The novel biomarkers in the diagnosis of prostate cancer

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Abstract: The prostate-specific antigen (PSA) as a biomarker for prostate cancer (PCa) diagnosis has been widely used in the clinic for several decades. However, PSA has a low specificity for PCa diagnosis, thereby several genes, blood, and urine-based biomarkers (such as sarcosine) underlying biology of PCa progression are being developed to improve the accuracy of PCa diagnosis. In the present review, we focus on novel PCa biomarkers, which are potentially superior to PSA in PCa screening and facilitate clinical PCa diagnosis. The early PCa screening with reliable biomarkers is critical in reducing the mortality of clinical PCa (high-risk PCa). For clinical insignificant PCa (low-risk PCa) patients and benign prostatic hyperplasia patients, biopsies should be avoided and disease progression should be monitored using non-invasive biomarkers.

Keywords: prostate cancer; biomarker; clinical diagnosis

1. Introduction
Prostate cancer (PCa) is one of the most common cancers in Chinese men, and the incidence of PCa is increasing rapidly, while the clinical stage of PCa patients is comparatively late with a lower survival rate. Moreover, it is hard to control when the disease metastasizes. It is reported that for about 30,000 men died of PCa and 690,000 men were diagnosed with PCa in 2014 [1]. The primary diagnosis of PCa currently used in the clinic is based on digital rectal inspection (DRE), clinical staging, Gleason score, serum prostate-specific antigen (PSA), and prostate biopsy. The gold standard for PCa diagnosis is biopsy examination [2].

The utility of DRE is limited to the detection of the tumor that can only be observed in the high-risk PCa. The patient who was highly PCa suspicious in DRE must receive a prostate biopsy. In addition, DRE comes with a strong sense of discomfort and only 40–50% of individuals with unusual findings in DRE are diagnosed as PCa according to the biopsy results. It is not highly specific in the diagnosis of PCa [3]. Although PSA also has low specificity for the high-grade cancer, PSA examination is more convenient and more acceptable by patients. Thereby, serum PSA remains the most widely used biomarker for screening and therapy patients with PCa or no PCa [4]. To date, there is no any biomarker or combination of biomarkers applied in the clinic as a reliable reference for PCa diagnosis.

In general, from diagnosis to management of PCa, there are few biomarkers used by physicians. Although a variety of body fluids including urine and blood are promising
sources for biomarker tests, identifying men with PCa through body fluids is indeed a challenge. In the present review, we highlight some important or potential biomarkers, and sarcosine, for example, that promise to enhance personality detection of PCa. These biomarkers, as shown in [Table 1], are promising in diagnosis of PCa.

1.1. Sarcosine

Cancer biomarkers are investigated from various fields including genomics, proteomics, and metabolomics. Sarcosine, a glycine metabolite, is considered as a potential biomarker for PCa first by Sreekumar et al. in 2009[9]. More than 112 metabolites originated from tissue, plasma, and urine were analyzed using gas chromatography/mass spectrometry (MS) and high-performance liquid chromatography/MS. It is reported that clinically insignificant PCa, clinical significance PCa, benign prostate hyperplasia, and benign prostate are able to be distinguished by metabolomic profiles. Sarcosine is included in the metabolomic profiles and is not only significantly increased in patients with malignant PCa tissues but also could be feasibly detected in urine. Levels of sarcosine have a significant elevation in urine sediments and supernatants from PCa patients compared with no PCa. There is a study investigated the specificity and sensitivity of urine sarcosine and serum PSA in subjects with PSA in a challenging range of 2–10 ng/ml[9]. It was found that the sarcosine area under the curve (AUC) was for sarcosine and PSA was 0.69 and 0.53, respectively. It is suggested that the accuracy of urinary sarcosine assay in the diagnosis of PCa is higher than serum PSA detection.

Key enzymes related to sarcosine metabolism in PCa progression are investigated[6,7]. The expression of glycine N-methyltransferase (GNMT), sarcosine biosynthetic enzyme, is elevated in PCa cells. In contrast, piperolic acid oxidase (PIPOX) and sarcosine dehydrogenase (SARDH), sarcosine metabolize enzymes, are reduced in PCa cells. Consistently, GNMT catalyzes the conversion of glycine to creatine which promoted the invasiveness of prostate cells. PIPOX and SARDH suppressed the development of PCa by metabolizing sarcosine. Moreover, treatment of sarcosine induced invasion and intravasation in the animal model of PCa. In addition, a study in PCa xenografts shows that GNMT knockdown or SARDH overexpression will control the development of PCa[6]. It is considered that sarcosine is a novel biomarker in the diagnosis of PCa; meanwhile, it was a key element of androgen secretion for the treatment of PCa development[7]. At the gray area criterion of PSA, a study has shown that sarcosine has a greater ability to delineate PCa and benign prostatic hyperplasia than PSA[9].

Prostarix was a post-DRE urine test developed by Metabolon Inc. The test detects metabolites including sarcosine, glycine, alanine, and glutamate, which are evaluated by a Prostarix risk score. Prostarix has been applied for clinical in America[9]. Sarcosine is a urine-based marker for PCa detection compared to other PCa biomarkers. The non-invasive procedure enables it more acceptable for patients than a standard biopsy.

Although there is no available cutoff value of sarcosine for PCa diagnosis so far, the advantage of using sarcosine as a PCa biomarker is that the detection of sarcosine is feasible and efficient, which may make it become an auxiliary biomarker of PSA or other biomarkers in PCa diagnosis.

1.2. PSA and PSA derivatives

Measuring the PSA level in asymptomatic men has been widely accepted for PCa screening[9,10]. The first study describing PSA was published in 1960[11]. With the development, PSA has been used as a diagnostic tool for PCa in a long time. PSA is an antigen produced by the epithelial component of the prostate gland both in normal and cancerous cells. More than half of metastatic PCa are accompanied by the development of metastasis especially osteoblastic metastasis. Moreover, PSA/ALK3 may be a risk factor for osteoblastic metastasis, which can promote the apoptosis of osteoclasts and the proliferation of osteoblasts[12]. The important role of PSA is the liquidation of the seminal coagulum by being released directly into the lumen, which releases of viable sperm.

In the normal condition, PSA from the prostate tissue remains a low concentration in blood, whereas some studies show that there is an increase of PSA under particular abnormal circumstances of the prostate. Men with prostatic diseases, including PCa and benign prostatic hyperplasia, may show a high level of PSA in circulation, which is resulted from the enhanced production of PSA and squeeze in the gland[14]. As high levels of PSA (10.0 ng/ml) are confirmed to have a high risk of PCa and companied with worse invasive disease; prostate biopsy should be considered according to the NCCN[15] and AUA[16] guidelines recommendation.

In the USA, PCa mortality rate declined nearly 30% during the 1990s. It may contribute to PSA screening. In the meantime, like changing the treatment, may also have played an crucial part in improving the effectiveness of PCa treatment[17]. The recurrence rate can be estimated by the PSA level and the survival rate.

However, there are some limitations for the PSA test in clinical application. Patients with PSA level at 3–10 ng/ml are in a gray zone with a high false negative rate, particularly for men at high risk for high-grade cancer based on PSA-negative factors[18]. Although PSA level at 4.0 ng/ml indicates a high risk of PCa, it comes with a series of problems, for example, overdagnosis and overtreatment. Although PSA screening can predict PCa up to 12 years before diagnosis; it is estimated[19] that there are approximately half of PCa patients with increasing PSA level, ages 55–67, have the non-important disease. A significant increasing of false negative can bring unnecessary biopsy on patients with no PCa and benign prostatic hyperplasia. Some studies[20,21] have shown that only 25–35% of men with 4–10 ng/mL of PSA in blood...
are diagnosed as PCa by prostate biopsy, and most of these tumors are in low-grade\cite{35}. Furthermore, approximately 15% of men with a PSA level under 4.0 ng/mL are diagnosed as PCa, and 15% of these display a high Gleason score\cite{23,24}.

Although some large prospective randomized clinical trials, for example, the European Randomized Study of Screening for PCa, show a positive result in reducing mortality rate, PCa screening with PSA remains controversial\cite{23-28}. There are two large trials, PLCO\cite{29,26} and ERSPC\cite{31}, showing different results in the effect of PCa screening on mortality rate. 76,693 men were assigned by PLCO Cancer Screening Trial in the USA to receive annual screening of PSA. The results show that the mortality of PCa was mild and there was no significant difference between the two study groups\cite{29,20}. In contrast, the European ERSPC trial\cite{31} recruited 182,000 men ages from 50 to 74 years, and the results show that PSA-based screening leads to a reduced death from PCa by 20%. However, both of the two trials pointed out that PSA diagnosis was associated with over-treatment over-diagnosis for low-risk PCa. In addition, due to prostate biopsy, the occurrence of infectious complications have increased in recent years\cite{32}.

To improve the specificity of PCa screening, some other derivative indicators of PSA are offered.

1.2.1. Free PSA (fPSA)

Most PSA is binding PSA and bind to 1-antichymotrypsin. Total PSA (tPSA) exists in free state and binding state. Another form of PSA, fPSA, is not bond to proteins\cite{33}. Compared with tPSA, the value of fPSA is more profound in PCa diagnosis, and the ratio of fPSA/tPSA mostly improves the PCa diagnostic specificity, particularly within the PSA level at 4–10 ng/mL. It reduces unnecessary biopsies with minimal damage to patients in detecting cancer.

fPSA is synthesized in non-malignant anterior glandular cells. If the prostate becomes cancerous, the tPSA level will be increased, whereas the fPSA level will remain unchanged. It is suggested that the fPSA/tPSA ratio will be decreased and this ratio can be used for clinical screening\cite{34}. Furthermore, many evidences indicate that a low percentage of fPSA is possibly associated with the aggressive of PCa\cite{35,36}. The studies in patients with PSA values in the grey zone show that it is more effective of f/T PSA compared with tPSA\cite{37}. Receiver operating characteristic curves value for tPSA and fPSA/tPSA were 0.566 and 0.602, respectively. f/T PSA demonstrated a higher discriminative power compared with t PSA\cite{38}. However, the determination of cutoff value is complicated due to the partial dependence of the percentage of fPSA on prostate size, age, and PSA-positive tumors.

1.2.2. PSA velocity (PSAV)

In general, PSAV (v) >0.75 ng/mL/year\cite{34} as a criterion-the specificity of PSAV is 90%, but sensitivity is only 11%. One study demonstrates that if PSAV (v) >0.1 ng/mL/year as a criterion-the specificity and sensitivity of PSAV can achieve 81% and 50%\cite{39}.

Both the European and the National Comprehensive Cancer Network guidelines recommend the elevation of PSAV as a sign of PCa development and treatment is needed in this condition. And PSAV > 0.75 ng/mL/year as a sign of progression for PCa was defined by the National Comprehensive Cancer Network\cite{40}.

1.2.3. PSA density (PSAd)

PSAd represents the relationship of the PSA level to the size and weight (volume) of the prostate. It is a better parameter to distinguish patients with prostatic cancer from those with no PCa and benign prostatic hyperplasia compared with PSA level\cite{41}. PSAd <0.15\cite{12} may be used as a clinical reference for no requirement of prostate biopsy.

PSAd requires prostate volume and the estimated volume measured by trans rectal ultrasonography (TRUS) can be variable. To date, TRUS-based prostate volume measurements are used to conduct prostate biopsy\cite{40}. It was uncomfortable and unaccepted for many patients yet.

It was one of the greatest controversial experiments that the widespread adoption of PSA screening in modern medical history\cite{41}, although the sensitivity and specificity of the PSA test still need to be optimized, the PSA test will be retained as the most important diagnostic biomarker; meanwhile, screening rates are increasing in many countries.

Due to the limitations of PSA as a biomarker in the diagnosis of PCa, new effectively biomarkers are required urgently\cite{42}. Especially it can be used in distinguishing between low-risk and malignant PCa.

1.3. Human glandular kallikrein 2 (hK2)

hK2 is a proteolytic enzyme mainly secreted by prostate epithelial cells, and it is also expressed in thyroid, salivary glands, and female breast tissues\cite{43}. hK2 has already been used in the clinic as PCa biomarkers and is reported that hK2 can be detected in blood, semen, and urine. The amino acid sequence for hK2 is 80% similar to that for PSA also called hK3\cite{44}.

The increased circulating insulin-like growth factor (IGF) level is associated with metastasis and aggressive phenotypes in PCa\cite{45}. hK2 and PSA (hK3) can efficiently cleave IGFBP3, IGFBP2-5, IGFBP4, and IGFBP4-6\cite{46}, which can significantly elevate the risk of PCa.

Numerous studies\cite{47,48,12} indicate that fPSA, hK2, and their combinations are valuable in the diagnosis of minimal PCa. The tested samples were collected from PCa patient with a PSA range of 4–10 ng/mL. The results of fPSA, hK2, hK2/fPSA, and fPSA/TPSA, distinguished minimal and moderate/advanced PCa\cite{12}. In a study, 12,542 men were measured four kallikrein markers – hK2, free, total, and intact PSA and this study was followed for over
15 years. This combination model significantly increased the accuracy of prediction in metastasis and benign PCs compared with measurement of TPSA levels alone[47]. In addition, a test of 1012 patients shows that hK2, total, free, and intact PSA displayed an excellent ability to discriminate between individuals who are benign or metastatic PCs and those who are benign prostatic hyperplasia or no cancer. A number of potential prostate over biopsy have been reduced in this test[49].

The studies mentioned above investigated the value of hK2 combined with PSA in discriminating PCs, whereas a single hK2 as the indicator is controversial in clinical and there is a lack of validations.

1.4. Early prostate cancer antigen (EPCA)

According to the report, EPCA, a nuclear structural protein, can identify men with PCs earlier than PSA or DRE for at least 5 years[49]. EPCA is expressed throughout the prostate of individuals with PCs but not produced in normal prostate issues. EPCA can serve as an accessory diagnostic mode to the current clinical diagnostic approach for the patient who receives a prostate biopsy. In a study, localized PCs and EPCA staining show a positive result in the 94% PCs samples whereas there was no significant correlation between EPCA expression and the stage of PCs. In addition, EPCA positive cells were detected in noncancerous tissues near cancerous tissue in 86% of PCs[50]. These evidence suggest that EPCA expression is considered as an early event in the pathogenesis of PCs, and it possibly can be used for early diagnosis.

Some studies show that EPCA-2-a subtypes of EPCA, may contribute to the diagnosis of aggressive PCs[13,51]. The release of nuclear antigen into the circulation leads to cell death, and it was debatable whether the nuclear antigen could be a valid biomarker for early cancer diagnosis. In addition, the amount of EPCA in serum that arising from dying PCs cells is possibly not sufficient for measurement using common techniques, especially with small and localized tumors[52].

1.5. Prostate cancer gene 3 (PCA3)

PCA3 mRNA can be detected in urine, blood, and semen using reverse transcription polymerase chain reaction. It is a noncoding prostate specific gene that is over expressed in 95% of PCs cells[53]. In a clinical study, PCA3 mRNA was quantified in 443 patients. The results showed that the sensitivity of this method was 58% and specificity of PAC3 to PCs was 91% in a gray zone. With a PSA level >10 ng/mL, the sensitivity and specificity was 79% and 80%, respectively[54].

As a potential biomarker in the diagnosis of PCs, at the low PCA3 score of 20, the rate of misdiagnosis of high-grade (Gleason score >6) cancers was low; however, a high rate (13%) was observed in low-grade cancers. Although a high positive predictive value can get up to 80% if the PCA3 score cutoff was set to 60[55], neither the NCCN nor FDA suggests to use PCA3 as an indicator of whether a biopsy is performed. In addition, mRNA is easily degraded by RNA enzymes in urine which increases the difficulty in sample collection and leads to false result.

1.6. α-Methylacyl-CoA-racemase (AMACR)

AMACR is a new tumor marker of PCs discovered in recent years. It is encoded by P504S genes and highly expressed in PCs high-throughput immunoblot analysis revealed that AMACR measurement was more sensitive and specific in subjects with a PSA range of 4–10 ng/ml compared with PSA measurement in distinguishing sera of PCs patients (sensitivity and specificity of 77.8% and 80.6%; AUC of 0.789)[56]. Its worth noting that there was no significant correlation between androgen receptor and the expression of AMACR.

It was reported that AMACR is also positively expressed in other malignant tumor tissues such as endometrial clear cell carcinoma, breast cancer, renal cell carcinoma, and bladder urothelial carcinoma[57,58]. In addition, AMACR is expressed in a certain extent of normal prostate glands adjacent to prostate tumors, and AMACR intensity is negatively correlated with its distance from tumor tissues. It also suggests that there are early morphological changes of malignant transformation in adjacent tissues[59]. Moreover, there are many variations of AMACR[60], which are not conducive to the establishment of detection methodology. What is more, AMACR often requires simultaneous detection of p63 and CK34βE12 (Cytokeratin antibody) to distinguish between prostate intraepithelial neoplasm and PCs[61,62].

1.7. Other biomarker

Urokinase plasminogen activator[71],TMPRSS2:ERG[22], and prostate stem cell antigen[19] have also been investigated as PCs biomarkers. To date, the researches of these biomarkers stay in the theoretical stage, and there are no commercial products developed yet, and further evaluation is needed.

2. Clinical Scenarios

In clinical, there is no single indicator showing excellent performance in PCs diagnosis currently. Although PSA is unreliable and cannot accurately discriminate between PCs and other disease conditions and many patients are currently misdiagnosis and over-biopsy based on PSA measurement[63], it is still the most widely used biomarker in PCs diagnosis. Many PSA-based biomarkers (PSA, PSAD, and PSAV) were investigated to improve the accuracy in PCs diagnosis. Unfortunately, PSA-based biomarkers do not significantly optimize diagnostic efficiency.

Therefore, some novel biomarkers for PCs diagnosis have emerged. It has been suggested that some urine
biomarkers (sarcosine and PCA3) and serum biomarkers (EPCA, PSA, and hK2) are probably superior to PSA in specificity and sensitivity and are highly valued by clinicians. However, these tumor markers lack a unified judgment boundary value, accompany with non-standardized detection method. These biomarkers are also limited in clinical verifications, which also bring a series of problems for clinical diagnosis and treatment of PCa.

The ideal biomarker for clinical use should be safely and easily measured, and the specimen should be collected in a less or non-invasive way. It should have a high sensitivity and specificity in the diagnosis of PCa. Those improve decision-making abilities in conjunction with clinic-pathological parameters\(^6\). The combination of multiple biomarkers measurement is promising in PCa prediction, screening, diagnosis, and prognosis. In the future, the development of biomarkers will improve management of this disease, especially in the diagnosis of benign PCa, metastatic PCa, and benign prostatic hyperplasia.

Although due to the detection and treatment of PCa can increase the survival rate, PCa screening remains controversial, which is mainly involved in over diagnosis and overtreatment. PCa can be simply divided into two categories: Clinical insignificant PCa and clinical PCa. There is no doubt that clinical PCa has a high risk of mortality and invasiveness; the efficient treatment is needed. However, in patients with slow progress of clinically insignificant PCa which have a low-risk of death, PCa screening increases the rate of biopsy-induced complications before PCa progression. The American Cancer Society statement of guidelines for early detection of PCa was supported by the American urological association, while some other organizations do not recognize the benefits of PCa screening\(^6\). For low-risk PCa, insignificant PCa, active surveillance has minimal incident with effective treatment. Although therapies may lead to an impact on erectile function, it provides a clear treatment strategy\(^6\). However, there is no convincing evidence from any clinical trials and research that early screening of PCa affects mortality, and the standard treatment for EPCA is associated with more frequent side effects. Biopsies possibly induce more serious problems, due to increasing risks of complications includes Escherichia coli infections, urinary tract infection, epididymitis, orchitis, prostatitis, and sepsis. In addition, the risk of rectal bleeding, vasovagal episodes, hematuria, hematospermia, fever, and dysuria also increase\(^5,6\), although the mortality of PCa is strongly associated with the progression of the disease\(^6\). Compare to Europe and the United States, there is a low rate of early diagnosis of PCa in China, which leads to a high mortality rate. Reduction in PCa mortality is most likely resulted from early detection, assessment and effective treatment options of PCa\(^7\). Treatment of EPCA has shown benefits, in terms of reductions in PCa mortality, local tumor progression, and metastases\(^7\). Early diagnosis provides a more conservation, non-invasive approach to cancer than clinical symptoms of the disease. Once PCa metastasis or tolerance to hormone therapy occurs, it will be difficult to control. There is a large and growing population of individuals diagnosed with PCa all over the world, and they should be monitored for PCa progression. To choose a minimum damage treatment\(^6\).

In summary, early diagnosis by biomarkers is important in improving the patient’s life quality and reducing the mortality of PCa. It helps to avoid overdiagnosis and overtreatment. It also helps in patient selection for prostate

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**Table 1. Different biomarkers signatures**

| Name          | Definition                                  | Sample       | Clinical evidence                                                                 |
|---------------|---------------------------------------------|--------------|----------------------------------------------------------------------------------|
| Sarcosine     | Amino acid, a glycine metabolite            | Urine        | The sarcosine AUC was for sarcosine and PSA was 0.69 and 0.53, respectively      |
| PSA           | An antigen produced by the epithelial component of the prostate gland | Serum        | Patients with PSA level at 3–10 ng/ml are in a gray zone with a high false negative rate. Some other derivative indicators of PSA are offered to improve the specificity |
| hK2           | A proteolytic enzyme secreted by prostate epithelial cells, thyroid, salivary glands, and female breast tissues | Serum        | fPSA, hK2, and their combinations are valuable in the diagnosis of metastasis and benign PCa compared with measurement of TPSA levels alone A single hK2 as the indicator is controversial in clinical and there is a lack of validations |
| EPCA          | A nuclear structural protein                 | Serum        | Localized PCa and EPCA staining show a positive result in the 94% PCa samples. It was debatable whether the nuclear antigen could be a valid biomarker for early cancer diagnosis |
| PCA3          | CaP-specific gene                           | Urine        | The sensitivity and specificity was 58% an 91% in a gray zone. With a PSA level >10 ng/mL, the sensitivity and specificity was 79% and 80% It has a high rate of misdiagnosis of PCa |
| AMACR         | A kind of racemase encoded by P504S genes   | Serum        | AMACR measurement was more sensitive and specific in subjects with a PSA range of 4–10 ng/ml AMACR is also positively expressed in other malignant tumor tissues |

PSA: Prostate-specific antigen, hK2: Human glandular kallikrein 2, EPCA: Early prostate cancer antigen, PCA3: Prostate cancer Gene 3, AMACR: α-Methyl acyl CoA racemase, AUC: Area under the curve, fPSA: Free prostate-specific antigen, PCa: Prostate cancer
biopsy and minimizing the potential harms[32]. There is a clinical need in the diagnostic indicators of PCAs with high sensitivity and specificity.

3. Conclusion
The diagnosis of PCAs through novel biomarkers improves the therapeutic effect and prognosis of PCAs. These new biomarkers potentially improve the specificity of diagnosis and reduce over-biopsy. Moreover, the analysis of multiple combined biomarkers for PCA screening probably is a promising approach in clinical applications.

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