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Prostate cancer markers: An update (Review)

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Abstract. As the most common noncutaneous malignancy in American men, prostate cancer currently accounts for 29% of all diagnosed cancers, and ranks second as the cause of cancer fatality in American men. Prostatic cancer is rarely symptomatic early in its course and therefore disease presentation often implies local extension or even metastatic disease. Thus, it is extremely critical to detect and diagnose prostate cancer in its earliest stages, often prior to the presentation of symptoms. Three of the most common techniques used to detect prostate cancer are the digital rectal exam, the transrectal ultrasound, and the use of biomarkers. This review presents an update regarding the field of prostate cancer biomarkers and comments on future biomarkers. Although there is not a lack of research in the field of prostate cancer biomarkers, the discovery of a novel biomarker that may have the advantage of being more specific and effective warrants future scientific inquiry.

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1. Introduction

Prostate cancer is the most common noncutaneous malignancy in American men. It currently accounts for 29% of all diagnosed cancers, and ranks second as the most common cause of cancer fatality in American men, accounting for 13% of all cancer fatalities (1). Recent studies have shown that ~220,800 men in the United States were diagnosed with prostate cancer, and ~27,540 men in the United States succumbed to prostate cancer (2).

Approximately 98% of prostate cancer cases are glandular in origin (3); the microscopic diagnosis of prostate adenocarinoma is based primarily on certain features of glandular formation and pattern. The most accepted and used grading protocol is the Gleason score; using this classification, prostate adenocarcinoma can be stratified on a histological basis to provide significant prognostic information for urologists. Prostate cancers are also multifocal in nature; the majority have an average of at least two geographically distinct foci of varying histological patterns and thus differing Gleason scores.

Prostatic cancer is rarely symptomatic early in its course, as the majority of malignancies arise in the peripheral portion of the gland away from the prostatic urethra. Symptomatic presentation often implies local extension or even metastatic disease. As the cancer begins to involve the urethra and/or bladder neck, obstructive voiding symptoms often develop; these can include hesitancy, slowing of the urinary stream and intermittent flow. Irritative voiding symptoms, like frequency and urgency, may also occur although these are more difficult to attribute to cancer, as they are also associated with benign prostatic hyperplasia (BPH). With tumor progression, patients may also notice sexual symptoms such as hematospermia and/or decreased ejaculatory volume secondary to ejaculatory duct obstruction. Erectile dysfunction may also be observed if there is local encroachment on neurovascular bundles.

Bony pain is often a sign of metastatic involvement of the skeleton; human prostate cancer is one of the rare cancers that repeatedly produces osteoblastic metastases to the bone in 95% of cases (4). Other signs of metastasis include anemia secondary to bone marrow involvement and lower body edema due to obstruction of local lymphatics and veins. However, in the last 15 years, the percentage of patients presenting symptoms has decreased in proportion to the patients diagnosed with prostate cancer largely due to the use of prostate specific antigen (PSA) screening.

2. Diagnostic and prognostic markers

Prostatic acid phosphatase (PAP). In terms of biochemical markers, the first to be used routinely in the diagnosing

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and staging of prostate cancer was PAP. PAP hydrolyzes esters under acidic conditions to yield inorganic phosphates. Although identified in numerous organs such as the liver, brain and lungs, the highest concentration of PAP is identified in the prostate. Prostate epithelial cells secrete PAP into the glandular lumen and can be measured by either immunoassay or enzymatic assay.

A 1938 study showed PAP to be elevated in the prostates of patients with metastatic prostate cancer (5). However, a subsequent study showed that PAP levels in prostate cancer tissue were only a fraction of those identified in patients with BPH (6). Furthermore, in a study that evaluated PAP levels in 102 patients, 84% of patients that had elevated PAP levels were subsequently found to have either extracapsular extension or metastatic disease (7). One study estimated that the probability of having surgically curable disease in patients with elevated PAP could be as low as 5% (8). With the advent of serum PSA testing, PAP level evaluation has become decreasingly informative. In a study involving 460 consecutive patients referred to Johns Hopkins (Baltimore, MD, USA) (9), PAP was found to be elevated in only 4.6% patients and provided useful staging information beyond that available from PSA measurements in only 0.9% of cases.

PSA. PSA, or human kallikrein 3 (hK3) is a 33-kDa serine-protease of the tissue kallikrein family that was identified in prostatic extracts in the 1970s (10). PSA was discovered in human sera by Wang *et al* (11) and later isolated from prostate tissue by Papsidero *et al* (12). PSA is primarily produced by the ductal and acinar cells of the prostatic epithelium, as well as male periurethral glands, and is secreted into seminal fluid; its physiological function is to liquefy seminal coagulum in the human ejaculate (13). PSA is produced by normal, hyperplastic and neoplastic prostate tissue; however, the highest concentrations are identified in the prostatic transition zone of BPH patients (14). The majority of PSA circulates in serum while bound to protease inhibitors, such as a_1-antichymotrypsin and a_2-macroglobulin, whilst the remaining PSA exists unbound or free. Processes, such as inflammation, hyperplasia and neoplasia, within the prostate lead to disruption of physiological barriers and increased basement membrane permeability and thus increased release of PSA into the circulation (15).

PSA is widely used to screen for prostate cancer. PSA screening is less expensive than transrectal ultrasound (TRUS), and it can detect more prostate cancers than digital rectal examination (DRE) or TRUS, and is more likely to be organ-confined compared to those cancers discovered by DRE alone (16-18). The current percentage of prostate cancers that are organ-confined is estimated to be 70-80%; this is compared to 20-30% prior to the use of PSA screening (19,20). However, it is not recommended to use PSA alone in screening for prostate cancer as this results in missing 18-28% cancers that would have been otherwise detected if using a cutoff PSA of 4 ng/ml in conjunction with DRE (20).

The analysis of multiple preoperative variables to predict the ultimate pathological stage of patients undergoing radical prostatectomy was first developed by Oesterling *et al* (21) in 1987, using PAP, Gleason score and clinical stage. Subsequently, PSA-based algorithms such as those developed by Partin *et al* and Blute *et al* (22-24) used the combination of Gleason score, clinical stage and serum PSA to predict organ-confined disease (with a concordance index of 0.76) and node-positive disease (with a concordance of 0.84). These algorithms aid urologists and patients in making decisions regarding definitive surgery by estimating disease recurrence following prostatectomy.

Although certain studies showed that PSA expression in prostate cancer tissue decreased with increasing Gleason score, serum PSA levels remained proportional to the volume, Gleason score and stage of the prostate cancer (25,26). This increase in serum PSA may be explained by an increased release of PSA secondary to increased disorganized prostatic epithelium in higher grade cancers. A multi-institutional study (22) involving >4,000 patients confirmed the linear association between PSA levels and tumor stage. While only 9% of patients with PSA >50 ng/ml had organ-confined disease, 64% patients with PSA <4 ng/ml had organ-confined disease.

Although PSA is the most popular biomarker for prostate cancer, it is one of the most controversial. A recent study recognized the disadvantage of PSA for the early detection of prostate cancer. It was found that multiple men must be screened, biopsied and diagnosed to prevent one fatality (27). This study sought to increase the specificity of screening for lethal prostate cancer at an early stage. The results suggested that screening for prostate cancer using PSA in men at ages 50-60 years should focus on those with PSA levels in the top quartile. It was noted that men in this group compromised the majority of subsequent cases of metastasis. Furthermore, it was recommended that men with elevated PSA levels should be tested for four kallikrein markers in order to aid in biopsy decision-making. This is one of numerous studies attempting to identify a way to increase the accuracy of PSA screening. The vast amount of literature on this subject has resulted in a number of differing opinions on the correct use of PSA and thus a more reliable marker is suggested.

hK2. hK2 is a member of the same family as PSA and exhibits ~80% homology in the amino acid sequence with PSA. Similar to PSA, the highest level of hK2 is in prostatic tissue (28). As opposed to PSA, hK2 exists mostly in a free, unbound state in the serum. In patients with PSA levels between 4 and 10 ng/ml, a higher percentage of free PSA (fPSA) signifies that the elevation in total PSA was more likely to be due to BPH and not cancer (29). Partin *et al* (30) reported that higher hK2 levels associated with lower fPSA levels increased the probability of identifying prostate cancer. In particular, in men with fPSA <25% and an hK2/fPSA ratio >0.18, there was an increase of 13-62% in the detection of prostate cancer. A later study (31) compared the preoperative levels of hK2 and PSA in patients that underwent prostatectomy; the sensitivity and specificity of hK2 in detecting organ-confined disease were 37 and 100%, respectively, compared to a sensitivity and specificity of 14 and 100%, respectively, for total PSA.

A recent study reported on the use of a porous silicon antibody immunoassay platform for the detection of serum levels of total hK2 (32). This more effective method uses 15 μl of serum with a total assay time of ~3 h. It has been reported that either the level of hK2 alone or in combination with PSA may improve the prediction of prostate cancer. This new system could contribute to future clinical practice by allowing a more accurate diagnosis of prostate cancer.

Insulin-like growth factor-1 (IGF-1) and insulin-like growth factor binding proteins (IGFPBs). IGF-1 and IGFBPs are associated with tumor prognosis and progression in patients with colon, breast, lung and prostate cancers (33). While an early study (34) showed that IGF-1 was detectable in prostate cancer and could be used adjunctively with PSA in early detection protocols, another study (35) could not find any association between systemic IGF-1 levels and the presence of prostate cancer.

However, plasma concentrations of IGFBP-2 and IGFBP-3 were associated with disease progression; IGFBP-3 was found to be lowest in patients with bony metastasis, progressively higher in patients with localized disease and highest in healthy subjects (36). Conversely, higher levels of IGFBP-2 were associated with organ-confined disease and lower levels were associated with disease progression (33).

Transforming growth factor-β1 (TGF-β1). The cytokines in the TGF-β1 family of polypeptides have been implicated in numerous steps of tumor development and elevated levels of TGF-β1 have been found in patients with various cancers (37). Specific immunohistochemical studies have demonstrated increased expression of TGF-β1 in neoplastic prostatic epithelium when compared to normal prostate tissue (38). A number of studies (39,40) have documented the positive correlation between elevated plasma levels of TGF-β1 and prostate cancer progression. In another study (41), no correlation was identified between plasma levels of TGF-β1 and prostate cancer stage, but a direct correlation was demonstrated between urinary levels of TGF-β1 and prostate cancer stage. In a study conducted by Shariat *et al* (42), the preoperative plasma levels of TGF-β1 were measured in 302 consecutive patients who underwent radical prostatectomy for clinically localized disease. The study reported that TGF-β1 plasma levels were significantly elevated pre- and postoperatively in patients shown to have extraprostatic extension, seminal vesicle involvement and metastasis to lymph nodes.

Interleukin-6 (IL-6). IL-6 has a role in various cellular activities, including regulation of immune function and bone turnover. Studies using immunohistochemistry have demonstrated that protein concentrations of IL-6 are increased 18-fold in localized prostate cancer tissue when compared with normal prostate cancer tissue. In addition, concentrations of IL-6 receptors are increased 8-fold in prostate cancer tissue when compared with normal tissue (43). Another study measured the plasma levels of IL-6 and IL-6 soluble receptor in 120 consecutive patients with clinically localized prostate cancer that underwent prostatectomy (44). Plasma IL-6 levels were highest in those patients with metastatic cancer; IL-6 soluble receptor levels were highest in those with bone metastases, followed by those in patients with regional lymph node involvement. A previous study that evaluated TGF-β1 levels also assessed the pre- and postoperative plasma levels of IL-6 and IL-6 receptor in a group of 302 patients with localized disease undergoing prostatectomy (39). Preoperative circulating levels of IL-6 and its receptor were associated with tumor volume and metastasis to lymph nodes, and levels of these two markers were decreased significantly following prostatectomy, regardless of whether cure was achieved.

Reverse transcriptase-polymerase chain reaction (RT-PCR). PCR is a molecular technique that amplifies minute amounts of DNA using sequence-specific primers and heat-stable bacterial DNA polymerase. When RNA is the starting material, reverse transcriptase is used to transcribe it into DNA prior to initiating the reaction. RT-PCR is extremely sensitivity in detecting tissue-specific mRNA of tumor markers such, as PSA (45), hK2 (46), and more recently, prostate-specific membrane antigen (PSMA).

PSMA is a 100-kDa transmembrane glycoprotein identified in all types of prostatic tissue, but particularly elevated in carcinomas. PSMA levels have been used (47) to detect prostate cancer cells in the blood using RT-PCR and other modalities that employ radionuclide tagging (ProstaScint). However, another study (48) reported the detection of high PSMA mRNA levels in healthy donor bloods via RT-PCR. Further studies are required to assess the utility of using PSMA in the detection of prostate cancer.

Fatty acid synthase (FAS). Epstein *et al* (49) first studied the immunohistochemical staining for oncoantigen 519 (OA-519), an FAS in radical prostatectomy specimens, and suggested that OA-519 staining provided predictive information regarding pathological stage, beyond that provided by Gleason scores. In another study (50), 99 primary prostate cancer specimens were evaluated for OA-519 reactivity and the patients were followed for a mean of 4 years for disease progression. Patients who were positive for OA-519 reactivity were more likely to progress than their OA-519 negative counterparts, and furthermore, in patients with low to intermediate Gleason scores 2-7, OA-519 reactivity was shown in multivariate analyses to be the only significant predictor of cancer progression. Another study (51) examined frozen-needle prostate biopsies and identified that FAS signaling increased in the intensity from low-grade prostatic intraepithelial neoplasia (PIN) to high-grade PIN and to the highest intensity in invasive carcinoma. In addition, higher FAS signaling was associated with a higher proliferative index. The study proposed that FAS expression is an early event in the development of prostate cancer and may be used as a general prostate cancer marker.

Early prostate cancer antigen (EPCA). EPCA, a nuclear structural protein associated with prostate cancer, was identified (52) via immunohistochemistry to be expressed throughout the prostate in individuals with prostate adenocarcinoma. In a study involving 25 patients who had previously negative prostate biopsies and later found to have prostate cancer, EPCA staining intensity was assessed in the negative biopsy specimens, the subsequent positive biopsy specimens, and the eventual prostatectomy specimens. EPCA staining was found to have an 84% sensitivity and 85% specificity in detecting prostate cancer. The study suggest that EPCA immunohistochemistry has the potential to detect prostate cancer 5 years earlier than current protocols, as well as limit the number of biopsies performed as a result of increased PSA levels.

Secretory markers (urine-based diagnostics). In a study by Bolduc *et al* (53), it was found that urinary PSA may have potential in contributing to the differential diagnosis of prostate cancer and BPH. In particular, serum PSA levels between

2.5 and 10 ng/ml, and a low urinary PSA to serum PSA ratio are associated with prostate cancer.

Other promising research involves glutathione s-transferase (GSTP), a cytosolic enzyme that converts certain toxic compounds to glutathione (54). The detection of GSTP1 methylation in urine has shown potential as a biomarker in the diagnosis of prostate cancer. The methylation of GSTP may help distinguish patients with BPH from those with prostate cancer by improving the specificity of PSA.

A novel protein that has shown much evidence in the diagnosis of prostate cancer is PCA3. Multiple studies (55-57) have proven PCA3 to be a useful marker that can be used alongside PSA and DRE for a more accurate diagnosis, mainly due to its increased specificity (58). One study (59) suggests that as the PCA3 score appears to differentiate based on tumor volume and Gleason score, it may be used to select men with low-grade or low-volume cancer for clinical purposes and further research. Furthermore, PCA3 urine tests have been shown to improve specificity in prostate cancer diagnosis, and may help lower the number of prostate biopsies performed on individual patients (60). Although PCA3 has been useful as a diagnostic marker, it does not appear to have any prognostic value (61).

The analysis of multiple urinary proteins, rather than one specific protein, is also a novel idea. Instead of simply studying PSA or PCA3 levels, it has been proposed that combinations of proteins, such as TMPRSS2-ERG and PCA3, be analyzed for early detection (62). Laxman *et al* (63) suggest using a multiplex biomarker analysis of urine involving proteins such as golgi phosphoprotein 2 and serine protease inhibitor Kazal-type 1, as well as PCA3, to help detect prostate cancer in its early stages.

3. Tissue/cell-specific detection

Combining mulitple genes used as biomarkers, such as GalNac-T3, PSMA, hepsin and PCA3, in RT-PCR analysis can be a powerful new method to distinguish between prostate cancer and BPH (64).

Prostasomes have also recently been linked to the incidence of prostate cancer. A previous study has shown that malignant prostate cancer cells produce and secrete prostasomes (65). Secretion of prostasomes by prostate cancer cells is problematic (66) as they promote angiogenesis by producing and overexpressing tissue factor (67). Therefore, it is possible that monitoring the amount of prostasomes can be used as a potential marker to detect the presence of cancerous tissue.

4. Circulating markers (blood-based diagnostics)

While the PSA test is the current standard in blood-based diagnostic tests for prostate cancer, research is currently being performed to find an alternative and improved blood-based diagnostic test.

Chavarro *et al* (68) compared fatty acid levels in the blood to the risk of prostate cancer. Increased levels of trans-fatty acid in the blood is associated with nonaggressive prostate tumors, and increased polyunsaturated fatty acid levels in the blood are associated with a reduced risk of prostate cancer (69). Studying the levels of fatty acids within the blood can therefore be used as potential biomarkers in the prognosis of prostate cancer.

Gann *et al* (70) revealed that high levels of circulating testosterone, low levels of sex-hormone binding globulin and low levels of circulating estradiol may all contribute to an increased risk of prostate cancer, indicating that sex hormones circulating in the blood may also contribute to the occurrence of prostate cancer. However, another study reported that apart from testosterone, there is no significant correlation between the majority of sex hormones and the occurrence of prostate cancer (71). The influence of testosterone on prostate cancer is specific to the aggressive form of prostate cancer, and not the nonaggressive disease (72).

5. Future markers in development

The field of prostate cancer biomarkers is extensive; however, the search for a more rapid and accurate marker continues. Recent study has utilized genomic testing as a biomarker for aggressive prostate cancer. A recent discovery of the long noncoding RNA SChLAP1 in the prostate has provided a novel biomarker that not only adds to the ability to identify prostate cancer, but also to conventional risk stratification (73). SChLAP1 has been clinically validated for the prognosis of aggressive prostate cancer and integration of genomic tests may advance the diagnosis of prostate cancer through early identification of high-risk patients.

Exosomes have proved to be non-invasive cancer biomarkers as tumor-specific molecules can be found in exosomes isolated from biological fluids. A recent study has explored the proteome of urinary exosomes by utilizing mass spectrometry to identify proteins that are expressed differentially in prostate cancer patients (74). The study found that when comparing normal and prostate cancer samples of urinary exosomes, 246 proteins were differentially expressed. Of these, 221 were upregulated in exosomes from the prostate cancer patients. Although a number of the proteins exhibited high specificity and sensitivity as individual biomarkers for prostate cancer, when combined in a multi-panel test they had the potential for full differentiation of prostate cancer from non-disease controls. In conclusion, this study presents the potential of using urinary exosomes in the diagnosis and clinical management of prostate cancer.

6. Conclusion

The present review explored a portion of current and future biomarkers used in the detection of prostate cancer. A study from 1938 showed PAP to be elevated in the prostates of patients with metastatic prostate cancer, thus identifying the first biomarker used routinely in diagnosing and staging of prostate cancer. In the 1970s, PSA, also known as hK3, was discovered and today it remains the most widely used, and controversial, biomarker in prostate cancer.

The search for a more rapid, specific marker for the detection of prostate cancer has lead to numerous laboratories examining biomarkers. Although a number of markers have been acknowledged, there is yet to be one that is widely accepted and used. Research now concentrates on genomic factors. For example, SChLAP1 has been validated for the

prognosis of aggressive prostate cancer and it is suggested that the integration of genomic tests may advance the diagnosis of prostate cancer through early identification of high-risk patients. Furthermore, the potential of using urinary exosomes in the diagnosis and clinical management of prostate cancer has been explored. Understanding the biochemical and genetic aspects of prostate cancer biomarkers not only has the ability to more efficiently detect prostate cancer, but it may also provide an insight into how and why prostate cancer arises, and may suggest a method to manage or even cure it.

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