent and advanced castration-resistant PCa could be beneficial.
- Application of new biomarkers for detection of the disease, localized and metastatic PCa, could allow reduction in over-diagnosis and over-treatment, and guide early initiation of the appropriate intervention.
- Proteomics technologies enable high-throughput analyses providing the possibility to obtain large and high-resolution datasets.
- Urine appears to be the most relevant and promising bio-fluid for prostate biomarker research, among others, due to its physical proximity to the prostate gland.
- The high disease heterogeneity could be assessed by combination of biomarkers or, ideally, by applying multiple biomarker panels.
- Independent validation in a prospectively collected cohort would be required, in order for proteomic biomarkers to be translated into clinically applicable assays.

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Supplementary material available online
Tables S1 and S2