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Review Article

Urine And Blood Biomarkers That Could be used to Identify and Localise Prostate Cancer

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Abstract:

While serum PSA remains the mainstay screening for prostate cancer, appropriate use of biomarkers prevents the overdiagnosis of prostate cancer and over treatment of prostate cancer which is not clinically significant. Even though only PHI and PCA3 had been the only biomarkers which had been FDA approved, it is important that the clinician should be familial with other biomarkers that could provide advantages to a group of patients. PHI test and 4Kscore demonstrated similar capability to predict clinically significant cancer and both can reduce the number of unnecessary biopsies but PHI price is significantly lower. Select MdX and Exosome Dx do have encouraging results but would necessitate more studies for incorporation into the routine clinical practice. The largest limitation when deciding which biomarker to use is the lack of prospective head-to-head trials comparing the various tests. There are many biomarkers available for the detection of csPCa both in the initial and repeat biopsy setting. The largest limitation when deciding which biomarker to use is the lack of prospective head-to-head trials that compare the various tests. In addition, many studies had suggested that even within a given test the cutoff used in one population may not be the most appropriate cutoff for another population. In view of this, extensive validation in multiple diverse cohorts is critical to confirm the findings. Serum and urine biomarkers do improve the detection of csPCa reducing over-treatment and making treatment strategies more cost effective. Large prospective head-to-head comparisons of all biomarkers are required to fully assess the potential of incorporating biomarkers in routine clinical practice. At the moment, serum PSA determination, and digital rectal examination to identify abnormal findings within the prostate backed by radiology imaging of the prostate gland and radiology image-guided prostate biopsies generally enable the clinician to identify various grades and stages of prostate cancer. Nevertheless, biomarker and cytogenetics testing is now able the clinician to identify prostate cancers that would tend to portend indolent biological behaviour which would not need to be treated as well as aggressive tumours that need urgent treatment. There is however, a global need for further urine and blood biomarker studies that would convincingly demonstrate tumours that need not to be treated as well as those that need to be treated and their locations.

Keywords: prostate cancer; urine biomarker; blood-biomarker; early diagnosis; prostate biopsy

Introduction

Prostate cancer (PCa) is stated to be the most common malignant tumour of the urinary tract. [1] PCa is iterated to rank second in incidence and fifth in mortality among all malignant tumours. [1] The life risk of PCa diagnosis is reported to be one in nine men, but the risk of death might be as low as 2% [1] [2] The current recommendations for PCa diagnosis are based upon the guidelines of the European Association of Urology (EAU-ESTRO-SIOG), which entails the analysis of the serum concentration of prostate-specific antigen (PSA), as well as undertaking a digital rectal examination (DRE) for abnormalities. [3] Nevertheless, digital rectal examination (DRE) is stated to be associated with low sensitivity., [4] while serum PSA level is rather organ-, but not tumour-specific (low specificity), and has a low positive predictive value of about 30%). [5] Thus far, the final diagnosis of prostate cancer depends upon histopathology examination report of adenocarcinoma in the core biopsy of the prostate gland. False positive PSA test results, in patients with benign prostatic hyperplasia (BPH) and/or prostatitis, may result in systematic transrectal ultrasonography (TRUS) – controlled prostate biopsy (Bx). In addition, serum PSA – based screening might lead to over-diagnosis and potentially over-treatment of PCa, which would never be of clinical relevance. There is a clinically unmet necessitation for the development of biomarkers that would help control PCa treatment strategies. Many diagnostic tools are available on the PCa laboratory market. There are new biomarkers for serum, urine and even tissue samples. [6] New biological markers, such as TMPRSS2-ERG fusion gene, and the non-coding RNA (PCA3) [7] or kallikrein included in basic PHI (prostate health index) or 4K tests, [8] had been demonstrated to increase sensitivity and specificity PSA, potentially avoiding biopsy and reducing over-diagnosis. Modern biomarkers used in prostate diagnosis

have been listed in. Measurement of serum PSA levels entail an invasive procedure undertaking blood tests but if urine examination would determine the risk of prostate cancer, this would be beneficial to all patients. Recent tests have demonstrated the usefulness of urine biomarkers in ascertaining the risk of prostate cancer. The guidelines recommend utilising these tests, additionally to standard methods, as an effective diagnostic tools for cancer diagnosis. Irregularities resulting from the mentioned tests are an indication for prostate biopsy [3]. Lastly, risk calculators could be helpful in the determination (individually) of the potential risk of cancer; hence reducing the undertaking of a number of unnecessary biopsies. [9]

Hessels et al. [10] made the ensuing preamble iterations:

- It has been pointed out that annually, 241 740 men in the United States of America (USA) and 338 700 men within Europe are newly diagnosed with prostate cancer (PCa) and around 28 170 USA and 70 800 European men die from this disease. [11] [12]
- Early detection of PCa relies on serum prostate-specific antigen (PSA) testing or digital rectal examination (DRE).
- Since its first clinical application, serum PSA had been a valuable tool in the detection, staging and monitoring of prostate cancer.
- Even though the routine use of serum PSA testing had undoubtedly increased PCa identification, one of its main disadvantages has been its lack of specificity resulting in a high negative biopsy rate. [13]
- The early detection of many indolent prostate cancers (PCas) had resulted in treatment of tumours that would not have become life-threatening to an individual patient.
- Serum PSA has a low specificity because it is not a prostate cancer (PCa)-specific event; elevated levels could also be identified in men with benign prostatic hyperplasia (BPH) and prostatitis.
- Methods to enhance serum PSA specificity had assisted clinicians in deciding which patients should undergo prostate biopsy; nevertheless, have not necessarily improved diagnostic accuracy or facilitated optimal therapy decision-making.
- More accurate tests which can stratify patients according to their risk of developing prostate cancer (PCa), identify men who require repeat prostate biopsy and stratify men at risk for aggressive disease are needed.
- Many biomarkers had been identified and some of them are promising because of their specificity for the disease in tissue. Nevertheless, tissue is unsuitable as substrate for biomarker testing because of its invasiveness and expensiveness. Therefore, testing of disease-related biomarkers in body fluids that could be obtained in a non-invasive manner seems a good alternative as possible screening tool.
- Because of the ease of collection, and the fact that prostate cells are directly released into the urethra via prostatic ducts after DRE, urine has become the future for non-invasive biomarker testing.

The ensuing article on urine biomarkers that are being used for the assessment prediction of prostate cancer is divided into two parts: (A) Overview of prostate cancer and (B) Miscellaneous narrations and discussions related to urine and blood biomarkers in use for the prediction and assessment of prostate cancer.

Aim

To update the literature on urine and blood biomarkers associated with prostate cancer.

Methods

Internet databases were searched. The search words that were used included urine biomarkers for prostate cancer, urine biomarkers for adenocarcinoma of prostate, blood biomarkers for prostate cancer, and blood biomarkers for adenocarcinoma of prostate. – references were identified which were used to write the article which has been divided into two parts: (A) Overview of prostate cancer and (B) Miscellaneous narrations and discussions related to urine and blood biomarkers in use for the prediction and assessment of prostate cancer.

Results

[A] Overview

Definition / general iterations

- Adenocarcinoma of the prostate gland is known to be the most common malignancy of the prostate gland. [14]
- Adenocarcinoma of the prostate gland originates from prostatic secretory epithelium. [14]

Essential features

The ensuing summations had been made regarding the essential features of adenocarcinoma of the prostate gland: [14]

- Clinical and radiological features of adenocarcinoma of the prostate gland are stated to be neither sensitive nor specific for the diagnosis of adenocarcinoma of the prostate gland.
- Adenocarcinoma of the prostate gland is stated to be often diagnosed by non-targeted needle biopsies investigating raised serum prostate specific antigen (PSA) and the histopathology examination features of the biopsy specimen are used to establish the diagnosis.
- Absence of basal cell layer is iterated to be a pathognomonic histological feature of adenocarcinoma of the prostate gland.
- Pathognomonic diagnostic features of adenocarcinoma of the prostate gland include the ensuing: circumferential perineural invasion, glomerulations and collagenous micronodules (mucinous fibroplasia)
- Other histopathology examination features of adenocarcinoma of the prostate gland include the ensuing: infiltrative architecture, nucleolar prominence, amphophilic cytoplasm and some intraluminal contents (crystalloids, blue mucin, pink amorphous material)

Terminology

Terminologies that tend to be used for adenocarcinoma of the prostate gland include: [14]

- Prostate cancer
- Prostate adenocarcinoma
- Sub-types of prostatic adenocarcinoma include the ensuing: acinar adenocarcinoma, ductal adenocarcinoma, atrophic adenocarcinoma, pseudo-hyperplastic adenocarcinoma, microcystic adenocarcinoma, foamy gland adenocarcinoma, mucinous adenocarcinoma, signet ring variant of adenocarcinoma, pleomorphic giant cell adenocarcinoma, Sarcomatoid adenocarcinoma

Epidemiology

The epidemiology of adenocarcinoma of the prostate gland had been summated as follows: [14]

- Adenocarcinoma of the prostate gland is the second most common cancer and second leading cause of cancer related death in American men (SEER data). [15]

- 92% of U.S. cases of adenocarcinoma of the prostate gland is diagnosed in men aged 55+ years; 19.5% in men aged 75+ years (SEER data available at) [16]
- Adenocarcinoma of prostate gland is found at autopsy in 40% of men age 60+ years [17]
- Incidental prostate cancer is iterated to be reported in about 25% of cystoprostatectomies performed for treatment of bladder cancer [18]
- It has been iterated that globally, highest age standardized rates of adenocarcinoma of prostate gland is found in Oceania, North America, Europe [19]
- It has been pointed out that lower rates of adenocarcinoma of the prostate gland are reported in developing countries and this may be due to different screening programs and diagnostic pathways. [14]
- Higher incidence of adenocarcinoma of the prostate gland is reported in men of African heritage [19]

Sites

The sites of origin of primary adenocarcinoma of the prostate gland had been summated as follows: [14]

- Most adenocarcinoma of prostate gland tumours are multi-focal [20]
- 75% to 80% of adenocarcinomas of the prostate gland are within the posterior / posterolateral peripheral zone of the prostate gland. [14]
- Approximately 13% to 20% adenocarcinomas of the prostate gland are found within the transition (periurethral) zone [21] [22]
- Most clinically significant prostatic adenocarcinoma cancers arise within the peripheral zone that is sampled by needle biopsies. [14]
- Transition zone prostate cancer of the prostate gland is associated with favourable pathologic features and better recurrence free survival. [23]
- Less frequently, adenocarcinomas of the prostate gland involve the anterior prostate most likely due to inadequate sampling using standard biopsy approach. [24]

Pathophysiology

Pathophysiology of adenocarcinoma of the prostate gland had been summated as follows: [14]

- Germline variants can increase risk of adenocarcinoma of the prostate gland. [14]
- Somatic mutations in genes such as *ERG, ETV1/4, FLI1, SPOP, FOXA1, IDH1, PTEN, TP53, MYC, CDH1* are found in cases of adenocarcinoma of the prostate gland. [25]
- The most common somatic genomic rearrangement associated with adenocarcinoma is fusion of the androgen regulated gene *TMPRSS2* with a member of the *ETS* transcription family. [26]

Aetiology

The aetiology of adenocarcinoma of the prostate gland has been summarized as follows: [14]

- Obesity increases the risk of prostate cancer. [27]
- Non-modifiable risk factors for adenocarcinoma of the prostate gland include: age, race and family history [28]
    - Genetic susceptibility is linked to African heritage [28]
    - Increased risk of prostate cancer with first degree relative with prostate cancer. [28]
    - *BRCA2* mutations increase risk of prostate cancer by 5-fold; *BRCA2*-associated cancers occur at a lower age and have worse survival outcomes. [26] [29]
    - Additional germline variants associated with increased cancer risk occur in *HOXB13.* [26]
    - Increased risk of prostate cancer has been documented in Lynch syndrome. [30]
- Numerous single nucleotide polymorphisms (SNPs) that have a low to moderate effect on risk / progression of prostate cancer have been identified. [31]
- High levels of IGF1 may confer increased risk of prostate cancer. [32]

Clinical features

The clinical manifestations of prostate cancer had been summarized as follows: [14]

- Prostate cancer tends to be generally asymptomatic unless when it is locally advanced or metastatic.
- Prostate cancer is often diagnosed following investigation of non-specific lower urinary tract symptoms ensuing pathology examination of prostate biopsy specimen demonstrating features of prostate cancer.
- Digital rectal examination (DRE) in the scenario of prostate cancer demonstrates prostate gland that may feel normal or may be enlarged / asymmetrical / hard / or have a palpable nodule present

Diagnosis

The diagnosis of prostate cancer has been summarized as follows: [14]

- Prostate cancer is generally diagnosed by pathology examination of specimens of systematic trans-rectal ultrasound guided prostate biopsies.
- Trans-perineal needle biopsies are increasingly being undertaken as the procedures are associated with lower risk of infection.
- It has been pointed out that pre-biopsy MRI scan followed by systematic biopsies supplemented with targeted biopsies from any radiological abnormality leads to better identification of clinically significant prostate cancer than systematic prostate biopsy alone. [33]
- Incidental prostate cancer is sometimes diagnosed in transurethral resections following pathology examination of the prostate resected specimens.
- Immunohistochemistry staining studies with basal cell markers (HMWCK, p63) and AMACR are used to confirm the diagnosis in equivocal cases.

Laboratory tests

Salient points related to laboratory tests in prostate cancer had been summarised as follows: [14]

- Raised serum PSA levels constitute indications for prostate biopsy for pathology examination in the absence of clinical prostatitis and serum prostate specific acid tests are undertaken regularly in the follow-up assessment of individuals pursuant to treatment of prostate cancer.
- Different serum PSA cutoffs have been used to prompt prostate needle biopsy, depending upon the ages of the patients.
- Age specific cutoffs, PSA velocity (rate of change in PSA over time) and PSA density (PSA per unit prostate volume - ng/mL/cc) might increase the sensitivity and specificity of serum PSA testing. [34]
- U.S. Preventative Services Task Force (USPSTF) had recommended against serum PSA based screening for prostate cancer in men who are 70 years and older than 70 years.
    - It has been recommended that for men aged between 55 years and 69 years, periodic serum PSA based screening should be an individual choice.
    - It has been pointed out that screening in this age group offers a small potential benefit of reducing the chance of death from prostate cancer in some men; however, many men will experience potential harm. [35]
- It has also been pointed out that the American Urological Association (AUA) does not recommend PSA screening in men under age 40 years or in men aged 40 - 54 years at average risk:
    - It has been pointed out that for men who are aged between 55 years and 59 years, shared decision making is desirable.
    - It has been iterated that for men aged 70 years and over or men with less than 10 years to 15-year life expectancy, serum PSA screening is not recommended. [36
- It has been pointed out that potential urine biomarker for prostate cancer is PCA3 [26]

Radiology description

Summations related to various radiology-imaging features of prostate cancer had been summarised as follows: [14]

- It has been pointed out that ultrasound scan (USS) is generally used to guide the undertaking of prostate biopsies and that prostate cancer may appear hypoechoic on ultrasound scan but USS is neither sensitive nor specific for the diagnosis of prostate cancer.
- It has been explained that multiparametric MRI scan is commonly used for local tumour staging and that it may also be used to identify abnormalities for targeting during prostate biopsy.
- It has been pointed out that MRI scan abnormalities are generally reported using either PI-RADS (Prostate Imaging - Reporting and Data System) or Likert score
- It has been stated that CT scan can be used to identify metastatic disease within lymph nodes in the scenario of prostate cancer.
- Bone scan is used to detect bony metastases.
- PET scan is used to detect micro-metastatic disease in selected patients, such as men who have raised serum PSA levels following treatment of prostate cancer.

Prognostic factors

Factors of prognostication in the scenario of prostate cancer had been summarised as follows: [14]

- Biopsy: The ensuing pathology examination features of biopsy specimens of prostate cancer specimens include: Tumour extent (mm or percentage core involvement), grade (Gleason score and grade group), perineural invasion, extra-prostatic extension of the tumour.
- Radical prostatectomy: In radical prostatectomy specimens, the tumour size, Gleason score and grade group, stage, margin status of the tumour represent factors of prognostication.
- Cribriform morphology and intraductal carcinoma of the prostate gland associated with invasive prostate cancer are adverse prognostic indicators.
- Small cell carcinoma of the prostate component of prostate cancer is associated with aggressive clinical and biological behaviour and is treated differently.
- Some expert groups had recommended incorporating intraductal component of the prostate cancer into the Gleason score while others had recommended reporting it separately in a comment.

Treatment

The ensuing summations had been made regarding the assessment and treatment options for prostate cancer include the ensuing: [14]

- Pre-operative risk stratification of prostate cancer is based upon serum PSA, clinical stage, biopsy parameters (tumour extent, grade, cribriform morphology, intraductal carcinoma, perineural invasion) of the tumour.
- Primary treatment options of prostate cancer based upon pre-operative risk stratification include:
    - Active surveillance
    - Focal therapy (cryotherapy, high intensity ultrasound)
    - Radical prostatectomy
    - Brachytherapy
    - External beam radiotherapy
    - Hormone therapy (e.g., luteinizing hormone releasing hormone [LHRH] analogues, antiandrogens)
    - Orchidectomy (rare in contemporary practice)
    - Chemotherapy (for metastatic disease)

Other treatment options that could be used on rare occasions include: irreversible electroporation of prostate cancer, gamma knife surgery of prostate cancer, selective angiography and super-selective embolization of the branch of prostate artery supplying a bleeding prostate artery feeding the prostate cancer.

Immunotherapy is also used in combination with hormonal therapy and radiotherapy plus / minus chemotherapy.

- Post-prostatectomy options:
    - Generally, following radical prostatectomy serum PSA monitoring and early salvage therapy is undertaken if there is rising serum PSA
    - Less commonly adjuvant therapy is given for high stage disease or margin positivity of the tumour.

Gross description

- In the scenario of a number of cases of prostate cancer often grossly the tumour in the prostate gland specimens is inapparent and the diagnosis is established based upon pathology examination of the prostate specimen. [14]
- Gross examination of prostate cancer tumour specimen may demonstrate tumour which has formed a cream mass. [14]

Microscopic (histologic) description

Microscopy pathology examination features of prostate cancer specimens had been summated as follows: [14]

- Gleason grading is based upon the architecture of the tumour
- Gleason grades represent a morphological spectrum from well-formed glands (pattern 3) to increasingly complicated glandular proliferations (pattern 4) to almost no glandular differentiation (pattern)
- Glandular crowding and infiltrative growth pattern of the tumour.
- Nuclear enlargement, nucleolar prominence within the tumour.
- Round generally monomorphic nuclei are seen in the tumour.
- Amphophilic cytoplasm is seen in the tumour specimen.
- Mitoses are visualised upon microscopy pathology examination of the prostate cancer.
- Apoptotic bodies are seen upon microscopy pathology examination of the prostate cancer.
- Stromal desmoplasia is demonstrated within the tumour specimen.
- Intraluminal contents of the tumour upon microscopy examination demonstrate the ensuing: crystalloids, pink amorphous secretions, blue mucin
- Glomerulations, collagenous micronodules (mucinous fibroplasia) are seen during microscopy histopathology examination of the tumour.
- Absence of basal cell layer that is seen in specimens of prostate cancer generally requires immunohistochemistry staining study confirmation.

Cytology description

Cytology examination features of prostate cancer had been summated as follows:

- It has been pointed out that urine cytology for detecting prostate cancer has a very low sensitivity.
- It has been pointed out that urine cytology is not used clinically in the diagnosis of prostate cancer
- FNA of metastatic prostate cancer to a lymph node may show micro-acinar complexes / cell clusters / single cells with fragile cytoplasm and prominent nucleoli

Positive stains

Adenocarcinoma of prostate tumour specimens exhibit positive immunohistochemistry staining for the ensuing tumour markers: [14]

- **PSA**
- **NKX3.1**
- **AMACR** (P504S, racemase)
- **Prostein** (P501S)
- **PSMA**
- Rare prostate cancer tumours may have aberrant expression of **p63**

Negative stains

Adenocarcinoma of prostate tumour specimens exhibit negative immunohistochemistry staining for the ensuing tumour markers: [14]

- **CK7**
- **CK20**
- High molecular weight cytokeratins (**34 beta E12, CK5, CK5/6**)
- **p63**
- **CDX2**
- **GATA3**
- **TTF1**

Molecular / cytogenetics description

Molecular / cytogenetics features of prostate cancer have been summated as follows: [14]

- Prostate cancer is a heritable disease.
- Family history of a first degree relative with prostate cancer increases the risk of developing prostate cancer by 2-fold. [47]
- 30% to 40% of familial risk is due to genetic factors. [26]
- Genetic factors of prostate cancer include highly penetrable rare variants and more common low to moderate risk variants. [26]
- Highly penetrant variants occur in BRCA2 and HOXB13
- Over 280 SNPs have been identified as prostate cancer risk factors. [26]
- For most SNPs, the molecular mechanism of cancer association has been generally unknown, as they occur in noncoding regions of the genome. [26]
- Somatic mutations occur in genes such as *ERG, ETV1/4, FLI1, SPOP, FOXA1, IDH1, PTEN, TP53, MYC, CDH1*. [25] [26]
- Most common somatic genomic rearrangement is fusion of the androgen regulated gene *TMPRSS2* with a member of the *ETS* transcription family. [26]
- Somatic mutation profiles of prostate cancer are associated with clinical and pathological outcomes as follows: [14]
    - There are 7 major subtypes, which are defined by either specific gene fusions of *ETS* transcription family members (*ERG, ETV1, ETV4* and *FLI1*) or mutations (*SPOP, FOXA1, IDH1*)
- Different subtypes of prostate cancer have different molecular profiles, for example:
    - *ETS* subset (59% of cases) are enriched in *PTEN* mutations

- *SPOP* mutant subset (11%) of cases have distinct somatic copy number alteration profiles, including deletions of *CHD1*, 6q and 2q

Differential diagnoses

The differential diagnoses of prostate cancer include the ensuing: [14]

- **Benign prostate tissue** which has:
  - Pale cytoplasm
  - Corpora amylacea
  - No other intraluminal contents
  - Basal cell marker immunoreactivity
- **Prostatic atrophy** which has:
  - Lobular architecture
  - Scant cytoplasm
  - Basal cell marker immunoreactivity
- **Adenosis**:
  - Lobular architecture
  - Basal cell marker immunoreactivity (often scattered)
- **Atypical small acinar proliferation (ASAP):**
  - Small size
  - Lack of significant cytological atypia, including a lack of macro-nucleoli
- **High-grade prostatic intraepithelial neoplasia (HGPIN):**
  - Less architectural atypia
  - Maintained basal cells
- **Post-atrophy hyperplasia.**
  - Some glands atrophic
  - Basal cell marker immunoreactivity (often scattered)
- **Partial atrophy**:
  - Atrophic glands with abundant lateral pale cytoplasm
  - Irregularly distributed nuclei
  - Basal cell marker immunoreactivity (often scattered)
- **Radiation atypia**:
  - Glandular atrophy
  - Nuclear irregularity and pleomorphism
  - Atypical stromal cells
  - Basal cell marker immunoreactivity
- **Urothelial carcinoma**:
  - Nuclear irregularity and pleomorphism
  - Hyaline dense eosinophilic cytoplasm
  - Desmoplastic stromal reaction
  - Immunoreactivity for urothelial markers (**GATA3, CK7, p63**)
  - No expression of prostatic immunomarkers **(PSA, PSAP, NKX3.1)**

[B] Miscellaneous Narrations And Discusions From Some Case Reports, Case Series, And Discussions Related To Urine And Blood Biomarkers That Could Be Used To Identify And Localise Prostate Cancer

Porzycki et al. [1] stated that:

- The most common malignant tumour of the urinary tract is prostate cancer (PCa), which is a heterogeneous disease, that ranges from very slowly developing and slightly benign to progressing, aggressive, metastatic and fatal, even when it is properly treated.
- Existing, imperfect diagnostic methods often lead to over-diagnosis and over-treatment of PCa.
- This is why new, better prostate cancer (PCa) biomarkers are being developed**.**

Porzycki et al. [1] summated the current results of the most promising and clinically used PCa biomarkers, as well as which have the potential to create new diagnostic and prognostic tools, based upon the Web of Science (www.apps.webofknowledge.com) and Scopus (www.scopus) databases. com). Porzycki et al. [1] summated the results as follows:

- Limited specificity of the prostate-specific antigen (PSA) test has brought out a need to develop new and better diagnostic tools.
- In the last few years, new approaches for providing significantly better biomarkers, an alternative to serum PSA testing, had been introduced.
- Modern biomarkers show improvement in being used as not only a diagnostic procedure, but also for staging, evaluating aggressiveness and managing the therapeutic process.
- They had described the methods recommended in the diagnosis of PCa and new PCa molecular diagnostics technologies.
- Individual biomarkers are utilised in various stages of the PCa diagnostic process, which was presented on the developed diagnostic flowchart describing the role of biomarkers in prostate cancer management.

Porzycki et al. [1] concluded that:

- Given the diverse nature of PCa, one diagnostic test will not answer all questions, so the use of several diagnostic methods will enable physicians to provide patients with better, personalized clinical advice.

Fujita et al. made the ensuing iterations:

- The development of more specific biomarkers for prostate cancer and/or high-risk prostate cancer is necessary, due to the fact that the prostate-specific antigen test lacks specificity for the detection of prostate cancer and can lead to unnecessary prostate biopsies.
- Urine is a promising source for the development of new biomarkers of prostate cancer.
- Biomarkers that are derived from prostate cancer cells are released into prostatic fluids and then into urine.
- Urine after manipulation of the prostate gland is enriched with prostate cancer biomarkers, which include prostate cancer cells, DNAs, RNAs, proteins and other small molecules.
- The urinary prostate cancer antigen 3 test is the first Food and Drug Administration-approved RNA-based urinary marker, and it enables the identification of prostate cancer on repeat biopsy.
- The SelectMDx test is based upon messenger RNA detection of DLX1 and HOXC6 in urine after prostate massage, and enables the detection of high-risk prostate cancer on prostate biopsy.

- Exosomes are extracellular vesicles with a diameter of 30nm to 200 nm that are secreted from various types of cells.
- Urinary prostate cancer-derived exosomes also contain RNAs and proteins specific for prostate cancer (e.g. PCA3 and TMPRSS2-ERG), and could be promising sources of novel biomarker discovery.
- The ExoDx Prostate test is a commercially available test that is based upon the detection of three genes (PCA3, ERG and SPDEF) in urinary exosomes.
- Advancement of comprehensive analysis (microarray, mass spectrometry and next-generation sequencing) had resulted in the discovery of many urinary biomarkers.
- Non-invasive urinary markers could help in the decision to undertake prostate biopsy or in the design of a therapeutic strategy.

Ploussard, et al. stated the ensuing:

- The deficiencies of serum PSA as a prostate-cancer-specific diagnostic test are well recognized.
- Hence, the development of new biomarkers for prostate cancer detection remains an important and exciting challenge.
- Non-invasive urine-based tests are particularly attractive candidates for large-scale screening protocols, and biomarker discovery programs using urine samples have emerged for detecting and predicting aggressiveness of prostate cancer.
- Some new biomarkers already outperform serum PSA in the diagnosis of this disease.
- Currently, the *PCA3* (prostate cancer antigen 3) urine test is probably the best adjunct to serum PSA for predicting biopsy outcome, and had proven its clinical relevance by surpassing the predictive abilities of traditional serum biomarkers.
- New research methods had also been emerging, and high-throughput technologies will facilitate high-dimensional biomarker discovery.
- Future approaches would probably integrate proteomic, transcriptomic and multiplex approaches to detect novel biomarkers, and aim to identify combinations of multiple biomarkers to optimize the detection of prostate cancer.
- Additionally, an unmet need had remained for markers that differentiate indolent from aggressive cancers, to better inform treatment decisions.

Wu et al. made the ensuing iterations:

- Prostate cancer (CaP) is the most common cancer in men and the second leading cause of cancer deaths in males in Australia.
- Even though serum prostate-specific antigen (PSA) had been the most widely utilized biomarker in CaP detection for decades, serum PSA screening has limitations such as low specificity and potential association with over-diagnosis.
- Current biomarkers that are used in the clinic are not useful for the early detection of CaP, or monitoring its progression, and have limited value in predicting response to treatment.
- Urine is an ideal body fluid for the detection of protein markers of CaP and has been emerging as a potential source for biomarker discovery.
- Gene-based biomarkers in urine such as prostate cancer antigen-3 (PCA3), and genes for transmembrane protease serine-2 (TMPRSS2), and glutathione S-transferase P (GSTP1) had been developed and evaluated in the past decades.
- Among these biomarkers, urinary PCA3 is the only one approved by the FDA in the USA for clinical use.
- The study of urine microRNAs (miRNAs) is another burgeoning area for investigating biomarkers to achieve a pre-biopsy prediction of CaP to contribute to early detection.
- The development of mass spectrometry (MS)-based proteomic techniques had sparked new searches for novel protein markers for many diseases including CaP.
- Urinary biomarkers for CaP represent a promising alternative or an addition to traditional biomarkers.
- Future success in biomarker discovery would rely upon collaboration between clinics and laboratories.
- Furthermore, research efforts need to be moved from biomarker discovery to validation in a large cohort or separate population of patients and translation of these findings to clinical practice.

Persaud et al. made the ensuing iterations:

- In patients who have elevated serum PSA, the urinary MPS2 test had high accuracy for excluding high-grade prostate cancer requiring biopsy.
- The urinary 18-gene MyProstateScore 2.0 (MPS2) test could detect clinically significant prostate cancer and reduce the frequency of biopsies compared with the Prostate Cancer Prevention Trial risk calculator (PCPTrc), investigators report.
- "The MyProstateScore 2.0 test measures 18 cancer-associated and high-grade cancer-associated genes in urine in order to provide a percentage likelihood of detecting GG $\geq$ 2 cancer on biopsy," Jeffrey J. Tosoian, MD, MPH, of Vanderbilt University Medical Center in Nashville, Tennessee, and colleagues explained in The Journal of Urology.
- "Utilising urine obtained without [digital rectal examination (DRE)], the MPS2 test provides a highly accurate, personalized risk score to better identify patients who can confidently forego additional testing with MRI or biopsy."
- The team had previously validated the test using post-DRE urine. In their current study, they tested MPS2 as a first-line non-invasive test after serum PSA screening using first-catch, non-DRE urine. They tested 3 MPS2-based models: biomarkers alone (1), biomarkers and clinical data including, age, race, PSA, DRE findings, family history, and prior negative biopsy (2), and biomarkers combined with clinical factors and prostate volume (3).

They made the ensuing summations and discussions regarding the results:

- Among 266 men with a median serum PSA of 6.6 ng/mL, grade group 2 or higher cancer was eventually diagnosed in 103 men (39%), including 83, 9, and 11 men with GG2, GG3, and GG4-5 disease, respectively.
- Median MPS2 values were found to be significantly higher in patients with vs without GG 2 or higher cancer in model 1 (31% vs 15%), model 2 (36% vs 15%), and model 3 (41% vs 13%), Dr Tosoian's team reported. MRI use did not differ between groups with and without GG 2 or higher cancer: 18% vs 17%.
- The MPS2 models had outperformed serum PSA alone and the PCPT risk calculator, they added.
- The area under the curve (AUC) for the 3 MPS2 models was 71%, 74%, and 77%, respectively, compared with 57% for PSA and 62% for the PCPT risk calculator.
- Only MPS2 model 1 was found to be not significantly better than the PCPTrc. Model 3 appeared the most discriminative.
- Assuming a 92% rate of detecting clinically relevant prostate cancers at initial biopsy, use of MPS2 would have avoided 36% to 42% of unnecessary biopsies, as compared with 13% using the PCPTrc, the investigators reported.
- In the repeat biopsy setting, MPS2 would have avoided 44% to 53% of unnecessary biopsies, compared with only 2.6% using the PCPTrc, Dr Tosoian's team reported. Systematic biopsy with or without prebiopsy MRI was the reference standard for prostate cancer diagnosis, which is a limitation of their study.

- "Ultimately, the MPS2 tool's superior sensitivity and positive predictive value better stratify patients for invasive confirmatory testing, while sparing those without significant clinical risk," Shreya Thiagarajan and coauthors at Dell Medical School at the University of Texas at Austin wrote in an accompanying editorial.
- The team would be investigating MPS2 use in patients undergoing active surveillance for low-grade prostate cancer. If further validated, the MPS2 might enable at-home testing for clinically significant prostate cancer, which would especially benefit remote patients relying on telehealth care.

Becerra et al. made the ensuing iterations:

- Since the "prostate-specific antigen (PSA) era," they had seen an increase in unnecessary biopsies, which has ultimately led to an overtreatment of low-risk cancers.
- Given the limitations of serum prostate-specific antigen and the invasive nature of prostate biopsy several serum and urinary biomarkers had been developed.
- In their paper, they had provided a comprehensive review of the available biomarkers for the detection clinically significant prostate cancer namely PHI, 4Kscore, PCA3, MiPS, SelectMDx, ExosomeDX.
- Current literature had suggested that these biomarkers could improve the detection of clinically significant prostate cancer reducing overtreatment and making treatment strategies more cost-effective.
- Nevertheless, large prospective studies with head-to-head-comparisons of the available biomarkers are necessary to fully assess the potential of incorporating biomarkers in routine clinical practice.

Hessels et al. [10] made the ensuing iterations:

- Even though the routine use of serum prostate-specific antigen (PSA) testing has undoubtedly increased prostate cancer (PCa) detection, one of its main drawbacks is its lack of specificity.
- As a result, many men undergo unnecessary prostate biopsies or treatments for indolent tumours.
- PCa-specific markers are required for the early detection of the disease and the prediction of aggressiveness of a prostate tumour.
- Due to the fact that PCa is a heterogeneous disease, a panel of tumour markers is fundamental for a more precise diagnosis.
- Many biomarkers are promising due to their specificity for the disease in tissue. However, tissue is unsuitable as a possible screening tool.
- Since urine could be easily obtained in a non-invasive manner, it is a promising substrate for biomarker testing.

Robinson et al. [54] made the ensuing iterations:

- Recognizing the limitations of prostate-specific antigen (PSA) screening and the morbidity of prostate biopsies, many blood- and urine-based biomarkers had been proposed for pre-biopsy risk stratification.
- These assays do aim to reduce the frequency of the undertaking of unnecessary biopsies (i.e., negative or Grade Group 1 [GG1]) while maintaining highly sensitive detection of clinically significant cancer (GG ≥ 2) prostate cancer.

Robinson et al. [54] reviewed the literature describing the use of currently available blood- and urine-based biomarkers for detection of GG ≥ 2 cancer, including the Prostate Health Index (PHI), 4Kscore, MyProstateScore (MPS), SelectMDx, ExoDx Prostate Intelliscore (EPI), and IsoPSA. To facilitate clinical application, Robinson et al. [54] focused on the use of biomarkers as a post-PSA secondary test prior to biopsy, as proposed in clinical guidelines. The outcomes of Robinson et al. [54] included test performance measures—sensitivity, specificity, negative predictive value (NPV), and positive predictive value (PPV)—as well as clinical outcomes resulting from biomarker use (i.e., unnecessary biopsies avoided, GG ≥ 2 cancers missed). Robinson et al. summated the results as follows:

- Contemporary validation data (2015–2023) revealed that currently available biomarkers provide ~15–50% specificity at a sensitivity of 90–95% for GG ≥ 2 PCa.
- Clinically, this indicates that secondary utilisation of biomarker testing in men with elevated serum PSA could allow for avoidance of up to 15–50% of unnecessary prostate biopsies, while preserving detection of 90–95% of GG ≥ 2 cancers that would be detected under the traditional "biopsy all" approach.

Robinson et al. made the conclusions:

- The contemporary literature further supported the proposed role of post-PSA biomarker testing to reduce the use of invasive biopsy while maintaining highly sensitive detection of GG ≥ 2 cancer.
- Questions remain regarding the optimal application of biomarkers in combination or in sequence with multi-parametric magnetitic resonance imaging (mpMRI) scan.

Pavlovic, et al. stated that Prostate-Specific Antigen (PSA) based screening of prostate cancer (PCa) needs refinement. Pavlovic, et al. undertook a study was which was aimed at the identification of urinary biomarkers to predict the Prostate Imaging—Reporting and Data System (PI-RADS) score and the presence of PCa prior to prostate biopsy. Pavlovic, et al. collected urine samples from patients with elevated PSA prior to prostate biopsy (cohort = 99). The re-analysis of mass spectrometry data from 45 samples was undertaken to identify urinary biomarkers to predict the PI-RADS score and the presence of PCa. The most promising candidates, i.e. SPARC-like protein 1 (SPARCL1), Lymphatic vessel endothelial hyaluronan receptor 1 (LYVE1), Alpha-1-microglobulin/bikunin precursor (AMBP), keratin 13 (KRT13), cluster of differentiation 99 (CD99) and hornerin (HRNR), were quantified by ELISA and validated in an independent cohort of 54 samples. Pavlovic, et al. summated the results as follows:

- Various biomarker combinations demonstrated the ability to predict the PI-RADS score (AUC = 0.79).
- In combination with the PI-RADS score, the biomarkers improved the detection of prostate carcinoma-free men (AUC = 0.89) and of those with clinically significant PCa (AUC = 0.93).

Pavlovic, et al. concluded that:

- They had uncovered the potential of urinary biomarkers for a test that allows a more stringent prioritization of mpMRI use and improves the decision criteria for prostate biopsy, minimizing patient burden by decreasing the number of unnecessary prostate biopsies.

Kim et al. made the ensuing iterations:

- Even though prostate-specific antigen (PSA) has remained the most used test to detect prostate cancer (PCa), the limited specificity and an elevated rate of overdiagnosis are the main problems associated with PSA testing.
- Over the preceding three decades, a large body of evidence has indicated that PSA screening methods for PCa are problematic, even though serum PSA screening significantly reduces PCa-specific mortality.
- A number of new biomarkers had been introduced to overcome these limitations of PSA in the clinical setting.

- These biomarkers have shown an increased ability to select patients for biopsy and identify men at risk for clinically significant PCa.
- Even though a number of assays do need further validation, initial data are promising.
- Forthcoming results will ultimately ascertain the clinical utility and commercial availability of these assays.
- Extensive efforts have recently been made to identify and commercialize new PCa biomarkers for more effective detection of PCa, either alone or in combination with currently available clinical tools.

Farha et al. made the ensuing educative iterations:

- Even though prostate cancer (PCa) is the most commonly diagnosed cancer in men, most patients do not die from the disease.
- Serum prostate specific antigen (PSA), the most widely used oncological biomarker, has revolutionized screening and early detection, resulting in reduced proportion of patients presenting with advanced disease.
- Nevertheless, given the inherent limitations of serum PSA, additional diagnostic and prognostic tools are required to facilitate early detection and accurate risk stratification of disease.
- Serum, urine, and tissue-based biomarkers are increasingly being incorporated into the clinical care paradigm, but there is still a limited understanding of how to utilise them most effectively.

Areas of relevant biomarkers for prostate detection discussed by Farha et al. [57] included test characteristics and clinical performance data for both serum [4 K score, prostate health index (phi)] and urine [SelectMDx, ExoDx Prostate Intelliscore, MyProstateScore (MPS), and PCa antigen 3 (PCA3)] biomarkers to aid decisions regarding initial or repeat biopsies as well as tissue-based biomarkers (Confirm MDx, Decipher, Oncotype Dx, and Polaris) aimed at risk stratifying patients and identifying those patients most likely to benefit from treatment versus surveillance or monotherapy versus multi-modal therapy.

Wei et al. made the ensuing preamble iterations:

- The field of urology has been beset by many major trends that have affected the early detection of prostate cancer.
- These stem primarily from a backlash against overdiagnosis due to serum PSA based screening efforts, and are epitomized by the US Preventative Services Task Force giving PSA-based prostate cancer screening a 'D' recommendation.
- Consequently, the active surveillance strategy for low-risk prostate cancer has become commonplace, leading many clinicians to ask how best to follow these patients.
- More importantly, this public outcry has shifted the focus of early detection from an effort to diagnose any and all prostate cancers to an effort to diagnose only 'high-risk' cancer.
- Along with a trend for minimally invasive procedures, these forces have challenged the early detection field to more efficiently identify clinically significant prostate cancers at an early stage while limiting the number of biopsies.

Wei et al. documented the ensuing recent Findings:

- With FDA approval, PCA3 had emerged as the first bona-fide urinary biomarker for prostate cancer. Using the same platform, investigators have developed a second urinary test based on TMPRSS2:erg fusion.
- Recent literature has supported the use of these biomarkers as a combined panel that improves risk evaluation in the setting of prostate cancer detection.
- Early work for applying urinary biomarkers for active surveillance have been underway.
- Other biomarkers in the pipeline will need further pre-validation and validation work.

Wei et al. summated and concluded that:

- Recent literature would support that urinary biomarkers have a clear role to supplement risk evaluation for men undergoing prostate biopsy and for prognostication.

Nasimi et al. stated that:

- Prostate cancer (PCa) is a common male malignancy and early diagnosis is crucial for successful treatment.

Nasimi et al. undertook a study aims to validate results from a pilot study that demonstrated an inverse association between urine tyrosine and tryptophan levels and the severity of PCa. The study comprised a cohort of 97 patients with benign prostatic hyperplasia, 93 patients diagnosed with localized PCa, 75 patients diagnosed with locally advanced PCa, and 68 patients diagnosed with metastatic PCa. Nasimi et al. analyzed the tyrosine and tryptophan levels in the samples by liquid chromatography-tandem mass spectrometry (LC-MS/MS) and electrochemical sensors in accordance with the pilot to maintain uniformity for accurately evaluating the data. One-way ANOVA with post Tukey test as well as the Wilcoxon Rank Sum Test were undertaken. Analyzing 333 patients across PCa stages with consistent methods, Nasimi et al. observed no significant differences in tyrosine and tryptophan levels between PCa patients and controls, finally rejecting the use of tyrosine and tryptophan as PCa biomarkers. Nasimi et al. did, however, verify the strong correlation between the urinary concentrations of tyrosine and tryptophan found in the pilot study.

Chang et al. made the ensuing preamble iterations:

- There is no consensus on the role of biomarkers in determining the utility of prostate biopsy in men with elevated prostate-specific antigen (PSA).
- There are many biomarkers such as prostate health index, 4Kscore, prostate cancer antigen 3, ExoDX, SelectMDx, and Mi-Prostate Score which may be useful in this decision-making process.
- Nevertheless, it is not clear whether any of these tests are accurate and cost-effective enough to warrant being a widespread reflex test following an elevated serum PSA.
- Their goal was to report on the clinical utility of these blood and urine biomarkers in prostate cancer screening.

Chang et al. undertook a systematic review of studies published between January 2000 and October 2020 to report the available parameters and cost-effectiveness of the aforementioned diagnostic tests. Chang et al. focused on the negative predictive value, the area under the curve, and the decision curve analysis in comparing reflexive tests due to their relevance in evaluating diagnostic screening tests. Chang et al. summated the results as follows:

- Overall, the biomarkers are roughly equivalent in predictive accuracy.

- Each test has additional clinical utility to the current diagnostic standard of care, but the added benefit is not substantial to justify using the test reflexively after an elevated serum PSA.

Chang et al. made the ensuing conclusions:

- Their findings had suggested these biomarkers should not be used in binary fashion and should be understood in the context of pre-existing risk predictors, patient's ethnicity, cost of the test, patient life-expectancy, and patient goals.
- There are more recent diagnostic tools such as multi-parametric magnetic resonance imaging, polygenic single-nucleotide panels, IsoPSA, and miR Sentinel tests that are promising in the realm of prostate cancer screening and need to be investigated further to be considered a consensus reflexive test in the setting of prostate cancer screening.

Rigan et al. made the ensuing iterations:

- In order to successfully cure patients with prostate cancer (PCa), it is pivotal to detect the disease at an early stage.
- The existing clinical biomarkers for PCa are not ideal, due to the fact that they cannot specifically differentiate between those patients who should be treated immediately and those who should avoid over-treatment.
- Current screening techniques lack specificity, and a decisive diagnosis of PCa is based upon prostate biopsy.
- Even though PCa screening is widely utilized nowadays, two thirds of the biopsies performed are still unnecessary.
- Hence, the discovery of non-invasive PCa biomarkers remains urgent.
- Over recent years, the utilization of urine had emerged as an attractive option for the non-invasive detection of PCa.
- Moreover, a great improvement in high-throughput “omic” techniques had presented considerable opportunities for the identification of new biomarkers.

Hendriks et al. made the ensuing iterations:

- The diagnosis of prostate cancer (PCa) is currently based upon serum PSA testing and/or abnormal digital rectal examination and histopathology evaluation of prostate biopsies.
- The main drawback of serum PSA testing is the lack of specificity for PCa.
- To improve early detection of PCa more specific biomarkers are needed.
- Over the preceding few years, many new promising biomarkers had been identified; however, up to 2017, only a few had reached clinical practice.

Hendriks et al. reviewed and discussed new blood-based and urinary biomarker models that are promising for usage in PCa detection, follow-up and treatment decision-making. These include Prostate Health Index (PHI), prostate cancer antigen 3 (PCA3), four-kallikrein panel (4K), transmembrane protease serine 2-ERG (TMPRSS2-ERG), ExoDx Prostate Intelliscore, SelectMDx and the Mi-Prostate score. Only few head-to-head studies are available for these new fluid-based biomarkers and/or models. The blood-based PHI and urinary PCA3 are two US Food and Drug Administration-approved biomarkers for diagnosis of PCa. In the second part of the review, Hendriks et al. gave an overview of published studies comparing these two available biomarkers for prediction of (1) PCa upon prostate biopsy, (2) pathological features in radical prostatectomy specimen and (3) significant PCa in patients eligible for active surveillance. Hendriks et al. summated the results as follows:

- Studies showed opposing results in comparison of PHI with PCA3 for prediction of PCa upon initial and repeat prostate biopsy.
- PHI and PCA3 are able to predict pathology examination findings on radical prostatectomy specimen, such as tumour volume and Gleason score.
- Only PHI could predict seminal vesicle invasion and only PCA3 could predict multifocality.
- There is some evidence that PHI outperforms PCA3 in predicting significant PCa in an active surveillance population

Hendriks made the ensuing conclusion:

- Future research should focus upon independent validation of promising fluid-based biomarkers/models, and prospective comparison of biomarkers with each other.

Eskra et al. made the ensuing iterations:

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

Eskra et al. undertook a review of current literature on urinary biomarkers for prostate cancer. Eskra et al. evaluated the strengths and limitations of a variety of approaches that vary in sampling strategies and targets measured; discussed reported urine tests for prostate cancer with respect to their technical, analytical, and clinical parameters; and provided their perspectives on critical considerations in approaches to developing a urine-based test for prostate cancer. Eskra et al. summated the results as follows:

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

Eskra et al. concluded that:

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

Chan et al. made the ensuing iterations:

- Current diagnostic methods for prostate cancer are invasive and they do lack specificity towards aggressive forms of the disease, which could 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 were systematically reviewed and their potential for translation to clinical practice was evaluated.

Chan et al. undertook a systematic review of the literature 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. Chan et al. summated the results as follows:

- They had identified a total of 76 records.
- After screening and eligibility, they had included 14 articles and are discussed them in the paper. The biosensors were discussed based upon 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.

Chan et al. made the ensuing conclusions:

- Their review had highlighted the potential of urinary biosensors for non-invasive prostate cancer detection.
- Nevertheless, clinical studies had up to 2021 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, was that an adequate combination of biomarkers into multiplex biosensor platforms is required to achieve accurate diagnostic tests.
- Furthermore, whether such devices could discriminate between aggressive and indolent cancer had not yet been addressed, because it entails optimized biomarkers panels and long-term clinical trials.

Madu et al. made the ensuing iterations:

- Prostate cancer is the most frequently diagnosed malignancy in American men, and a more aggressive form of the disease is particularly prevalent among African Americans.
- The therapeutic success rate for prostate cancer could be tremendously improved if the disease is diagnosed early.
- Hence, a successful therapy for this disease depends heavily upon the clinical indicators (biomarkers) for early detection of the presence and progression of the disease, as well as the prediction after the clinical intervention.
- Nevertheless, the current clinical biomarkers for prostate cancer are not ideal as there remains a lack of reliable biomarkers that can specifically distinguish between those patients who should be treated adequately to stop the aggressive form of the disease and those who should avoid overtreatment of the indolent form.
- A biomarker is a characteristic which is objectively measured and evaluated as an indicator of normal biologic processes, pathogenic processes, or pharmacological responses to a therapeutic intervention.
- A biomarker reveals further information to presently existing clinical and pathological analysis.
- A biomarker facilitates screening and detecting the cancer, monitoring the progression of the disease, and predicting the prognosis and survival after clinical intervention.
- A biomarker can also be utilised to evaluate the process of drug development, and, optimally, to improve the efficacy and safety of cancer treatment by enabling physicians to tailor treatment for individual patients.
- The form of the prostate cancer biomarkers can vary from metabolites and chemical products present in body fluid to genes and proteins in the prostate tissues.
- Current advances in molecular techniques had provided new tools facilitating the discovery of new biomarkers for prostate cancer.
- These emerging biomarkers would be beneficial and critical in the development of new and clinically reliable indicators that will have a high specificity for the diagnosis and prognosis of prostate cancer.

Jin a study to identify and validate urine exosomal AMACR (UE-A) as a novel biomarker to improve the detection of prostate cancer (PCa) and clinically significant PCa (Gleason score ≥ 7) at initial prostate biopsy. Jin [65] collected a total of 289 first-catch urine samples after the digital rectal exam (DRE) from patients who underwent prostatic biopsy, and 17 patients were excluded due to incomplete clinical information. Urine exosomes were purified, and urinary exosomal AMACR (UE-A) was measured by enzyme-linked immunosorbent assay (ELISA). The diagnostic performance of UE-A was evaluated by Jin by receiver operating characteristic (ROC) analysis, decision curve analysis (DCA), and waterfall plots. Jin [65] summated the results as follows:

- The expression of AMACR in PCa and csPCa was significantly higher than that in BPH and non-aggressive ($p < 0.001$).
- The UE-A presented good performance in distinguishing PCa from BPH or BPH plus non-significant PCa (nsPCa) from csPCa with an area under the ROC curve (AUC) of 0.832 and 0.78, respectively.
- The performance of UE-A was further validated in a multi-centre cohort of patients with an AUC of 0.800 for detecting PCa and 0.749 for detecting csPCa.
- The clinical utility assessed by DCA showed that the benefit of patients using UE-A was superior to PSA, f/t PSA, and PSAD in both the training cohort and the validation cohort in terms of all threshold probabilities. Setting 95% sensitivity as the cutoff value, UE-A could avoid 27.57% of unnecessary biopsies, with only 4 (1.47%) csPCa patients missed.

Jin made the ensuing conclusions:

- They had demonstrated the great performance of UE-A for the early diagnosis of PCa and csPCa.
- UE-A could be a novel non-invasive diagnostic biomarker to improve the detection of PCa and csPCa.

Eyrich et al. made the ensuing iterations:

- The use of serum prostate-specific antigen (PSA) testing for early detection of prostate cancer appears to reduce cancer-specific mortality.
- In view of the limited specificity of PSA for clinically significant [Grade Group (GG) ≥2] cancer; nevertheless, screening carries substantial risks, including frequent unnecessary prostate biopsies and over-detection of non-aggressive cancers.
- To that end, serum and urine biomarkers with improved specificity for GG ≥2 cancer had been proposed for clinical use following PSA.

- They had presented clinical validation data for five such biomarkers: PHI, 4Kscore, SelectMDx, ExoDx, and MPS.
- For all studies, they had specified the study population (overall biopsy referral versus pre-specified PSA ranges), previous biopsy status (biopsy-naïve versus previous negative biopsy), and the proportion of subjects diagnosed with GG ≥2 cancer.
- The outcomes included test performance characteristics: sensitivity, specificity, negative predictive value (NPV), and positive predictive value (PPV).
- Published data were used to compute the number of unnecessary biopsies avoided and number of GG ≥2 cancers missed if the biomarker had been used clinically to select for prostate biopsy.

Teng et al. made the ensuing iterations:

- Prostate cancer is the second deadliest cancer among men and poses a threat to the health of elderly men.
- Current methods of diagnosing prostate cancer including digital rectal tests or determining the increase in prostate-specific antigen level in serum are still not effective and hence could lead to overtreatment.
- New prostate cancer biomarkers in blood, urine, or tissues are reported and the methods for their accurate detection are being pursued.
- They had presented a comprehensive review of the recent literature reporting the biosensors for prostate cancer detection.
- The focus of their review was to evaluate and compare the design and performance of biosensors based upon single and/or multiple biomarkers.
- The continual emergence of new biomarkers promotes the specificity of biosensors. And the joint detection of multiple biomarkers promotes the accuracy of biosensors.
- Nevertheless, it is necessary to correctly screen the biomarker types and combinations because having more biomarkers does not necessarily guarantee improved biosensing performance.
- Furthermore, their review especially had highlighted the potential of artificial intelligence and machine learning tools and methodologies in prostate cancer biosensing due to their ability to recognize weak and complex signals, which will effectively improve the specificity, sensitivity, and accuracy of biosensors.
- The combination of machine learning and multiple biomarkers biosensors is a trend in the development of prostate cancer diagnosis.
- Nevertheless, most of the current work still focuses on the classification of non-cancer and cancer.
- Utilisation of linear regression and other tools for quantification to distinguish different stages of cancer is urgently required for development.

Xue et al. made the ensuing iterations:

- Urinary biomarkers offer a non-invasive and easily accessible means of assessing an individual's health and susceptibility to various diseases.
- Urine biomarkers have advantages in no need or mechanism for stability, specific biomarkers produced by tubules, and non-invasive nature compared with serum biomarkers.
- Urine biomarkers could provide critical insights into an individual's predisposition to certain conditions, disease progression, and therapeutic response.

Xue et al. in their review of the literature, summarized the currently reported urinary biomarkers that outperformed serum biomarkers, including urinary protein biomarkers, gene biomarkers, urinary metabolites, electrolytes, and urinary extracellular vesicles. Xue et al. made the ensuing educative conclusions:

- Combining urinary and serum biomarkers can offer a more comprehensive approach to disease diagnosis, monitoring, and personalized medicine.
- Despite some challenges in standardization and expanding the repertoire of diseases that can be diagnosed using urinary biomarkers, urinary biomarkers hold immense promise in improving patient outcomes and transforming healthcare.

Wood et al. made the ensuing iterations:

- Urine is an ideal body fluid for the detection of protein markers produced by urological cancers as it can be sampled noninvasively and contains secreted and directly shed proteins from the prostate, bladder and kidney.
- Major challenges of working with urine include high inter-individual and intra-individual variability, low protein concentration, the presence of salts and the dynamic range of protein expression.
- Despite these challenges, significant progress is being made using modern proteomic methods to identify and characterize protein-based markers for urological cancers.
- The development of robust, easy-to-use clinical tests based on novel biomarkers has the potential to impact upon diagnosis, prognosis and monitoring and could revolutionize the treatment and management of these cancers.

Wood et al. summated the ensuing Key Points:

- Urine is an accessible body fluid that can be utilized for the discovery of prognostic, diagnostic and monitoring biomarkers for urological cancers
- Proteomics has the potential to identify the key molecules in urine that are involved in the development and spread of urological cancers and might have roles as biomarkers
- Increasing numbers of potential biomarkers are being discovered for prostate cancer, bladder cancer and renal cell carcinoma using urine-based proteomic studies
- Urinary exosomes are a promising source of biomarkers for cancer diagnosis
- Consideration needs to be given to how biomarkers from laboratory-based studies are validated and evaluated to ensure effective and timely translation into clinical use

Boehm et al. made the ensuing iterations:

- Within the United States of America, prostate cancer (CaP) has remained the second leading cause of cancer deaths in men.
- CaP is predominantly indolent at diagnosis, with a small fraction (25% to 30%) representing an aggressive subtype (Gleason score 7–10) that is prone to metastatic progression.
- This fact, coupled with the criticism encompassing the role of prostate specific antigen in prostate cancer screening, has

demonstrated the current need for a biomarker(s) which could identify clinically significant CaP and avoid the undertaking of unnecessary biopsy procedures and psychological implications of being diagnosed with low-risk prostate cancer.

- Even though many diagnostic biomarkers are available to clinicians, very few comparative trials have been performed to assess the clinical effectiveness of these biomarkers.
- They had noted; however, that a majority of these clinical trials have been over-represented by men of Caucasian origin, despite the fact that African American men have a 1.7 times higher incidence and 2.1 times higher rate of mortality from prostate cancer.
- Biomarkers for CaP diagnosis based upon the tissue of origin include urine-based gene expression assays (PCA3, Select MDx, ExoDx Prostate IntelliScore, Mi-Prostate Score, PCA3-PCGEM1 gene panel), blood-based protein biomarkers (4K, PHI), and tissue-based DNA biomarker (Confirm MDx).
- Another potential direction that had emerged to aid in the CaP diagnosis include multi-parametric magnetic resonance imaging (mpMRI) and bi-parametric magnetic resonance imaging (bpMRI), which in conjunction with clinically validated biomarkers may provide a better approach to predict clinically significant CaP at diagnosis.
- In the review, they had discussed some of the adjunctive biomarker tests along with newer imaging modalities that are currently available to help clinicians decide which patients are at risk of having high-grade CaP on prostate biopsy with the emphasis on clinical utility of the tests across African American (AA) and Caucasian (CA) men.
- Prostate cancer is the second most common male cancer worldwide demonstrating the highest rates of incidence in Western Europe.
- Even though the measurement of serum prostate-specific antigen levels is the current gold standard in PCa diagnosis, serum PSA-based screening is not regarded to be a reliable diagnosis and prognosis tool due to its lower sensitivity and poor predictive score which led to a 22% to 43% overdiagnosis, unnecessary biopsies, and over-treatment.
- These major limitations together with the heterogeneous nature of the disease had made PCa a very unappreciative subject for diagnostics, resulting in poor patient management; hence, it urges to identify and validate new reliable PCa biomarkers which can provide accurate information with regard to disease diagnosis and prognosis.
- Researchers had explored the analysis of microRNAs (miRNAs), messenger RNAs (mRNAs), small proteins, genomic rearrangements, and gene expression in body fluids and non-solid tissues in search of lesser invasive yet efficient PCa biomarkers.
- Even though the presence of miRNAs in body fluids like blood, urine, and saliva initially sparked great interest among the scientific community; their potential use as liquid biopsy biomarkers in PCa is still at a very nascent stage with respect to other well-established diagnostics and prognosis tools.
- Up to 2023, many studies had been undertaken in search of PCa miRNA-based biomarkers in whole blood or blood serum; nevertheless, only a few studies had investigated their presence in urine samples of which less than two tens involve the detection of miRNAs in extracellular vesicles isolated from urine.
- Additionally, there exists some discrepancy around the identification of miRNAs in PCa urine samples due to the diversity of the urine fractions which can be targeted for analysis such as urine circulating cells, cell-free fractions, and exosomes.

Plas et al. made the ensuing iterations:

- Prostate cancer (PCa) screening primarily relies on Prostate-Specific Antigen (PSA), which has low specificity and therefore leads to unnecessary biopsies.
- Consequently, there is a growing need for, ideally, non-invasive biomarkers.
- Liquid biopsy, a diagnostic approach analyzing circulating tumor components in body fluids, has emerged as a promising diagnostic tool for various cancers, including PCa.

Plas et al. evaluated recent evidence on urine-based biomarkers for the detection of PCa, they undertook a systematic review in accordance with the PRISMA guidelines. Their literature search identified a total of 286 studies, of which 66 met their inclusion criteria (men suspected of PCa with no prior history of PCa). After assessing the risk of bias using the QUADAS-2 tool, studies on five distinct urinary biomarker tests were included for further analysis. Plas et al. summated the results as follows:

- Tests that do not rely upon digital rectal examination (non-DRE), such as Exosome Dx Prostate IntelliScore (EPI) and Protexam Prostate Status Management (PSM)/Prostate Check-Up (PSU), had demonstrated strong performance in detecting PCa, particularly clinically significant PCa. Meanwhile, the MyProstateScore test (MPS) showed the highest efficacy among tests utilizing urine samples collected post-DRE.
- Unfortunately, the performance of the biomarker test with the most available studies, PCA3 ProGensa® Score, was underwhelming with only moderate sensitivity and specificity.

Plas et al. made the ensuing conclusions:

- Despite promising results from various urine-based biomarker tests, they were currently unable to recommend one specific test for implementation into clinical practice.
- The broad heterogeneity of the studies undertaken had hindered the ability to undertake a meta-analysis, and prospective randomized trials providing clinical evidence are still lacking.

Singh made the ensuing iterations:

- Prostate cancer is a heterogeneous disease process with a wide spectrum of clinicopathologic variables which impact diagnosis, risk stratification, and management.
- To improve diagnostic accuracy and to better inform clinical decision making, the development of molecular biomarkers had undergone considerable discovery and clinical validation in the past decade.
- Prostate cancer is no longer seen as a single disease entity but one with considerable heterogeneity existing between tumours and between patients.
- Biomarkers now enable for more personalized and precision-based approaches to management that otherwise would have depended on applying clinical algorithms alone.

Yu et al. investigated the use of urinary exosomal mRNA as a potential biomarker for the early detection of prostate cancer (PCa). Yu et al. used

next-generation sequencing to analyse exosomal RNA from 10 individuals with confirmed PCa and 10 individuals without cancer. Subsequent validation through qRT-PCR in a larger sample of 43 PCa patients and 92 healthy controls revealed distinct mRNA signatures associated with PCa. Yu et al. [73] summated the results as follows:

- Notably, mRNAs for *RAB5B*, *WWP1*, *HIST2H2BF*, *ZFY*, *MARK2*, *PASK*, *RBM10*, and *NRSN2* showed promise as diagnostic markers, with AUC values between 0.799 and 0.906 and significance p values.
- Combining *RAB5B* and *WWP1* in an exoRNA diagnostic model outperformed traditional PSA tests, achieving an AUC of 0.923, 81.4% sensitivity, and 89.1% specificity.

Yu et al. made the ensuing conclusion:

- These findings had highlighted the potential of urinary exosomal mRNA profiling, particularly focusing on *RAB5B* and *WWP1*, as a valuable strategy for improving the early detection of PCa.

Filippo Martignano et al. made the ensuing iterations:

- Prostate cancer (PCa) is the commonest malignancy in the male population worldwide.
- Serum prostate specific antigen (PSA) test is the most important biomarker for the detection, follow-up and therapeutic monitoring of PCa.
- Defects in serum PSA specificity have elicited research for new biomarkers to improve early diagnosis and avoid false-positive results.

Filippo Martignano et al. evaluated urinary RNA-based biomarkers. They stated the following in their discussion:

- Urine is a versatile body fluid for non-invasive biomarker detection in case of urological malignancies.
- The importance of RNA-based biomarkers had been demonstrated by the current use of PCA3, a long non coding RNA biomarker already approved by the Food and Drugs Administration.
- Over the years, other urinary RNA biomarkers had been evaluated, including the well-known TMPRSS2:ERG transcript, as well as many messenger RNAs, long non coding RNAs and micro-RNA.
- Validation of a specific urinary RNA-based marker or an algorithm of different biomarkers levels as diagnostic markers for PCa could be useful to avoid unnecessary prostate biopsies.

Sequeira-Antunes et al. made the ensuing iterations:

- Biosensing and microfluidics technologies are transforming diagnostic medicine by accurately detecting biomolecules in biological samples.
- Urine is a promising biological fluid for diagnostics due to its non-invasive collection and wide range of diagnostic biomarkers.
- Point-of-care urinalysis, which integrates biosensing and microfluidics, has the potential to bring affordable and rapid diagnostics into the home to continuing monitoring, but challenges still remain.
- As such, they had undertaken a review to provide an overview of biomarkers that are or could be used to diagnose and monitor diseases, including cancer, cardiovascular diseases, kidney diseases, and neurodegenerative disorders, such as Alzheimer's disease.
- In addition, they had reviewed the different materials and techniques for the fabrication of microfluidic structures along with the biosensing technologies often used to detect and quantify biological molecules and organisms.
- They had discussed in their review the current state of point-of-care urinalysis devices and highlights the potential of these technologies to improve patient outcomes.

Sequeira-Antunes made the ensuing conclusions:

- Traditional point-of-care urinalysis devices require the manual collection of urine, which may be unpleasant, cumbersome, or prone to errors.
- In order to overcome this issue, the toilet itself can be used as an alternative specimen collection and urinalysis device.

Coric et al. made the ensuing iterations:

- In view of the fact that the use of prostate-specific antigen (PSA) in prostate cancer (PC) management is limited, the search for novel biomarkers useful for early detection and monitoring, especially from non-invasive biofluids is an important pursuit.
- Urine has become one of the most attractive biofluids in clinical practice.
- Even though none of the novel biomarkers up to 2024 had been used in common everyday clinical practice, they may bestow a strategy to resolve the dilemma of early detection and monitoring of PC.
- Diagnostic and monitoring biomarkers may be assessed as standalone tests or be merged, and consequently used as risk stratification tools for detection and management of PC.
- Novel biomarkers, classified as urine, blood or tissue-based, may represent a component of new risk calculators and can be computed independently or merged with data obtained from imaging procedures.
- On the other hand, novel techniques in the field of omics, exsosomal science and nanomedicine represent a new kind of intervention biomarker identification and application.
- The future path of biomarker discovery will rely on the active collaboration among clinics and laboratories in defining the standards and establishing guidelines in order to facilitate the discoveries by providing a framework for specimen collection, processing, analysis, significance and data reduction.

. Lawisch et al made the ensuing iterations:

- Prostate cancer (PCA) is the second most common type of cancer in the world.
- However, diagnosis of PCA is still based upon non-specific methods, or invasive methods which makes clinical decision and diagnosis difficult, generating risk of both underdiagnosis and overdiagnosis.
- Given the high prevalence, morbidity and mortality of PCA, new strategies are required for its diagnosis.

Lawisch et al undertook a review of the literature on available biomarkers for PCA, using the following terms: prostate cancer AND marker OR biomarker. The search was carried out in Pubmed, Science Direct, Web

of Science and Clinical Trial. A total of 35 articles were used, and PHI (Prostate Health Index) and the 4Kscore tests were identified as the best well-established serum markers. These tests had been based upon the evaluation of expression levels of several molecules. For analysis of urine samples, Progensa, ExoDXProstate, and Mi Prostate Score Urine Test were available. All these tests have the potential to help diagnosis, avoiding unnecessary biopsies, but they were used only in association with digital rectal examination and PSA level data. Lawisch et al concluded that:

- The search for biomarkers that can help in the diagnosis and therapeutic management of PCA is still in its initial phase, requiring more efforts for an effective clinical application.

Nilsson, et al. described a novel approach in the search for prostate cancer biomarkers, which relies upon the transcriptome within tumour exosomes. As a proof-of-concept, Nilsson et al. [78] showed the presence of two known prostate cancer biomarkers, *PCA-3* and *TMPRSS2:ERG* the in exosomes isolated from urine of patients, showing the potential for diagnosis and monitoring cancer patients status.

Malode et al. made the ensuing iterations:

- Malignant growth is the most dangerous illness with the most elevated mortality rate.
- Early identification of malignant growth could be fundamental in effective treatment.
- One of the most promising early symptomatic tools for tracking infection spread and subsequent disease treatment is biomarker-based malignant growth screening.
- Prostate cancer (PCa) is the most widely recognized reason for death in men and is the following driving reason for death from the disease worldwide.
- Serum prostate-specific antigen (PSA) is a fundamental biomarker for diagnosing PCa.
- Despite signs against its utilization in massive populace tests, serum PSA concentrates most on PCa biomarkers.
- This biomarker does remain an essential determinant in the treatment of prostate malignant growth due to its different structures and its inclusion in rehashed plans with other biomarkers.

Eggener et al. documented a guideline which provided recommendations for available tissue-based prostate cancer biomarkers geared toward patient selection for active surveillance, identification of clinically significant disease, choice of postprostatectomy adjuvant versus salvage radiotherapy, and to address emerging questions such as the relative value of tissue biomarkers compared with magnetic resonance imaging. Eggener et al. reported that an ASCO multidisciplinary Expert Panel, with representatives from the European Association of Urology, American Urological Association, and the College of American Pathologists, had undertaken a systematic literature review of localized prostate cancer biomarker studies between January 2013 and January 2019. Numerous tissue-based molecular biomarkers were evaluated for their prognostic capabilities and potential for improving management decisions. The Panel made recommendations regarding the clinical use and indications of these biomarkers. Eggener et al. summated the results as follows:

- Out of 555 studies identified, 77 were selected for inclusion plus 32 additional references selected by the Expert Panel.
- Few biomarkers had rigorous testing involving multiple cohorts and only 5 of these tests are commercially available currently: Onco*type* Dx Prostate, Prolaris, Decipher, Decipher PORTOS, and ProMark.
- With various degrees of value and validation, multiple biomarkers have been shown to refine risk stratification and can be considered for select men to improve management decisions.
- There was a paucity of prospective studies assessing short- and long-term outcomes of patients when these markers are integrated into clinical decision making.

Eggener et al. made the ensuing recommendations:

- Tissue-based molecular biomarkers (evaluating the sample with the highest volume of the highest Gleason pattern) may improve risk stratification when added to standard clinical parameters, but the Expert Panel endorses their use only in situations in which the assay results, when considered as a whole with routine clinical factors, are likely to affect a clinical decision.
- These assays are not recommended for routine use as they have not been prospectively tested or shown to improve long-term outcomes—for example, quality of life, need for treatment, or survival.

Tosoian et al. emphasised the ensuing Key Points in their articles:

- **Question** - Can a new 18-gene urinary test for high-grade prostate cancer (ie, grade group [GG] 2 or greater) improve prostate-specific antigen (PSA) screening outcomes relative to existing biomarker tests?
- **Findings** - In this diagnostic study including 761 men in the development cohort and 743 men in the validation cohort, novel cancer-specific and high-grade cancer-specific genes were identified from RNA sequencing data and optimally modelled in a development cohort, yielding an 18-gene test for high-grade prostate cancer. Applying a testing approach with 95% sensitivity for high-grade prostate cancer to an external validation population, use of the 18-gene test would have reduced the number of unnecessary biopsies performed relative to current guideline-endorsed tests.
- **Meaning** - The new 18-gene prostate cancer test may reduce more burdensome additional testing (eg, imaging and biopsy) while maintaining highly sensitive detection of high-grade cancer in patients undergoing PSA screening.

Tosoian et al. stated that:

- Benefits of prostate cancer (PCa) screening with prostate-specific antigen (PSA) alone are largely offset by excess negative biopsies and over-detection of indolent cancers resulting from the poor specificity of PSA for high-grade PCa (for example grade group [GG] 2 or greater).

Tosoian et al. undertook a study to develop a multiplex urinary panel for high-grade PCa and validate its external performance relative to current guideline-endorsed biomarkers. Tosoian et al. reported that RNA sequencing analysis of 58 724 genes had identified 54 markers of PCa, including 17 markers uniquely overexpressed by high-grade cancers. Gene expression and clinical factors were modelled in a new urinary test for high-grade PCa (MyProstateScore 2.0 [MPS2]). Optimal models were developed in parallel without prostate volume (MPS2) and with prostate volume (MPS2+). The locked models underwent blinded external validation in a prospective National Cancer Institute trial cohort. Data were collected from January 2008 to December 2020, and data were analysed from November 2022 to November 2023. Protocolized blood and urine collection and transrectal ultrasound-guided systematic prostate biopsy were undertaken. Tosoian et al. summarised the main outcomes and measures as follows:

- Multiple biomarker tests were assessed in the validation cohort, including serum PSA alone, the Prostate Cancer Prevention

Trial risk calculator, and the Prostate Health Index (PHI) as well as derived multiplex 2-gene and 3-gene models, the original 2-gene MPS test, and the 18-gene MPS2 models.

- Under a testing approach with 95% sensitivity for PCa of GG 2 or greater, measures of diagnostic accuracy and clinical consequences of testing were calculated.
- Cancers of GG 3 or greater were assessed secondarily.

Tosoian et al. summated the results as follows:

- Out of 761 men included in the development cohort, the median (IQR) age was 63 (58-68) years, and the median (IQR) PSA level was 5.6 (4.6-7.2) ng/mL; of 743 men included in the validation cohort, the median (IQR) age was 62 (57-68) years, and the median (IQR) PSA level was 5.6 (4.1-8.0) ng/mL.
- In the validation cohort, 151 (20.3%) had high-grade PCa on biopsy.
- Area under the receiver operating characteristic curve values were 0.60 using PSA alone, 0.66 using the risk calculator, 0.77 using PHI, 0.76 using the derived multiplex 2-gene model, 0.72 using the derived multiplex 3-gene model, and 0.74 using the original MPS model compared with 0.81 using the MPS2 model and 0.82 using the MPS2+ model.
- At 95% sensitivity, the MPS2 model would have reduced unnecessary biopsies performed in the initial biopsy population (range for other tests, 15% to 30%; range for MPS2, 35% to 42%) and repeat biopsy population (range for other tests, 9% to 21%; range for MPS2, 46% to 51%).
- Across pertinent subgroups, the MPS2 models had negative predictive values of 95% to 99% for cancers of GG 2 or greater and of 99% for cancers of GG 3 or greater.

Tosoian et al. made the ensuing conclusions including iteration of point of relevance as follows:

- In their study, a new 18-gene PCa test had higher diagnostic accuracy for high-grade PCa relative to existing biomarker tests.
- Clinically, use of this test would have meaningfully reduced unnecessary biopsies performed while maintaining highly sensitive detection of high-grade cancers.
- These data support use of this new PCa biomarker test in patients with elevated PSA levels to reduce the potential harms of PCa screening while preserving its long-term benefits.

Kohaar et al. made the ensuing iterations:

- Prostate cancer is the most prevalent non-skin cancer in men and is the leading cause of cancer-related death.
- Early detection of prostate cancer is largely determined by a widely used prostate specific antigen (PSA) blood test and biopsy is performed for definitive diagnosis.
- Prostate cancer is asymptomatic in the early stage of the disease, comprises of diverse clinical-pathological and progression features, and is typified by a large subset of the indolent cancer type.
- In view of this it is critical to develop an individualized approach for early detection, disease stratification (indolent versus. aggressive), and prediction of treatment response for prostate cancer.
- There had been remarkable progress in prostate cancer biomarker discovery, largely through advancements in genomic technologies.
- A rich array of prostate cancer diagnostic and prognostic tests had emerged for serum (4K, phi), urine (Progensa, *T2-ERG*, ExoDx, SelectMDx), and tumour tissue (ConfirmMDx, Prolaris, Oncoytype DX, Decipher).
- The development of these assays has created new opportunities for the improvement of prostate cancer diagnosis, prognosis, and treatment decisions.
- While opening exciting opportunities, these developments also pose unique challenges in terms of selecting and incorporating these assays into the continuum of prostate cancer patient care.

Lih et al. made the ensuing iterations:

- Majority of patients with indolent prostate cancer (PCa) could be managed with active surveillance.
- Therefore, finding biomarkers for classifying patients between indolent and aggressive PCa is essential.

Kohaar et al. in their study, investigated urinary marker panels composed of urinary glycopeptides and/or urinary prostate-specific antigen (PSA) for their clinical utility in distinguishing non-aggressive (Grade Group 1) from aggressive (Grade Group ≥ 2) PCa. Urinary glycopeptides acquired via data-independent acquisition mass spectrometry (DIA-MS) were quantitatively analysed by Kohaar et al. where prostatic acid phosphatase (ACPP), clusterin (CLU), alpha-1-acid glycoprotein 1 (ORM1), and CD antigen 97 (CD97) were selected to be evaluated in various combinations with and without urinary PSA. Targeted parallel reaction monitoring (PRM) assays of the glycopeptides from urinary ACPP and CLU were investigated along with urinary PSA for the ability of aggressive PCa detection. The multi-urinary marker panels, combined via logistic regression, were statistically evaluated using bootstrap resampling and validated by an independent cohort. Kohaar et al. [83] summated the results as follows:

- Majority of the multi-urinary marker panels (for example, a panel consisted of ACPP, CLU, and Urinary PSA) achieved area under the curve (AUC) ranged from 0.70 to 0.85.

Kohaar et al. concluded that:

Multi-marker panels investigated in the study showed clinically meaningful results on aggressive PCa detection to separate Grade Group 1 from Grade Group 2 and above warranting further evaluation in clinical setting in future.

Dinges et al. made the ensuing iterations:

- Urinary tests have been used as non-invasive, cost-effective tools for screening, diagnosis and monitoring of diseases since ancient times.
- As we progress through the 21st century, modern analytical platforms had enabled effective measurement of metabolites, with promising results for both a deeper understanding of cancer pathophysiology and, ultimately, clinical translation.
- The first study to measure metabolomic urinary cancer biomarkers using NMR and mass spectrometry (MS) was published in 2006 and, since then, these techniques have been used to detect cancers of the urological system (kidney, prostate and bladder) and nonurological tumours including those of the breast, ovary, lung, liver, gastrointestinal tract, pancreas, bone and blood.

- This growing field warrants an assessment of the current status of research developments and recommendations to help systematize future research.

Dinges et al. summated the ensuing Key points from their study:

- Initial NMR and mass spectrometry (MS) studies of human urine identified biomarkers that can distinguish patients with cancer from healthy controls and outperform many current clinical markers, possibly enabling early detection.
- Biomarker panels can be used to identify a single type of cancer, stratify grade and stage, differentiate between multiple cancer types and perform longitudinal evaluations.
- A similar set of urinary metabolites (hippurate, creatine, tyrosine, citrate, isoleucine, phenylalanine, putrescine, succinate, tryptophan and valine) can indicate malignancy of various organs, possibly reflecting the global metabolic effects of cancer.
- The lack of specificity means that caution must be exercised and that many biomarkers could be too nonspecific for clinical application.
- Methodological variations impair comparability of existing studies, highlighting the need for guidelines.
- The expense of NMR and MS instrumentation means that a centralized testing hub might provide the best solution for eventual clinical implementation of cancer urinary biomarkers.

Svensen et al. made the ensuing iterations:

- Urine proteins can serve as viable biomarkers for diagnosing and monitoring various diseases.
- A comprehensive urine proteome database, generated from a variety of urine samples with different disease conditions, can serve as a reference resource for facilitating discovery of potential urine protein biomarkers.

Swensen et al. presented a urine proteome database generated from multiple datasets using 2D LC-MS/MS proteome profiling of urine samples from healthy individuals (HI), renal transplant patients with acute rejection (AR) and stable graft (STA), patients with non-specific proteinuria (NS), and patients with prostate cancer (PC). Swensen et al. summated the results as follows:

- A total of ~28,000 unique peptides spanning ~2,200 unique proteins were identified with a false discovery rate of <0.5% at the protein level.
- More than one third of the annotated proteins were plasma membrane proteins and another one third were extracellular proteins according to gene ontology analysis.
- Ingenuity Pathway Analysis of these proteins had revealed 349 potential biomarkers in the literature-curated database.
- Forty-three percentage of all known cluster of differentiation (CD) proteins were identified in the various human urine samples.
- Interestingly, pursuant to comparisons with five recently published urine proteome profiling studies, which applied similar approaches, there are still ~400 proteins which are unique to this current study. These may represent potential disease-associated proteins. Among them, several proteins such as serpin B3, renin receptor, and periostin had been reported as pathological markers for renal failure and prostate cancer, respectively.

Swensen et al. concluded that:

- Taken together, our data should provide valuable information for future discovery and validation studies of urine protein biomarkers for various diseases.

Smelik et al. made the ensuing iterations:

- Early cancer diagnosis is crucial but challenging owing to the lack of reliable biomarkers that can be measured using routine clinical methods.
- The identification of biomarkers for early detection is complicated by each tumour involving changes in the interactions between thousands of genes.
- In addition to this staggering complexity, these interactions could vary among patients with the same diagnosis as well as within the same tumour.
- They had postulated that that reliable biomarkers that can be measured with routine methods could be identified by exploiting three facts: (1) the same tumour can have multiple grades of malignant transformation; (2) these grades and their molecular changes can be characterized using spatial transcriptomics; and (3) these changes can be integrated into models of malignant transformation using pseudo-time.

Svensen et al. constructed Pseudo-time models based upon spatial transcriptomic data from three independent prostate cancer studies to prioritize the genes that were most correlated with malignant transformation. Svensen et al. identified the genes that were associated with cancer grade, copy number aberrations, hallmark pathways, and drug targets, and they encoded candidate biomarkers for prostate cancer in mRNA, immunohistochemistry, and proteomics data from the sera, prostate tissue, and urine of more than 2,000 patients with prostate cancer and controls. Svensen et al. summated the results as follows:

- Machine learning-based prediction models had revealed that the biomarkers in urine had an AUC of 0.92 for prostate cancer and were associated with cancer grade.

Svensen et al. concluded that:

- Overall, their study had demonstrated the diagnostic potential of combining spatial transcriptomics, pseudo-time, and machine learning for prostate cancer, which should be further tested in prospective studies.

Conclusions

- There is an urgent need to identify more accurate non-invasive tests for the diagnosis of prostate cancer and to enable the stratification of patients who have life-threatening prostate cancer.
- In view of the ease of collection, and the fact that prostate cells are directly released into the urethra via prostatic ducts after digital rectal examination (DRE) or prostate massage, urine has now become the future of non-invasive biomarker testing.
- Many studies have demonstrated the feasibility of urine for the non-invasive detection of prostate cancer.
- It had been demonstrated that RNA biomarkers could also be identified in urinary exosomes, making them promising for biomarkers research as well.
- Biomarker research is in focus at many laboratories and many biomarkers are promising due to their specificity for the disease in tissue.
- Only few of these biomarkers had been shown to be useful as urinary marker.

- Two prostate-specific RNA-based biomarkers have been found (PCA3 and TMPRSS2-ERG gene fusions).
- The recent FDA approval of PCA3 had led to its introduction in clinical practice and the combination of both markers has been marketed for clinical use as well.
- In comparison with single biomarkers, the combination of many biomarkers considerably improves the prediction of prostate cancer within urine samples which is consistent with the heterogeneity of the disease.
- In the era of individualized therapy, the biomarker combinations are necessary to not only predict prostate cancer at biopsy; nevertheless, also the aggressiveness of the cancer.
- Preliminary results had shown that the prostate cancer (PCa)-specific TMPRSS2-ERG gene fusion could be indicative of aggressiveness of cancer upon biopsy, although further studies are warranted.
- In PCa biomarker development, the greatest unmet need has remained: a biomarker that stratifies men at risk of aggressive PCa, eventually leading to a reduction in the undertaking of unnecessary interventions.

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