Biomarkers in Prostate Cancer Diagnosis

Subjects: Oncology

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Early detection of prostate cancer (PC) is largely carried out using assessment of prostate-specific antigen (PSA) level; yet it cannot reliably discriminate between benign pathologies and clinically significant forms of PC. Exosomes are extracellular vesicles that are secreted from all mammalian cells and virtually detected in all bio-fluids, thus allowing their use as tumor biomarkers.

prostate cancer biomarkers metabolomics exosomes early diagnosis

1. Biomarkers in Prostate Cancer: Current Limitations

Prostate cancer (PC) is the most commonly diagnosed cancer in men, principally affecting men over 50 years old, and is the leading cause of cancer-related deaths in men [1]. Furthermore, PC and subsequent treatments have a high impact on both functional and psychological status, significantly affecting patients' quality of life (QoL) [2]. Early detection of PC is largely carried out using assessment of prostate-specific antigen (PSA) level in blood complemented by digital rectal examination (DRE). Regrettably, PSA cannot reliably discriminate between benign prostatic hyperplasia (BPH) or prostatitis and clinically significant forms of PC, due to its limited sensitivity and specificity [3].

In 2012, the US Preventive Services Task Force (USPSTF) released a recommendation against PSA screening [4], which resulted in a reduction in the use of PSA for early detection. This strategy and recommendation led to a rise in the incidence of advanced disease and, possibly, PC cancer-related mortality after 2012 [5][6]. In 2018, the USPSTF published an updated statement suggesting that men aged 55–69 should be informed about the benefits and harms of PSA-based screening, discouraging this program for men over 70 years old [7]. A comparison of systematic and opportunistic screening suggested over-diagnosis and mortality reduction in the systematic screening group, compared to a higher over-diagnosis in the opportunistic screening regimen [8].

Over the past few years, the urgency to find an alternative approach for an early and non-invasive detection of PC, as well as for a proper discrimination between PC and several prostatic benign pathologies, has become clear. PC is a highly heterogeneous neoplasm, with many men presenting with an indolent course, while others present with a rapidly progressive disease. Due to the clinical heterogeneity of PC present in clinical practice, the analysis of the metabolic profile of PC samples is highly dispersed. Indolent PC cases with a Gleason score (GS) of 6 can display a low aggressiveness and low propensity for growth and progression; it is possible that the metabolic profile of these indolent PC cases is closer to that of BPH cases. On the contrary, clinically significant PC (csPC) cases, and in particular those with a GS of 8 or higher, often show rapid growth and progression, probably sustained by a

different metabolic profile. Moreover, patients with PC usually have various extents of concurrent BPH in the transition and periurethral zones of the prostate. The determination in bio-fluids of current markers, such as PSA and its derivates, continue to be unable to properly discriminate between these two coexisting entities. The analysis of possible biomarkers, enclosed in extracellular nanovesicles released in the same bio-fluids (exosomes) rather than freely circulating, could increase their specificity and accuracy in discriminating between neoplastic and benign hyperplastic prostatic modifications [9].

2. Role of Different Bio-Fluids on PC Biomarkers

2.1. Urinary or Serum Biomarkers: Which Are Better?

In recent years, new urinary and serum biomarkers have been developed, with the goal of overcoming the current limitations of PSA, mainly represented by a low specificity, which has led to unnecessary biopsies and over-diagnosis and over-treatment of indolent PC cases [10][11][12]. Ideally, to be useful in clinical practice, a tumor biomarker should present the following characteristics: first and most importantly, it should be relatively specific for PC, and not affected by other benign conditions; second, it should be useful in all steps of the natural history of the disease (i.e., from diagnosis to follow-up after initial and subsequent therapy) and, in this context, it should be accurate in distinguishing csPC from indolent cases. Finally, biomarkers should be cost-effective, and not invasive in the method of collection [13]. In the last ten years, a better knowledge of the genetic and epigenetic mechanisms involved in PC biology has led to the availability of new urinary and plasma markers in clinical practice [14]. Although several biomarkers have been explored in various scenarios and patient settings—with the aim to identify more sensitive and specific biomarkers for detecting and monitoring PC—to date, specific guidelines with a high level of evidence on the use of these markers are lacking, mainly due to limitations inherent to both plasma and urine samples. Moreover, before widely implementing them in the different phases of patient care, there are several open questions waiting to be answered: What are the advantages of blood and urinary routes, respectively? What are the scenarios in which one biomarker is more useful than another? What is the impact—and its magnitude—of the interplay with other decision tools, such as imaging?

2.2. Urinary Biomarkers

Recent advances in metabolomic, genomics, and proteomics have made new potential biomarkers available, virtually in all fields of oncology. In the field of PC, these advances have led to a renewed interest in urine as a valuable biomaterial source of new markers [15]. Indeed, PC cells or substances derived from PC cells can be found in prostatic fluids—and therefore in urine samples—both directly and after prostatic massage by DRE. Therefore, urine can represent a source of prostate cells, proteins, DNA, and RNA, with the potential to serve as markers for the detection and follow-up of PC [16]. Urine has become one of the most attractive bio-fluids in clinical proteomics. Compared with other clinical biological specimens, such as blood samples, urine provides many advantages for the determination of both diagnostic and prognostic biomarkers (Table 1).

Table 1. Advantages and limitations of serum and urinary biomarkers.

Advantages	Critical Issues	Availability	Potential Clinical Utility
Serum Biomarkers: PHI, 4K scores			
Easy to perform Reproducible	High risk of confounding factors Include PSA for interpretation Include clinical variables (4Kscore) Uncertain reference range and ethnic variability (PHI)	PHI: FDA-approved 4K: CLIA-certified	Primary Diagnosis (biopsy-naïve/repeat biopsy) Diagnosis of csPC AS
Urinary Biomarkers: PCA3, SelectMDx, MiPS, ExoDx			
Easy to collect Large quantities Reproducible Fewer confounding elements	Need DRE (not ExoDx) Visit to a health-care provider to obtain the urine sample (not ExoDx) Difficult to collect cells derived from PC Include clinical variables (SelectMDx) Uncertain cut-off value (PCA3)	PCA3: FDA-Approved SelecMDx, MiPS, and ExoDx: CLIA-certified	Primary Diagnosis (biopsy-naïve/repeat biopsy) Diagnosis of csPC AS

Abbreviations list: PHI = Prostate Health Index; 4K = four-kallikrein; PCA3 = Prostate Cancer Antigen 3; PSA = prostate-specific antigen; DRE = digital rectal examination; PC = prostate cancer; csPC = clinically significant PC; AS = active surveillance; FDA = Food and Drug Administration; CLIA = Clinical Laboratory Improvement Amendments.

First, urine is easy to collect—recurrently and in large quantities—without any risk or harm to the patient [17]. In addition, since it is not associated with significant proteolytic degradation and has a less complex composition compared to serum or plasma, the presence of fewer confounding elements facilitates the isolation process and thus the evaluation of biomarkers [16]. With regard to its application as a source of biomarkers for localized and

early-stage PC, urine may be more appropriate than blood, as it contains markers from virtually all human tissues [16]. Moreover, urine does contain materials coming directly from the prostate gland, and it does not require crossing of blood–tissue barriers. Despite the advantages of urinary flow, only a few biomarkers are currently available and approved by regulatory authorities. The first and only FDA-approved urinary biomarker for PC is the Progensa Prostate Cancer Antigen 3 (PCA3) assay, which measures the concentration of PCA3 and PSA messenger RNAs (mRNA) levels by transcription-mediated amplification, using 2.5 mL of post-DRE urine. A PCA3 score is generated by calculating the ratio of PCA3:PSA mRNA, the latter being used as a method of normalizing for the amount of prostate material within the total volume of urine [18]. Since its introduction into clinical practice, it has shown promising results for PC detection, staging, and prognosis [19]. A recent meta-analysis showed that the sensitivity of the PCA3 test was 46.9–82.3%, and the specificity was 56.3–89% for primary diagnosis, and similar results were reported for csPC [20]. Moreover, PCA3 has proven to be useful in the context of active surveillance (AS), in which the PCA3 scores obtained at the first biopsy and during AS protocol were significantly higher in patients with Gleason grade reclassification than in those without [21]. Despite the clinical scenarios in which it has been tested, at present, PCA3 is only approved for patients with a previous negative biopsy, probably due to the fact that the definition of the best discriminating cut-off value is controversial—which has made the available studies very heterogeneous, especially in the setting of biopsy-naïve patients [22]. Indeed, several studies have highlighted the fact that PCA3 does not work well with a single threshold, showing a high NPV below a low threshold, and a high PPV above a high threshold, with a gray zone in between—which is reflective of the reality of PC biology [22][23]. These limitations might be in part overcome by combining multiple gene analysis, such as SelectMDx or Mi Prostate score [15]. SelectMDx measures post-DRE mRNA transcripts from the HOX6 and DLX1 genes in combination with other risk factors, such as age, DRE, PSA, PSA density, and family history [24]. SelectMDx has shown promising results for the initial diagnosis, with an AUC of 0.90 in the diagnosis of csPC [25]. Similar results in terms of specificity and sensibility were reported for Mi Prostate Score, which combines PCA3 with TMPRSS2-ERG and serum PSA [26]. ExoDx Prostate IntelliScore is a test that measures PCA3 and ERG RNA expression in exosomes in voided urine, without the need for a prior DRE—and thus without the need of a health-care provider to obtain the sample [27][28]. It was found to provide additional predictive accuracy above a clinical model to predict csPC, with an AUC of 0.80 [27]. In a prospective series, the addition of the gene expression model increased diagnostic performance of csPC significantly, compared to the current standard of care (AUC 0.73) [28].

Given the promising results of these urinary markers, to implement their use in clinical practice, some critical issues should be resolved in the future. First and most importantly, to overcome PSA limitations, future studies on new urinary biomarkers should be more focused on the diagnosis of csPC, since a biomarker that merely detects any PC will not be sufficient to improve patient care. Second, the role of DRE for the collection of urinary samples should be further investigated; yet it still remains debated, adding an element of variability among clinical studies.

2.3. Serum Biomarkers

Compared with urinary biomarkers, in the last years only a few blood biomarkers have been proposed and tested in PC patients, and only one is approved in clinical practice by the Food and Drug Administration (FDA). This could be partially explained by the fact that a serum biomarker should have specific characteristics, yet blood contains

markers from all tissues—with a high risk of confounding factors. Moreover, blood should contain substances exclusively produced by the prostate, like PSA, and ideally not conditioned by other pathologies that can affect the prostate itself. Finally, it should be more specific than PSA for csPC. Currently, many of the new serum markers under investigation include the use of total PSA (tPSA) or free PSA (fPSA) in the analysis and interpretation of the results, leading to the issue of whether these new markers may suffer from the same limitations as PSA [14]. Ideally, optimal PC screening risk stratification requires molecular subtyping to yield information on disease biology, prognosis, and treatment benefits [29]. The prostate health index (PHI) assay and four-kallikrein (4Kscore) test have been recently developed and tested in several clinical studies, including primary diagnosis and monitoring after therapy [30]. Both tests use combinations of different serum PSA isoforms and/or related proteins to increase PC-specific sensitivity. PHI was the first FDA-approved new blood serum assay, which combines the levels of tPSA, fPSA, and p2PSA (a PC-specific fPSA isoform) [31]. Following FDA approval, several studies have focused on the comparison between the diagnostic performance of PHI and the f/tPSA ratio in different clinical settings. In the context of primary diagnosis, a large multicenter study involving more than 800 patients with PSAs between 2 and 10 ng/mL, PHI showed an AUC of 0.70 for the detection of any PC and 0.72 for csPC, highlighting its potential clinical utility for AS [31]. In this context, PHI is not recommended by scientific societies as a diagnostic tool for predicting biopsy reclassification in men under AS. However, a recent metanalysis showed a pooled sensitivity of 0.90 and specificity of 0.17 for PHI in the detection of high-grade PC [32]. The four-kallikrein (4Kscore) test is a Clinical Laboratory Improvement Amendments (CLIA) certified serum-based test that combines the levels of tPSA, fPSA, intact PSA, human kallikrein 2 (KLK2), and clinical information to obtain a risk stratification index indicating whether the patient has a csPC [33]. Similar to PHI, 4Kscore showed good accuracy for both primary diagnosis and prediction of csPC [34].

Table 2 summarizes data on diagnostic performance of new markers in various clinical scenarios reported from previous studies.

Table 2. Diagnostic performance of new markers in various clinical scenarios.

Biomarkers	Primary Diagnosis of PC (No. of pts, Inclusion Criteria, AUC Results)	Primary Diagnosis Repeat Biopsy (No. of pts, Inclusion Criteria, AUC Results)	Diagnosis of csPC (No. of pts, Inclusion Criteria, AUC Results)	Active Surveillance (No. of pts, Inclusion Criteria, AUC Results)
Serum				
PHI	No. pts 892	No. pts 95	No. pts 658	No. pts 253

Biomarkers	Primary Diagnosis of PC (No. of pts, Inclusion Criteria, AUC Results)	Primary Diagnosis Repeat Biopsy (No. of pts, Inclusion Criteria, AUC Results)	Diagnosis of csPC (No. of pts, Inclusion Criteria, AUC Results)	Active Surveillance (No. of pts, Inclusion Criteria, AUC Results)
	PSA 2–10 ng/mL	AUC 0.72 [35]	PSA 4–10 ng/mL	AUC 0.65 for GR [36]
	AUC 0.72 [31]		AUC 0.71 [37]	
	No. pts 658	No. pts 391	No. pts 769	
	PSA 4–10 ng/mL	PSA 2–10 ng/mL	PSA 2–10 ng/mL	
	AUC 0.71 [37]	AUC 0.78 [38]	AUC 0.72 all (0.68 initial biopsy, 0.78 repeat biopsy) [38]	
	No. pts 300	No. pts 110		
	PSA 2–10 ng/mL	PSA 2–20 ng/mL		
	AUC 0.77 [39]	AUC 0.69 [40]		
4K Score	No. pts 749	No. pts 925	No. pts 749	No. pts 718
	PSA > 3 ng/mL	PSA > 3 ng/mL	PSA > 3 ng/mL	AUC 0.78 for GR [41]
	AUC 0.69 including age and DRE [42]	AUC 0.68 including age,	AUC 0.78 including age and DRE [42]	

Biomarkers	Primary Diagnosis of PC (No. of pts, Inclusion Criteria, AUC Results)	Primary Diagnosis Repeat Biopsy (No. of pts, Inclusion Criteria, AUC Results)	Diagnosis of csPC (No. of pts, Inclusion Criteria, AUC Results)	Active Surveillance (No. of pts, Inclusion Criteria, AUC Results)
		PSA, DRE [43]		
	No. pts 531		No. pts 531	
	PSA 3–15 ng/mL		PSA 3–15 ng/mL	
	AUC 0.69 including age [34]		AUC 0.71 including age [34]	
	No. pts 740		No. pts 740	
	PSA > 3 ng/mL		PSA > 3 ng/mL	
	AUC 0.83 including age, PSA, DRE [44]		AUC 0.90 including age, PSA, DRE [44]	
			No. pts 925	
			PSA > 3 ng/mL	
			AUC 0.87 including age, PSA, DRE [43]	
		Urinary		

Biomarkers	Primary Diagnosis of PC (No. of pts, Inclusion Criteria, AUC Results)	Primary Diagnosis Repeat Biopsy (No. of pts, Inclusion Criteria, AUC Results)	Diagnosis of csPC (No. of pts, Inclusion Criteria, AUC Results)	Active Surveillance (No. of pts, Inclusion Criteria, AUC Results)
PCA3	No. pts 300	No. pts 48	No. pts 497	No. pts 552
	PSA 2–10 ng/mL	PSA 2.5–6.5 ng/mL	PSA > 3 ng/mL	AUC for GR 0.61 [45]
	AUC 0.73 [39]	AUC 0.79 [46]	AUC 0.53 [47]	
	No. pts 497	No. pts 470	No. pts 905	No. pts 294
	PSA > 3 ng/mL	Any PSA	PSA > 3 ng/mL	AUC for GR 0.58 [48]
	AUC 0.72 [47]	AUC 0.65 [49]	AUC 0.65 [25]	
	No. pts 578 PSA <50 ng/mL AUC 0.75, PSA 4–10 ng/mL, AUC 0.74 [50]	No. pts 103 Any PSA AUC 0.64 [51]	No. pts 138 PSA 4–20 ng/mL AUC 0.55 [52]	
SelectMDx	No. pts 52		No. pts 114	No. pts 125
	PSA > 3 ng/mL		PSA > 3 ng/mL	AUC for GR 0.70 [53]

Biomarkers	Primary Diagnosis of PC (No. of pts, Inclusion Criteria, AUC Results)	Primary Diagnosis Repeat Biopsy (No. of pts, Inclusion Criteria, AUC Results)	Diagnosis of csPC (No. of pts, Inclusion Criteria, AUC Results)	Active Surveillance (No. of pts, Inclusion Criteria, AUC Results)
	AUC 0.92 [54]		AUC 0.67 [55]	
	[62][63]		No. pts 905 PSA > 3 ng/mL AUC 0.76 [25]	[58][59][60][61]
MiPS	No. pts 1225 PSA > 3 ng/mL AUC 0.75 [26]		No. pts 1225 PSA > 3 ng/mL AUC 0.7 [26]	
ExoDX	[64] [65] No. pts 195 [65] PSA 2–10 ng/mL AUC 0.73 [27]		No. pts 195 PSA 2–10 ng/mL AUC 0.80 [27]	
			No. pts 519 PSA 2–10 ng/mL	

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preparation, such as separation or derivatization. Another strength of this technique is the high reproducibility; however, although the sensitivity of NMR spectroscopy has increased enormously, this remains a weak point for NMR compared with MS. MS-based metabolomics provides an excellent approach that can offer a combined

Biomarkers	Primary Diagnosis of PC (No. of pts, Inclusion Criteria, AUC Results)	Primary Diagnosis Repeat Biopsy (No. of pts, Inclusion Criteria, AUC Results)	[66][67] Diagnosis of csPC (No. of pts, Inclusion Criteria, AUC Results)	Active Surveillance (No. of pts, Inclusion Criteria, AUC Results)
			AUC 0.73 [28]	

complex biological sample. The MS-based metabolomics approach is the method of choice for targeted analysis compared to the NMR-based approach. Instead, untargeted approaches provide the most appropriate route to detect unexpected changes in metabolite concentrations, maximizing the number of identified metabolites. In fact, in untargeted analysis, it is possible to detect hundreds to thousands of metabolites. Moreover, no laborious sample preparation is required compared to targeted analysis.

Untargeted approaches are usually employed when observational studies are performed, with the purpose of determining still unraveled possible biomarkers. These studies are generally performed on relatively small, but statistically significative, sets of samples. By limiting the manipulation of the samples, the broadest variety of compounds is considered. Due to the extreme complexity of biological samples, however, several minor compounds are consistently masked by high-abundance species. In a recent paper by Cerrato et al., an untargeted metabolomics study of zwitterionic and positively charged compound was set up thanks to a prior sample pretreatment step [68]. A cornerstone of any metabolomics study is the acquisition of high-quality data. This involves careful planning of experiments, analytical measurements, data processing, and statistical/chemometric analysis. Chemometrics is fundamental for obtaining reliable results after NMR and MS analyses, which provide a large amount of data. Statistical modelling, such as univariate statistical testing, multivariate regression methods (i.e., principal components analysis, partial least squares, or orthogonal projections to latent structures), cluster analysis, machine learning techniques, and non-linear methods are commonly employed for classification purposes, and selection of under—or over—expressed compounds associated with two different sets of samples [69]. Unsupervised approaches, e.g., principal component analysis, are employed for data overview for revealing outliers, groups, and trends in the groups. Conversely, supervised approaches, e.g., partial least square discriminant analysis and orthogonal projections to latent structures, are employed for building models and highlight the putative biomarkers [69]. Whenever supervised approaches are employed, particular attention on model validation must be paid to make sure that the model is not overfitted [70]. In a recent paper by Amante et al. [71], untargeted mass spectrometric data were processed by partial least square discriminant analysis in repeated double-cross validation. Untargeted approaches, therefore, counterbalance the use of small sets of samples with the need for high-performance instrumentation, multiple expertise, extensive database, and manual interpretation of the spectra. Conversely, targeted approaches are performed on generally larger patient cohorts, with a particular attention to compounds that are suspected to be linked to PC. A previous large-scale targeted study of 188

selected metabolites was performed on 777 patients, highlighting that lysophosphatidylcholines were associated with overall risk of PC [72].

PC is a disease of great interest from a metabolomics perspective for prediction, diagnosis, progression, and prognosis. A number of studies focused on the characterization of the specific PC metabolic phenotype using different experimental approaches have been recently reported (Table 3). Moreover, metabolomics approaches have been employed for determining biomarkers of PC recurrence [73]. In particular, choline phosphate has been identified as a major indicator of PC recurrence in a work by Maxeiner et al. [74]. Similarly, thioamino acid derivatives, namely cysteine, homocysteine, and cystathionine, were found to provide an increased ability in detecting recurrence over the sole clinical indices [75].

Table 3. Metabolomics studies focused on the analysis of bio-fluids to identify clinically relevant prostate cancer biomarkers.

Source	Experimental Approach	Sample Cohort	Main Findings	Ref
Tissue	HR-MAS combined with multivariate analysis (PLS, PLS–DA) and absolute quantification (LCModel)	no. pts = 48	Low levels of spermine and citrate are correlated with PC aggressiveness.	[76]
Prostatic fluid	1H NMR spectroscopy coupled to multiple regression analysis	no. pts = 38	Significance differences between citrate and spermine ratio in PC.	[77]
Serum	1H NMR spectroscopy coupled to multivariate analysis	no. pts = 210	Glycine, sarcosine, alanine, creatine, xanthine, and hypoxanthine were able to determine abnormal prostate (BPH + PC).	[78]
Tissue, urine, and plasma	UHPLC-MS and GC–MS	no. pts = 110	Sarcosine and N-methyl derivative of glycine were highly elevated during PC progression to metastasis.	[79]
Tissue	1H HR-MAS spectroscopy	no. pts = 20	High choline and phosphocholine levels, along with an increase in the glycolytic products lactate and alanine in PC.	[80]

Source	Experimental Approach	Sample Cohort	Main Findings	Ref
Urine	UHPLC-MS/MS coupled to ROC curve analysis	no. pts = 148	Kynurenic acid was found a promising biomarker for PC detection. Sarcosine was not found as significant biomarker for the diagnosis of PC.	[81]
Serum and urine	LC–ESI–MS/MS technique and the aTRAQ reagent couple to ROC and multivariate (PLS–DA) analyses	no. pts = 89	Ethanolamine, arginine markers for PC.	[82]
Urine	ID GC/MS couple to PCA and ROC analyses	no. pts = 48	Sarcosine has no statistical difference between the PC group and in the non-PC group. Decreased urinary levels of glycine, threonine, and alanine was observed in PC group.	[83]
Urine	HPLC–TOF/MS in positive and negative polarity as well as GC–QqQ/MS couple to PCA and PLS–DA analyses	no. pts = 64	Altered levels of urinary metabolites involved in such biochemical pathways like AA, purine and glucose metabolism as well as urea and TCA cycle may be considered as potential markers of PC.	[84]
Serum	LC–MS and GC–MS	no. pts = 400	PC risk was correlated with the levels of α-ketoglutarate, thyroxine, TMAO, and erucoyl-sphingomyelin; metabolites involved in the metabolism of nucleotides, steroid hormones, and tobacco were associated with non-aggressive PC.	[85]

Abbreviations list: Ref = reference; no. pts = number of patients; PC = prostate cancer; pts = patients; HR-MAS = high resolution magic angle spinning MRS; UHPLC-MS = high throughput liquid mass spectrometry; GC–MS = gas chromatography-based mass spectrometry; NMR = nuclear magnetic resonance; ROC = receiving operating characteristics; PLS–DA = partial least squares–discriminant analysis; PCA = principal component analysis; ID

2021, 71, 7–33.

2. Maggi, M.; Gentilucci, A.; Salciccia, S.; Gatto, A.; Gentile, V.; Colarieti, A.; Von Heland, M.; Busetto, G.M.; Del Giudice, F.; Sciarra, A. Psychological impact of different primary treatments for prostate cancer: A critical analysis. Andrologia 2019, 51, e13157.

3. Etzioni, R.; Gulati, R.; Cooperberg, M.R.; Penson, D.M.; Weiss, N.S.; Thompson, I.M. Limitations of basing screening policies on screening trials: The US Preventive Services Task Force and Prostate Cancer Screening. Med. Care 2013, 51, 295–300.

GC/MS = isotope dilution gas chromatography/mass spectrometry; UPLC-TOF/MS = high performance liquid chromatography coupled with time of flight mass spectrometry; GC–QqQ/MS = gas chromatography coupled with triple quadruple mass spectrometry; BPH = benign prostate hypertrophy; RP = radical prostatectomy; PSA = prostate-specific antigen; US = ultra-sound; TMAO = trimethylamine oxide; TCA = tricarboxylic acid.

4. Can Exosomes Analysis Improve PC Biomarkers Performance?

Exosomes (Exos) are a broad and heterogeneous group of small membrane-limited extracellular vesicles (EVs) (40–180 nm in diameter) that are released from almost all mammalian cells, in both normal and pathological processes, and are thus virtually detected in all bio-fluids, including plasma and urine [86][87][88][89]. Exos are generated from the membrane invagination of endosomes and are secreted in the microenvironment after multivesicular body (MVB) fusion with the plasma membrane [86][87][90]. For this reason, Exos show specific markers obtained by budding from the endosome membranes, such as tetraspanins (CD63, CD9, and CD81), heat shock proteins (HSP70), and compounds from the Rab family, Tsg101 and Alix [86][87][88][91][92], and also other markers obtained during the process of fusion with the plasma membrane [86][87]. Moreover, Exos show a particular lipid bilayer membrane and they contain nucleic acids (i.e., DNAs, mRNAs, and microRNAs (miRNAs)) [86][87]. Cells from several organ systems (e.g., hematopoietic, gastrointestinal, neurons), as well as cancerous cells, can secrete vesicles extracellularly [91][93]. A growing body of evidence has highlighted the role of Exos as mediators of cell-to-cell communications [86][87][88][94][95][96], as well as in modulating microenvironments. Because it has been demonstrated that Exos act in the pathophysiology of different human pathologies—including cancer—they have become a promising source of disease biomarkers [86][87][97][98].

To date, nanoparticle tracking analysis (NTA), immune captured based technologies, and nanoscale flow cytometry (NFC) represent new technologies to analyze EVs, which could allow valid information—both quantitative and qualitative—for clinical application [9][99].

Different neoplasms have shown some common features, such as hypoxic conditions, low nutrient supply, and extracellular acidosis [100][101][102]. Strikingly, it has been demonstrated that, independent of the tumor histology and type, Exos are secreted in larger quantities, as well as with a smaller size, when cultured in vitro under acidic pH (6.5) compared to a physiological pH (7.4) [89]. This phenomenon is comparable to the increased plasmatic Exo levels detected in PC patients when compared to inflammatory conditions of BPH patients [99]. Given this evidence, it is possible that tumor microenvironmental acidity is responsible for the increased Exo release in cancer conditions.

In both preclinical studies and pilot clinical trials on PC, Exo, under acidic conditions express ions transporters, such as Carbonic Anhydrase IX (CA-IX), which on Exo exerts a full enzymatic function [103]. Increased CA-IX expression has been detected in Exo from plasma of PC patients [104], suggesting that the quantification of its exosomal expression and activity may be used as a potential cancer biomarker.

4. Moyer, V.A. U.S. Preventive Services Task Force. Screening for prostate cancer: U.S. Preventive Services Task Force recommendation statement. Ann. Intern. Med. 2012, 157, 120–134.
5. Kelly, S.P.; Anderson, W.F.; Rosenberg, P.S.; Cook, M.B. Past, Current, and Future Incidence Rates and Burden of Metastatic Prostate Cancer in the United States. Eur. Urol. Focus 2018, 4, 121–127.
6. Hu, J.C.; Nguyen, P.; Mao, J.; Halpern, J.; Shoag, J.; Wright, J.D.; Sedrakyan, A. Increase in Prostate Cancer Distant Metastases at Diagnosis in the United States. JAMA Oncol. 2017, 3, 705–707.
7. Grossman, D.C.; Curry, S.J.; Owens, D.K.; Bibbins-Domingo, K.; Caughey, A.B.; Davidson, K.W.; Doubeni, C.A.; Ebell, M.; Epling, J.W., Jr.; Kemper, A.R.; et al. Screening for Prostate Cancer: US Preventive Services Task Force Recommendation Statement. JAMA 2018, 319, 1901–1913.
8. Arnsrud Godtman, R.; Holmberg, E.; Lilja, H.; Stranne, J.; Hugosson, J. Opportunistic testing versus organized prostate-specific antigen screening: Outcome after 18 years in the Göteborg randomized population-based prostate cancer screening trial. Eur. Urol. 2015, 68, 354–360.
9. Logozzi, M.; Angelini, D.F.; Giuliani, A.; Mizzoni, D.; Di Raimo, R.; Maggi, M.; Gentilucci, A.; Marzio, V.; Salciccia, S.; Borsellino, G.; et al. Increased Plasmatic Levels of PSA-Expressing Exosomes Distinguish Prostate Cancer Patients from Benign Prostatic Hyperplasia: A Prospective Study. Cancers 2019, 11, 1449.
10. Cooperberg, M.R.; Carroll, P.R.; Dall'Era, M.A.; Davies, B.J.; Davis, J.W.; Eggener, S.E.; Feng, F.Y.; Lin, D.W.; Morgan, T.M.; Morgans, A.K.; et al. The State of the Science on Prostate Cancer Biomarkers: The San Francisco Consensus Statement. Eur. Urol. 2019, 76, 268–272.
11. Sciarra, A.; Voria, G.; Monti, S.; Mazzone, L.; Mariotti, G.; Pozza, M.; D'Eramo, G.; Silverio, F.D. Clinical understaging in patients with prostate adenocarcinoma submitted to radical prostatectomy: Predictive value of serum chromogranin A. Prostate 2004, 58, 421–428.
12. Sciarra, A.; Maggi, M.; Salciccia, S.; Nicolai, A.; Tortorella, E.; Giantulli, S.; Magliocca, F.M.; Silvestri, I.; Taglieri, L.; Cattarino, S.; et al. Tissue Expression of Androgen Receptor Splice Variant 7 at Radical Prostatectomy Predicts Risk of Progression in Untreated Nonmetastatic Prostate Cancer. Oncology 2021, 18, 1–5.
13. McShane, L.M.; Altman, D.G.; Sauerbrei, W.; Taube, S.E.; Gion, M.; Clark, G.M. Statistics Subcommittee of the NCI-EORTC Working Group on Cancer Diagnostics. REporting recommendations for tumour MARKer prognostic studies (REMARK). Br. J. Cancer 2005, 93, 387–391.
14. Hendriks, R.J.; van Oort, I.M.; Schalken, J.A. Blood-based and urinary prostate cancer biomarkers: A review and comparison of novel biomarkers for detection and treatment decisions. Prostate Cancer Prostatic Dis. 2017, 20, 12–19.

15. Fujita, K.; Nonomura, N. Urinary biomarkers of prostate cancer. Int. J. Urol. 2018, 25, 770–779.
16. Eskra, J.N.; Rabizadeh, D.; Pavlovich, C.P.; Catalona, W.J.; Luo, J. Approaches to urinary detection of prostate cancer. Prostate Cancer Prostatic Dis. 2019, 22, 362–381.
17. Hamade, M.; Davis, J.; Esquinas, V. Current state of the art for enhancing urine biomarker discovery. Expert Rev. Proteom. 2016, 13, 609–626.
18. Salagierski, M.; Verhaegh, G.W.; Jannink, S.A.; Smit, F.P.; Hessels, D.; Schalken, J.A. Differential expression of PCA3 and its overlapping PRUNE2 transcript in prostate cancer. Prostate 2010, 70, 70–78.
19. Auprich, M.; Bjartell, A.; Chun, F.; de la Taille, A.; Freedland, S.J.; Haese, A.; Schalken, J.; Stenzl, A.; Tombal, B.; van der Poel, H. Contemporary role of prostate cancer antigen 3 in the management of prostate cancer. Eur. Urol. 2011, 60, 1045–1054.
20. Luo, Y.; Gou, X.; Huang, P.; Mou, C. Prostate cancer antigen 3 test for prostate biopsy decision: A systematic review and meta analysis. Chin. Med. J. 2014, 127, 1768–1774.
21. Tosoian, J.J.; Patel, H.D.; Mamawala, M.; Landis, P.; Wolf, S.; Elliott, D.J.; Epstein, J.I.; Carter, H.B.; Ross, A.E.; Sokoll, L.J.; et al. Longitudinal assessment of urinary PCA3 for predicting prostate cancer grade reclassification in favorable-risk men during active surveillance. Prostate Cancer Prostatic Dis. 2017, 20, 339–342.
22. Luo, Y.; Gou, X.; Huang, P.; Mou, C. The PCA3 test for guiding repeat biopsy of prostate cancer and its cut-off score: A systematic review and meta-analysis. Asian J. Androl. 2014, 16, 487–492.
23. Wei, J.T.; Feng, Z.; Partin, A.W.; Brown, E.; Thompson, I.; Sokoll, L.; Chan, D.W.; Lotan, Y.; Kibel, A.S.; Busby, J.E.; et al. Can urinary PCA3 supplement PSA in the early detection of prostate cancer? J. Clin. Oncol. 2014, 32, 4066–4072.
24. Leyten, G.H.; Hessels, D.; Smit, F.P.; Jannink, S.A.; de Jong, H.; Melchers, W.J.; Cornel, E.B.; de Reijke, T.M.; Vergunst, H.; Kil, P.; et al. Identification of a candidate gene panel for the early diagnosis of prostate cancer. Clin. Cancer Res. 2015, 21, 3061–3070.
25. Van Neste, L.; Hendriks, R.J.; Dijkstra, S.; Trooskens, G.; Cornel, E.B.; Jannink, S.A.; de Jong, H.; Hessels, D.; Smit, F.P.; Melchers, W.J.; et al. Detection of high-grade prostate cancer using a urinary molecular biomarker-based risk score. Eur. Urol. 2016, 70, 740–748.
26. Tomlins, S.A.; Day, J.R.; Lonigro, R.J.; Hovelson, D.H.; Siddiqui, J.; Kunju, L.P.; Dunn, R.L.; Meyer, S.; Hodge, P.; Groskopf, J.; et al. Urine TMPRSS2:ERG Plus PCA3 for Individualized Prostate Cancer Risk Assessment. Eur. Urol. 2016, 70, 45–53.
27. Donovan, M.J.; Noerholm, M.; Bentink, S.; Belzer, S.; Skog, J.; O'Neill, V.; Cochran, J.S.; Brown, G.A. A molecular signature of PCA3 and ERG exosomal RNA from non-DRE urine is predictive of initial prostate biopsy result. Prostate Cancer Prostatic Dis. 2015, 18, 370–375.

28. McKiernan, J.; Donovan, M.J.; O'Neill, V.; Bentink, S.; Noerholm, M.; Belzer, S.; Skog, J.; Kattan, M.W.; Partin, A.; Andriole, G.; et al. A Novel Urine Exosome Gene Expression Assay to Predict High-grade Prostate Cancer at Initial Biopsy. JAMA Oncol. 2016, 2, 882–889.

Table 4. Summary of the clinical data obtained in prostate cancer patients using exosomes as a source of biomarkers.

Exosomal Biomarkers	Source	Isolation Method	Potential Use	Ref
PSA	Plasma	UC	Screening/Early Diagnosis	[9][99]
CA IX	Plasma	UC	Diagnosis	[104]
Survivin	Plasma	UC	Early Diagnosis	[107]
Exosomes levels	Plasma	UC	Diagnosis/Prognosis/Disease surveillance	[108]
PTEN	Plasma	UC	Diagnosis	[109]
miR-141, miR-375	Serum	FCE	Diagnosis/Stage Determination	[110]
miR-1290, miR-375	Plasma	PP	Prognosis	[111]
miR-141	Serum	PP	Diagnosis	[112]

35. Scattoni, V.; Lazzeri, M.; Lughezzani, G.; De Luca, S.; Passera, R.; Bollito, E.; Randone, D.; Abdollah, F.; Capitanio, U.; Larcher, A.; et al. Head-to-head comparison of prostate health index and urinary PCA3 for predicting cancer at initial or repeat biopsy. J. Urol. 2013, 190, 496–501.
36. Schwen, Z.R.; Mamawala, M.; Tosoian, J.J.; Druskin, S.C.; Ross, A.E.; Sokoll, L.J.; Epstein, J.I.; Carter, H.B.; Gorin, M.A.; Pavlovich, C.P. Prostate Health Index and multiparametric magnetic resonance imaging to predict prostate cancer grade reclassification in active surveillance. BJU Int. 2020, 126, 373–378.

Abbreviations list: PSA = prostate-specific antigen; CA IX = carbonic anhydrase; PTEN = phosphatase and tensin homolog; FCE = filtration-based capture of exosomes; PP = polymeric precipitation; UC = ultracentrifugation; miR = microRNAs.

In addition to the promising results, further research is still needed in order to validate the implementation in clinical practice of either plasmatic- or other bio-fluid-derived Exos. That would potentially allow considerable advantages for both patients and clinicians, by avoiding or limiting unnecessary invasive procedures and, hopefully significantly reducing the healthcare costs.

37. Loeb, S.; Sanda, M.G.; Broyles, D.L.; Shin, S.S.; Bangma, C.H.; Wei, J.T.; Partin, A.W.; Klee, G.G.; Slawin, K.M.; Marks, L.S.; et al. The prostate health index selectively identifies clinically significant prostate cancer. J Urol. 2015, 193, 1163–1169.

5. Conclusions

38. Boegemann, M.; Stephan, C.; Cammann, H.; Vincendeau, S.; Houlgatte, A.; Jung, K.; Blanchet, J.S.; Semjonow, A. The percentage of prostate-specific antigen (PSA) isoform [-2]proPSA and the Prostate Health Index improve the diagnostic accuracy for clinically relevant prostate cancer at

The biomarker field in PC has exploded in recent years. Moreover, the available set of PC biomarkers is constantly growing (numerous novel tests, different biomaterial sources, improved analytical measurements, and statistical

promising, yet only a few of them have been approved by regulatory authorities. Although the current data on PC biomarkers show great potential in aiding decision making and improved patients care, further investigation is warranted. For an optimal implementation of these tools in clinical practice, trials should be specifically designed to answer key clinical questions, and to explore whether a biomarker can actually improve PC management. Finally, combining mpMRI with biomarkers would be of particular value; yet the interplay of these tools is still uncertain, as the optimal sequence and timing remains to be determined.

initial and repeat biopsy compared with total PSA and percentage free PSA in men aged ≤65 years. BJU Int. 2016, 117, 72–79.

39. Ferro, M.; Bruzzese, D.; Perdonà, S.; Marino, A.; Mazzarella, C.; Perruolo, G.; D'Esposito, V.; Cosimato, V.; Buonerba, C.; Di Lorenzo, G.; et al. Prostate Health Index (Phi) and Prostate Cancer Antigen 3 (PCA3) significantly improve prostate cancer detection at initial biopsy in a total PSA range of 2–10 ng/ml. PLoS ONE 2013, 8, e67687.

40. Stephan, C.; Jung, K.; Semjonow, A.; Schulze-Forster, K.; Cammann, H.; Hu, X.; Meyer, H.A.; Bögemann, M.; Miller, K.; Friedersdorff, F. Comparative assessment of urinary prostate cancer antigen 3 and TMPRSS2:ERG gene fusion with the serum [-2]proprostate-specific antigen-based prostate health index for detection of prostate cancer. Clin. Chem. 2013, 59, 280–288.

41. Lin, D.W.; Newcomb, L.F.; Brown, M.D.; Sjoberg, D.D.; Dong, Y.; Brooks, J.D.; Carroll, P.R.; Cooperberg, M.; Dash, A.; Ellis, W.J.; et al. Canary Prostate Active Surveillance Study Investigators. Evaluating the Four Kallikrein Panel of the 4Kscore for Prediction of High-grade Prostate Cancer in Men in the Canary Prostate Active Surveillance Study. Eur. Urol. 2017, 72, 448–454.

42. Braun, K.; Sjoberg, D.D.; Vickers, A.J.; Lilja, H.; Bjartell, A.S. A Four-kallikrein Panel Predicts High-grade Cancer on Biopsy: Independent Validation in a Community Cohort. Eur. Urol. 2016, 69, 505–511.

43. Gupta, A.; Roobol, M.J.; Savage, C.J.; Peltola, M.; Pettersson, K.; Scardino, P.T.; Vickers, A.J.; Schröder, F.H.; Lilja, H. A four-kallikrein panel for the prediction of repeat prostate biopsy: Data from the European Randomized Study of Prostate Cancer screening in Rotterdam, Netherlands. Br. J. Cancer 2010, 103, 708–714.

44. Vickers, A.J.; Cronin, A.M.; Aus, G.; Pihl, C.G.; Becker, C.; Pettersson, K.; Scardino, P.T.; Hugosson, J.; Lilja, H. A panel of kallikrein markers can reduce unnecessary biopsy for prostate cancer: Data from the European Randomized Study of Prostate Cancer Screening in Göteborg, Sweden. BMC Med. 2008, 6, 19.

45. Newcomb, L.F.; Zheng, Y.; Faino, A.V.; Bianchi-Frias, D.; Cooperberg, M.R.; Brown, M.D.; Brooks, J.D.; Dash, A.; Fabrizio, M.D.; Gleave, M.E.; et al. Performance of PCA3 and TMPRSS2:ERG urinary biomarkers in prediction of biopsy outcome in the Canary Prostate Active Surveillance Study (PASS). Prostate Cancer Prostatic Dis. 2019, 22, 438–445.

46. Auprich, M.; Augustin, H.; Budäus, L.; Kluth, L.; Mannweiler, S.; Shariat, S.F.; Fisch, M.; Graefen, M.; Pummer, K.; Chun, F.K. A comparative performance analysis of total prostate-specific antigen, percentage free prostate-specific antigen, prostate-specific antigen velocity and urinary prostate cancer gene 3 in the first, second and third repeat prostate biopsy. BJU Int. 2012, 109, 1627–1635.

47. Leyten, G.H.; Hessels, D.; Jannink, S.A.; Smit, F.P.; de Jong, H.; Cornel, E.B.; de Reijke, T.M.; Vergunst, H.; Kil, P.; Knipscheer, B.C.; et al. Prospective multicentre evaluation of PCA3 and TMPRSS2-ERG gene fusions as diagnostic and prognostic urinary biomarkers for prostate cancer. Eur. Urol. 2014, 65, 534–542.
48. Tosoian, J.J.; Loeb, S.; Kettermann, A.; Landis, P.; Elliot, D.J.; Epstein, J.I.; Partin, A.W.; Carter, H.B.; Sokoll, L.J. Accuracy of PCA3 measurement in predicting short-term biopsy progression in an active surveillance program. J. Urol. 2010, 183, 534–538.
49. Haese, A.; de la Taille, A.; van Poppel, H.; Marberger, M.; Stenzl, A.; Mulders, P.F.; Huland, H.; Abbou, C.C.; Remzi, M.; Tinzl, M.; et al. Clinical utility of the PCA3 urine assay in European men scheduled for repeat biopsy. Eur. Urol. 2008, 54, 1081–1088.
50. Ochiai, A.; Okihara, K.; Kamoi, K.; Oikawa, T.; Shimazui, T.; Murayama, S.; Tomita, K.; Umekawa, T.; Uemura, H.; Miki, T. Clinical utility of the prostate cancer gene 3 (PCA3) urine assay in Japanese men undergoing prostate biopsy. BJU Int. 2013, 111, 928–933.
51. Wu, A.K.; Reese, A.C.; Cooperberg, M.R.; Sadetsky, N.; Shinohara, K. Utility of PCA3 in patients undergoing repeat biopsy for prostate cancer. Prostate Cancer Prostatic Dis. 2012, 15, 100–105.
52. Seisen, T.; Rouprêt, M.; Brault, D.; Léon, P.; Cancel-Tassin, G.; Compérat, E.; Renard-Penna, R.; Mozer, P.; Guechot, J.; Cussenot, O. Accuracy of the prostate health index versus the urinary prostate cancer antigen 3 score to predict overall and significant prostate cancer at initial biopsy. Prostate 2015, 75, 103–111.
53. Pepe, P.; Dibenedetto, G.; Pepe, L.; Pennisi, M. Multiparametric MRI Versus SelectMDx Accuracy in the Diagnosis of Clinically Significant PCa in Men Enrolled in Active Surveillance. In Vivo 2020, 34, 393–396.
54. Busetto, G.M.; Del Giudice, F.; Maggi, M.; De Marco, F.; Porreca, A.; Sperduti, I.; Magliocca, F.M.; Salciccia, S.; Chung, B.I.; De Berardinis, E.; et al. Prospective assessment of two-gene urinary test with multiparametric magnetic resonance imaging of the prostate for men undergoing primary prostate biopsy. World J. Urol. 2020.
55. Wysock, J.S.; Becher, E.; Persily, J.; Loeb, S.; Lepor, H. Concordance and Performance of 4Kscore and SelectMDx for Informing Decision to Perform Prostate Biopsy and Detection of Prostate Cancer. Urology 2020, 141, 119–124.
56. Mottet, N.; van den Bergh, R.C.N.; Briers, E.; Conford, P.; De Santis, M.; Fanti, S.; Gillessen, S.; Grummet, J.; Henry, A.M.; Lam, T.B.; et al. EAU-ESTRO-SIOG Guidelines on Prostate Cancer. 2019. Available online: (accessed on 9 February 2021).
57. NCCN Clinical Practice Guidelines in Oncology-Prostate Cancer Early Detection. 2019. Available online: (accessed on 9 February 2021).

58. Sciarra, A.; Panebianco, V.; Ciccariello, M.; Salciccia, S.; Cattarino, S.; Lisi, D.; Gentilucci, A.; Alfarone, A.; Bernardo, S.; Passariello, R.; et al. Value of magnetic resonance spectroscopy imaging and dynamic contrast-enhanced imaging for detecting prostate cancer foci in men with prior negative biopsy. Clin. Cancer Res. 2010, 16, 1875–1883.
59. Sciarra, A.; Panebianco, V.; Salciccia, S.; Cattarino, S.; Lisi, D.; Gentilucci, A.; Alfarone, A.; Mariotti, G.; Passariello, R.; Gentile, V. Modern role of magnetic resonance and spectroscopy in the imaging of prostate cancer. Urol. Oncol. 2011, 29, 12–20.
60. Panebianco, V.; Sciarra, A.; Marcantonio, A.; Forte, V.; Biondi, T.; Laghi, A.; Catalano, C. Conventional imaging and multiparametric magnetic resonance (MRI, MRS, DWI, MRP) in the diagnosis of prostate cancer. Q. J. Nucl. Med. Mol. Imaging 2012, 56, 331–342.
61. Maggi, M.; Panebianco, V.; Mosca, A.; Salciccia, S.; Gentilucci, A.; Di Pierro, G.; Busetto, G.M.; Barchetti, G.; Campa, R.; Sperduti, I.; et al. Prostate Imaging Reporting and Data System 3 Category Cases at Multiparametric Magnetic Resonance for Prostate Cancer: A Systematic Review and Meta-analysis. Eur. Urol. Focus 2020, 6, 463–478.
62. Busetto, G.M.; De Berardinis, E.; Sciarra, A.; Panebianco, V.; Giovannone, R.; Rosato, S.; D'Errigo, P.; Di Silverio, F.; Gentile, V.; Salciccia, S. Prostate cancer gene 3 and multiparametric magnetic resonance can reduce unnecessary biopsies: Decision curve analysis to evaluate predictive models. Urology 2013, 82, 1355–1360.
63. Sciarra, A.; Panebianco, V.; Cattarino, S.; Busetto, G.M.; De Berardinis, E.; Ciccariello, M.; Gentile, V.; Salciccia, S. Multiparametric magnetic resonance imaging of the prostate can improve the predictive value of the urinary prostate cancer antigen 3 test in patients with elevated prostate-specific antigen levels and a previous negative biopsy. BJU Int. 2012, 110, 1661–1665.
64. Fiehn, O. Metabolomics-the link between genotypes and phenotypes. Plant Mol. Biol. 2002, 48, 155–171.
65. Chen, Z.; Li, Z.; Li, H.; Jiang, Y. Metabolomics: A promising diagnostic and therapeutic implement for breast cancer. Onco Targets Ther. 2019, 12, 6797–6811.
66. Emwas, A.H. The strengths and weaknesses of NMR spectroscopy and mass spectrometry with particular focus on metabolomics research. Methods Mol. Biol. 2015, 1277, 161–193.
67. Sciarra, A.; Panebianco, V.; Ciccariello, M.; Salciccia, S.; Lisi, D.; Osimani, M.; Alfarone, A.; Gentilucci, A.; Parente, U.; Passariello, R.; et al. Magnetic resonance spectroscopic imaging (1H-MRSI) and dynamic contrast-enhanced magnetic resonance (DCE-MRI): Pattern changes from inflammation to prostate cancer. Cancer Investig. 2010, 28, 424–432.
68. Cerrato, A.; Bedia, C.; Capriotti, A.L.; Cavaliere, C.; Gentile, V.; Maggi, M.; Montone, C.M.; Piovesana, S.; Sciarra, A.; Tauler, R.; et al. Untargeted metabolomics of prostate cancer zwitterionic and positively charged compounds in urine. Anal. Chim. Acta 2021, 1158, 338381.

69. Madsen, R.; Lundstedt, T.; Trygg, J. Chemometrics in metabolomics--a review in human disease diagnosis. Anal. Chim. Acta 2010, 659, 23–33.

70. Kos, G.; Lohninger, H.; Krska, R. Validation of chemometric models for the determination of deoxynivalenol on maize by mid-infrared spectroscopy. Mycotoxin Res. 2003, 19, 149–153.

71. Amante, E.; Salomone, A.; Alladio, E.; Vincenti, M.; Porpiglia, F.; Bro, R. Untargeted Metabolomic Profile for the Detection of Prostate Carcinoma-Preliminary Results from PARAFAC2 and PLS-DA Models. Molecules 2019, 24, 3063.

72. Röhnisch, H.E.; Kyrø, C.; Olsen, A.; Thysell, E.; Hallmans, G.; Moazzami, A.A. Identification of metabolites associated with prostate cancer risk: A nested case-control study with long follow-up in the Northern Sweden Health and Disease Study. BMC Med. 2020, 18, 187.

73. Kelly, R.S.; Vander Heiden, M.G.; Giovannucci, E.; Mucci, L.A. Metabolomic Biomarkers of Prostate Cancer: Prediction, Diagnosis, Progression, Prognosis, and Recurrence. Cancer Epidemiol. Biomarkers Prev. 2016, 25, 887–906.

74. Maxeiner, A.; Adkins, C.B.; Zhang, Y.; Taupitz, M.; Halpern, E.F.; McDougal, W.S.; Wu, C.L.; Cheng, L.L. Retrospective analysis of prostate cancer recurrence potential with tissue metabolomic profiles. Prostate 2010, 70, 710–717.

75. Stabler, S.; Koyama, T.; Zhao, Z.; Martinez-Ferrer, M.; Allen, R.H.; Luka, Z.; Loukachevitch, L.V.; Clark, P.E.; Wagner, C.; Bhowmick, N.A. Serum methionine metabolites are risk factors for metastatic prostate cancer progression. PLoS ONE 2011, 6, e22486.

76. Giskeødegård, G.F.; Bertilsson, H.; Selnæs, K.M.; Wright, A.J.; Bathen, T.F.; Viset, T.; Halgunset, J.; Angelsen, A.; Gribbestad, I.S.; Tessem, M.B. Spermine and citrate as metabolic biomarkers for assessing prostate cancer aggressiveness. PLoS ONE 2013, 8, e62375.

77. Lynch, M.J.; Nicholson, J.K. Proton MRS of human prostatic fluid: Correlations between citrate, spermine, and myo-inositol levels and changes with disease. Prostate 1997, 30, 248–255.

78. Kumar, D.; Gupta, A.; Mandhani, A.; Sankhwar, S.N. NMR spectroscopy of filtered serum of prostate cancer: A new frontier in metabolomics. Prostate 2016, 76, 1106–1119.

79. Sreekumar, A.; Poisson, L.M.; Rajendiran, T.M.; Khan, A.P.; Cao, Q.; Yu, J.; Laxman, B.; Mehra, R.; Lonigro, R.J.; Li, Y.; et al. Metabolomic profiles delineate potential role for sarcosine in prostate cancer progression. Nature 2009, 457, 910–914.

80. Swanson, M.G.; Zektzer, A.S.; Tabatabai, Z.L.; Simko, J.; Jarso, S.; Keshari, K.R.; Schmitt, L.; Carroll, P.R.; Shinohara, K.; Vigneron, D.B.; et al. Quantitative analysis of prostate metabolites using 1H HR-MAS spectroscopy. Magn. Reson. Med. 2006, 55, 1257–1264.

81. Gkotsos, G.; Virgiliou, C.; Lagoudaki, I.; Sardeli, C.; Raikos, N.; Theodoridis, G.; Dimitriadis, G. The Role of Sarcosine, Uracil, and Kynurenic Acid Metabolism in Urine for Diagnosis and

Progression Monitoring of Prostate Cancer. Metabolites 2017, 7, 9.

82. Dereziński, P.; Klupczynska, A.; Sawicki, W.; Pałka, J.A.; Kokot, Z.J. Amino Acid Profiles of Serum and Urine in Search for Prostate Cancer Biomarkers: A Pilot Study. Int. J. Med. Sci. 2017, 14, 1–12.
83. Wu, H.; Liu, T.; Ma, C.; Xue, R.; Deng, C.; Zeng, H.; Shen, X. GC/MS-based metabolomic approach to validate the role of urinary sarcosine and target biomarkers for human prostate cancer by microwave-assisted derivatization. Anal. Bioanal. Chem. 2011, 401, 635–646.
84. Struck-Lewicka, W.; Kordalewska, M.; Bujak, R.; Yumba Mpanga, A.; Markuszewski, M.; Jacyna, J.; Matuszewski, M.; Kaliszan, R.; Markuszewski, M.J. Urine metabolic fingerprinting using LC-MS and GC-MS reveals metabolite changes in prostate cancer: A pilot study. J. Pharm. Biomed. Anal. 2015, 111, 351–361.
85. Mondul, A.M.; Moore, S.C.; Weinstein, S.J.; Karoly, E.D.; Sampson, J.N.; Albanes, D. Metabolomic analysis of prostate cancer risk in a prospective cohort: The alpha-tocolpherol, beta-carotene cancer prevention (ATBC) study. Int. J. Cancer 2015, 137, 2124–2132.
86. Yáñez-Mó, M.; Siljander, P.R.; Andreu, Z.; Zavec, A.B.; Borràs, F.E.; Buzas, E.I.; Buzas, K.; Casal, E.; Cappello, F.; Carvalho, J.; et al. Biological properties of extracellular vesicles and their physiological functions. J. Extracell Vesicles 2015, 4, 27066.
87. Théry, C.; Witwer, K.W.; Aikawa, E.; Alcaraz, M.J.; Anderson, J.D.; Andriantsitohaina, R.; Antoniou, A.; Arab, T.; Archer, F.; Atkin-Smith, G.K.; et al. Minimal information for studies of extracellular vesicles 2018 (MISEV2018): A position statement of the International Society for Extracellular Vesicles and update of the MISEV2014 guidelines. J. Extracell Vesicles 2018, 7, 1535750.
88. Spugnini, E.P.; Logozzi, M.; Di Raimo, R.; Mizzoni, D.; Fais, S. A Role of Tumor-Released Exosomes in Paracrine Dissemination and Metastasis. Int. J. Mol. Sci. 2018, 19, 3968.
89. Logozzi, M.; Mizzoni, D.; Angelini, D.F.; Di Raimo, R.; Falchi, M.; Battistini, L.; Fais, S. Microenvironmental pH and Exosome Levels Interplay in Human Cancer Cell Lines of Different Histotypes. Cancers 2018, 10, 370.
90. Zhang, Y.; Liu, Y.; Liu, H.; Tang, W.H. Exosomes: Biogenesis, biologic function and clinical potential. Cell Biosci. 2019, 9, 19.
91. van der Pol, E.; Böing, A.N.; Harrison, P.; Sturk, A.; Nieuwland, R. Classification, functions, and clinical relevance of extracellular vesicles. Pharmacol. Rev. 2012, 64, 676–705.
92. Sciarra, A.; Lichtner, M.; Autran, G.A.; Mastroianni, C.; Rossi, R.; Mengoni, F.; Cristini, C.; Gentilucci, A.; Vullo, V.; Di Silverio, F. Characterization of circulating blood dendritic cell subsets DC123+ (lymphoid) and DC11C+ (myeloid) in prostate adenocarcinoma patients. Prostate 2007, 67, 1–7.

93. Properzi, F.; Logozzi, M.; Fais, S. Exosomes: The future of biomarkers in medicine. Biomark Med. 2013, 7, 769–778.
94. Camussi, G.; Deregibus, M.C.; Bruno, S.; Grange, C.; Fonsato, V.; Tetta, C. Exosome/microvesicle-mediated epigenetic reprogramming of cells. Am. J. Cancer Res. 2011, 1, 98–110.
95. Zhao, H.; Achreja, A.; Iessi, E.; Logozzi, M.; Mizzoni, D.; Di Raimo, R.; Nagrath, D.; Fais, S. The key role of extracellular vesicles in the metastatic process. Biochim. Biophys. Acta Rev. Cancer 2018, 1869, 64–77.
96. Fais, S.; Logozzi, M.; Lugini, L.; Federici, C.; Azzarito, T.; Zarovni, N.; Chiesi, A. Exosomes: The ideal nanovectors for biodelivery. Biol. Chem. 2013, 394, 1–15.
97. Cappello, F.; Logozzi, M.; Campanella, C.; Bavisotto, C.C.; Marcilla, A.; Properzi, F.; Fais, S. Exosome levels in human body fluids: A tumor marker by themselves? Eur. J. Pharm. Sci. 2017, 96, 93–98.
98. Huang, T.; Deng, C.X. Current Progresses of Exosomes as Cancer Diagnostic and Prognostic Biomarkers. Int. J. Biol. Sci. 2019, 15, 1–11.
99. Logozzi, M.; Angelini, D.F.; Iessi, E.; Mizzoni, D.; Di Raimo, R.; Federici, C.; Lugini, L.; Borsellino, G.; Gentilucci, A.; Pierella, F.; et al. Increased PSA expression on prostate cancer exosomes in in vitro condition and in cancer patients. Cancer Lett. 2017, 403, 318–329.
100. Pillai, S.R.; Damaghi, M.; Marunaka, Y.; Spugnini, E.P.; Fais, S.; Gillies, R.J. Causes, consequences, and therapy of tumors acidosis. Cancer Metastasis Rev. 2019, 38, 205–222.
101. Fais, S.; Venturi, G.; Gatenby, B. Microenvironmental acidosis in carcinogenesis and metastases: New strategies in prevention and therapy. Cancer Metastasis Rev. 2014, 33, 1095–1108.
102. Peinado, H.; Zhang, H.; Matei, I.R.; Costa-Silva, B.; Hoshino, A.; Rodrigues, G.; Psaila, B.; Kaplan, R.N.; Bromberg, J.F.; Kang, Y.; et al. Pre-metastatic niches: Organ-specific homes for metastases. Nat. Rev. Cancer 2017, 17, 302–317.
103. Logozzi, M.; Capasso, C.; Di Raimo, R.; Del Prete, S.; Mizzoni, D.; Falchi, M.; Supuran, C.T.; Fais, S. Prostate cancer cells and exosomes in acidic condition show increased carbonic anhydrase IX expression and activity. J. Enzym. Inhib. Med. Chem. 2019, 34, 272–278.
104. Logozzi, M.; Mizzoni, D.; Capasso, C.; Del Prete, S.; Di Raimo, R.; Falchi, M.; Angelini, D.F.; Sciarra, A.; Maggi, M.; Supuran, C.T.; et al. Plasmatic exosomes from prostate cancer patients show increased carbonic anhydrase IX expression and activity and low pH. J. Enzym. Inhib. Med. Chem. 2020, 35, 280–288.
105. Campanella, C.; Rappa, F.; Sciumè, C.; Marino Gammazza, A.; Barone, R.; Bucchieri, F.; David, S.; Curcurù, G.; Caruso Bavisotto, C.; Pitruzzella, A.; et al. Heat shock protein 60 levels in tissue

and circulating exosomes in human large bowel cancer before and after ablative surgery. Cancer 2015, 121, 3230–3239.

106. Logozzi, M.; De Milito, A.; Lugini, L.; Borghi, M.; Calabrò, L.; Spada, M.; Perdicchio, M.; Marino, M.L.; Federici, C.; Iessi, E.; et al. High levels of exosomes expressing CD63 and caveolin-1 in plasma of melanoma patients. PLoS ONE 2009, 4, e5219.
107. Khan, S.; Jutzy, J.M.; Valenzuela, M.M.; Turay, D.; Aspe, J.R.; Ashok, A.; Mirshahidi, S.; Mercola, D.; Lilly, M.B.; Wall, N.R. Plasma-derived exosomal survivin, a plausible biomarker for early detection of prostate cancer. PLoS ONE 2012, 7, e46737.
108. Nawaz, M.; Camussi, G.; Valadi, H.; Nazarenko, I.; Ekström, K.; Wang, X.; Principe, S.; Shah, N.; Ashraf, N.M.; Fatima, F.; et al. The emerging role of extracellular vesicles as biomarkers for urogenital cancers. Nat. Rev. Urol. 2014, 11, 688–701.
109. Gabriel, K.; Ingram, A.; Austin, R.; Kapoor, A.; Tang, D.; Majeed, F.; Qureshi, T.; Al-Nedawi, K. Regulation of the tumor suppressor PTEN through exosomes: A diagnostic potential for prostate cancer. PLoS ONE 2013, 8, e70047.
110. Bryant, R.J.; Pawlowski, T.; Catto, J.W.; Marsden, G.; Vessella, R.L.; Rhees, B.; Kuslich, C.; Visakorpi, T.; Hamdy, F.C. Changes in circulating microRNA levels associated with prostate cancer. Br. J. Cancer 2012, 106, 768–774.
111. Huang, X.; Yuan, T.; Liang, M.; Du, M.; Xia, S.; Dittmar, R.; Wang, D.; See, W.; Costello, B.A.; Quevedo, F.; et al. Exosomal miR-1290 and miR-375 as prognostic markers in castration-resistant prostate cancer. Eur. Urol. 2015, 67, 33–41.
112. Li, Z.; Ma, Y.Y.; Wang, J.; Zeng, X.F.; Li, R.; Kang, W.; Hao, X.K. Exosomal microRNA-141 is upregulated in the serum of prostate cancer patients. Onco Targets Ther. 2015, 9, 139–148.